The effect of maternal fish oil supplementation during lactation on body composition and blood pressure in 13-year old children

Master Thesis in Human Nutrition

by

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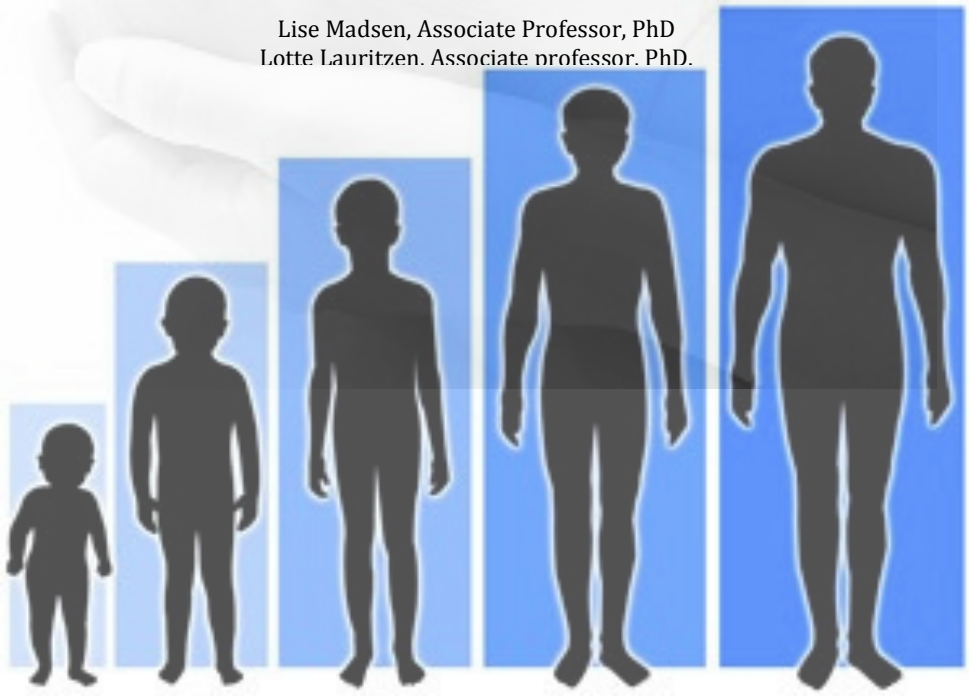

Foreword;

The present 13-year follow-up is based on a double-blinded randomized placebo-controlled intervention trial which included two previous follow studies at 2.5-years and at 7 years. The intervention trial was designed to contribute to novel insights of infant nutrition and later health and was conducted from February 1999 to May 2000 at the Department of Human Nutrition, the Faculty od Life Sciences, Copenhagen University, Frederiksberg. The present 13-year follow up study was conducted from October 2012 to January 2013 and included those mothers who were recruited from the intervention trial and their 13-year old children. The mothers from the intervention trial were supplemented with fish oil or olive oil during the first four months of lactation. In order to investigate the potential long-term effects on blood pressure and body composition of fish oil supplementation during the four first months of lactation, I performed a series of examinations collecting data on anthropometric measurements and of blood pressure on the 13-year old children. The children filled out a food frequency questionnaire in order to collect dietary data from the children, and the children also completed a puberty test (Tanner Scales) in order to assess the child's pubertal maturation stage.

Data collection involved a structured and flexible time plan in order to achieve as many examinations as possible, which were carried out either at Department of Human Nutrition or at the home of the child. During the approximately three months of examinations I managed to collect data from a total of 58 children. It has been a challenge to administer the practical work and data collection on my own, but indeed very educational. It has especially been a pleasure to work with the participating families who have made the whole process a good experience.

Thanks to Lotte Laurtizen who kindly welcomed me all the way from Bergen and were willing assist me during the process.

Abstract

Background: It has long been recognized that the long-chain n-3 polyunsaturated fatty acid (n-3 LCPUFA), docosahexaenoic acid (DHA) accumulates in the brain during the first years of life and that this is potentially affected by dietary intake. Breast-feeding has been linked to several beneficial health outcomes, several of which have been suggested to be due to the presence of n-3 LCPUFA in breast milk. Based on data from rodents, perinatal intake of n-3 LCPUFA has also been speculated to play a programming role in relation to later body composition and blood pressure.

Objective: To investigate the long-term effect of supplementation with fish oil during the first four months of lactation on body composition and blood pressure in 13-year old children.

Design: In a randomized controlled trial mothers with a low fish intake (below the population median) were administered with either fish oil or olive oil during the first four months of lactation. The trial also included a high-fish intake reference group. Of the 149 children who completed the intervention period a total of 60 children were followed up at 13 years with 21 from the HF-group, 20 from the FO-group, and 19 from the OO-group. Blood pressure and anthropometric measurements was measured, diet was assessed by an electronic food frequency questionnaire and pubertal stage was assessed by Tanner Scales. Diet and puberty are factors that are considered to affect body composition and blood pressure.

Results: In body composition, no significant differences were found between the two randomized groups, when adjusting for ponderal index at birth and sex. However, the FO group had a significantly larger non-adjusted mean head circumference compared to the OO-group ($p=0.029$). Significant group*sex interactions were found in MAP ($p=0.038$) and DBP ($p=0.019$). The girls from the FO-group had a tendency towards a lower MAP compared to the girls from the OO-group ($p=0.079$. A significant positive association between MAP and maternal RBC-DHA ($p=0.028$) and between DHA in maternal breast milk and MAP ($p=0.043$) were found in boys A tendency towards a positive association between maternal RBC-DHA and DBP in boys was found ($p=0.05$).

Conclusion: The significant difference in blood pressure observed between the boys from the two randomized groups at 7-year was no longer evident at 13-year. The difference in body composition observed at 2.5 years, was at 7 years diminished, and now we observed no significant difference in body composition at 13 years. I found a significant sex*group interaction in mean arterial and diastolic blood pressure, which indicated that the effect of the intervention differs between boys and girls. Due to a

small sample size in the randomized groups, the results might be inaccurate, and further studies are need to confirm these findings,

Resume in Danish

Baggrund; Man har længe kendt til at den langkædede polyumættede fedtsyre docosahexaensyre (DHA) akkumulerer I hjernen i det første leveår hvilket sandsynligvis er påvirket af kostindtag. Amning har været forbundet med flere gavnlige sundhedsmæssige tilstande, hvoraf flere er blevet foreslået at skyldes tilstedeværelsen af n-3 LCPUFA i modermælk. Baseret på data fra gnavere, har perinatal indtag af n-3-LCPUFA også blevet spekuleret at spille et programmerings rolle i forhold til senere kropssammensætning og blodtryk.

Formål: At undersøge langtidseffekten af et dagligt tilskud af fiskeolie til mødre I de første fire måneder af amme perioden på kropssammensætning og blodtryk i 13-årige børn

Design: I et dobbelblindet interventionsstudie blev ammende mødre med et lavt fiskeindtag randomiseret til tilskud med 4.5g/d fiskeolie eller olivenolie. Derudover blev en referencegruppe af mødre med et højt fiskeindtag inkluderet I studiet. Ud af de 149 børn som gennemførte interventionsperioden deltog 60 børn I dette 13-års opfølgningsstudie, hvoraf 20 fra fiskeolie-gruppen, 19 fra olivenolie gruppen og 21 fra referencegruppen. Ved 13 år målte jeg vækst, kropssammensætning og blodtryk. Kostoplysninger var samlet ind med et madfrekvens-spørgeskema og pubertetsstatus var vurderet ved brug af Tanner Scales. Kost og pubertet har begge en potential indvirkning på kropssammensætning og blodtryk.

Resultater; Ingen signifikante forskelle var fundet i kropssammensætning mellem de to randomiserede grupper efter justering for ponderal index ved fødslen og køn. FO-gruppen et signifikant større hovedomkreds sammenlignet med OO-gruppen (p=0.029). En signifikant gruppe*køn interaktion blev fundet i middel arterielt blodtryk (p=0-038) and diastolisk blodtryk (0.019). Pigerne fra FO-gruppen havde en tendens til et lavere blodtryk sammenlignet med pigerne fra OO-gruppen (p=0.079). En signifikant positiv association mellem middel arterielt blodtryk og indholdet af DHA I moderens røde blodceller (0.028) og mellem indholdet af DHA I moderens brystmælk og middle arterielt blodtryk (p=0.043) var fundet I drengene. Derudover havde OO-gruppen et signifikant større energiindtag end FO-gruppen (p=0.002).

Konklusion: Den signifikante forskel I blodtryk observeret mellem drengene fra de to randomiserede grupper ved 7 år var ikke længere synlig ved 13 år. Forskellen I kropssammensætning observeret ved 2.5 år var ved 7 år reduceret og ikke længere synlig ved 13 år. I stedet fandt jeg signifikant gruppe*køn interaktion i middel arterielt og diastolisk blodtryk, som indikerer at effekten af interventionen er forskellig mellem køn. Stikprøven for dette opfølgningsstudie var imidlertid lille for de to randomiserede grupper, og flere fremtidige studier er nødvendige for at bekræfte disse resultater.

Abbreviations

AA	**Arachionic Acid**
ALA	**alpha-linolenic acid**
ANOVA	**Analysis of variance**
ANCOVA	**Analysis of covariance**
BMI	**Body mass index**
cAMP	**Cyclic Adenosine monophosphate**
CVD	**Cardiovascular disease**
DBP	**diastolic blood pressure**
DHA	**Docosahexaenoic acid**
EI	**energy intake**
EPA	**Eicosapentaenoic acid**
FFQ	**Food frequency questionnaire**
FO	**fFsh oil**
LA	**Linolenic acid**
LCPUFA	**Long-chain polyunsaturated fatty acid**
MAP	**Mean arterial blood pressure**
MUFA	**Monounsaturated fatty acid**
OO	**Olive oil**
PUFA	**Polyunsaturated fatty acid**
RBC	**Red blood cells**
SBP	**Systolic blood pressure**
SD	**Standard deviation**
SE	**Standard error**
SEM	**Standard error of the mean**

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Introduction

Nutritional influences during critical periods of pre-and postnatal human development may have long-term programming effect on health conditions in later life.

It has long been recognized that the long-chain n-3 polyunsaturated fatty acid (n-3 LCPUFA), docosahexaenoic acid (DHA) accumulates in the brain during the first years of life and that this is affected by dietary intake (Lauritzen, Hansen et al. 2001). Dietary n-3 LCPUFAs are required for normal conception, growth and development of the fetus. Based on data from rodents, perinatal intake of n-3 LCPUFA has been speculated to play a programming role in relation to later body composition (Ailhaud 1992) and blood pressure (Armitage, Pearce et al. 2003). From animal studies it is seen that n-3 LCPUFAs as a nutritional programming factor in pre-and postnatal life may affect the proliferation and differentiation of pre-adipocytes and prevent excessive adipose tissue development (Kim, Della-Fera et al. 2006). n-3 LCPUFAs may also have a beneficial long-term effect on blood pressure (Forsyth, Willatts et al. 2003), which is supported from findings from animal studies (Wyrwoll, Mark et al. 2006). However, scientific evidence from human trials are scarce, and studies have been performed with the purpose to investigate whether increased content of DHA in breast milk, increased via maternal fish oil supplementation, can affect body composition and blood pressure in infants. Breast-feeding may have long-term favorable effects on body composition (Owen, Martin et al. 2005) and blood pressure (Martin, Gunnell et al. 2005; Innis 2011) which could be explained by the presence and beneficial effect of n-3 LCPUFA in breast milk.

A Danish fish oil supplementation study has followed children's growth and development through their first year of life and conducted follow-up studies at 2½ and 7 years. In order to explore further the potential long-term effect of early intake of n-3 LCPUFA on risk of obesity in children another follow-up examination of the children from the maternal fish oil supplementation trial was performed with the now 13-year old children.

Objective - Research question

The purpose of the 13-year follow-up study was to investigate if supplementations with fish oil (and fish intake in general) in lactating mothers had any long-term effects on the child's body composition and blood pressure. The present follow-up study aims to investigate the following two research questions:

a) Does maternal fish oil supplementation in the first four months of lactation have detectable long-term effects on offspring body composition and if so, is the effect affected by intake of carbohydrates and protein?
b) Does maternal fish oil supplementation in the first 4 months of lactation have detectable long-term effects on the blood pressure of the offspring?

Furthermore, statistical analyses will be performed in order to investigate the following sub-questions:

- Do body composition, blood pressure and dietary intake measured at 13 years differ between the two originally randomized groups?

- Furthermore, when data from all three groups (including the reference groups) are combined, is there a correlation between intake of DHA in infancy (assessed in breast-milk and on maternal status) and later on body composition and blood pressure?

- Is the effect of early n-3 LCPUFA intake on fat deposits modified by dietary composition (especially by a high intake of protein relative to carbohydrates)?

- Can potential effects on body composition be explained by diet and could that in turn also explain any observed long-term effects of the maternal fish oil intervention on blood pressure?

- Does the effect of early n-3 LCPUFA intake on blood pressure, dietary intake, and body composition differ between girls and boys?

Background

Does early nutrition in infancy affect our adult health?

Programming and tracking

Does early nutrition in the peri-and/or postnatal period of life have long-term effects in terms of human health outcomes? Such theory is connected to the concept of ”programming” – which defines the process whereby the early development is affected by endogenous or exogenous factors which generate or ”program” long-term structural or functional changes in an individual organism (Lucas 1991; Lucas 1998; Innis 2011). Furthermore, such long-term effects might only arise, if the ”programming-stimulus” is applied at a critical or sensitive period in development. Such critical periods may occur *during embryonic, fetal or early infant development* according to the development of the specific health outcome of interest, e.g. blood pressure or development of fat mass (Lucas 1998). For this reason, programming seen in a nutritional perspective in the developing infant is considered to be of great importance because it may predispose later health by metabolic adaption, altered cell differentiation or epigenetic changes (Innis 2011).
A lot of focus revolves around programming in the prenatal period, a time with high plasticity making the intra uterine life vulnerable to environmental changes (Koletzko, Brands et al. 2012). However, recent research has also suggested that early-life nutrition in the peri- and postnatal period may be of great importance with respect to different health implications in later life (Srinivasan and Patel 2008; Tamashiro and Moran 2010)

Poor nutrition during early life may result in health implications that, when maintained over time, might be observed in adult life, and therefore can be considered to have been “tracked” into adulthood (Lane and Gill 2004). Analysis of tracking is often expressed as a correlation coefficient, in which the size of a specific outcome of interest is analyzed over time to identify if an association in size of the outcome can be detected between early life and into adult life. Tracking of a specific health implication from early life into adult life might be due to several factors that maintain this health implication over time. These factors include genetic, dietary or environmental factors. For instance, BMI might track from childhood into adulthood due to maintenance (or tracking) of dietary habits (Li and Wang 2008).

Growth and body composition

Growth velocity and fat mass development during childhood and infancy

Growth velocity in infants into late childhood is comprised of a number of phases. The first phase of growth takes place in the first year of life, and especially the first couple of months (Michaelsen 1997) when the adipose tissue significantly increases due to the extensive proliferation and differentiation of adipose precursor cells (Soriguer Escofet, Esteva de Antonio et al. 1996). The increase in fat mass typically reaches a postnatal adiposity peak around the age of 6-9 months with a percentage in body fat at approximately 25 % (Fomon, Haschke et al. 1982). The time period after the first growth phase the increase in adipocytes diminishes and the child's body length continues to increase, which result in a more lean body mass in which the child's BMI reaches a maximal leanness. At that point where body fatness declines to a minimum is designated as the adiposity rebound (AR), which occurs usually around the age of 5-7 years, however the exact age of AR varies greatly among children (Fomon, Haschke et al. 1982). The age at AR is defined as the lowest BMI between age range from 3 to 9 years, calculated form a pediatric growth chart {(Ohlsson, Lorentzon et al. 2012). In the second phase of growth, an expansion in number and size of adipocytes takes place and a gradual increase in BMI occurs throughout adolescence and most of adulthood.

Figure 1 illustrates the BMI growth curves of girls from 0-20 years and shows the approximate timing of a nadir BMI indicating the occurrence of AR (Rolland-Cachera, Deheeger et al. 1984). Much interest has revolved around the timing of the occurrence of AR as it has been suggested that an early incidence of AR might predict obesity in adolescence and adulthood (Rolland-Cachera, Deheeger et al. 2006; Addo and Himes 2010)

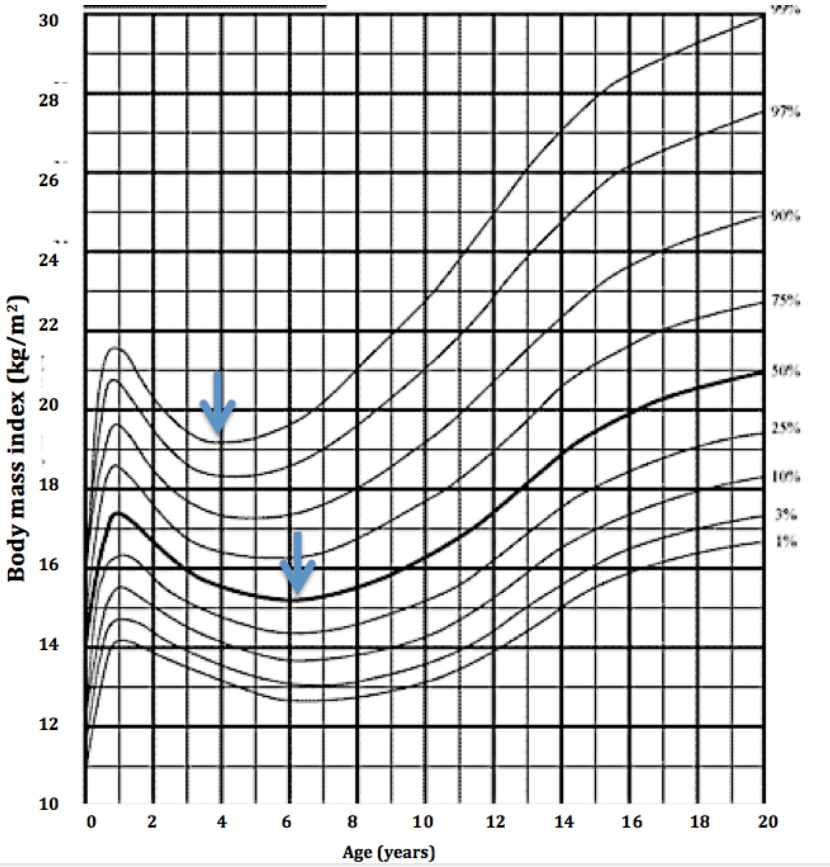


Figure 1. Danish reference curves for body mas index for girls. The blue arrows indicate time of adiposity rebound at different ages according to the specific BMI-percentile. (Figure borrowed from Danish Pediatric Society)

Age related changes that occur in AR could be shown by different anthropometric measurements other than BMI, including skin-fold thickness. Measurements of the thickness of skinfolds are considered as an important and valid measurement of subcutaneous fat, and are used as an anthropometric indicator of regional and total body fatness (Rolland-Cachera, Cole et al. 1991; Addo and Himes 2010). In addition, measurement of waist circumference is used to estimate central obesity together with measurements of the waist-height ratio, both being component predictors of risk factors of cardiovascular disease in children (Savva, Tornaritis et al. 2000)

Statistic population surveys of overweight and obesity

BMI is a validated measure of adiposity and is recommended as a measure of fatness in adults, however, due to the height and weight changes that occur during growth, which also varies with sex and age, it is complicated to make a stable interpretation of BMI from approximately 5-18 years of age (Freedman and Sherry 2009). For this reason overweight and obesity in children are determined through international and sex related BMI cut-off values that are used as reference in age-and gender specific groups (Cole, Bellizzi et al. 2000). Table 1 shows the BMI-cut off values for children in the aged 12-14 years.

Table 1. BMI cut-off values for children ages 12-14 years

	Overweight		Obese	
Age	Boys ($kg/m^{2)}$	Girls ($kg/m^{2)}$	Boys ($kg/m^{2)}$	Girls ($kg/m^{2)}$
12	21.22	21.68	26.02	26.67
12.5	21.56	22.14	26.43	27.24
13	21.91	22.58	26.84	27.76
13.5	22.27	22.98	27.25	28.20
14	22.62	23.43	27.63	28.57

The BMI cut-off values are defined as values corresponding to a BMI of 25 and 30 kg/m^2 by the age of 18, representing overweight and obesity, respectively (Cole, Bellizzi et al. 2000); (Nysom, Molgaard et al. 2001).

