

Table of contents

PREFACE	5
SUMMARY	8
SAMMENDRAG	10
ABBREVIATIONS	12
1 INTRODUCTION	13
2 BACKGROUND	15
2.1 n-3 LCPUFA essentiality	15
2.1.1 Metabolism	15
2.1.2 Physiological functions	17
2.2 n-3 LCPUFA roles in brain development and function	20
2.2.1 Summary and potential implications for brain function in children	21
2.3 Neuropsychological functions.....	22
2.3.1 Cognitive functions	23
2.3.2 Socioemotional function.....	27
2.3.3 Stress	29
2.4 Sex differences in brain development and neuropsychological function	30
2.5 n-3 LCPUFA and neuropsychological function	32
2.5.1 Effects of n-3 LCPUFA on cognition and socioemotional function.....	32
2.5.2 Effects of n-3 LCPUFA on stress.....	40
2.6 Fish as a source of n-3 LCPUFA	42
2.6.1 Recommendations and intake of fish in Danish children.....	42
3 OVERALL AIM, SPECIFIC OBJECTIVES AND HYPOTHESES.....	44
4 METHODS.....	46
5 OVERVIEW OF PAPERS.....	47

6 PAPER I	49
7 PAPER II	84
8 PAPER III	116
9 GENERAL DISCUSSION	141
9.1 Effects of n-3 LCPUFA on cognition, emotions and behavior in healthy children	141
9.1.1 Potential sex-specificity in effects.....	142
9.1.2 Consistency in results from observational and intervention studies	143
9.2 Effects of n-3 LCPUFA on stress in healthy children	144
9.3 Overall effect of n-3 LCPUFA on neuropsychological function	145
9.4 Strengths and limitations	148
9.5 Methodological considerations	148
9.5.1 Dose and duration	148
9.5.2 Fish vs. n-3 LCPUFA supplementation	149
9.5.3 Choice of control.....	150
9.5.4 Outcome measures	151
10 CONCLUSION AND FUTURE PERSPECTIVES	154
ACKNOWLEDGEMENTS	156
LIST OF ADDITIONAL PUBLICATIONS	158
REFERENCES	159

Summary

Background: Long-chain n-3 polyunsaturated fatty acids (n-3 LCPUFA), especially docosahexanoic acid (22:6n-3, DHA), are accreted in the brain and likely play important roles in brain development and function. The accretion is substantial during early infancy and continues throughout childhood. However, randomized clinical trials (RCTs) in children show inconsistent effects of n-3 LCPUFA on neuropsychological functions such as cognition, emotions and behavior and no trials in children investigated effects on stress. Inconsistencies might arise from differences in the tests used and moreover, some studies indicate potential sex-specificity in the effects which could contribute to inconsistencies. Thus, assessment of overall patterns in the effects and potential sex differences might be important. Furthermore, only few of the studies investigated effects of fish *per se*, which likely resemble real life effects of fish consumption better than studies with n-3 LCPUFA supplements.

Objective: The main objective of this thesis was to investigate effects of n-3 LCPUFA provided through oily fish on cognition, emotions and behavior in healthy children, and secondly to investigate the effects on stress. These effects were investigated with a focus on potential sex differences. To substantiate interpretation of effects on cognition, emotions and behavior, we investigated overall patterns in performance.

Methods: The objectives were investigated in the FiSK Junior study, which was a two-arm parallel RCT in which 199 healthy Danish 8-9-year-old children were randomized to consume ~300 g/week oily fish or poultry (control) for 12±2 weeks. At baseline and endpoint, we assessed cognitive functions including attention, processing speed, executive functions, and memory, as well as emotions and behavior with a large test battery of tests and questionnaires. We used principal component analysis to explore overall patterns in cognitive performance as well as socioemotional traits (emotions and behavior) and the generated principal components were used as scores of overall function. We furthermore assessed the physiological response to cold-induced stress by measuring blood pressure, heart rate variability (HRV) and salivary cortisol and assessed general stress level by measuring cortisol released in the hair during the last ~3 months. Erythrocyte n-3 LCPUFA was used as biomarker for fish intake.