It has been reported that a significant prevalence of overweight and obesity among schoolchildren from the Copenhagen area in the period from 1947-2003 has emerged (Pearson, Olsen et al. 2005). The most recent data from the Danish Health Behavior in School-aged Children (HBSC) suggest that approximately 8% and 11 % of girls and boys, respectively, is overweight (Due 2011). However, these data were collected based on self-reported height and weight measurements. Data from another recent study with physicians conducting the weight and height measurements from 7541 15-year old children found that 12.9 % of the girls was overweight and 8.2% characterized as obese and 15.2 % of the boys were overweight and 14.1% was characterized as obese – suggesting that a much higher number of overweight and obesity in Danish school children (Krue and Coolidge 2010)

Prediction of obesity in later life; Adiposity rebound as predictor of later obesity?

AR defines the period where the second growth spurt initiates and is characterized by adipocytes hypertrophy and hyperplasia (Rolland-Cachera, Deheeger et al. 1984). It has been reported that children displaying an earlier AR, typical around the age of 3, gain body fat at an increased rate compared to children displaying AR at a later age typically around the age of 5-7 years (Rolland-Cachera, Deheeger et al. 2006; Williams and Goulding 2009; Dietz 1994). In a study observing the growth development of 13-year old children it was found that those children that were characterized as overweight at 13 years of age had an earlier AR compared to their normal-weight peers (Lagstrom, Hakanen et al. 2008). Thus, a rapid fat accumulation in children displaying an early AR may be considered as a simple indicator predicting the risk of developing obesity later in life years (Rolland-Cachera, Deheeger et al. 2006; Williams and Goulding 2009). Furthermore, studies have suggested that a sexual dimorphism in the relation to displaying AR might exist (Taylor, Goulding et al. 2004).

Determinants of growth in infancy and childhood

The control of growth during infancy, puberty and into adolescence is controlled by a number of interacting mechanism involving metabolic pathways that regulate growth at different age-specific stages. Whereas nutritional factors may have the most pronounced role on height growth in infancy, hormonal regulation is among the main factors regulating height growth in adolescence (Liu, Jalil et al. 1998; Veldhuis, Roemmich et al. 2005)

Infant growth

Genetic inheritance has shown to be of great importance in postnatal growth after 1 month (Touwslager, Gielen et al. 2011). During the first postnatal year of life the child develops a pronounced change in the central nervous system, which provides the child with fundamental neurological sources for further development of the organism. Regarding the terms of growth, a great expansion of bodily growth happens in the first postnatal year of life (app. 25 cm/y) (Taylor 2010). This growth is highly nutrition-dependent and is accelerated by the growth promoting effect of growth factor 1 (IGF 1) (Stanfield, C. 2008). Bodily tissues are therefore strongly dependent on proper nutrition during this intense growth period of the organs. Both breast-feeding and formula feeding can provide nutrition in the postnatal life. It is recommended to exclusively breastfeed the first six months of life and this is mainly due to the content of substances like n-3 and n-6

LCPUFAs, hormones, growth factors and immunoglobin, besides the important macronutrients, which are provided as important nutrients for the development of the child (Hornell, Lagstrom et al. 2013). The recommendations for intake of n-3 PUFA in infancy are listed in table 5.

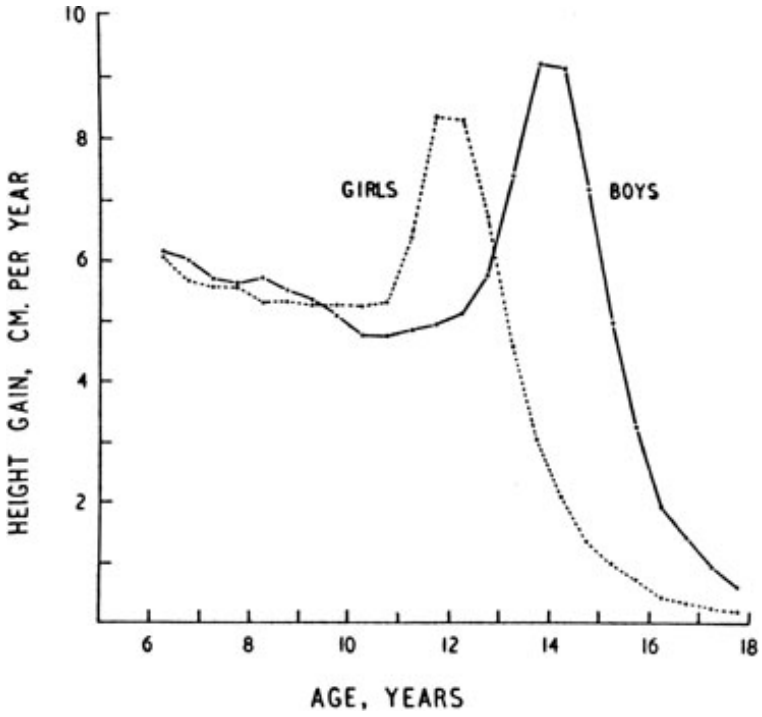
Childhood Growth

Growth during childhood is constantly affected by bodily changes that are regulated by the actions of growth hormone (GH), thus the growth rate of the child is dependent of the amount of GH present. Other hormones that need to be present to ensure normal growth are insulin, thyroid hormones and the androgens and estrogens (Stanfield 2008). In general a child grows 5-6 cm each year until puberty after the age of 2 years (Rogol, Clark et al. 2000). The recommended daily energy intakes of macronutrients (E%) are listed in table 3 and excessive or deficient intake deviating from these recommendations might affect childhood growth (Noh, Song et al. 2011). Furthermore, different outcomes between girls and boys is seen with regard to body fat percentage, due to the higher muscle gain and lean tissue in males as opposed to girls who gain fat at a more rapid rate than boys (Ogden, Li et al. 2011).

Puberty

Pubertal development is accompanied by a rapid physical growth which is further associated with several changes in physiological properties including an increase in blood pressure (Shankar, Eckert et al. 2005). A significant difference in sexual dimorphism is associated with pubertal development with the girls displaying an earlier onset of pubertal development than boys. Figure 2 shows the growth velocity, illustrating the difference between the growth spurt during adolescents for boys and girls.

Figure 2. Growth velocity from birth through 18 years of life. The pattern of pubertal growth spurt in boys and girls are illustrated (Tanner JM:1962)



During puberty the gametogenesis mature and gonadal hormones are secreted which is accompanied by final development of reproductive functions (Bordini and Rosenfield 2011). In girls the ovaries increases the production of female hormones including estrogen, which is accompanied by the initiation of the menstruation cycle and breast development. In boys an increased production of testosterone happens in the testicles. The timing of puberty vary according to different physiological outcomes, but mean values from Scandinavian children is estimated to be 13.5 and 11.6 years for boys and girls respectively (1 y ±SD in both sexes) (Liu, Wikland et al. 2000). However, the exact age a child enters puberty is depended of several factors including genes, nutrition, and gender. (Bordini and Rosenfield 2011)

The rapid changes in physical body composition during puberty is associated with a higher energy intake in both genders, however this increase in energy intake have been shown to be significantly higher in boys during puberty compared to girls (Shomaker, Tanofsky-Kraff et al. 2010)

An increased prevalence of obesity has been observed among children (Pearson, Olsen et al. 2005). A high fat diet results in a high-energy intake and will lead to a positive energy balance and increase the risk of obesity if the energy expenditure is not increased as well. Thus, the increase in prevalence of obesity among children may be due to decreased energy expenditure in form of physical activity (Deckelbaum and Williams 2001). However, it has also been suggested that the quality composition of dietary fatty acids fats have changed the last decades, and may play a role in the prevalence of obesity among children (Ailhaud, Massiera et al. 2006). Table 2 and 3 shows the recommended values for total daily energy intake (Sundhedsstyrelsen 2009) and recommended macronutrient E%, respectively (Trumbo, Schlicker et al. 2002).

Table 2. Recommended daily energy intake.

Age	**Boys**	**Girls**
10-13	9.8 MJ	8.6 MJ
14-17	12.3 MJ	9.6 MJ

Table 3. Recommended daily macronutrient E%

Macronutrient	
Carbohydrate E%	50-60%
Fat E%	20-30%
Protein E%	15-20%

Blood pressure.

Growth and increasing body size correlate positively with an increase in systolic and diastolic blood pressure during infancy into childhood and adolescence in both sexes (NHBPEP, 1996). The most prominent increase in blood pressure is during early infancy between the age of 2 days and 6 weeks (Voors, Webber et al. 1978). In late childhood blood pressure is furthermore correlated with height and the sexual maturation of the child (Kotchen, McKean et al. 1982) and may accelerate during

puberty (Reckelhoff, 2001;Shankar Eckert, et al. 2005). A gender-associated difference in blood pressure may also persist in puberty and it is seen that the increase in blood pressure during puberty in males is significantly greater than in females. This may be explained by the effect of testosterone that is suggested to increase blood pressure opposite to estrogen that may have a blood pressure lowering effect (Reckelhoff, 2001;Shankar Eckert, et al. 2005). This has also been verified from animal studies (Orshal and Khalil 2004). Meanwhile, it has been stated that an increase in blood pressure among children and adolescents has emerged, which is thought to be due to the increased prevalence of obesity (Muntner, He et al. 2004). Table 4 shows the oscillometric blood pressure percentiles in 13-year-old children.

Table 4; Oscillometric blood pressure percentiles in 13-year-old children

	*Oscillometric blood pressure percentiles in 13 year old **girls** (mmHg)*		
	10th	**Median**	**90th**
SBP	104	114	125
DBP	53	62	72
MAP	78,5	88	98,5
	*Oscillometric blood pressure percentiles in 13 year old **boys** (mmHg)*		
	10th	**Median**	**90th**
SBP	107	118	129
DBP	52	62	71
MAP	79,5	90	100

Values are presented as median and 10th-90th percentiles. U.S standards of blood pressure in children aged 7 years, adapted from a cohort of 7208 children at age 5-17 years. Modulated from Park et al. 2005. SBP; systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure.

Determinants of blood pressure

Physiologic factors;

The body regulates blood pressure through a complex pattern of interactions between physiological mechanisms that involves vascular functions, hormonal regulation and neural systems (Stanfield 2008). The renal system affects blood pressure by the stimulation of the renin-angiotensin-system and aldosterone. Both angiotensin and aldosterone increase the kidneys reabsorption of sodium and

thus expands extracellular fluid compartments affecting arterial compliance, endothelial function and therefor cause an increased blood pressure. Furthermore, constriction and dilation of blood vessels is under neural control and involves regulation from the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis (Stanfield 2008). Also, emotional responses such anxiety and anger are mechanisms known to affect the physiological factors controlling heart rate and thereby blood pressure as well (Howell, Rice et al. 2007).

Environmental and genetic factors

The development of specific blood pressure phenotypes might be established in fetal life under the influence of epigenetic factors (Ritz, Amann et al. 2011). Furthermore, the role of genetic inheritance influencing blood pressure is well recognized and may account for as much as 23-50% of the variation in blood pressure in the general population (Miall and Oldham 1963)

Nutrition is an important factor influencing blood pressure. High dietary intake of sodium chloride is known to increase blood pressure, and it has been reported that dietary restriction of salt during the first year of life decrease the rise in blood pressure in later childhood (Geleijnse, Hofman et al. 1997). Increased dietary intake of high-glycemic index carbohydrates have been associated with adverse effects on blood pressure (Gopinath, Flood et al. 2012), whereas low glycemic index carbohydrates, such as fiber may have a beneficial effect on blood pressure (Gopinath, Flood et al. 2012). Furthermore, high-protein diets have also been associated with a beneficial effect on blood pressure (Rebholz, Friedman et al. 2012), (Obarzanek, Velletri et al. 1996). Different types of dietary fat may also affect blood pressure. From animal studies it is seen that increased intake of saturated fat results in elevated blood pressure compared to a diet high in unsaturated fatty acids (Kaufman, Peterson et al. 1994; Niinikoski, Jula et al. 2009). In addition, restriction of dietary saturated fat from infancy to 15 years has been suggested to decrease blood pressure in childhood and adolescence (Niinikoski, Jula et al. 2009). The interplay and relative amounts of consumed macronutrients may also be a factor influencing blood pressure (Soenen, Bonomi et al. 2012).

The biology n-3 long chain polyunsaturated fatty acids

Chemistry

Polyunsaturated fatty acids contains more than one double bond in their backbone of the hydrocarbon carboxylic acid chain, and are classified as a omega-3 (n-3) or omega-6 (n-6) PUFA dependent on the location of the last double bond in relation to the methyl end of the fatty acid molecule. Thus, for n-3 PUFAs the double bond is found after the third carbon atom from the CH3-end of the carbon chain (Drevon 1992). All PUFAs from the n-3 family is described by a short nomenclature that describes the number of carbon atoms in the acyl chain, number of double bonds and the position of the first double bond from CH3-end of the acyl chain. The most simple n-3 PUFA is ala-linoelic acid (ALA, [C18:3n-3]), the dietary precursor of the n-3 series of fatty acids. Synthesis of ALA involves desaturation catalyzed by the enzymes Δ-15 and Δ-12 desaturase. However, the mammalian cell lacks the desaturase enzymes, Δ-15 and Δ-12 and therefore has not got a metabolic pathway to synthesize ALA. Thus, ALA is considered an essential fatty acid in the mammalian organism that must be provided in the diet.

When ALA is ingested in the diet, mammalian cells are capable of metabolizing it into other n-3 long chain unsaturated fatty acids (LCPUFA). The biosynthesis of n-3 LCPUFAs involves a series of linked desaturation and elongation reactions that mainly takes place in the liver (Innis 1991). The desaturation reactions introduce further double bonds, processed by delta 5 and 6 desaturase, and elongation reactions lengthening the acyl chain, processed by elongase. Figure 3 illustrates the biosynthesis of n-3 LCPUFA. Thus, the metabolic pathway for biosynthesis of n-3 LCPUFAs in mammals is dependent on Δ5 and 6 desaturase and elongase, which convert ALA (18:3 n-3) to EPA (20:5 n-3) and further on to DHA (22:6 n-3).

The same series of enzymes that is used to metabolize the n-3 PUFA into its n-3 LCPUFAs is used to metabolize linoelic acid, (LA, C18:2n-6) the precursor of the n-6 series of fatty acids into its corresponding active long-chain metabolite, arachionic acid (AA, 20:4*n*–6). Thus, the endogen synthesis of LCPUFAs is influenced by the competition between n-3 PUFA family and n-6 PUFA family. Δ6 desaturase enzyme is considered the rate-limiting step in the pathway of biosynthesis of LCPUFA (Nakamura and Nara 2003)

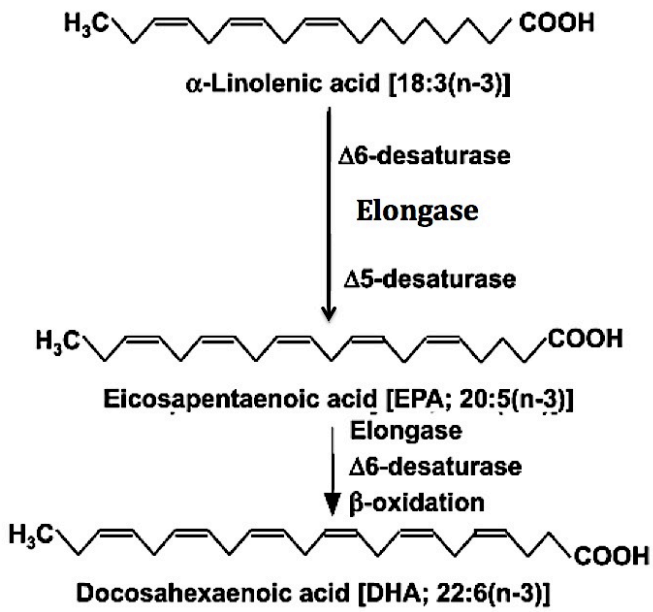


Figure 3; Biosynthesis of n-3 LCPUFA. (Figure; own construction, inspired from (Calder 2012)

n-3 PUFA and essentiality

In general, an essential nutrient may be defined as a substance whose metabolic absence has critical or fatal consequences, so-called deficiency symptoms, which can be reversed if the nutrient is replaced again (Shils 2006). Due to the fact that n-3 PUFA is distributed at a much lower level compared to n-6 PUFAs in mammalian tissues, and many of the functions that is carried out by n-3 PUFAs also is carried out by n-6 PUFAs it has been difficult to demonstrate the essential need for n-3 PUFA (Shils 2006). However, the distribution of the n-3 LCPUFA DHA is concentrated mainly in the retina, testes and CNS in mammals, thus the essentiality of n-3 LCPUFAs is primarily related to the development of neural tissue, visual and reproductive function. It has been possible to demonstrate the essential role of DHA in phospholipids of the brain and retina in a-linolenic acid ALA deficient monkeys. It was demonstrated that monkeys who were fed a diet with the sole source of fat from safflower oil (n-6:n-3 ratio of 255:1) during gestation gave birth to offspring, weaning on the same diet, with loss of visual acuity and decreased concentrations of n-3 LCPUFA in plasma phospholipids of brain and retina (Neuringer and Connor 1986). However, feeding the offspring a diet rich in fish oil reversed the loss of visual activity. Thus, with regard to n-3 PUFA

deficiency, this may be of greatest concern during the last trimester of pregnancy and first months of life when accumulation of especially DHA takes place in the brain and retina.

Dietary sources

As opposed to mammals, plants and algae are able synthesize and alfa-linoelic acid because they posses the Δ-12 and Δ-15 desaturase enzymes (Williams and Burdge 2006). Plant oils like linseed oil and perilla oil are rich in ALA (Eckert, Franke et al. 2010). However, only ALA is found in these plant oils, and long-chain highly polyunsaturated fatty acids like EPA and DHA is mainly found in fish oil and fat from other marine organisms. EPA and DHA are synthesized by certain types of marine plankton that serve as the base of the food chain for marine life. Especially fish such as mackerel and salmon are rich in EPA and DHA (Williams and Burdge 2006). Thus the predominant sources of n-3 LCPUFA in the human diet are fish and fish oils.

The conversion of ALA to its long-chain metabolites in the human body is weak, and it is estimated that approximately 8-20 % of ALA is converted to EPA and even less ALA is converted to DHA, around 0.5-9 % (Burdge 2006), (DeFilippis and Sperling 2006). Furthermore, DHA and EPA are incorporated into cell membranes phospholipids where they serve several important biological functions. However, this incorporation of n-3 LCPUFA into phospholipids happens a lot more efficiently when ingested directly from the diet than after synthesis from ALA (Burdge and Wootton 2002; Burdge 2006). This emphasizes the need of dietary intake of EPA and DHA to provide the sufficient level of n-3 LCPUFA for human health.

Requirement and recommended intake of n-3 PUFA during infancy

The infant have limited endogenous ability to synthesize DHA from the dietary precursor ALA, which provide a significant need for n-3 PUFAs, especially DHA, during gestation and perinatal period for the development of neurophysiologic brain structures in the fetus (Shils 2006). It is estimated that 50 to 60 mg/day of maternal DHA stores are transferred to a fetus via the placenta during the last trimester of gestation (Shils 2006). The brain undergoes a rapid growth spurt during the first year of life (Lauritzen, Hansen et al. 2001). This growth spurt is particular pronounced in the first months of postnatal life (Dobbing and Sands 1979), where especially DHA accumulates in the brains phospholipids (Innis 1992; Innis 1994), and thus reflects the higher need for n-3 PUFA in this period (see table 5). Most of the DHA that the brain requires during this period is supplied through the maternal milk during breast-feeding. The maternal milk reflects the nutritional status of maternal diet, thus maternal nutrition plays an important role in providing essential nutrients, such

as n-3 LCPUFAs, to the developing offspring. Breast milk in the Danish population contains approximately 0,4 % DHA of total fatty acids (Lauritzen, Hansen et al. 2001), which is reflected in the RBC-DHA of the mother and the infant. Maternal diet deficient in n-3 PUFAs during gestation and lactation may have long-term health consequences for adult brain plasticity in the offspring (Bhatia, Agrawal et al. 2011). It is believed that n-3 LCPUFAs works as a programming factor to the health beneficial effects of breast-feeding.

The recommended intake of n-3 PUFA is listed in table 5. In general, it is recommended that n-3 fatty acids contribute with minimum 0,5 % of the daily energy intake (E%).