Results: Fish intake dose-dependently improved the overall score of cognitive performance. This was driven by beneficial tendencies in several cognitive domains and by improvements in single measures of attention and cognitive flexibility. The fish intervention furthermore reduced a score of parent-rated socioemotional difficulties, mainly due to a reduction in emotional problems, and this was reflected in a tendency to reduction in the overall score of socioemotional problems. Some effects appeared to differ for boys and girls in the attention domain and the reduction in emotional problems was twice as strong in girls compared to boys. In contrast to the effects of the fish intervention, the analyses of baseline associations with erythrocyte n-3 LCPUFA indicated a positive association with processing speed and negative association with a single measure of attention, and no association with the overall cognitive performance score. Moreover, we observed no overall or gender-specific associations for the socioemotional scores. The fish intervention furthermore appeared to reduce stress responsiveness reflected by changes in HRV that indicated increased parasympathetic activity during the cold-induced stress and a tendency to improved endurance to the stress-stimulus. Interestingly, we observed a marked HRV response during recovery from the stress-stimulus with a sympathetic peak followed by a parasympathetic peak of which the latter appeared higher in the fish group. The effect on general stress tended to be modified by sex with a decrease in hair cortisol among boys but increase among girls in response to fish intake, although these effects were not significant.

Conclusion: Our findings suggested overall beneficial effects of oily fish on neuropsychological function in healthy children. Thus, our results substantiate the importance of n-3 LCPUFA for optimal brain function and recommendations of fish consumption in children. The effects of n-3 LCPUFA on specific cognitive domains remains inconclusive, possibly due to differences in the measured outcomes as we observed lack of correlation between measures supposed to reflect the same function, especially for the more complex cognitive domains. Thus, a deeper understanding of the functions underlying performance in different tests is needed. Likewise, there is a need for better characterization of a typical response to stress induced by various methods. This would be relevant to explore along with perceived stress in future studies. Furthermore, the indicated potential sex-specificity in effects of n-3 LCPUFA on some neuropsychological functions needs to be investigated in studies that are powered to perform sex-stratified analyses.

Sammendrag

Baggrund: n-3 langkædede flerumættede fedtsyrer (n-3 LCPUFA), især docosahexansyre (22: 6n-3, DHA), indbygges i hjernen og spiller sandsynligvis vigtige roller i hjernens udvikling og funktion. Indbygningen er betydelig i de første leveår og fortsætter gennem hele barndommen. Randomiserede forsøg med børn viser imidlertid inkonsistente effekter af n-3 LCPUFA på neuropsykologiske funktioner såsom kognition, følelser og adfærd, og ingen forsøg med børn har undersøgt effekter på stress. Uoverensstemmelserne kan skyldes forskelle i de anvendte tests, og endvidere indikerer nogle studier potentiel kønsspecificitet i virkningerne, hvilket kan bidrage til inkonsistensen. Derfor er undersøgelse af de overordnede mønstre i effekter og potentielle kønsforskelle muligvis vigtig. Endvidere har kun få studier undersøgt virkningerne af fisk, hvilket sandsynligvis afspejler de virkelige effekter af fiskeindtag bedre end studier med n-3 LCPUFA tilskud.

Formål: Hovedformålet med denne afhandling var at undersøge virkningerne af n-3 LCPUFA givet gennem fed fisk på kognition, følelser og adfærd hos sunde børn og dernæst at undersøge virkningerne på stress. Disse effekter blev undersøgt med fokus på potentielle kønsforskelle. For at underbygge fortolkningen af effekter på kognition, følelser og adfærd undersøgte vi de overordnede mønstre i præstationer.

Metoder: Vi undersøgte formålene i FiSK Junior studiet, som var et to-arms parallelt randomiseret forsøg, hvor 199 sunde danske 8-9-årige børn blev randomiseret til at indtage ~ 300 g/uge fed fisk eller fjerkræ (kontrol) i 12 ± 2 uger. Ved baseline og endpoint undersøgte vi kognitive funktioner inklusive opmærksomhed, processerings-hastighed, eksekutive funktioner og hukommelse samt følelser og adfærd med et stort testbatteri af test og spørgeskemaer. Vi brugte principalkomponent analyse til at undersøge overordnede mønstre i kognitiv præstation såvel som socioemotionelle træk (følelser og adfærd) og de genererede principalkomponenter blev anvendt som scorer for overordnet funktion. Vi undersøgte endvidere det fysiologiske respons på kulde-induceret stress ved at måle blodtryk, hjerterytme variabilitet (HRV) og spytcortisol og vurderede det generelle stressniveau ved at måle cortisol frigivet i håret i de seneste ~ 3 måneder. Erythrocyt n-3 LCPUFA blev anvendt som biomarkør for fiskeindtag.

Resultater: Fiskeindtaget forbedrede den overordnede score for kognitiv præstation dosisafhængigt. Dette var drevet af gavnlige tendenser inden for flere kognitive domæner og af forbedringer i enkelte mål for opmærksomhed og kognitiv fleksibilitet. Fiskinterventionen reducerede endvidere en score for forældre-vurderede socioemotionelle vanskeligheder, hovedsageligt på grund af en reduktion i følelsesmæssige problemer, og dette blev afspejlet i en tendens til reduktion i den overordnede score for socioemotionelle problemer. Nogle effekter syntes at variere for drenge og piger inden for opmærksomhedsområdet, og reduktionen i følelsesmæssige problemer var dobbelt så stærk i piger i forhold til drenge. I modsætning til effekterne af fiskeinterventionen indikerede analyser af baseline associationer med erythrocyt n-3 LCPUFA en positiv association med processerings-hastighed og negativ association med et enkelt mål for opmærksomhed og ingen association med den overordnede kognitive præstations-score. Endvidere observerede vi ikke nogen overordnede eller kønsspecifikke associationer for de socioemotionelle scorere. Fiskinterventionen syntes også at reducere stressfølsomhed afspejlet ved ændringer i HRV, som indikerede forøget parasympatisk aktivitet under kulde-induceret stress og en tendens til forbedret udholdenhed under stress-stimuleringen. Vi observerede et interessant markant HRV-respons under hvilefasen efter stress-stimuleringen med en sympatisk top efterfulgt af en parasympatisk top, hvoraf sidstnævnte syntes højere i fiskegruppen. Virkningen på generel stress tenderede til at være modificeret af køn med et fald i hårcortisol blandt drenge, men stigning blandt piger efter fiskeindtag, skønt disse effekter ikke var signifikante.

Konklusion: Vores resultater tydede samlet set på gunstige virkninger af fed fisk på neuropsykologisk funktion hos sunde børn. Vores resultater understøtter således vigtigheden af n-3 LCPUFA for optimal hjernefunktion og anbefalinger for fiskeindtag hos børn. Virkningerne af n-3 LCPUFA på specifikke kognitive domæner forbliver ukonklusiv, muligvis på grund af forskelle i de anvendte mål, da vi observerede manglende korrelation mellem mål, der antages at afspejle den samme funktion, især for de mere komplekse kognitive funktioner. Således er det nødvendigt med en dybere forståelse af de funktioner, der bidrager til præstation i forskellige test. Ligeledes er der behov for bedre karakterisering af en typisk respons på stress induceret ved forskellige metoder. Dette ville være relevant at udforske sammen med den selvopfattede stress i fremtidige studier. Desuden skal den indikerede potentielle kønsspecificitet i effekter af n-3 LCPUFA på visse neuropsykologiske funktioner undersøges i studier med power til at udføre køns-stratificerede analyser.

1 Introduction

Nutrition plays an important role in children's brain development (1). Intake of n-3 long-chain polyunsaturated fatty acids (n-3 LCPUFA), especially docosahexanoic acid (22:6n-3, DHA), is considered particularly important for neuronal development due to a substantial accretion of DHA in brain cell membranes (2). This incorporation of DHA affects cell function, among others cell signaling, and might thus affect brain function (3). Intake of n-3 LCPUFA has therefore been suggested to affect neuropsychological functions, including cognition, emotions, behavior and stress responsiveness (4), which are known to be linked to specific brain regions and pathways.

Due to the rapid growth and development of the brain from gestation up until two years of age, most research within the area of n-3 LCPUFA and neuropsychological function has focused on this time period. During the early brain growth spurt, a substantial amount of DHA is accreted in the brain (2), but despite this, the evidence regarding n-3 LCPUFA and neurodevelopment during infancy remains inconclusive (5-7) although recent meta-analyses suggest some beneficial effects on mental and psychomotor development (8, 9). The accretion of DHA in the brain continues throughout childhood, especially in the frontal cortex important for cognitive functions such as executive functions, attention and memory as well as emotional regulation, impulse control, social behavior and stress responsiveness (10, 11). However, randomized trials in school-aged children show inconsistent effects of n-3 LCPUFA on neuropsychological functions and to my knowledge, no trials in children investigated effects on stress responsiveness. The inconsistencies might be due to methodological differences, especially the use of different neuropsychological tests and questionnaires. Furthermore, sex differences in the effects may contribute to inconsistencies as some studies have reported sex-specific associations between n-3 LCPUFA and cognitive performance in children (12-14).