Table 5

	n-3 PUFA E%
6-11 months	>1
12-23 months	>0.5
>24 months	0.5
Pregnant/lactating women	1

Table 5. Recommended intake of n-3 PUFA The recommended intake of n-3 PUFAs varies with age, and the dietary need is higher during growth and development, as well in pregnancy and lactating (Based on values from the NNR project group, 2004

Individual dietary recommendations for ALA, EPA and DHA have been estimated based on evidence from several randomized controlled trials and epidemiologocal studies (Gebauer, Psota et al. 2006). The adequate intake of EPA+DHA varies greatly among studies, but have been suggested to be 0.3E% of a 2000 kcal diet (Simopoulos 2000).

Physiological roles and health benefit of n-3 PUFA

n-3 PUFA have the ability to act as ligands or precursors of ligands for transcriptions factors (Tai and Ding 2010). Changes in the composition of polyunsaturated fatty acids incorporated in cell membranes phospholipids can affect cell signaling pathways, which can lead to altered transcription factor activity and thus changes in gene expression (Tai and Ding 2010). Virtually all of these functions are mediated by the interactions and balance between dietary intake of n-3 PUFAs and n-6 PUFAs. n-3 and n-6 PUFA in cell membranes acts as intracellular cell mediators of signal transduction or as modulators of cell-cell interaction and thereby modulate cell function. Actions like these are initiated by phospholipases, which also is the main factor in the biosynthesis of eicosanoids (Calder 2012). Eicosanoids exert different physiological effects in blood pressure, capillary permeability, inflammatory reactions, and blood platelet functions (DeFilippis and Sperling 2006) and also have important functions in modulating triglyceride and cholesterol metabolism (Harris 1996). Overproduction of pro-inflammatory eicosanoids from the n-6 PUFA

series may cause excessive inflammation and may be involved in different health implications involving obesity and hypertension (CVD) (Patterson, Wall et al. 2012)

It is by now a well known fact that a significant increase in n-6:n-3 ratio of the Western diet have occurred during the last decades (Ailhaud, Massiera et al. 2006). An increase in dietary n-6 PUFAs have been linked to several health implications e.g. development of childhood obesity (Ailhaud, Massiera et al. 2006) and other lipid-disorders (Simopoulos 2002). Furthermore, a corresponding increase of the n-6 PUFA linoleic acid (LA) of maternal breast milk has been observed, which reflects the mothers diet (Ailhaud and Guesnet 2004). From animal studies it is demonstrated that mice fed with n-6 PUFAs during pregnancy and lactation resulted in obese offspring (Massiera, Saint-Marc et al. 2003). Another recent study in mice showed that an increase of dietary n-6 PUFAs from 1% (recommended daily intake) to 8 % (reflecting todays current energy intake, western diet) 14 weeks form weaning was associated with elevated adiposity the level of AA on induced obesity in mice (Alvheim, Malde et al. 2012).

n-3 PUFA and programming

Body composition

A number of observational and cohort studies have documented that breast-feeding have beneficial effects on infant growth and body composition (Owen, Martin et al. 2005; Beyerlein and von Kries 2011). Compared to breast-fed infants it is seen that formula-fed infants have significantly higher levels of plasma insulin that is believed to stimulate fat deposition and early development of obesity (Lucas, Sarson et al. 1980). Furthermore, the duration of breastfeeding may be a highly important factor with respect to body composition, and a recent literature review described that exclusive breastfeeding for 4 months minimum was associated with a slower increase in fat mass after six months in the postnatal period (Hornell, Lagstrom et al. 2013). Other studies have also suggested an inverse relationship between the duration of breastfeeding and BMI in later life (O'Tierney, Barker et al. 2009; Yin, Quinn et al. 2012)

From studies in animals (p. 28) and human studies there is a growing body of evidence on the increased intake of n-3 LCPUFAs having a fat-reducing effect (Buckley and Howe 2010). This thesis, however, focuses on the *long-term* effect of n-3 LCPUFA supplementation in early life in humans. However from human studies, relatively sparse evidence exists in relation to the long-term effect of n-3 PUFAs on body composition (Table 6).

Two recent follow-up studies administering n-3 LCPUFA during gestation/lactation and solely during gestation found no effect on offspring body composition at age 7 and 19-years, respectively (Helland, Smith et al. 2008; Rytter, Bech et al. 2011). Another follow-up study by Bergmann, Bergmann et al. 2012 et al., found, that offspring to mothers supplemented with n-3 LCPUFA during pregnancy and lactation had lower BMI at 21 months compared to the control group, however this difference in BMI were no longer evident at 6 years. Hauner et al., supplemented women during pregnancy/lactation with n-3 LCPUFA and neither found any effect on fat mass development in infants at 1 year. Andersen et al supplemented 9 months old children for 9 months with fish oil and did not find any effect on body composition parameters. A previous follow-up study at 2.5 years from this present study showed that children receiving n-3 LCPUFA during lactation had increased body weight (Lauritzen, Hoppe et al. 2005), thus this observation together with the finding from Bergmann at 21 months indicate that there is conflicting evidence regarding the effects of maternal n-3 LCPUFA supplementation on the body composition of offspring during the early years of life. Table 6 shows human randomized controlled trials assessing the effects of increased intake of n-3 LCPUFA in early life on body composition

Table 6; Human randomized controlled trials assessing the effects of increased intake of n-3 LCPUFA in early life on body composition

Author/study design	Study population	Intervention groups	Participation of the intervention	Outcome
Rytter, 2011 Randomized controlled 19-year follow up trial	Pregnant women randomized to FO, OO or NO during the last trimester of pregnancy until delivery and their healthy term offspring(19y)	1) Fish oil; 2·7 g/d n-3 LCPUFA 2) Olive oil (OO) 3) No oil (2+3= control group)	19-year old offspring had their BMI and waist circumference measured (n= 243)	N0 effect of fish-oil supplementation during pregnancy on offspring BMI in 19-year old offspring
Hauner et al, 2012 Open-label randomized control led trial	Pregnant and lactating women; n-3 LCPUFA supplementation + reduction in n-6:n-3 ratio from 15 week of pregnancy until four mo. of lactation, and their healthy term infants (1y)	1) Fish oil; 1.2 g n-3 LCPUFA (1.02 g DHA and 0.18 g EPA) + dietary reduction inn-6: n-3 ratio 2) Dietary counseling on healthy balanced diet.	Infant fat mass estimated by skinfold thickness (SFT) (n=165)	No effect of fish-oil supplementation on infant fat mass the first year of life´
Helland et al, 2008 Randomized double-blinded controlled 7 year follow up trial	Pregnant and lactating women; n-3 LCPUFA supplementation from week 18 of pregnancy until 3 mo after delivery, and their their healthy term infants (7y).	1) Cod liver oil; 10 mL/d; 2494 mg/10 mL total n-3PUFAs (1183 mg/10 mL of DHA, 803 mg/10 mL of EPA) 2) Corn oil; 4747 mg/10 mL of LA and 92 mg/10 mL of ALA.	Child height and weight measured at 7 years (corn oil, n=61; cod liver oil, n=82)	No effect of n-3 LCPUFA supplementation on child growth at 7-years
Ulbak et al, 2005; Asserhoj et al. 2007 Randomized double-blinded controlled (2.5 and 7 year) follow up trial	Lactating mothers with n-3 PUFA <0.4 g/d or >0.82 g/d and their healthy term infants (2.5 y and 7 y)	1) 4.5 g fish oil (1.5/d n-3 LCPUFA) (0.62 g/d EPA, 0.79 g/d DHA) 2) 4.5 g olive oil daily (0% EPA and DHA) 3) Reference group (high fish intake)	BMI, waist+head circumference, tricep-and subscapularskinfold thickness and weight measured at 2.5 year (n=101) and 7 year (n=98)	FO-group have significant higher BMI than OO at 2.5 years No effect of fish oil on BMI at 7 years
Bergmann et al, 2012 Randomized controlled double-blinded 6-yearfollow-up trial	Pregnant and lactating women; n-3 LCPUFA supplementation from week 21 of pregnancy until 3 mo after delivery, and their healthy term infants (6y).	1) Fish oil (200 mg DHA + 60 mg EPA).* 2) prebiotic fructooligosaccharide (FOS), 3) vitamins, minerals (2+3 = control group)	Length, weight, and head circumference, tricep- and subscapular skin-fold thickness was measured in 6-year old children (115)	No effect of fish oil on BMI in 6-year old children

Animal studies;

A number of studies in animals have documented a fat-reducing effect in the offspring with supplementation with either ALA or n-3 LCPUFA (Massiera, Saint-Marc et al. 2003; Wyrwoll, Mark et al. 2006; Okuno, Kajiwara et al. 1997; Korotkova, Gabrielsson et al. 2002). Other studies have demonstrated a negative long-term effect of insufficient ALA in postnatal life on adiposity in adult life (Pouteau, Aprikian et al. 2010).

Still, the evidence has not been consistent and a recent a study in rats by Muhlhausler, Miljkovic et al., found that supplementation during pregnancy and lactation was not associated with a decrease in fat mass at weaning, but instead an increase in fat mass in offspring at 6 weeks of age. This finding is consistent with a study that found that feeding n-3 PUFA-enriched diet in the perinatal period caused a higher body weight in female offspring at 28 weeks compared to a diet rich in n-6 PUFA (Korotkova, Gabrielsson et al. 2005). The latter study could indicate a sex-specific long-term effect of n-3 PUFA. Furthermore, it has been well established that n-3 PUFA have beneficial effects on adiposity by the prevention of proliferation and differentiation of *mature* adipocytes (Raclot, Groscolas et al. 1997; Ruzickova, Rossmeisl et al. 2004; Okuno, Kajiwara et al. 1997. However, if n-3 PUFA supplementation during the perinatal alone have a fat-reducing long-term effect is uncertain (Muhlhausler, Miljkovic et al. 2011)

Blood pressure

It have been demonstrated that the duration of breast feeding is inversely associated with blood pressure in childhood (Taittonen, Nuutinen et al. 1996; Owen, Whincup et al. 2003; Martin, Ness et al. 2004; Martin, Gunnell et al. 2005). Furthermore, studies from animals have shown that perinatal n-3 PUFA deficiency may affect blood pressure later in life (Armitage, Pearce et al. 2003) and from a study in rats it has been reported that high intake of n-3 PUFAs in the post-natal diet prevents programmed hypertension (Wyrwoll, Mark et al. 2006). This finding is consistent with evidence from two human studies that have reported that exclusively formula-fed children, with no supplement of n-3 LCPUFA, showed to have a significant higher blood pressure than breastfed children (Wilson, Forsyth et al. 1998; Forsyth, Willatts et al. 2003). Based on these findings an author suggested the beneficial effects of breastfeeding on blood pressure later in life might emerge from the presence of LCPUFA in breast milk (Das 2001).

Few studies have evaluated the long-term effect of n-3 PUFA supplementation in early on blood pressure in humans and the results have been inconsistent (Table 7). Forsyth et al. and colleagues demonstrated that infants fed with formula enriched with n-3 LCPUFA had a lower blood pressure at age 6 compared to children receiving no supplement with n-3 LCPUFA. Another study did not find a difference in blood pressure between control group and in children who have received supplementation with fish oil from time of weaning to age 8 (Ayer, Harmer et al. 2009). Another follow-up study in 19-year old offspring evaluated the long-term effect of n-3 LCPUFA supplementation during the last trimester of pregnancy, but did not find any effect on blood pressure (Rytter, Christensen et al. 2012). However, a recent study performed by van Rossem, Wijga et al. showed that children who received breast-milk with a high content of n-3 LCPUFA (above the median) had a lower blood pressure at age 12 years compared to never-breastfed children. Furthermore, results from previous follow-up studies at 2.5 and 7 years from this present study showed that children receiving maternal fish oil supplementation in the four first months of lactation, did not have a lower blood pressure compared to the control group receiving olive oil at 2.5 years (Ulbak, Lauritzen et al. 2004). However, the blood pressure from the children in the FO-group was reported to be higher than the control group at the age of 7 years (Asserhoj, Nehammer et al. 2009).

Table 7. Blood pressure outcome in human studies administering n-3 PUFA to either formula, or to lactating mothers, during pregnancy/lactation or during pregnancy solely

Author/type of study	Study population	Intervention groups	Participation of the intervention	Outcome
Ayer et al., 2009 Randomized controlled trial	Healthy children receiving dietary supplement intervention from weaning and through the 5 first years of postnatal life.	1) Tuna oil supplemented the time at weaning, containing 135 mg DHA and 32 mg EPA and 6% n−6 2) Sunola oil (0.3% n−3 and 7% n−6 FA)	Blood pressure assessed in children receiving intervention diet (n=312) or control diet (n=304) at age 8 years.	No effect of n-3 LCPUFA on blood pressure the 8 y of age,
Ulbak et al, 2005; Asserhoj et al. 2007 Randomized double-blinded controlled (2,5 and 7 year) follow up trial	Lactating mothers with n-3 PUFA <0.4 g/d or >0.82 g/d and their healthy term infants (2.5 y and 7 y)	1) 4.5 g fish oil (1.5/d n-3 LCPUFA) (0.62 g/d EPA, 0.79 g/d DHA) 2) 4.5 g olive oil daily (0% EPA and DHA) 3) Reference group (high fish intake)	Blood pressure assessed at 2.5 year (n=101) and 7 year (n=98)	No effect of fish oil on blood pressure at 2.5 years Significant higher blood pressure in boys from FO-group at 7 years
Forsyth et al., 2003 Randomized controlled trial followed up at 6 years	Healthy term infants fed formula with or without supplement with n-3 LCPUFA	1) Formula supplemented with n-3 LCPUFAs (EPA; 0.3 to 0.4 g/d, DHA; 0.15 to 0.25 g/d) 2) Formula without n-3 LCPUFAs	Blood pressure assessed in the formula fed children with (n=71) or without (n=76) supplement with n-3 LCPUFA at age 6 years	Children fed formula with n-3 LCPUFA-supplementation had significantly lower mean blood pressure and diastolic blood pressure than the non-supplementation group.
Rytter et al., 2012 Randomized controlled 19 years follow up trial	Pregnant women randomized to FO, OO or NO during the last trimester of pregnancy until delivery and their healthy term offspring(19y)	1) Fish oil; 2·7 g/d n-3 LCPUFA 2) Olive oil (OO) 3) No oil (2+3= control group)	Blood pressure was assessed in 19-year old offspring (n= 180)	No effect of fish-oil supplementation during pregnancy on blood pressure in 19-year old offspring
Van Rossem et al., 2012 Observational birth cohort study- Follow up at 12 years	Non-breast feeding women or breastfeeding women with relatively high content of n-3 LCPUFA in breast milk, and their term offspring (12y)	) Infants fed human milk with n-3 LCPUFA above the median, i.e., ≥0.51 wt%). 2) Infants exclusive formula fed (contained no n-3 LCPUFA)	Blood pressure was assessed in children at age 12 years (n=250).	Breast-fed infants with a relatively high content of n-3 LCPUFA had a significantly lower systolic blood pressure aat age 12y. .

Mechanisms of action

n-3 PUFA and gene expression in adipocytes

Obesity is associated with an expansion in adipose tissue by hypertrophy or hyperplasia of adipocytes. An increase of adipocyte number (hyperplasia) is due to the proliferation and differentiation of pre-adipocytes into mature adipocytes (Jo, Gavrilova et al. 2009). From studies in rats it has been demonstrated that a high fat diet stimulates adipocyte proliferation (Ellis, McDonald et al. 1990) and that n-3 LCPUFAs have shown to exert a protective effect on adipose tissue accumulation by limiting the hyperplastic and hypertrophic growth of adipose tissue in adult rodents (Raclot, Groscolas et al. 1997; Ruzickova, Rossmeisl et al. 2004; Massiera, Guesnet et al. 2006; Okuno, Kajiwara et al. 1997). More specific, DHA have shown to inhibit fat cell proliferation in mice (Massiera, Saint-Marc et al. 2003), which also is verified from in vitro studies showing that DHA was able to inhibit adipocyte differentiation, and induced apoptosis in preadipoctytes (Madsen, Petersen et al. 2005).

It have been suggested that n-3 LCPUFAs modulate adipose tissue metabolism through the action of peroxisome-proliferator-activated receptors (PPAR) that are tissue-specific transcription factors, regulating gene expression several cellular processes, including lipid metabolism (Berger and Moller 2002). PPARs are expressed in several metabolically active tissues, however stimulation of gene expression regulating metabolic processes involved in adipocyte differentiation is dependent on the activation of PPAR-y {Berger, 2002). A close allied of the function of PPAR-y is CCAAT/enhancer-binding protein (C/EBP) that is important for the early phase of adipogensis and stimulate expression of PPAR-y and also regulate the maturation of adipocytes during adipogenesis (figure 4). Furthermore, n-3 LCPUFAs are also known to down regulate the expression of the sterol regulatory element-binding proteins (SREBPs), a transcription factor that suppresses lipogenic gene transcription. Down regulation of SREBP-1 causes a reduced accumulation of lipid by decreasing the expression of downstream lipogenic genes, including fatty acid synthetase (FAS) and glycerol 3-phosphate (Madsen et al., 2005; Xu, Nakamura et al. 1999; Tai and Ding 2010). When SREBP-1 is expressed it is able to increase the activity of PPAR-y (Kim, Wright et al. 1998). See figure 4

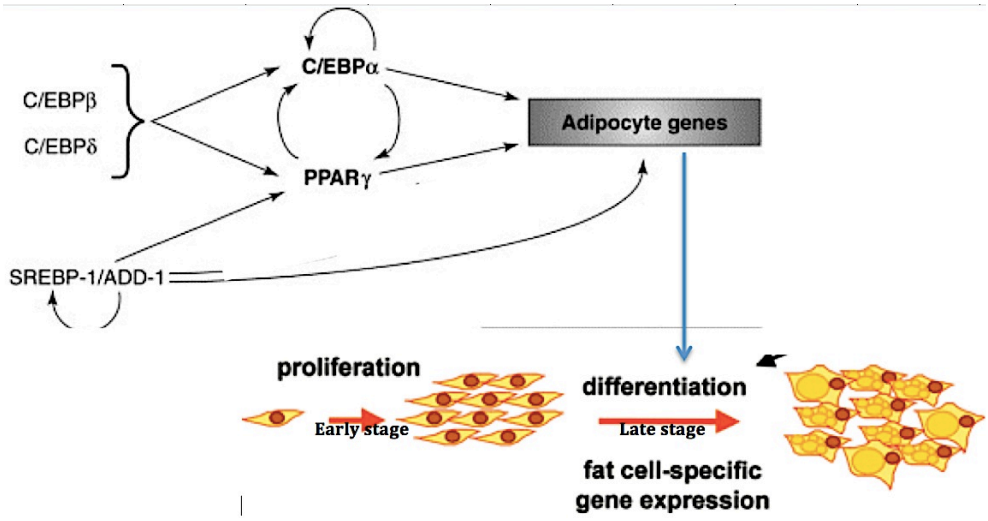


Figure 4; Transcriptional regulation of adipocyte differentiation; CCAAT/enhancer-binding protein (C/EBP)β/δ (during early phase of differentiation) activate CRE/Bα and PPAR-y that stimulate adipocyte genes. SREBP-1 may stimulate/repress gene expression independently or through PPAR-y (Kim, Wright et al. 1998), Figure; Own construction, inspired by (MacDougald and Mandrup 2002)

In vitro studies have demonstrated that both preadipocytes and adipocytes produce a significant amount of prostacyclins (PGI_2) from the n-6 PUFA series (Vassaux, Gaillard et al. 1992;). PGI_2 up regulates C/EBP that enhances the process of pre-adipose cell differentiation and proliferation by activation of PPAR-y. PGI_2 released by differentiated adipocytes exerts an autocrine function in which it is able to induce differentiation in preadipocytes (Darimont, Vassaux et al. 1994; Fujimori 2012) (figure 5). In vitro studies (Kim, Della-Fera et al. 2006) and animal studies (Okuno, Kajiwara et al. 1997) have shown that n-3 LCPUFA might prevent adipocyte differentiation by down-regulating the synthesis and action of prostacyclins from the n-6 PUFA series. Furthermore, since PGI_2 is the major metabolite of AA in adipose tissue, it is thought that AA and its metabolic products act more adipogenic by stimulating preadipocyte proliferation and adipogenesis, compared to EPA and DHA. This assumption has also been demonstrated in vitro (Gaillard, Negrel et al. 1989; Massiera, Saint-Marc et al. 2003). Consistent with this, it has also been seen that the expression of genes involved in the proliferation of adipocytes is less expressed in diets rich in n-3 LCPUFA are more expressed in diets rich in n-6 LCPUFAs (Muhlhausler, Cook-Johnson et al. 2010).

Arbejde 12/5/13 21.20

Kommentar [1]: ilhaud G., Massiera F., Weill P., Legrand P., Alessandri J. M., Guesnet P. (2006). Temporal changes in dietary fats: role of n-6 polyunsaturated fatty acids in excessive adipose tissue development and relationship to obesity. Prog. Lipid Res. 45, 203–236. doi: 10.1016/j.plipres.2006.01.003. [PubMed] [Cross Ref]

Massiera F., Guesnet P., Ailhaud G. (2006). The crucial role of dietary n-6 polyunsaturated fatty acids in excessive adipose tissue development: relationship to childhood obesity.

{Fujimori, 2012 }
PGI2, derived from arachidonic acid (AA) from the n-6 PUFA series {Sessler, 1998}.

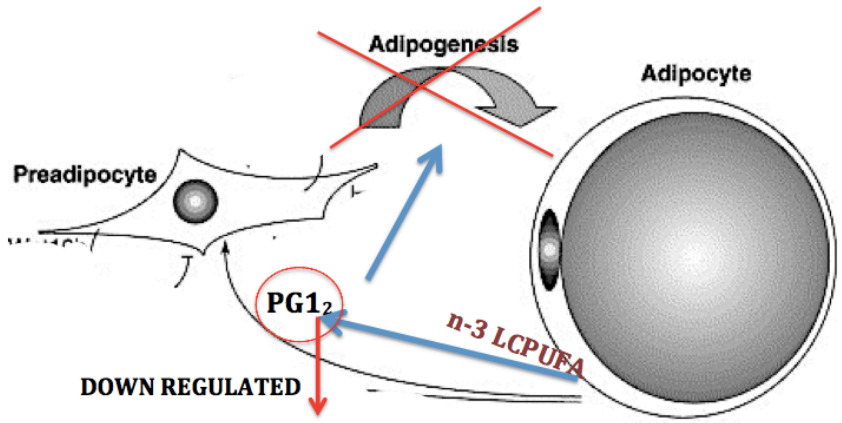


Figure 5; Possible anti-obesity mechanism of n-3 PUFA; competition with n-6 PUFA in cell signaling pathways may abrogate the adipogenic effect of n-6 PUFA by slowing down biosynthesis of prostacyclins (PGI_2). Potential feedback mechanism in the development of mature adipocytes from preadipocytes is decreased. Figure; own condtruction, inspired from (MacDougald and Mandrup 2002)

In vitro studies have reported that n-6 PUFA might act as both anti-and adipogenic modifiers. This might be due to dietary interactions of the background diet in that the ratio between carbohydrate and protein might determine the adipogenic potential of n-6 PUFAs through the presence of insulin secretion and cAMP-signaling (Madsen, Pedersen et al. 2008).

Appetite signaling

From animal studies it have been demonstrated that nutritional programming affects the development of the hypothalamus, in that nutritional changes during critical periods in peri- and post natal life have an impact on hypothalamic development (Lucas 1998), which have long-term effects on the feeding metabolism (Cripps, Martin-Gronert et al. 2005). From studies in rats it is reported that a sufficient supply of n-3 LCPUFA is necessary during perinatal life in the development of neural systems that regulate appetite and that n-3 LCPUFA deficiency in the perinatal period contributes to increased appetite signaling (Mathai, Soueid et al. 2004). Insufficient intake of n-3 LCPUFA during the critical period of neural development of systems that regulate appetite would decreases the content of DHA in membranes phospholipids, which results in altered membrane physiological properties. Because it is seen from in vitro studies that n-3 LCPUFA can limit the synthesis of pro-inflammatory cytokines, like the neuron damaging cytokine tumor necrosis factor-α, a dietary deficient intake of n-3 LCPUFA may lead to damaging of the neurons in

the hypothalamus (Cripps, Martin-Gronert et al. 2005) that might lead to an increased appetite in adult life.

Osmoregulatory functions

Deficiency of dietary n-3 PUFA during development in rats have also shown to result in a significant increase in sodium appetite in later life (Weisinger, Armitage et al. 2010; and Weisinger et al., 2001). This finding may be explained by an influence of n-3 PUFA on gene transcription and the functioning of membrane-bound G-protein coupled receptors, such as ANG II type 1 receptors, involved in sodium homeostasis (Weisinger, Denton et al. 1988). Furthermore, an abnormality in sodium homeostasis is believed to contribute to the development of hypertension (Weisinger, Armitage et al. 2010);Begg,et al., 2010). n-3 PUFA deficiency have shown to have a long-term long term negative effect on blood pressure in animals (Begg et al., 2010b and Weisinger et al., 2001). Neural control of blood pressure is regulated by the hypothalamus and by interactions of the renin-angiotensin system that also controls sodium appetite. From a recent study in rats it has been demonstrated that n-3 PUFA deficiency-induced hypertension was associated with changes in gene expression involved in blood pressure regulation (Begg,et al., 2010)

Subjects and Methods

Study design, intervention and ethics

The present 13-year follow-up is based on a double-blinded randomized controlled intervention trial (Lauritzen, Jorgensen et al. 2004) which included two previous follow studies at 2.5-years {Ulbak, Lauritzen et al, 2004} and at 7 years (Asserhoj, Nehammer et al. 2009). The intervention trial and both follow-up studies were approved by the scientific-ethical Committees for Copenhagen and Frederiksberg (KF 01-300/98, KF 01-183/01, KF 11321572). The present 13 year-old follow-up study did not include any blood samples and did not require specific approval by the Scientific Ethical Committees for Copenhagen and Frederiksberg. Both parents in custody of the child had to sign an informed written consent in order to participate in the study.

Description of intervention trial and previous follow-up studies

- *The intervention trial*

Participants:

The intervention trial (KF 01-300/98) recruited 122 healthy, Danish pregnant women living in the greater Copenhagen area from the Danish National Birth Cohort (DNBC) during December 1998 to November 1999 (Olsen, Melbye et al. 2001). The selection of the women to be part of the randomized controlled trial was based on their fish intake, which had to be below the population median (equivalent to a fish intake of 12.3 ± 8.2 g/d and less than 0.40 g/d of n-3 LCPUFA) and participated as a low fish group. In addition, women who a fish intake above the population median (equivalent to a fish intake of 55.2 ±26.7 g/d or 0.82 g/d of n-3 LCPUFA) was asked to participate as a high fish reference group (HF-group). A total of 919 women were invited to participate in the low fish group and 554 women were invited to participate as the high fish reference group.

A number of recruitment criteria had to be met by the mother and their newborn in order to participate in the trial;

Criteria for the woman	Criteria for the newborn
Uncomplicated pregnancy	Born term (34-37 of gestation)
Body mass index during pregnancy <30kg/m^2	Had to be singleton
No metabolic disorders	No health complications (no admission to neonatal department)
Intention to exclusively breastfeed for ≥ 4 months	Normal weight for gestation (Greisen and Michaelsen 1989)
Start intervention supplements within < 2 weeks after birth	Apgar Score > 7 at 5 minutes after delivery

Design of intervention

After giving birth, the women with fish intake below the population median were randomly supplemented with either fish oil (FO) or olive oil (OO) for the first four months of lactation. 62 women were allocated to the FO-group, designated the experimental group and 60 women were allocated to the OO-group, designated the control group. Investigators as well as the families were blinded to the randomization during data collection and analysis. The mothers were told after 1 year which group they were allocated to.

The supplementation of OO and FO was administered as müslibars with encapsulated OO and FO. The amount of FO supplement given to the women in the FO-group corresponded to the fish intake of the women in the high fish intake group. The FO-group was given 17 g/d of deodorized microencapsulated FO powder, containing 4.5 g of FO and 1.5 g n-3 LCPUFA of which was 0.62 g EPA and 0.79 g DHA. The control group was given a similar amount of OO. The fatty acid compositions of the two supplements are shown in table 8. The supplementation started 9 ± 3 days after delivery and the women were instructed to consume two 35 g müsli-bars daily for the subsequent four months of the lactation period. Through the intervention period the women were not given any dietary instructions but were told not to take any oil supplements during the trial.

Self-reported compliance, expressed as the percentage of oil in müslibars taken relative to the intended dose, was in both groups was on average 88 % (Lauritzen, Jorgensen et al. 2004).

Table 8.
Fatty acid composition of microencapsulated oils used for the intervention (Lauritzen, Jorgensen et al. 2004)

	Olive oil	Fish oil
Total SFA	13.6	30.2
Total MUFA	70.9	21.4
18:2n-6	7.4	1.3
20:4n-6	—	1.7
Total n-6 PUFA	7.4	4.0
18:3n-3	0.6	0.5
20:5n-3	—	10.0
22:5n-3	—	1.7
22:6n-3	—	22.8
Total n-3 PUFA	0.6	38.3

Values are based on gas chromatography analysis and are expressed as a percentage of the total fatty acid content {Lauritzen et al., 2004}. Abbreviations; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids

150 women completed the four months trial period, which included 53 women from the FO-group, 47 women from the OO-group and 50 women from the HF-group. Furthermore, 107 mothers reported to have exclusively breastfed their newborns during the four months of intervention. Women who had not exclusively breast-fed during the four months intervention period were not excluded from the trial, but the degree of breast-feeding was taken into account when performing the statistical analysis (Lauritzen, Jorgensen et al. 2004).

Protocol

In order to collect a 10 ml blood sample for baseline analysis of the fatty acid composition in maternal RBCs, a pre-enrollment visit was made in week 36.4 +/- 1.5 of gestation (Lauritzen, Jorgensen et al. 2004). Demographic and social information together with parental education was also collected at the visit. Another visit was made 9±3 days after birth in order to collect a maternal breast milk sample to determine fatty acid composition of maternal milk at baseline (Lauritzen, Jorgensen et al. 2004). At the visit the infant's head-circumference was measured and birth data on length and height were collected from the hospital journal. Furthermore, supplements were given in two rounds for two months at a time. Supplement for the first two months were given at the pre-enrollment visit, and supplements for the last two months were dispensed at the two-months visit.
During the supplementation period the infant together with the mother was assessed two times, at 2 and 4 months, respectively. Subsequently two follow-up examinations were made at the age of 9

months and 2.5 years, respectively. The examinations at the age of 2, 4 and 9 months took place at Department for Human Nutrition at Frederiksberg and included anthropometric measurements in terms weight, length and head circumference of the infant. Furthermore, the mother delivered a milk sample and was interviewed about her fish intake the day before and the previous month (Lauritzen, Jorgensen et al. 2004). The overall fish intake of the mother during the intervention period was assessed by a food frequency questionnaire. In addition, at the examination at 4 months, a blood sample was collected from the mother to determine the fatty acid composition of maternal RBC-DHA. This procedure was also carried out for the child.

2.5-year follow-up study

150 families were invited to participate in the 2.5-year follow up study, in which a total of 101 children ended up participating The group allocations of the 101 children were; 42 from FO-group, 30 from the OO-group and 29 from the HF-group (Ulbak, Lauritzen et al. 2004); Lauritzen et al., 2005). The following anthropometric measurements were collected the examination visit; height, weight, waist circumference and triceps- and subcapsular skinfold thickness. Oscillometric blood pressure measurements were also assessed two times at the examination visit with the child sitting in up-right position. The first blood pressure measurement was assessed shortly after arrival and the second one approximately one hour later. The second measurement was assessed after 30 minutes of rest, and this measurement was used in the final data analysis (Ulbak, Lauritzen et al. 2004), (Larnkjaer, Christensen et al. 2006). Furthermore, a blood sample was collected and the fatty acid composition of the RBC was detected (Larnkjaer, Christensen et al. 2006). Registration of diet was assessed for 7 consecutive days by using a pre-coded dietary questionnaire, from which the average daily nutrient intake was calculated. Other measurements investigated at the visit included pulse wave velocity, heart rate, heart rate viability, and electrocardiogram by a 2-channel tape recorder (Larnkjaer, Christensen et al. 2006).

7 year follow up study

149 families were invited to participate in the 7-year follow up study of which 98 children ended up participating. The 98 children were distributed in the following experimental groups: 36 from FO-group, 28 from OO-group and 34 from HF-group. The 7-year follow up study included two visits, one information meeting and a following examination visit. Following anthropometric measurements were collected at the examination visit: head circumference, waist circumference, hip circumference, height, weight and tricep- and subcapsular skinfold thickness. Blood pressure was

assessed using a Boso-medicus Prestige unit with a cuff with circumference size of 16-22 cm, and the child was told to lie minimum 5 minutes before the measurement of SDP and DBP. Blood pressure was measured three consecutive times with the average of second and third measurement used in data analysis. In addition, MAP was calculated as DBP + 1/3 * (SDP-DBP). Dietary intake was assessed by four-day weighed dietary record. Physical activity of the child was assessed in two ways; the first included a movement monitor (ActiReg®) with sensors placed on the chest and the thigh that was worn for four days expect during nighttime, showering and swimming. The second included answering of the Habitual Activity Estimated Scale (HAES)-questionnaire. Furthermore the parents were also asked to complete two questionnaires: a Strenght and Difficulties Questinnaire (SDQ) and a questionnaire concerning the health of the child.

The present 13-year follow-up study

Study design

The 13-year follow-up study included an examination visit carried out either at Department of Human Nutrition at Frederiksberg or at the home of the child. At the examination visit a number of anthropometric measurements and blood pressure were assessed and the child and the parent individually answered four questionnaires used to assess the mental wellbeing of the child. Furthermore, the child was instructed in using a movement monitor (ActiGraph®) and introduced to an electronic food-frequency questionnaire, which was sent to the e-mail of the parent after the examination visit and subsequently filled out by the child.

In remuneration the child received 200 Danish kroners for participation after end examination. The main researcher, Sara Elisabeth Eriksen carried, was blinded to the randomization throughout the trial period and until all basic statistical analyses were completed. The families were not blinded to the randomization in this present follow-up study.

Study population – recruitment

In the present follow-up study all parents of the 13-year-old children, who had not actively withdrawn their participation from the study, was invited to participate. The current addresses of the participants who had moved since last follow-up at 7 years were identified through the Danish National Registry. A total of 140 families were invited to participate in the study. The families were

contacted by postal letter containing 1) a informative letter describing the background of the study as well as introducing the details regarding the physical examination (App. 1), 2) a brochure about the rights of the participant as a test participant (App. 2) and 3) a statement of consent to be signed by both parents (App. 3). A week after sending out the letters, the families were contacted by phone to see if they were interested in participation. If the parents and/or child expressed a need to receive a more specified description about the examination, a thoroughly oral explanation was given on the phone. Furthermore, if the families were willing to participate an arrangement of date and time for the examination visit was scheduled. The examination visit was arranged to take place either at Department of Human Nutrition at Frederiksberg or at the home of the families, a decision solely made by the families, depending on what suited them best. The families were told to bring a signed statement of consent to the examination visit.

The recruitment of families and examinations was initiated in October 2012 and ended in January 2013 and were solely carried out by the main researcher. The purpose was to recruit as many families as possible during this period of time, however, due to the time limit of the present thesis, recruitment and examinations had to end in January 2013.

In general, 6 participants were excluded because they no longer were Danish residents, 5 participants did not wish to participate due to personal reasons and 13 participants showed interest in participating but did not manage to arrange an available date and time for the examination. Furthermore, it was not possible to make contact by postal letter or phone to the final 51 participants. In total 65 families agreed to participate in the present 13-year follow-up study, but due to sickness (n=4) and sudden death in the family (n=1), compelled these families to cancellation of the examination visit. Therefore, a total of 60 Danish children participated in the present follow-up study from which data were collected. Figure 6 illustrates the trial profile during the randomized intervention and follow-up examinations at 2.5, 7 and 13-years.

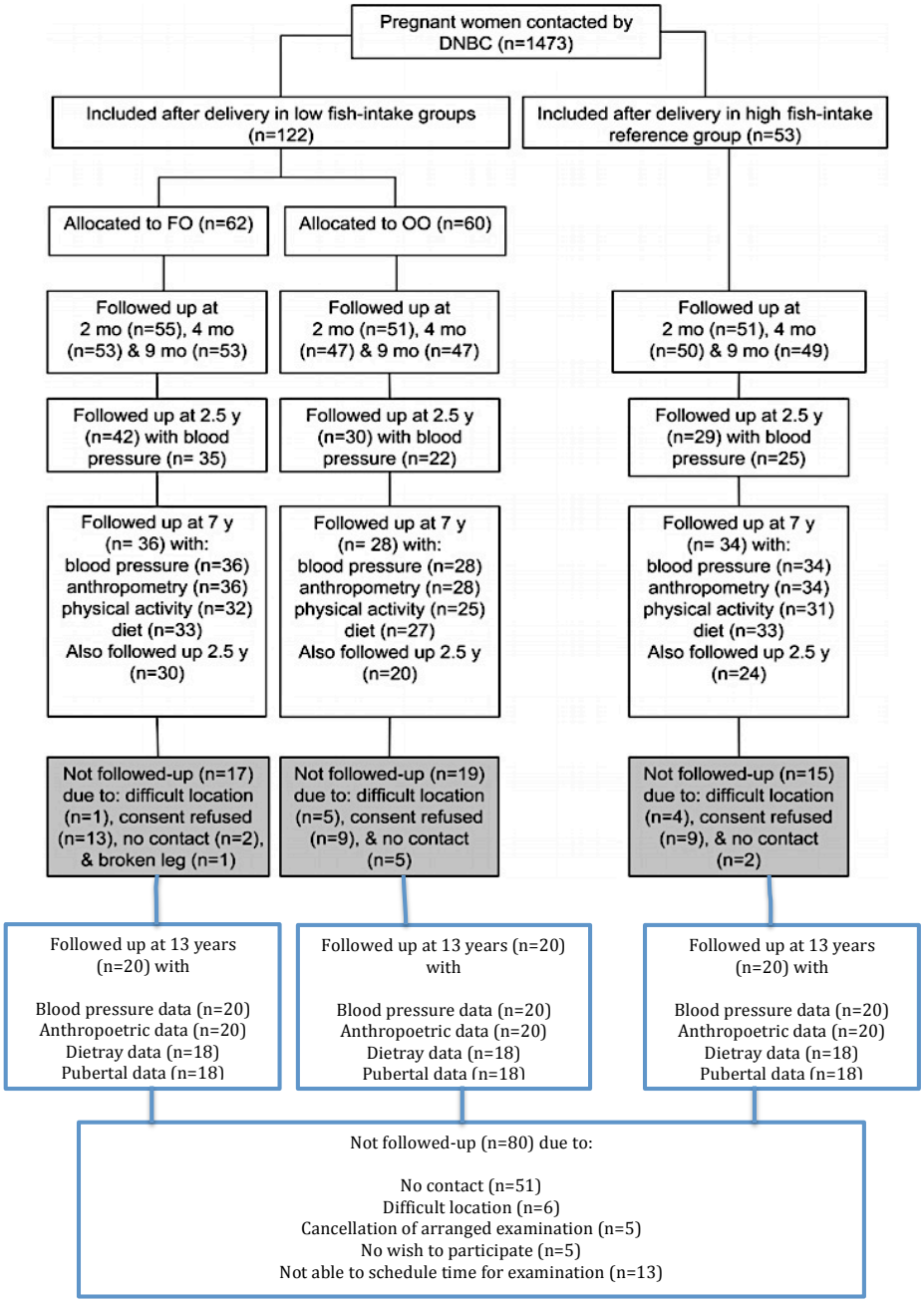


Figure 6. Trial profile illustrating participant flow during the randomized intervention and follow-up examinations for the three groups, with focus on the 13-year follow-up study. DNBC, Danish National Birth Cohort; FO, fish oil; OO, olive oil; HF, high fish

The examination

At the examination the child and parent were firstly informed about the procedure of the examination, and then to make the child more at ease, he or she was presented to the different devices used during the examination. Careful consideration had been put into deciding the order of examination procedure, beginning with the least "intensive" measurements (height and circumference measurements) and finishing off with assessing blood pressure, with the intention that the child would be more at ease after a while of examination. During examination of the child, the parent was given four questionnaires concerning the well-being and general health of the child. After the child had completed all anthropometric measurements he or she was asked to answer four questionnaires and a puberty test.