Fish, especially the oily types, is the primary dietary source of n-3 LCPUFA, and dietary recommendations therefore encourage fish consumption, but only very few studies investigated the effects of fish *per se*. Two studies showed dose-dependent overall beneficial effects of fish consumption on cognitive performance in school children and preschool children, respectively (13, 15). The study in school children, which compared effects of a school meal intervention high in fish to habitual lunch, did however find a reduction in attention, and interestingly also increases

in impulsivity and reading performance specifically in boys (16). Furthermore, one study in adolescents found that an oily fish intervention improved processing speed, reduced attention and had no effect on mental health (17, 18). A lack of effect on mental health was likewise observed in the aforementioned study with preschool children (19). Moreover, another study in preschool children found improvement in some tests of fluid intelligence, but not general intelligence in response to oily fish intake (20). However, more evidence is needed to evaluate the effect of n-3 LCPUFA, especially when consumed as fish, on neuropsychological function in children.

The overall aim of this thesis was to investigate effects of n-3 LCPUFA intake on neuropsychological functions, including cognitive functions, emotions, behavior and stress in healthy children. In our study, this was investigated through an oily fish intervention in 8-9-year-old Danish children that had a low baseline consumption of fish in line with Danish children in general. The effects were investigated with a focus on potential sex differences and to substantiate the overall interpretation of findings, we investigated overall patterns in performance and correlations between measures within specific domains. To our knowledge, this is the first study to investigate the effects of a pure fish intervention on neuropsychological outcomes in this age group of children.

5 Overview of papers

Table 2. Overview of the papers included in this PhD thesis

	Title	Design	Research objectives	Main findings
I	“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	Cross-sectional	To explore overall patterns in cognitive and socioemotional function, including basic sex differences, and assess correlation within domains. To explore associations between n-3 LCPUFA status and these functions, including potential sex differences.	The principal components that explained most of the variation in cognitive scores reflected overall cognitive performance and secondly speed-accuracy trade off. For socioemotional scores they reflected overall problems followed by the nature of problems (externalizing vs. internalizing problems). Boys were faster, had lower attention and higher externalizing problem scores than girls. Only the cognitive measures within processing speed, attention and impulsivity domains correlated and parent-rated scores for externalizing and internalizing problems correlated strongly, whereas correlations with child-rated scores were weak. n-3 LCPUFA status associated positively with processing speed and negatively with attention, but except for processing speed, scores within specific domains did not consistently associate with n-3 LCPUFA. Some sex differences were indicated for associations with attention and impulsivity.
II	“Effects of oily fish intake on cognitive and socioemotional function in healthy 8-9-year-old children: The FiSK Junior randomized trial”	RCT	To investigate effects of oily fish on overall scores as well as domain-specific scores of cognitive and socioemotional function, including potential sex differences.	Oily fish dose-dependently improved the overall cognitive performance score driven by beneficial tendencies in several cognitive domains and improvement in single measures of attention and cognitive flexibility. The fish intervention furthermore reduced a parent-rated score of total socioemotional difficulties, mainly due to reduction in internalizing

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problems, and this was reflected in tendency to reduction in the overall socioemotional problems score.

Paper	"Effects of oily fish	consumption on acute and	long-term stress in healthy	8-9-year-old children: The	FISK Junior randomized	trial"	Draft
III	To investigate effects of oily	fish on acute stress response	and long-term stress,	including potential sex	differences.	Only fish had few effects that suggested reduced stress responsiveness to	acute cold-induced stress, including increased HRV HF and tendency to
							reduced HR as well as tendency to improved stress endurance.
						Interestingly, the strongest HRV response was observed after the test,	where there appeared to be group differences that might reflect stronger
						PNS recovery in the fish group. There was a tendency to a sex modification	of the fish-induced effect on long-term stress measured by hair cortisol with
						a decrease in boys but increase in girls.	

HF, high frequency power; HRV, heart rate variability; n-3 LCPUFA, n-3 long-chain polyunsaturated fatty acids; PNS, parasympathetic nervous system; RCT, randomized clinical trial