Standard procedure of the examinations

Anthropometric Measurements

Standard Operating Procedures (SOP´s) (2. Version, valid from 1.9.2009) for measurements of height, subscapular and triceps skinfold thickness and blood pressure was carefully studied prior to the examinations to assure the quality and integrity of data collection from the anthropometric measurements. All measurements made during the examinations were listed onto a datasheet (App. 4). All measurements were carried out in the following order;

1. **Height** was measured using on a portable height measure (The Leicester Height Measure, Child Growth Foundation, London United Kingdom) with a resolution of 0.1 cm. During the measurement the child was barefooted, and height was assessed two times with the child stepping out from the height measure between the measurements.
2. **Head, waist and hip circumference** was assessed in triplicate with a circumference measuring tape with a button to retract the measuring tape. Head measurements were assessed placing the measuring tape across the forehead and measured around the full circumference of the head. Waist measurements were done on the bare skin of the stomach in a line around the belly button. Hip circumference was measured around the widest portion of the buttocks in the child's underpants to make the most accurate estimation of the measurement. If the child felt

uncomfortable during this procedure the measurement was assessed with the child wearing his or hers trousers.

3. **Triceps and subscapular skinfold-thickness** was assessed with a resolution of 0.1 mm in triplicate with a Harpenden skinfold caliper. When measuring the triceps skinfold-thickness the child was told to bent the non-dominating arm 90 degrees, and the skinfold measurement of the triceps was made midway between the shoulder and elbow. In order to measure the subscapular skinfolds, the child was asked to place the non-dominating hand behind the back. In this position the shoulder blade pointed out making it more assessable to measure in an angle of 135 degrees two centimeters diagonally below the inferior angle.
4. **Weight and body fat percentage** were assessed on a Body fat monitor scale (Model Omron BF511). Using a four-sensor measuring system (one at each hand and foot) the body fat monitor could estimate the percentage of body fat by a bio-impedance method in the whole of the body. This device also assessed the child's weight. Body fat measurements and weight were carried out two continuous times.
5. **Blood pressure** was measured with a Boso-medicus Prestige unit, measuring blood pressure by the oscillometric principle. The child was asked to rest for 10 minutes in the supine position before measurement. Also, the circumference of the child's overarm was measured with a measuring tape to ensure to use the right cuff size for the measurement. Two different cuff sizes were available; small (12-22 cm) and medium (22-26 cm). Blood pressure was measured with a cuff around the child's non-dominating arm, and three consecutive measurements of SBP and DBP and heart rate were assessed. During measurement the child was asked not to talk, except if the measurement were causing discomfort. For this study the mean of the second and third measurement was used in data analysis, which is a common procedure to predict the most accurate blood pressure measurement (Park, Menard et al. 2005). In addition, MAP was calculated as DBP + 1/3 * (SDP-DBP), to estimate the average blood pressure of the child.

Physical activity

Physical activity was assessed by a movement monitor (ActiReg®). The ActiReg® monitor was placed on the right or left hip of the child in elastic band and registered movements of the body. The child was asked to wear the ActiReg® for 7 consecutive days, expect during nighttime and at swimming and showering. At the examination the child and parent were informed about how to use the ActiReg® and was given an instruction about the ActiGraph to bring home.

Due to a time limit, data of the physical activity level (PAL) of the children obtained from ActiReg® was not available and will therefore not be further analyzed in the present thesis.

Questionnaires; After assessing the child's blood pressure, the child was asked to answer four questionnaires concerning the general health and well being of the child, and a puberty-test designated Tanner Scales. It was important that the child answered the questionnaires in private and was not disturbed or distracted by the presence of the parent, and was seated in a separate room next to the room of examination. If the child felt uncomfortable sitting in a room alone, it was possible to arrange the child to sit in the same room as the parent and the examiner while answering the questionnaires. The questionnaires concerning the general health and well being of the child were not relevant for this present thesis and will not be further described.

- *Tanner Scales*

Pubertal maturity was assessed using Tanner Scales (App. 5). The Tanner Scales is developed to determine the child's pubertal maturation and consists of pictures that portray different stages of pubertal development rated on a 5-point scale (I, II, III, IV, V). Stage I is classified as prepubertal and stage V is classified as fully matured. The boys were asked to identify their genital development and pubic hair growth, and girls were asked to identify their breast development. In addition, the girls were furthermore asked to note if their first menarche has occurred, and note date of month if it was current.

Diet

The dietary intake of the child was assessed by a food frequency questionnaire (FFQ) called SOFT (Spørgeskema Om Fødevare Frekvens for Teenagere) that is specifically developed to address adolescents (App. 6). Based on the age of the children it was assumed that it might be difficult to complete a focused weighed dietary record, which could result in inaccurate dietary data collection. The use of a FFQ was considered as a valid and manageable method for the registration of diet of the 13-year old children (Rimm, Giovannucci et al. 1992), (Cade, Burley et al. 2004). In the FFQ the child was asked to fill out what he or she´s food habits had been for the last 12 months. The FFQ was divided into 13 categories;

1) Examples	2) Personal data	3) Beverages	4) Dairy Products	5) Cereals and bread	6) Cold cuts	7) Cold and hot dishes
8) Garnishes for cold and hot dishes	9) Fruit and vegetables	10) Snacks and desserts	11) Food habits	12) Activity	13) Help to answer the questionnaire?	

The parent and child were presented to the FFQ at the end of the examination where they were explained about the construction of the FFQ and how to fill it out. To illustrate how the FFQ was supposed to be filled out, I brought a copy with three examples of typical dietary questions from the FFQ to the examination. The child was asked to answer the FFQ mainly by itself, but that the parents were allowed to help if needed. In order have the results from the FFQ returned within a decent time range, the child were asked to answer the FFQ during the 7-day time period he or she was wearing the ActiReg®. The parent and child received the FFQ via a link sent to the parents (or the child's own) e-mail address. This e-mail also had a personal code specific to the child, such that when the child's answer was registered in our own database, I could identify the individually children by this code. Dietary information was obtained from the database containing the collected SOFT data and the average daily energy intake (including macronutrient E%) and distribution of macronutrients in the diet was calculated from the dietary data for each child.

In order to get familiar with the FFQ before I introduced it to the parents and the child I did at test-run before initiation of the examinations. I had two 14-old interns to answer the FFQ, which was advantageous in order to evaluate how long it would take to answer the FFQ and also have the opportunity to evaluate the pros and cons associated with the FFQ seen from a peer's perspective relative to the 13-year old children.

Pilot studies

Prior to initiation of the examinations I attended an examination from the Småbørns Kost Og Trivsel (SKOT) research project at Department of Human Nutrition. This was done in order to observe the standard examination procedure as well as the connection and communication between researcher and test persons. Furthermore, I performed a test-examination on a 10-year old boy in the presence of an academic competent observer. This was done in order to test the planned examination procedure and thereby evaluate if any changes need to be done before initiation of the examinations of the present 13-year old follow-up study.

Statistics

The analysis of the intervention was planned to be performed as raw group comparisons with ANCOVA/multiple linear regression analysis that take various confounders into consideration.

Data consideration

In order to perform linear (multiple) regression analyses continuous data were tested for normal distribution, independent residuals and equal variance before analyzing data and choosing appropriate tests

Normal distribution: Continuous data were always checked for normal distribution by histograms and Shapiro-Wilks normality tests with a p-value > 0.05 to accept data being normally distributed. Natural log transformation of those parameters that did not appear to be normally distributed were carried out to meet the assumption for normal distribution for analysis of co-variance. Parameters of the present follow-up study that were not normally distributed included; skinfold thickness, waist circumference and waist:height-ratio.

Equal variance:

Equal variance among the groups of comparison was always tested by a two-group comparisons test, testing the homogeneity of variance. A p-value >0.05 was also here accepted.

Residual plots: To ensure independency among residuals and avoid systematic errors, residual plots were made on all parameters to ensure that a pattern was not present in the plots.

Sample size

This study was a follow-up study and power calculating was not part of the study design as the purpose was to recruit as many participants as possible. A total of 60 children participated in this follow up study, with 39 from the two randomized groups. When performing subgroup analysis between the randomized groups, the number of subjects included in the analysis became even smaller. Furthermore, the sample size varied due to few missing values among the included covariates, and stratification on one or more covariates with missing values was not considered feasible due to the small sample size of the study.

Detection for outliers;

In order to detect for potential outliers both BoxPlots and residual-vs-fitted values plots were made. BoxPlot illustrated if any values were located below or above the 95-confidence interval.

A result was considered statistical significant if a p-value < 0.05 was obtained. All data was measured using STATA/IC version 12.1 for Mac (64-bit Intel).

Data not normally distributed are presented as median; 10th - 90th percentile. Normally distributed data are presented as mean ± SD. Non-adjusted and adjusted mean differences are presented as estimate ±SE. Results obtained from correlation analysis are presented as the correlation coefficient and the p-value.

Group comparisons

Comparison of OO-, FO- and HF-group;

The non-randomized HF-group was included as reference group when discussing results found between the two randomized groups. Furthermore, the HF-group was also included in the analyses of baseline data from the intervention (Table 11). When comparing normally distributed data between all three groups analysis of variance (ANOVA) was applied, and when comparing nonparametric data between all three groups the Kruskall-Wallis H test was applied. Furthermore, nominal variables was compared by χ^2-analysis and given as ratios.

Comparison of the two randomized groups; OO- and FO group

Normally distributed non-adjusted continuous variables were compared using a Student t-test. When comparing not normally distributed continuous variables the Mann- Whitney U test was applied. Furthermore, nominal variables was compared by χ^2-analysis and given as ratios.

For comparison of normally distributed adjusted continuous variables an analysis of covariance (ANCOVA)/multiple linear regression analysis was performed (see below).

Possible confounders

When considering stratification for possible covariates the small sample size of the study was taken into account and the number of covariates included in the statistical models was restricted to the most strongest effect modifiers.

Different possible confounders were considered when investigating the results from the group comparisons. In order to detect for group effects from other continuous explanatory normally distributed variables on the continuous outcome ANCOVA was performed. The randomization was stratified according to group level, sex and other confounding variables. Possible confounding

variables were considered to affect the dependent variable based on the literature, and were included in the extended statistical model for body composition and blood pressure (table 9).

Table 9. Possible covariates investigated with respect to parameters of body composition and blood pressure.

	Body composition	**Blood pressure**
Factors		
	Group (OO/FO)	Group (OO/FO)
	Sex (Male/female)	Sex (Male/female)
		Waist:height-ratio
Possible covariates		
	Pubertal score	Pubertal score
	Energy Intake	Place of examination
	Ponderal index at birth	Protein:Carbohydrate-ratio
		Energy intake

Each possible covariate was tested on the dependent variable in the statistical regression model with group and sex being the systematic factor in the model. Covariates included in the model that appeared to have a high significance level and prominently decreased the explanatory degree (adjusted R^2) were excluded from the final regression model. Covariates and factors being significant at the level of <0,05 were included in the final statistical analysis for blood pressure and body composition. However, some factors were kept in the statistical model due to their theoretical effect. Furthermore, all regression models were tested if an interaction between sex and group was significant at level of <0.05, and if so, sub groups analysis was performed for sexes separately.

Blood pressure and confounding variables

When analyzing all three blood pressure parameters the waist:height-ratio was found to have a significant effect on all the results when included in the regression model for every blood pressure parameter. Furthermore, a significant interaction between group and sex was found in diastolic blood pressure and mean arterial blood pressure, whereupon the two sexes were analyzed separately. The final model for diastolic blood pressure and mean arterial blood pressure, sexes separately, included waist:height-ratio. In systolic blood pressure no interaction between group and sex were found and were only adjusted for waist:height-ratio and sex.

Excluded confounding variables for blood pressure;

- Place of examination and protein/carbohydrate-ratio

When including place of examination in the statistical model, it did not have any significant effect on the results, probably due to unequal distribution of home examinations (n=9) versus examinations at Department at Human Nutrition (n=51), and were excluded as a covariate in the regression model for blood pressure. Furthermore, in order to detect for any dietary effect on blood pressure, protein/carbohydrate-ratio was included as a possible covariate. However, no significant effect was found on neither of the blood pressure parameters, and protein/carbohydrate-ratio was excluded as covariate from the regression model.

Body composition and possible confounders

The regression model of every outcome for body composition included a sex*group interaction which were excluded from the final regression model if it was insignificant. If a significant interaction between group and sex were found, the analyses were performed separately in gender subgroups.

None of the possible covariates for body composition showed significant effects when included in the regression model. The final regression model when analyzing parameters for body composition included ponderal index at birth that is known to be a determinant for body composition in later life (Valdez, Greenlund et al. 1996). Ponderal index at birth appeared to be significantly correlated with growth parameters for skinfold thickness, body fat percentage, waist circumference, hip circumference, waist/height-ratio. Furthermore, growth parameters for head circumference, weight and height were adjusted for head circumference at birth, birth weight and birth length, respectively. Ponderal index did not appear have any significant effect in regression model for head circumference, weight and length and were not included in the regression model for these body composition parameters.

Excluded confounding variables in blood pressure and body composition;

- *Energy intake and pubertal score*

Both pubertal score and energy intake was excluded from the final regression model for both blood pressure and body composition. It is a known fact that energy intake have showed to be an important explanatory factor for outcomes in both blood pressure (Rasmussen, Vessby et al. 2006) and body composition (Farnsworth, Luscombe et al. 2003), and therefore were considered as a

covariate for these outcomes. However when including energy intake in the regression model for blood pressure and body composition it appeared to have a high significance level, accompanied by a decrease in explanatory factor (R^2) and for this reason it was considered without any statistical effect and were excluded. Furthermore, the stage of pubertal development is also considered to have an effect on blood pressure (Shankar, Eckert et al. 2005) and body composition (Bordini and Rosenfield 2011), but also appeared to have no effect when included in the regression model for body composition and blood pressure, and were also excluded.

Possible dose-response parameters

Dose-response analysis were performed for all three blood pressure parameters and BMI and waist/height-ratio. Regression model with either adjusted BMI or waist/height-ratio as a dependent variable and either maternal RBC-DHA at 4 months or DHA in maternal breast milk at 4 months as independent variables were performed to analyze for any effect of either maternal RBC-DHA or DHA in maternal breast milk on BMI or waist/height-ratio. The same dose-response analysis was performed for all of the three blood pressure parameters. Since the degree of breast-feeding explains the reliability of the dose-response parameters this parameter was also used in the regression models.

Maternal RBC-DHA at 4 months was considered the most reliable of the dose-response parameters since it reflects the maternal diet over a longer period of time. DHA in maternal milk at 4 months, reflects the maternal diet but is also a questionable variable in that it varies according to dietary intake prior to the breast milk sample, however this variable has a higher degree of variance than maternal RBC-DHA. Infant RBC-DHA at 4 months was also a possible covariate to include in the dose-response analysis, but this factor was excluded from the dose-response analysis due several missing values in the dataset covering infant RBC-DHA.

Results

Characteristics of the participants

60 children participated the present follow-up study. The group distribution of the participants were HF=21, OO = 19, FO =20, which resulted in a follow-up rate of 32,8 % (HF), 31,7 % (OO) and 32.3 % (FO) respectively.

Nine out the 60 examinations were carried out in the home of the child and fifty-one examinations were carried out at the Department of Human Nutrition. The main researcher, Sara Elisabeth Eriksen, carried out fifty-eight examinations and two examinations were carried out by a nutrition student. The characteristics of the study participants are given in Table 10.

Table 10. Characteristics of the participants in the trial.

	High fish	**Olive oil**	**Fish oil**
Number of Children	21	19	20
Gender Distribution (Boy:Girl) [a]	9:12	7:12	12:8
Birth weight (kg)	3.56±0.5 (21)	3.63±0.43 (19)	3.7±0.4 (20)
Birth length (cm)	52.38±2.13 (21)	52.37±2.19 (19)	52.9±1.97(20)
Head circumference at birth (cm)	35.79±1.44 (20)	36.84±1.40(16)	36.52±1.32 (17)
Ponderal index at birth (kg/m^3)	24.85±2.25 (21)	25.25±2.33(19)	24.97±1.82 (20)

Values in group columns are given as SD± (number of subjects included (n)). Differences between groups were tested with a Student t-test.

[a]Values are given as ratios. Difference between groups are tested with a χ^2 -test

Fifty-four children were turned 13 years at the time of examination; the remaining six children all had their 13th birthday within one month of the examination.

Breast-feeding descriptions and intervention outcome are given in table 11. No significant differences were observed in duration of breastfeeding and the degree of breastfeeding between all three groups, as well as compliance between the two randomized groups. Table 11 describes the breast-feeding and intervention outcomes.

Table 11. Breast feeding descriptions and intervention outcome

	High fish	Olive oil	Fish oil
Degree of breastfeeding during the 4 months intervention (%)[a]	98.86,99-100 (21)	96.55, 82.5-100 (19)	92.58, 68.75-100 (20)
Total duration of breastfeeding (weeks) [a]	28.07,21.7-36.89 (8)	28.31, 19.53-36.89 (11)	24.68, 3.26-32.55 (8)
> 9 months of breastfeeding (yes:no)[b]	13:8 (21)	8:11 (19)	12:8 (20)
Maternal n-3 LCPUFA intake during intervention period (g/d)[a]	0.97,0.43-1.76 (21)	0.26,0.03-0.51 (19)	1.18, 1.07-1.27 (18)
Maternal RBC-DHA at 4 months (FA%)	7.32±1.38(21)	5.58±1.04 (19)	9.31± 0 .94 (20)
Maternal milk-DHA at 4 months b	0.72,0.4-1.2 (21)	0.48,0.21-0.77 (19)	1.39, 0.82-1.81 (19)
Compliance (% taken of intended dose)[a]	-	93.41,81.29-99.6 (19)	92.80, 83.83-100 (18)
Infant RBC-DHA at 4 months	7.56±1.50 (19)	6.18±1.42(16)	8.88±1.81(13)

Values in group columns are given as SD± (number of subjects included (n)). Differences between groups were tested with a Student t-test.

[a]Data are not normally distributed. Values are given as median, 10th-90th percentile (n). Differences between groups tested with a Man Whitney U test or Kruskal-Wallis test

[b]) Values are given as ratios.

Infant RBC-DHA at 4 months; Significant differences were observed between OO-FO (p=<0.001), and HF-FO (p=0.032) and HF-OO (0.008).

*Maternal RBC-DHA at 4 months***;** Significant differences between OO-FO (p=.<0.001), HF-FO (p<0.001), OO-HF (p<0.001).

*Maternal milk-DHA at 4 months***;** OO-FO (p<0.001), HF-FO (p<0.001), HF-OO (p=0.0073)

Maternal n-3 LCPUFA intake during intervention period; FO-OO (p<0.001), FO-HF (p<1.2)

Between the two randomized groups, a significant difference was identified with respect to maternal n-3 LCPUFA intake during lactation (p<0.001), DHA in maternal breast milk at 4 months (p<0.001), maternal RBC-DHA at 4 months (p<0.001) and infant RBC-DHA at 4 month (p<0.001), that all were significant higher in the FP-group compared to the OO-group. These results describe the successful effect of the intervention on the mother and the child.

Results from the present 13-year follow-up study

Diet

54 children completed the electronic FFQ that was used to collect dietary data. 6 children did not complete the FFQ, and this number was distributed equally in the three groups with 2 from HF-group, 2 from OO-group and 2 from FO-group, respectively.

When analyzing the data obtained from *macronutrients* E% all values satisfied the general dietary recommendations (Table 3) and were included in the dietary analysis. However, when analyzing the results from energy intake (EI) several variables exceeded the general recommendations (Table 2) in such unrealistic range that exclusion of these values was considered necessary. Thus, in total 5 values for EI, lying more than double above or below the recommended intake, were excluded and a more accurate data of EI was included in the final statistical dietary analyses. Its believed that common lack of concentration or the fact that several children have filled out the FFQ by themselves may have caused these unrealistically high or low values. The final outcome of the dietary analysis on EI, protein E%, carbohydrate E% and Fat E% are shown in table 12

Table 12. Energy and macronutrient intake of boys and girls measured by the self-reported electronic FFQ

		Randomized groups			
	High fish (n=19)	**Olive oil** (n=17)	**Fish oil** (n=18)	Non-adjusted mean difference (p-value)	Adjusted mean difference (p-value)
EnergyIntake (MJ/d)	10.3±3.1 (n=18)	11.9±3.6 (n=15)	8.4±2.5 (n=16)	3.4±1.1 (0.005)	-3.6±1.0 (0.002)
Protein (E%)	15.4±2.3	16.2±2.7	14.9±2.0	1.3±0.79 (0.10)	-1.09±0.76 (0.16)
Fat (E%)	30.7±4.4	31.4±4.7	31.5±4.5	-0.15±1.5 (0.92)	-0.23±1.52 (0.88)
Carbohydrate (E%)	53.9±5.3	52.4±4.6	53.6±3.7	-1.17±1.4(0.41)	1.32±1.43 (0.36)
Protein:Carbohydrate ratio	0.29±0.06	0.32±0.66	0.28±0.037	0.03±0.018 (0.069)	-0.03± 0.018 (0.098)

Values in group columns are given as ±SD, non-adjusted and adjusted mean differences ±SE (fish oil group-olive oil group)(number of subjects included (n)). The p-value non-adjusted mean difference describe the difference level between the two randomized groups, tested by a t-test. The p-value for the adjusted mean differences describe the significance level between the two randomized groups, tested by ANCOVA, adjusted for sex.

Non-adjusted mean results

A significant difference in energy intake appeared between the two randomized groups, with a higher energy intake in the OO-group compared to the FO-group (p=0.005). Furthermore a tendency towards a higher Protein/Carbohydrate-ratio (P/C-ratio) was observed in the OO-group compared to the FO-group (p=0.069). No significant differences were found on protein E%, carbohydrate E% or fat E% between the randomized groups.

Adjusted mean differences

No significant interactions between group and sex were found in regressions analysis on EI and macronutrients E%.

A significant adjusted mean difference in EI was observed between the two randomized groups, showing that the FO group had a significant lower EI intake then the OO-group (p=0.002). No significant mean adjusted differences was observed between the randomized groups in macronutrient E%, only a minor tendency appeared in the group variable when analyzing P/C-ratio (p=0.098), suggesting that the FO-group has a smaller P/C-ratio compared to the OO-group.

Growth and Body Composition

Anthropometric measurements were collected for all 60 children. Growth and body composition of 13-year-old children are shown in table 13

Table 13. Growth and body composition of 13-year old children

		Randomized groups			
	High fish n=21	**Olive oil** n=19	**Fish oil** n=20	Non-adjusted mean difference n=39 (p-value)	Adjusted mean difference n=39 (p-value)
Head circum.	54.8±1.5	54.0±1.2	55.1±1.7	-1.08±0.48(0.029)	0.20±0.52(0.32)[c]
Subs. Skinfold [a]	7.5, 5.03-10.17	8.1, 5.1-11.43	7.4, 5.1-10.88	0.09 ± 0.094 (0.34)[b]	-0.02±0.08(0.753) [b]
Tric. Skinfold [a]	10.8, 6.87-15.77	13.1, 6.7-18.73	12.9, 7.6-18.6	0.037 ± 0.12 (0.76)[b]	0.02±0.12(0.84) [b]
Body fat %	15.1±7.4	17±7.1	16.8±5.6	0.25±2.03(0.90)	1.11±1.83 (0.55)
Waist circum.	69.6±5.6	69.1±5.4	69.8±7.7	-0.7±2.15(0.75)	0.55±2.26(0.81)
Waist circum.[a]	69.5, 65.5-76.0	69.1, 62.9-75.5	69.8, 62.3-77.2	-0.007±0.27 (0.8)[b]	0.004±0.03(0.89) [b]
Hip circum.	82.8±6.9	84.6±6	82.7±7.3	1.91±2.15(0.38)	-0.3±1.96(0.88)
Waist:height-ratio [a]	0.42, 0.39-0.47	0.43, 0.38-0.47	0.44, 0.40-0.49	-0.02±0.03(0.42)[b]	0.02±0.03(0.56) [b]
Height (cm)	164.0±7.7	161.6±5.7	159.3±6.5	2.29±1.96(0.25)	-2.42±1.80 (0.187)[d]
Weight (kg)	49.2±6.7	48.9±6.3	47.9±7.6	0.95±2.24(0.67)	-0.80±2.18 (0.714)[e]
BMI	18.2±2	18.7±2.0	18.8±2.2	-0.11±0.67(0.87)	0.27±0.67(0.69)
Puberty score	3.33±0.75(18)	3.35±0.66[f](18)	2.96±0.86[g](18)	0.39±0.26 (0.14)	-0.29±0.25 (0.26)

Values in group columns are given as ±SD, non-adjusted and adjusted mean differences ±SE (fish oil group-olive oil group). The p-value non-adjusted mean difference describe the difference level between the two randomized groups, tested by a t-test. The p-value for the adjusted mean differences describe the significance level between the two randomized groups, tested by ANCOVA, adjusted for sex and ponderal index at birth.

[a] Variables not normally distributed, values are given as median, 10th-90th in percentiles.

[b] Ln transformed (natural logarithms) to adjust values and make them similar to normal distribution for ANCOVA

[c] Head circumference adjusted for head circumference at birth and sex (n=33)

[d] Height adjusted for birth length and sex
[e] Weight adjusted for birth weight and sex
[f] Puberty Score mean values assessed by Tanner Scales in OO-group, n=18
[g] Puberty Score mean values assessed by Tanner Scales in FO-group, n=18
Abbreviations; BMI, body mass index; cirum., circumference; Subs., subscapularis; Tric., Triceps;

Non-adjusted results.

The only significant mean difference appeared in the head circumference between the two randomized groups (p=0.029), with the FO-group having a significant larger head circumference than the OO-group.

The OO-group appeared to have higher mean value in puberty score compared to the FO-group, however the difference was not significant when analyzing the mean non-adjusted values (p=0.14). Based on the assumption that girls tend to have an earlier onset of pubertal development compared to boys (Bordini and Rosenfield 2011), the puberty score between girls and boys from the two randomized groups, independent of group allocation, were analyzed. It was found that girls had a strong tendency towards a higher pubertal score than the boys (p=0.05) (not shown), and when including the children from the HF-group the difference in puberty between sexes became even more significant (p=0.02) (not shown).

According to the age-dependent BMI cut-off values (Cole, Bellizzi et al. 2000) none of the children included in the trial was characterized as obese. However, three children were characterized as being overweight; one girl from the OO-group and a boy and a girl from the FO-group. Taking together, this results in a rate of overweight children of only 5 % of total number of children included in the 13-year follow up study.

Adjusted mean difference

No significant interactions between group and sex were found in regressions analysis for anthropometric parameters. Furthermore, no significant mean adjusted differences were found between the two randomized groups in any of the anthropometric measurements, thus the significant mean difference between the randomized groups in head circumference was no longer evident after adjustment (p=0.32).

BMI for 2.5, 7 and 13 years

Table 14 shows mean BMI for all three follow up studies conducted at 2.5, 7 and 13 years, respectively and the mean difference between the two randomized groups for non-adjusted and adjusted values, boys and girls separately. In order to explore a possible relationship in the development of BMI from 2.5 to 13 years, non-adjusted and adjusted mean differences are also specified for the BMI-ratio between 2.5 years and 13 years.

Table 14. Mean BMI and mean differences in BMI between the two randomized groups among boys and girls from 2.5 to 13 years

	High fish	**Olive Oil**	**Fish Oil**	Non-adjusted mean difference (p=value)	Adjusted mean difference (p=value)
BMI2.5					
Girls + boys	16.1±1.06	15.8±0.77(13)	16.7±1.1(18)	-0.83±0.35 (0.02)	0.89±0.37 (0.024)
Boys	16.3±1.08 (8)	15.8±0.91(5)	16.8±1.2(11)	-1.0±0.60 (0.12)	1.27±0.60 (0.054)
Girls	15.8±1.05 (5)	15.9±0.74(8)	16.5±0.34 (7)	-0.63±0.43 (0.16)	0.64±0.45 (0.18)
BMI7					
Girls +boys	15.6±1.57	15.97±1.43(16)	16.0±0.28(18)	-0.23±0.50 (0.62)	0.46±0.47 (0.33)
Boys	15.5±1.25(12)	14.84±0.94(5)	16.0±0.98(12)	-1.15±0.52 (0.04)	1.24±0.50 (0.03)
Girls	15.8±2.0(8)	16.22±0.44(11)	16.1±1.63 (6)	0.16±0.77 (0.83)	-0.2±0.79 (0.8)
BMI13					
Girls + boys	18.2±2	18.7±2 (19)	18.8±2.2	-0.11±0.67(0.87)	0.27±0.67(0.69)
Boys	17.9±2.06(12)	18.0±0.53(7)	19.0±1.8(12)	-1.06±0.79 (0.20)	1.05±0.74 (0.17)
Girls	18.7±1.93(9)	19.1±2.2(12)	18.5±0.54(8)	0.62±1.12 (0.58)	-0.4±1.11 (0.69)
2.5/13-BMIratio					
Girls + boys	0.86±0.09	0.85±0.09 (13)	0.89±0.11(18)	-0.04±0.04 (0.25)	0.03±0.04 (0.38)
Boys	0.89±0.08(8)	0.87±0.1 (5)	0.88±0.09 (11)	-0.003±0.5 (0.9)	0.002±0.05 (0.97)
Girls	0.80±0.08(5)	0.83±0.07 (8)	0.9±0.14(7)	-0.07±0.06 (0.21)	0.07±0.06 (0.26)

Values in group columns are given as ±SD, non-adjusted and adjusted mean differences ±SE (fish oil group-olive oil group). The p-value non-adjusted mean difference describe the difference level between the two randomized groups, tested by a t-test. The p-value for the adjusted mean differences describes the significance level between the two randomized groups, tested by ANCOVA, adjusted for sex and ponderal index at birth

2.5 and 7-year non-adjusted and adjusted results

A significant non-adjusted mean difference in BMI between the randomized groups was observed at 2.5 years (p=0.002), with the FO-group having a higher BMI compared to the OO-group. When adjusting for ponderal index and sex this significant difference was still evident (p=0.024). No significant non-adjusted mean difference appeared between the two groups when analyzing the sexes separately. However, a tendency towards a higher BMI in boys from the FO-group compared

to boys from the OO-group was found in the adjusted mean difference (p=0.054). No significant mean adjusted difference was evident in girls between the randomized groups (p=0.18)

No significant non-adjusted or adjusted difference was observed between the two randomized groups in 7-year old children. When analyzing the sexes separately, however, a significant non-adjusted (p=0.04) and adjusted (p=0-03) mean difference was observed between the boys from the randmoized groups, with the boys from the FO-group having a significantly higher BMI compared to the OO-group. No significant difference was observed between the girls.

13-year non-adjusted and adjusted mean results

When analyzing the mean differences in BMI in the 13-year old children no significant differences were observed between the two randomized groups, also no significant mean differences were observed when analyzing the sexes separately..

Tracking BMI 2.5→ 13

No correlation was found between BMI at 2.5 year and BMI at 13 year in the children from the two randomized groups (p=0.46, r=0.14). No significance was also observed within the sexes separately; girls (p=0.81, r=0.07) and boys (p=0.34,r=0.23).

Furthermore, in order to further investigate the relationship between BMI from 2.5 years to 13 years the ratio comparing the BMI at 2.5 years and 13 years (2.5/13-BMI ratio) was analyzed between the randomized groups. However, no significant difference in 2.5/13-BMI ratio was observed between the randomized groups, neither when analyzing sexes separately (see table 14 and figure 7)

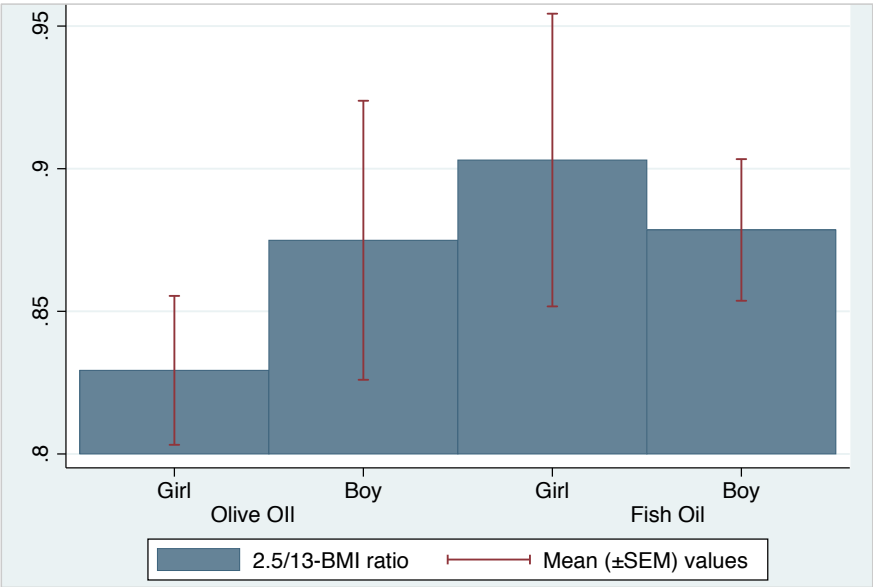


Figure 7. The association between the 2.5/13-BMI ratio between the two randomized groups, sexes separately. Values are given as ±SEM values

Dose-response Analysis

No significant associations were observed between maternal RBC-DHA at 4 months or maternal milk RBC-DHA at 4 months and BMI or W/H-ratio in the 13-year old children in the randomized groups. However, a weak tendency towards a positive association between DHA in breast milk and 2.5/13BMI-ratio was observed (p=0.098) in children from the randomized groups. Interestingly, when analyzing the sexes separately, it appeared that there was a significant association between DHA in breast milk and 2.5/13-BMI ratio in girls (p=0.038), indicating that increased maternal milk DHA was associated with a higher 2.5/13-BMI ratio only was associated with the girls, not the boys (p=0.64). Furthermore, no significant association was found between 2.5/13-BMI ratio and maternal RBC-DHA at 4 months. Figure 8Aand 8B portrays the regression analysis between adjusted 2.5/13-BMI ratio and DHA in breast milk in girls (figure 8A) and boys (figure 8B) from the randomized groups.

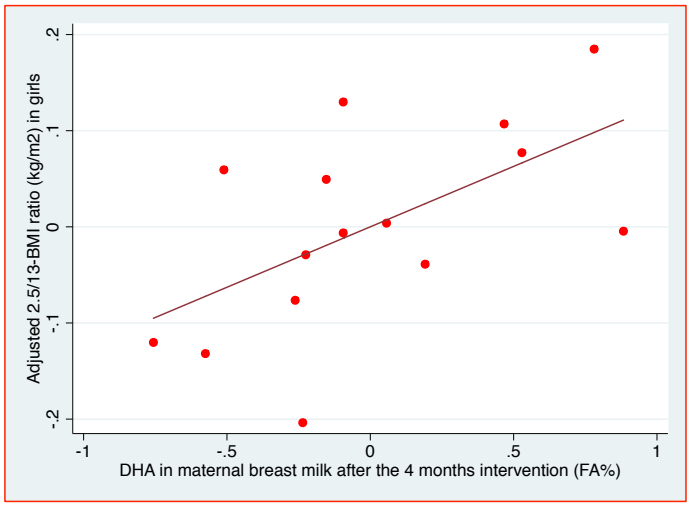


Figure 8A.
Association between adjusted 2.5/13-BMI ratio and DHA in maternal breast milk at 4 months in girls from the randomized groups
(p=0.038)
(n=15)

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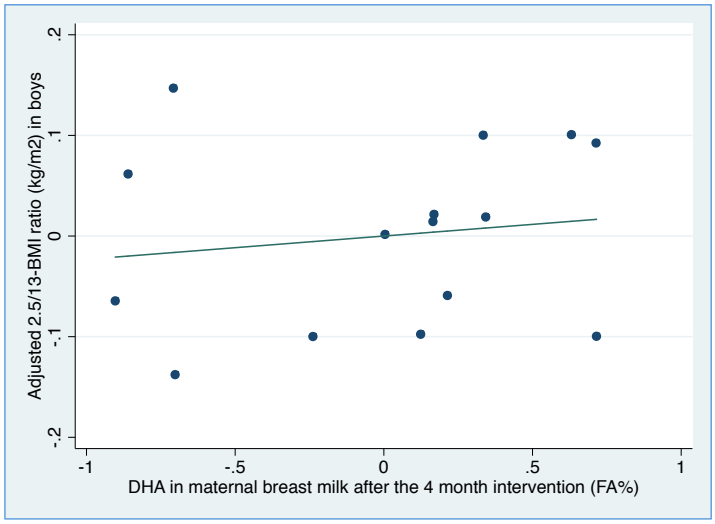


Figure 8B.
Association between adjusted 2.5/13-BMI ratio and DHA in maternal breast milk at 4 months in boys from the randomized groups
(p=0.64)
(n=15)

Dietary interactions

As a small tendency towards a higher P/C-ratio was observed in the OO-group compared to the FO-group (p=0.098), I investigated the potential effect of P/C-ratio on BMI in the 13-year old children. However, the P/C-ratio neither had an effect on BMI in general nor when analyzed for each sex separately in the randomized groups (figure 9-10).

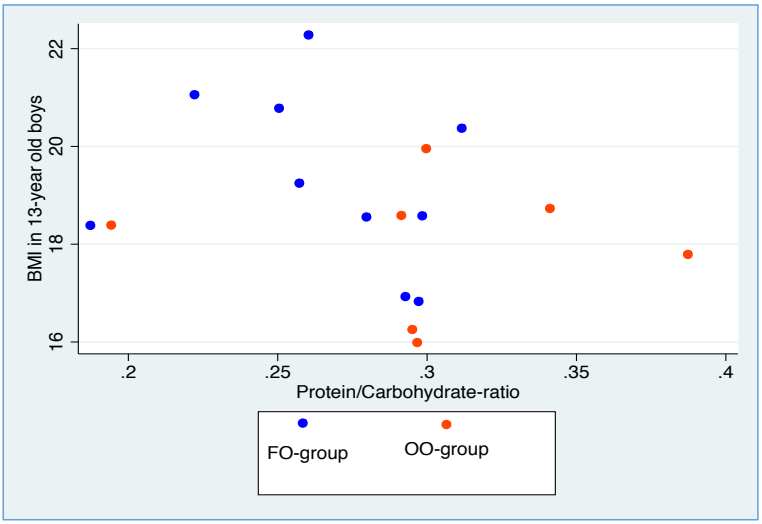


Figure 9;

Relationship between BMI and Protein/carbohydrate-ratio in 13-year old ***boys*** from the randomized groups. FO-group (blue, n= 10);OO-group (red, n=7).

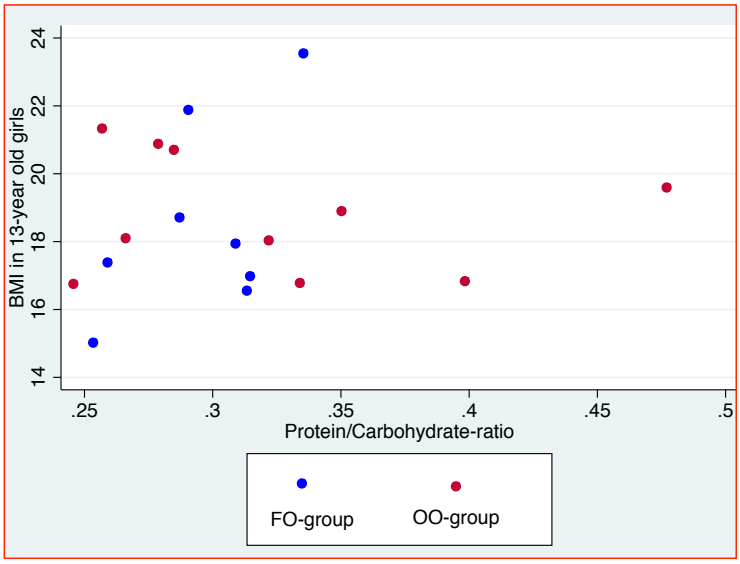


Figure 10; Association between BMI and Protein/carbohydrate-ratio in 13-year old ***girls*** from the randomized groups. FO-group (blue, n= 8); OO-group (red, n=10)

Blood pressure

Systolic and diastolic blood pressure measurements were assed in all 60 children. The results are shown in table 15

Table 15. Blood pressure in 13-year old children.

	HF (n=21)	**OO** (n=19)	**FO** (n=20)	Non-adjusted mean difference	Adjusted mean difference
SDP	106.4±7.1	107.2±6.2	106.6±7.9	0.61±2.28(0.79)	-1.67±2.25(0.46)
DBP					
Girls +boys	64.7±5.7	64.34±5.3	63.8±4.0	0.52±1.50 (0.73)	Group*sex (0.038)[a]
Boys[c]	64.4±6.67	61.57±4.14	64.54±3.94	-2.97±1.91(0.14)	2.8 ± 1.75 (0.12)[b]
Girls[d]	65.1±4.37	65.96±5.35	62.75±4	3.21±2.22 (0.17)	-3.26±2.31* (0.18)
MAP					
Girls+Boys	78.6±5.5	78.6±4.5	78.1±4.4	0.55±1.42(0.7)	Group*sex (0.019)[b]
Boys [c]	78.82±6.32	76.62±4.76	79.35±3.2	-2.73±1.81 (0.15)	2.64±1.71(0.14)
Girls[d]	78.26±4.42	79.78±4.06	76.15±5.39	3.63±2.11 (0.10)	-3.95± 2.11 (0.079)

Values in group columns are given as ±SD, non-adjusted and adjusted mean differences ±SE (fish oil group-olive oil group). The p-value non-adjusted mean difference describe the difference level between the two randomized groups, tested by a t-test. The p-value for the adjusted mean differences describe the significance level between the two randomized groups, tested by ANCOVA, adjusted for sex and waist:heigth-ratio.
[a]Significant intaeraction between group and sex in DBP (p=0.038)
[b]Significant interaction between group and sex in MAP (0.019)
[c] n=20 (number of boys from the randomized groups), [d] n=19 (number of girls from the randomized groups)

Non-adjusted mean results

Though insignificant, it appeared that the boys from the FO-group had higher non-adjusted mean values in MAP (2.73±1.81 mmHg, p=0.15) mmHg and DBP (2.97±1.81 mmHg, p=0.14) than the boys from the OO-group. While still insignificant, it was interesting that the opposite observation was made from the girl's perspective, where OO-girls had higher MAP (3.63±2.11, p=0.10) and DBP (3.21±2.22, p=0.17) than the girls from the FO-group.

Adjusted mean results

The final model for blood pressure included adjustment for W/H-ratio, and because of a insignificant group-sex interaction in SBP, analysis of this parameter also included the adjustment for sex.

Contrary to this we found a significant group*sex interaction in both DBP (p=0.038) and MAP (p=0.019), which is highly interesting and indicates that the effect of the intervention differs significantly between the sexes.

Furthermore, when analyzing the sexes separately I only observed a tendency towards a direct effect of the intervention on the MAP of the girls, where the FO group had a tendency towards a lower MAP compared to the OO group (p=0.079) (figure 11). This could be due to the small individual sample sizes for boys (n=19) and girls (n=20). No significant difference was observed in MAP in boys between the two randomized groups (p=0.14)

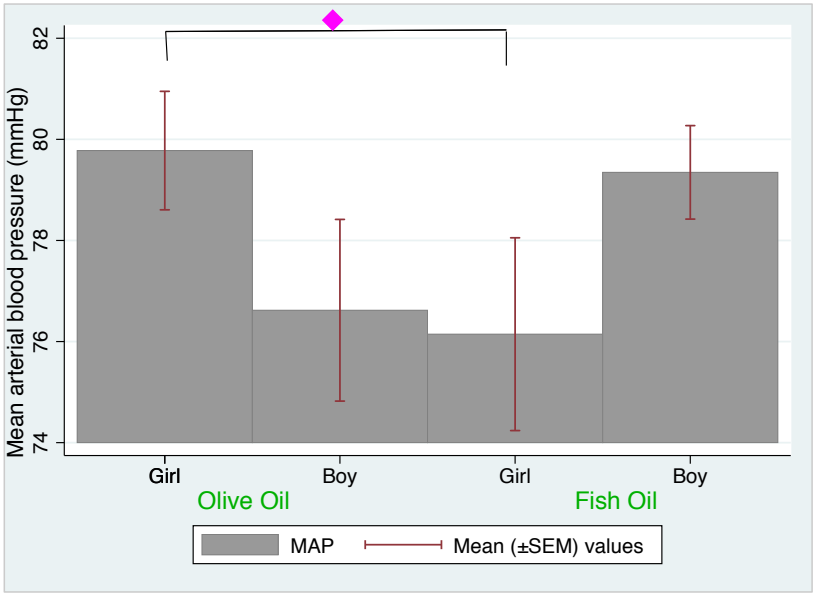


Figure 11. Difference in MAP between girls from the randomized groups and between boys from the randomized groups. Values are given as ±SEM. The black diamond indicates a tendency towards a lower blood pressure in girls from the FO-group compared to the OO-group (p=0.079).

Dose-response analysis

No significant correlations were found between the adjusted SBP and any of the dose-response parameters. Due to a group-sex interaction in DBP and MAP pressure sexes were analyzed separately.

- *Diastolic blood pressures*

For DBP in boys a strong tendency towards a positive association with maternal RBC-DHA at 4 months was found (p=0.05, n=19) No significant association was found when adjusting fro DHA in maternal breast milk at 4 months

- *Mean arterial blood pressure*

A significant positive association was found between maternal RBC-DHA at 4 moths and mean arterial blood pressure in boys (p=0.028) (figure 12). Furthermore, a significant association was also found between DHA in maternal breast milk at 4 months and mean arterial blood pressure in boys (p=0.043).

Including the HF-group

In order to expand the number of subjects in the dose-response analyses, the children from the HF-reference group was included in the dose response analysis of the 13-year old children from the randomized groups. No significant difference was found between the adjusted model for blood pressure (MAP, SDP, DBP) and adiposity parameters and maternal RBC-DHA and milk DHA and infant RBC.

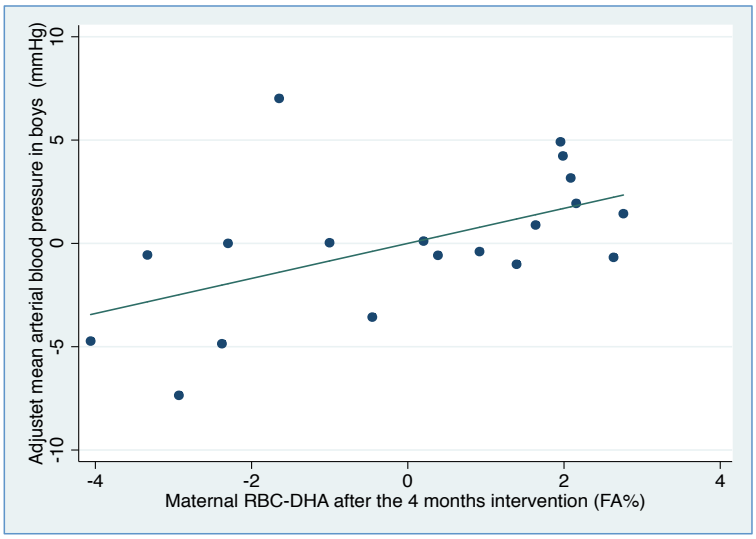


Figure 12; Association between adjusted mean arterial blood pressure and maternal RBC-DHA at 4 months in boys from the randomized groups (p=0.028, n=19)

Discussion

This study was the third follow-up study in a series of three studies. The two other follow-up studies were performed at 2.5 years and 7 years. A follow-up study at 13 years gives an excellent opportunity to detect long-term effects. To recapitulate on the results no effect of FO on blood pressure was found in the children at 2.5 year (Ulbak, Lauritzen et al. 2004), but a significantly higher blood pressure was found in the boys from the FO-group at 7 years (Asserhoj, Nehammer et al. 2009). Regarding body composition measurements, a significant difference in BMI was found in the children from the FO-group at 2.5 year (Lauritzen, Hoppe et al. 2005), but this difference was no longer detectable at 7 years (Asserhoj, Nehammer et al. 2009). In this follow up study at 13 years we found no significant difference in BMI or W/H-ratio. However we did find a significantly higher non-adjusted head circumference in the FO-group compared to the OO-group. This observation is in accordance with the prior 2.5 and 7-year follow-up study. However, in the present study this effect disappeared when adjusting for head circumference at birth and sex. A very interesting finding in the present follow up study was a significant group*sex interaction in both DBP and MAP, indicating that the intervention has a different effect in boys and girls However, when analyzing the sexes separately these significant differences were no longer evident, only a tendency towards a lower MAP in girls from the FO-group was still present (p=0.079).

Puberty

Due to lack of statistical power in the pubertal score, puberty was ruled put from several regression models as covariate. However, as the children at 13 years of age are about to enter or have entered puberty, the effect of this developmental stage will be taken into consideration in the discussion. The 13-year old boys were found to be between Tanner stages 2.7-3.2 and the 13-year old girls were found to be between Tanner stages 3.3-.3.4 (not shown). According to the Tanner stages the increment of testosterone in boys is steepest between stage 2-3 (Knorr, Bidlingmaier et al. 1974) and estradiol concentrations are significantly increased in girls at Tanner stage 3-4 (Rapkin, Tsao et al. 2006). This influence of sex hormones is considered highly relevant when discussing outcomes in blood pressure and body composition.

Body composition

A significantly larger head circumference was found in the FO-group in previous follow-up studies at 2.5 and 7 years.(Lauritzen, Hoppe et al. 2005{Asserhoj et al., 2009). This was also evident in the non-adjusted mean values in this present follow-up study at 13 years, though the effect disappeared when adjusting for head circumference at birth and sex. No other significant differences were observed in body composition parameters between the two randomized groups.

The dose of n-3 LCPUFA in this study (1.5 g/d) is comparable to two recent randomized controlled follow-up studies administrating n-3 LCPUFA; 1) a 19 year-follow up study by Rytter, Bech et al. where pregnant women were administrated app. 2.7 g/d total n-3 LCPUFA (app.0.6 g DHA and 0.8 g EPA) from the last trimester of pregnancy until delivery, and 2) in a 7-year follow up study where Helland, Smith et al. supplemented from week 18 of pregnancy until 3 months after delivery with app. 1.98 g/d n-3 LCPUFA (app.1.2 g DHA and 0.8 g EPA). However, none of these studies found an effect on growth in the healthy term infants. Furthermore, in another recent follow up study Bergmann et al. administrated 0.2 g DHA and 0.6 g EPA to women from week 21 of pregnancy until 3 months after delivery and fund no effect on growth in the 6-year old offspring. Previously, Bergmann et al., found a lower BMI in the supplemented group compared to the control group at 21 months of age, which is inconsistent with our finding in the previous follow-up study at 2.5 years, where children from the FO-group had a significantly higher BMI compared to the OO-group. However, the dose used by Bergmann et al., is notably lower than the dose used in this present study, which makes it difficult to compare the study by Bergmann et al., 1to this present study. In general, differences in duration of supplementation, the use of control oils and the source of n-3 LCPUFA make the comparison with other follow-up studies complex.

In this 13-year follow up study I did not find any significant difference in BMI or W/H-ratio between the two randomized groups. This finding is consistent with a 19-year follow up study by Rytter, Bech et al. did not find any effect of fish-oil supplementation during pregnancy on offspring BMI or W/H-ratio. Rytter, Bech et al. supplemented with 2.7g/d n-3 LCPUFA, which makes it comparable to the dose used in the present study. Furthermore, to my knowledge, the 19-year follow up study by Rytter, Bech et al. is the only randomized controlled follow-up study including children older than 7 years, which makes it more comparable to the present study age-wise. However, no previous follow-up studies on body composition have been conducted from this intervention, which makes it difficult to analyze the course of development of growth in these 19-year old offspring. Furthermore, supplementation was only administered during pregnancy, which makes the comparison between Rytter, Bech et al., and this present study further difficult due to the

fact that the time window of supplementation could be of importance and have a potential programming effect on body composition. Due to different developmental stages during gestation and in peri/postnatal period, n-3 LCPUFA exposure in early life might have different long-term outcomes when exposed during gestation compared to peri/postnatal life. In addition, Helland, Smith et al., and Bergman et al., both supplemented during pregnancy and lactation, which makes the overall comparison between these studies difficult due to the time duration and period of supplementation, which is discussed in the following.

Sex-specific differences in n-3 PUFA metabolism in relation to long-term effect on body composition

In the previous follow-up study at 2.5 years it was observed that the children in the FO-group had a significantly higher BMI than the children in the OO-group. From follow-up data at age 7 years this significant difference was reported to no longer be evident (Asserhoj, Nehammer et al. 2009). However, the authors analyzing BMI in the 7-year follow-up study only investigated the difference between the randomized groups and performed no analyses of the sexes separately. In this present study, when analyzing the sexes separately a significant adjusted mean difference between BMI at 7 years between boys from the two randomized groups was observed (p=0.003) (Table 14). Recently, focus have evolved around the possibility that there might exist a sex-dependent differential responses to exposure of n–3 PUFA during the perinatal period (Jen, Church et al. 2009), and the need of conducting subgroup analysis in sexes separately when analyzing possible long-term effects of n-3 LCPUFA supplementation have been suggested (Decsi and Kennedy 2011). This suggestion was mainly due to the fact that there might exist sex-specific differences in essential fatty acids metabolism and that females tend to have a higher conversion rate of α-linolenic acid to n-3 LCPUFA compared to males (Decsi and Kennedy 2011) . This is further supported by the fact that female rats have showed to have a higher expression of Δ-6 and Δ-5 desaturase accompanied by higher proportions of AA and DHA in liver and plasma phospholipids compared to male rats (Childs et al., 2010). Furthermore, this sex-specific difference in fatty acid metabolism might cause potential different programming effects of n-3 LCPUFAs (Sardinha, Fernandes et al. 2013). Evidently, the fact that Bergmann et al and Helland, Smith et al. did not analyze the sexes separately further complicates comparison of results obtained from these studies with this present study. In the study from Rytter, Bech et al. stratification of for sex were performed before analyzing the results, and indicated that differential effects for males and females, but the differences, however were not significant.

Critical period of programming effects of n-3 LCPUFA on body composition

An interesting, and important aspect of evaluating the long-term effect of n-3 LCPUFA on body composition is the time duration and period of which these PUFAs have been applied to the maternal diet. In this present follow-up study the women were administered n-3 LCPUFA during lactation. However, as outlined in table 6, different dietary approaches have been performed in recent studies investigating the long-term effect of n-3 LCPUFA; i.e during pregnancy (Rytter, Bech et al.), during pregnancy/lactation (Helland, Smith et al., Bergmann et al.), during lactation (Lauritzen 2004 et al.; Pedersen, Lauritzen et al. 2012) or early postnatal diet (Andersen, Michaelsen et al. 2011). However, none of these studies have found any long-term effect of early exposure of high levels of n-3 LCPUFA on body composition.

Because different stages in fat cell development in humans could be affected according to when the dietary intervention is applied, it could be possible that the different choices of interventional dietary approaches could result in different long-term effects on child body composition outcomes. Evidence from animal studies would give the best indication of the most effective dietary approach to reduce fat mass development in later life, however the results have been inconsistent. It have been demonstrated that exposure to ALA during the prenatal period resulted in decreased body fat in rat offspring at 3 week (Korotkova, Gabrielsson et al. 2002) and another study (Muhlhausler, Miljkovic et al. 2011) showed that a diet rich in DHA during lactation actually *increased* body fat in 6 weeks old offspring. Furthermore, those studies in animals that have demonstrated a fat-reducing effect from supplementation in the lactation/gestation period have further weaned the offspring on the same diet high in n-3 PUFA, which makes it difficult to determine if the fat-reducing effect arises from the perinatal period alone or the results simply is due to a fat-reducing effect on mature adipocytes. Thus the need for more animal studies investigating the effect n–3 long LCPUFA during the perinatal period alone, on body fat mass in the offspring to help clarify this have been emphasized (Muhlhausler, Miljkovic et al. 2011)

Mechanisms;

The fat-lowering effect of n-3 PUFA may be modulated by an extensive regulation of lipid metabolism through the action of transcription factors involving PPAR-y and SREBP-1 (Madsen, Petersen et al. 2005), that regulate adipogenic and lipogenic gene expression.

It has been demonstrated that feeding mice with a diet rich in LA compared to a diet rich in ALA during gestation and suckling period, resulted in a greater fat mass development in mice offspring receiving high levels of LA compared to high levels of ALA, and that this increase in fat mass persisted even though mice were fed a standard diet after weaning (Ailhaud, Massiera et al. 2006).

Thus, Ailhaud, Massiera et al, emphasizes the importance of the dietary fatty acid composition during gestiaion/suckling period in terms of fat mass development in later life. On the molecular level, these findings might be explained by an increased dietary intake of n-3 PUFA inducing changes in the composition of polyunsaturated fatty acids incorporated in the cell membrane. This might in turn affect cell-signaling pathways, which can lead to altered transcription factor activity and thus changes in gene expression. Furthermore, prostacyclins, a metabolic product of arachidonic acid from the n-6 PUFA-family, have been shown be adipogenic and influence the differentiation of pre-adipocytes into mature adipose cells by activation of PPAR-y. Thus, when increasing the dietary level of n-3 PUFA relative to n-6 PUFA, the synthesis of prostacyclin might be down regulated and adipocyte proliferation inhibited (fig 5). However, in this present study the children from the FO-group showed a significantly higher n-3 LCPUFA status in infant RBCs at 4 months compared to the OO-children, which reflected the impact of the intervention. Therefore, one would expect that this higher level of n-3 LCPUFA preferentially could suppress the differentiation of adipocytes by down regulating AA-mediated effects on adipocyte development. However, this was not the case in our study, and the children from the FO-group at 2.5 year even showed an increased fat mass compared to the OO-group. Interestingly, Muhlhausler, Miljkovic et al. found that that supplementation during pregnancy and lactation was not associated with a decrease in fat mass at weaning, but an increase in subcutaneous fat mass in rat offspring at 6 weeks of age. Surprisingly, no change in the expression of adipogenic or lipogenic genes was demonstrated. DHA have showed to suppress SREBP-1 in fat-induced adult rats (Ailhaud 1992), however this was obvious not the case in the study by Muhlhaulser.

However, Muhlhausler also found that n-3 LCPUFA supplementation did *not* affect the n-6 PUFA concentration. Interestingly, a number of studies have speculated that reducing n-6 PUFA intake during gestation/lactation might be a more effective approach in reducing fat mass in offspring than supplementation with n-3 PUFA (Moon, Harvey et al. 2013). Much, Brunner et al. also shares these speculations, and evidence from this study in pregnant women receiving a dietary intervention in order to reduce the n-6:n-3-ratio showed that n-3 LCPUFA might promote prenatal growth rather than serve as postnatal growth factors. Thus the increase in fat mass in offspring demonstrated Muhlhausler, Miljkovic et al. could be explained by the impact of n-6 PUFA level. The lactating women from this present follow-up study did not receive any dietary counseling to reduce the n-6:n-3 ratio, therefore it can not be ruled out that potential high levels of n-6 PUFA could have affected the outcome on body composition in this present study.

Dose-response analysis

If the dietary intervention of exposure of n-3 LCPUFA in early might have a potential programming effect on body composition, it would be expected that this would be explained by a dose-response effect. Much, Brunner et al. found a significant positive association between maternal RBC-DHA, EPA, AA in the last trimester of pregnancy on length, weight and lean body mass at birth. However, these findings were no longer evident at later stages of development up to first year of life. Much, Brunner et al., proposes that n-3 LCPUFAs may serve as prenatal growth factors rather than have a long-term effect on postnatal growth suggesting that the effect of DHA may be time dependent on the growth development in the child. This is supported by findings by Bergman et al.; the differences observed at 21 months also were no longer evident at 6 years. Bergmann et al also suggests that n-3 LCPUFA supplementation during the prenatal period may be of great importance regarding the programming effect of DHA on growth and the authors speculate that the long-term effect of DHA on growth in children may only be temporary, and that the child eventually will return to its intended size after the interventional effect of the supplement has subsided. In this study we observed a significant difference in body composition at 2.5 years in the FO-group compared to the OO-group, and that this also was significantly correlated with maternal RBC-DHA at 4 months (Lauritzen, Hoppe et al. 2005). Furthermore, outcome on body composition at 7 years (Asserhoj, Nehammer et al. 2009) or in this present study at 13 years was *not* explained by any effect of dose-response parameters. Thus, it could be a possibility that n-3 LCPUFA supplementation during lactation might only have a temporary effect. So, although Much, Brunner et al and Bergmann et al suggests that a potential temporary effect might arise from n-3 LCPUFA in the prenatal period, our findings from this study could also indicate n-3 LCPUFA supplementation during lactation also only persist temporarily.

Interestingly, I did find a significant effect of DHA in maternal breast milk on the 2.5/13-BMI ratio in girls (p=0.038). This may be an interesting finding, and will be discussed in the following.

Hypothesis; BMI ratio 2.5y/13y

In the preceding text I have speculated whether the lack of effect from the intervention on body composition in the 13-year old children may be due to a temporal effect of n-3 LCPUFA early in life or the supplementation solely during lactation might be a possible reason. However, a significant effect of DHA in maternal breast milk was found on the 2.5/13-BMI ratio, and consistent with other studies (Bergmann et al, 2012; Pedersen, Lauritzen 2012) that have found an association between DHA in maternal breast milk, this may reflect a programming long-term effect might be present.

Due to the fact that the differentiation and proliferation of adipocyte precursor cells might peak during the first year of the postnatal period {Ailhaud, Massiera et al, 2006), increased intake of n-3 LCPUFA may have the ability to alter adipocyte differentiation and perhaps slow the rate of fat mass development. Therefore, it could be speculated that those children administrated n3 LCPUFA during lactation might have a slower fat mass development and thereby display a later adiposity rebound compared to the OO-children. As seen in figure 1, the BMI curve during infancy and childhood increases in the first year of life and subsequently decrease until AR has been reached and further increase during pubertal development into adulthood. The greater fat mass in the children from the FO-group at age 2.5 might have evolved at a later stage compared to the children from the OO-group, thus the BMI-curve illustrating the growth development of the children from the FO-group might be shifted to the right compared to the OO-children, which would cause AR at a later age and a lower BMI at age 13 compared to the OO-children (figure 13). Thereby, would the children from the FO-group expected to have a higher 2.5/13-BMI-ratio than the children from the OO-group, reflecting a higher BMI at 2.5 years versus a lower BMI at age 13 years in children from the FO-group.

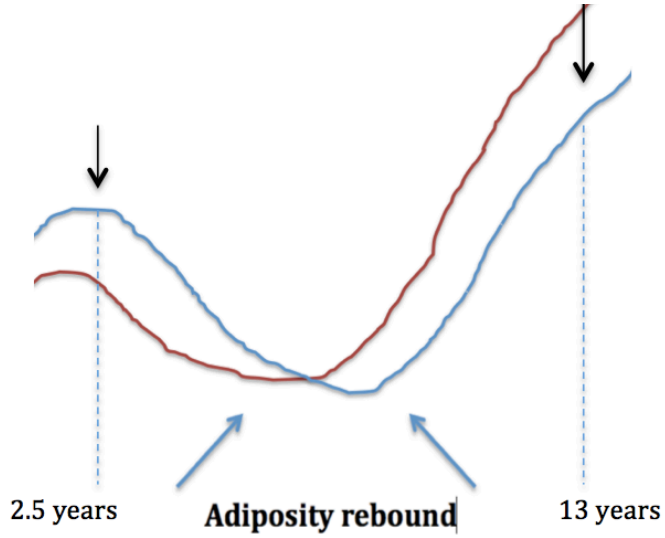


Figure 13. Hypothesis; the BMI-curve reflecting the children from the FO-group is shifted towards the right compared to the OO-children, meaning that the FO-children display a higher BMI at 2.5 years corresponding to a later occurrence of adiposity rebound and perhaps a leaner BMI at 13 years.
Red curve, children from the OO-group; Blue curve, children from the FO-group. Figure; Own construction.

No significant difference in the 2.5/13-BMI ratio between the two randomized groups were found. However, a significant positive association was found between DHA in breast milk and BMI-ratio

in girls (p=0.038), though this was not found in boys (p=0.64). Thus, an indication of a long-term effect of the intervention might be present, reflecting the effect of DHA in maternal breast milk on BMI development from 2.5 to 13 years specific to the girls. Interestingly, recent research have made the same suggestions of a later AR due to a long-term effect of DHA in breast milk; Pedersen, Lauritzen et al. 2012 found a positive significant association between the level of DHA in maternal breast milk and the age at adiposity rebound in girls, not the boys. The sex-specific difference between boys and girls in the study by is in agreement with the observation in this present study and further emphasizes the possibility that there might exists a sex-specific long-term effect of the intervention in girls. Furthermore, another recent study found a slower rate of fat mass development in children whose mothers had been administrated n-3 LCPUFA from mid pregnancy to the first three months of lactation, and likewise suggested that the observation might be an occurrence of delayed adiposity rebound (Bergmann, 2012 et al.)

Unfortunately, age at adiposity rebound has not been established in this follow-up study, making it difficult to estimate if the BMI indeed increased at a slower rate in the FO-group compared to the OO-group. Furthermore, BMI at age 13 would likewise be expected to be lower in the FO-group compared to the OO-group, but no significant difference appeared in BMI at 13 years between the two randomized groups, or between the sexes individually. Moreover, it should be noted that maternal RBC-DHA at 4 months did not appear to be associated with the 2.5/13-BMI ratio. It would have been expected that this dose-response parameter also would be associated with the BMI-ratio given that this is a more stabile parameter compared to DHA in maternal breast milk, thus it could not be ruled out that the observation might have been a chance finding.

Dietary interactions

It have been suggested that the adipogenic effect of n-6 PUFAs are dependent on the macronutrient composition of the diet and the ratio between carbohydrate and protein might determine the adipogenic potential of n-6 PUFAs through the presence of insulin secretion and cAMP-signaling (Madsen, Pedersen, et al. 2008). Furthermore, it has been suggested that high levels of n-3 PUFAs protects against high fat diet-induced obesity and inflammation in adipose tissue. However, as it has been determined that the amount of protein and carbohydrate in the background diet determine the adipogenic potential of n-6 PUFA, it has also ben demonstrated in mice that high glycemic index carbohydrates abrogates the anti-obesity effect of n-3 PUFAs (Hao, Lillefosse et al. 2012). Thus, the background diet has an influence on both the adipogenic potential of n-6 PUFAs and the anti-

obesity effect of n-3 PUFAs (Madsen, Pedersen et al. 2008). As a small tendency towards a higher P/C-ratio was observed in the OO-group compared to the FO-group (p=0.098), and as the amount of protein relative to (high glycemic) carbohydrates seems to determine the adipogeneic action of both n-3 and n-6 PUFAs it was interesting to investigate the potential effect of P/C-ratio on BMI in the 13-year old children from the two randomized groups. However, the P/C-ratio showed no effect on BMI in general or when analyzed for each sex separately either, in the randomized groups.

Appetite;

It should be noted that dietary intake also could be influenced by a long-term programming effect of n-3 LCPUFA (Mathai, Soueid et al. 2004). From studies in rodents it has been demonstrated that a diet deficient in n-3 LCPUFAs induced alterations in gene expression during perinatal development that increases neural appetite signaling (Mathai, Soueid et al. 2004). Furthermore, diets high in n-3 LCPUFA results in suppressed appetite in rats (Dziedzic, Szemraj et al. 2007), and mouse switched from a high-saturated fat diet to a diet with high levels on n-3 PUFA have shown to have reduced food intake (Wang, Storlien et al. 2002). Thus, these findings suggest that a diet rich in n-3 PUFA might have a role in regulation of appetite (Wang, Storlien et al. 2002). Furthermore, if these findings were to be coupled with the intervention of the present study it would be expected to see a lower energy intake in the FO-group compared to the OO-group, which is consistent with the fact that the OO-group indeed had a significantly higher energy intake compared to the FO-group (p=0.002). From the 7-year follow-up a significant higher energy intake was observed in the boys from the FO-group compared to the OO-group, and was mainly due to a higher consumption of fat and lower intake of carbohydrate. However, in this present study the overall macronutrient E% intake between the two randomized groups appeared to be very similar. Only a tendency towards a higher P/C-ratio was observed in the OO-group, indicating that the OO-group consumed more protein and less carbohydrate than the FO-group (p=0.098).

Blood pressure

An interaction between group and sex in DBP (p=0.038) and MAP (p=0.019) was found indication that the effect of the intervention differs between boys and girls. However, no significant difference between groups were found when analyzing sexes separately, which most likely is due to the small individual sample size of boys and girls. Still a tendency towards a lower blood pressure in girls

from the FO-group compared to the OO-group was observed (p=0.079).

In the last 7-year follow-up study the boys in the FO-group had significantly higher blood pressure than the boys of the OO-group. Furthermore, six boys from the FO-group appeared to have blood pressure above the 90th percentile from the 7-year follow-up study. Based on the knowledge that blood pressure tracks from childhood into adulthood, it was suggested that the boys from the FO-group were in greater risk of developing hypertension in later life (Asserhoj, Nehammer et al. 2009). However, no significant difference in blood pressure was found between the boys in our follow-up study at 13 years, and according to the oscillometric blood pressure standards in children (Table 4), no boys from the present study showed blood pressure over the 90th percentile.

In an observational cohort study children receiving breast-milk with high content of n-3 PUFA (above the median) was compared to formula fed babies, with no added supplement of n-3 LCPUFA, and were followed up at 12 years to investigate for any long-term effect in blood pressure (van Rossem, Wijga et al. 2012). Van Rossem, Wijga et al. found that breast-fed children had a significant lower systolic blood pressure than formula-fed children at the age of 12 years. Furthermore, in a randomized controlled trial with infants receiving formula with a high n-3 LCPUFA and n-6 PUFA or formula without n-3 supplementation found significant lower systolic and diastolic blood pressure at the age of 6 years in children supplemented with n-3 LCPUFA by (Forsyth, Willatts et al. 2003). No significant reduction in blood pressure was found in children from the FO-group in this present 13-year follow up study or previous studies at 2.5 and 7 year, which clearly contradicts the results obtained in the studies by Forsyth, Williams et al and van Rossem, Wijga et al.

However, several aspects should be taken into account; first of all, no individual doses of EPA and DHA was estimated in Van Rossem, Wijga et al, and second of all the doses used in the study by Forsyth, Williams et al. were considerably small compared to the one used in this present study (app. 0.6g/d vs. 1.5g/d n-3 LCPUFA, respectively). Such dosage-dependent differences make the comparison between these studies and the present study difficult, due to fact that the blood pressure lowering effect may be dependent of the dose of EPA and DHA and amount applied (Bonaa, Bjerve et al. 1990), (Appel, Miller et al. 1993). Furthermore, a noteworthy aspect to keep in mind is that the control groups used by Van Rossem, Wijga et al and Forsyth, Williams et al did not receive any n-3 LCPUFA in the formula during lactation that could result in a great incidence for n-3 PUFA deficiency in these infants. From studies in rats a deficiency in n-3 LCPUFA during the pre-and postnatal period increases blood pressure later in life (Armitage, Pearce et al. 2003) thus the

differences in blood pressure reported by Van Rossem, Wijga et al. and Forsyth, Williams et al could be due to a higher blood pressure in the control group due to a deficiency in n-3 LCPUFA. This observation was further supported by the fact that Van Rossem, Wijga et al found no association between RBC-n-3 LCPUFA status and blood pressure in the 12-year old children, indicating that the lower blood pressure in the group receiving high levels od n-3 LCPUFA during lactation probably wasn't due to the intervention.

In a recent follow up study, similar to this present study, blood pressure was assessed in 19-year old offspring from mothers who were randomized into groups receiving either fish oil (receiving 2.7 LCPUFA; app.0.6 g/d DHA and 0.8 g/d EPA), olive oil or no oil (the two last being control groups) in the last trimester of pregnancy (Rytter, Christensen et al. 2012). In accordance with this present study, FO supplementation was not associated with significant effect on blood pressure between the randomized groups in 19-year old offspring. However, a major difference between this study and our study is that the supplementation was only applied during the pregnancy period. Administering n-3 LCPUFA at different stages in development, i.e. in prenatal or peri/postnatal may have different long-term effects on blood pressure, and for this reason it is difficult to compare this study to the present study. Furthermore, another randomized study supplementing infant in the postnatal period from infants from the time of weaning until the age of 5, with the intention to increase the n-3/n-6 PUFA-ratio, did not find any effect of the intervention on blood pressure at the age of 8 either (Ayer, Harmer et al. 2009).

Sex-specific differences)

A very interesting finding in the present follow up study was a significant group*sex interaction in both DBP and MAP, indication that the intervention has a different effect in boys and girls. However, when analyzing the sexes separately only a tendency towards a lower MAP in girls from the FO-group was still present (p=0.079). In a 10-year follow up study of a preterm cohort, it was found that blood pressure in girls randomized to LCPUFA-supplemented formula had increased blood pressure at age 9-11 years, and no effects were observed in boys (Kennedy, Ross et al. 2010). However, this study included preterm children, and therefore may not be suitable for comparison with this present study. Nevertheless, these findings may suggest that blood pressure may be affected by differential programming effects of n-3 LCPUFA in boys and girls.

A sex-specific difference in blood pressure could due to the presence of hormones, and sex hormones have shown to modulate blood pressure. According to the Tanner stages (Knorr, Bidlingmaier et al. 1974) (Rapkin, Tsao et al. 2006) the influence of sex hormones might be highly relevant in these 13-year old children. Studies in rats have showed that estrogen, rather than the male hormone androgen, affects blood pressure probably due to promotion of vasodilation by estrogen (Orshal and Khalil 2004). Furthermore, estrogen may also have a regulatory effect that stimulate the conversion of essential fatty acids into their long-chain metabolites, which suggest that a higher conversion of α-linoelic acid to long chain PUFA in women that in men (Burdge and Wootton 2002). Furthermore, the fact that the girls from the FO-group display a lower blood pressure compared to the girls from the OO-group might be explained by that the conversion of α-linoelic acid to long chain PUFA might be further enhanced in the girls from the FO-group due to a long-term effect from the intervention. In fact, the role of sex hormones in modulation of blood pressure directly or indirectly by a modification of the n-3 LCPUFA metabolism could easily explain the different outcome in blood pressure observed between the girls from the randomized groups (Decsi and Kennedy 2011). Interestingly, it has been demonstrated that blood pressure of 7-year old children persisted until age of 15 in boys and until the age of 13 in girls, but that this association no longer was evident after puberty (Katz, Hediger et al. 1980). Katz, Hedinger 1980, et al suggested that if any possible tracking in blood pressure might persist from childhood into adulthood, it should be evaluated after puberty of sexually matured young adults due to the fact that puberty is a period with confounding effects of growth and maturation. If the presence of sex hormones could influence a potential long-term effect of the intervention on blood pressure, it only emphasizes the relevance of conducting another follow up study with these children after puberty. This would be necessary in order to make a firm conclusion regarding blood pressure development and if the outcome before and during puberty is still related to blood pressure of sexually mature adults.

Diet and blood pressure;

A number of studies have found that a high dietary intake of protein is associated with a lower blood pressure (Ulbak, Lauritzen et al. 2004). This is especially beneficial when increasing dietary protein intake relative to dietary intake of carbohydrate (Rebholz, Friedman et al. 2012). A tendency towards a higher P/C-ratio in the OO-group compared to the FO-group was observed ($p=0.098$), reflecting a higher protein intake versus carbohydrate intake in the OO-group compared to the FO-group. Recently a study in adolescents found that the quantity and quality of dietary carbohydrate also was associated with blood pressure (Gopinath, Flood et al. 2012). Furthermore, it

was suggested that dietary intake of foods with high glycemic index and a high glycemic load was associated with a higher blood pressure compared to those consuming a higher amount of dietary fiber. A high dietary intake of fiber was found to be associated with a lower blood pressure. This observation could help explain the observed lower blood pressure in boys from the OO group, which have a P/C ratio compared to the boys from the FO-group.

It has been suggested that fish-intake in general, rather than just consumption of n-3 LCPUFA from fish oil, is beneficial in lowering the risk of cardiovascular disease due to other beneficial nutritional components in fish like vitamin D, Iodide, Selene and proteins in fish (Dahl, Bjorkkjaer et al. 2006). For this reason, it would be interesting to compare the two randomized groups, which had a low habitual fish consumption, with the HF-group that had a fish-intake over the normal habitual fish intake. Comparing the blood pressure of the HF-group to the two randomized group it is seen that it primarily is found to be an intermediate of the blood pressure of the two other groups with a slightly closer resemblance to the FO-group than the OO-group, and thus, support the fact that a programming effect of n-3 LCPUFA may be present. However, it should be emphasized that the HF-group is not part of the randomization and therefore could deviate from the two randomized groups.

Critical period for programming effects of n-3 LCPUFA on blood pressure?

When Armitage et al. and colleagues demonstrated that n-3 LCPUFA deficiency during the perinatal period resulted in increased blood pressure later in life, it emphasized that the timing of n-3 LCPUFA exposure early in life might be of great importance for the programming effect on blood pressure. Studies discussed in this thesis of relevance to outcome in blood pressure have all administered n-3 PUFA during lactation (Forsyth, Williams et al, Van Rossem, Wigja, et al., Lauritzen 2004 et al.), only Rytter, Christensen et al administered n-3 LCPUFA during the third trimester of gestation and Ayer, Harmer et al. induced a dietary intervention during the 5 first years of postnatal life. Furthermore, only Forsyth, Williams et al. and Van Rossem, Wijga et al. have reported a beneficial effect of exposure to n-3 LCPUFA during lactation on blood pressure. Rytter, Christensen et al., Ayer, Harmer et al., and as well as this present study did not find any beneficial effect on blood pressure. Due to the fact that n-3 LCPUFA accumulates in the brain of the fetus during the third trimester of pregnancy and during the first year of life it would be expected that any potential programming effects of n-3 LCPUFA on blood pressure would be inducible in this period. However, Rytter, Christensen et al., as the only study that supplemented through pregnancy did not find any long-term effect on blood pressure. Ayer, Harmer et al. did not find any effect on blood

pressure in 8-year old offspring that had received dietary intervention with increased n-3 LCPUFA the first 5 years of postnatal life, and the authors questioned if the results reflected absence of any biological effect of the intervention. To my knowledge, Rytter, Christensen et al. is the only human study investigating n-3 LCPUFA supplementation during pregnancy on offspring blood pressure and no human studies have administered n-3 LCPUFA during gestation *and* lactation, which would give a broader perspective on the programming effect of n-3 LCPUFA exposure on blood pressure.

Dose response

It is noteworthy, that the two studies that did observe a reduction in blood pressure as a long-term effect of n-3 LCPUFA exposure during lactation were not explained by any effect of dose-responses. Forsyth and colleagues did not report any association between the observed reduction in blood pressure and plasma fatty acid concentration, thus the observed effect cannot be explained by the dietary intervention of n-3 LCPUFA. Furthermore, Van Rossem, Wigja et al that found a decreased blood pressure in 12-year old children, but this was not explained by infant fatty acid status at 12 years.

In this present study I found that adjusted DBP and MAP in boys were positively associated with maternal RBC-DHA ($p=0.05$) and ($p=0.028$), respectively. Furthermore, DHA in maternal breast milk were also associated with MAP in boys ($p=0.043$). Thus, these results indicate that the outcome of blood pressure in boys may be explained by the dose-responses. This is particular emphasized in MAP in boys since both DHA in maternal milk and RBC was positively associated with this measurement. However, no significant difference was found in blood pressure between the boys in the randomized groups that could be related to the effect of the dose-response analysis. However, it is indeed noteworthy that the intervention had different effect in boys and girls in MAP and DBP, and the fact that the dose-responses only showed a significant effect in boys only further emphasizes that possible sex-specific long-term effect of the intervention is present in blood pressure. Furthermore, no significant association were found between DBP or MAP and any of the dose-response parameters in girls, indicating that the observed tendency towards a lower blood pressure in girls from the FO-group ($p=0.079$) were not be explained by maternal RBC-DHA or DHA in maternal breast milk.

Mechanism

A way by which n-3 LCPUFA may affect blood pressure may be from their position in cell membranes wherefrom their synthesis of eicosanoids may act as vasodilators (Nestel, Shige et al.

2002) or as components in endothelium by increasing endothelial membrane fluidity. However, this finding does not explain the long-term programming effect of n-3 LCPUFA, in that a rapid turnover of fatty acids in cells membranes occur as well as the eicosanoid synthesis, which hardly would induce any long term effect. Instead it should be noted that exposure of n-3 PUFA in early life could induce potential programming effects on blood pressure which most likely involve modulation of gene expression. It have been demonstrated that insufficient intake of n-3 LCPUFA during perinatal life resulted in exaggerated sodium appetite in rats and it was suggested that this finding was due to enhanced activation of the renin–angiotensin system, which also controls blood pressure (Weisinger, Armitage et al 2010). Thus perinatal insufficient exposure of n-3 LCPUFA may alter the activity of the renin–angiotensin–aldosterone system that may be involved in the long-term regulation of blood pressure (and sodium appetite) (Weisinger, Armitage et al. 2001), (Begg, Sinclair et al. 2010). However, this present study I was not able to collect data on specific nutrients consumed by the children, and no data on sodium intake could be analyzed.

Possible confounding variables in blood pressure and body composition

When examining blood pressure in the children, place of examination, puberty, P/C-ratio, and W/H-ratio were included as possible covariates in the statistical model. Measurements of blood pressure tends to be lower at home measurements compared to institutional due to the fact that the child might feel more at ease in familiar environment, which also have been described elsewhere (Howell, Rice et al. 2007). However, when including place of examination in the statistical model, it did not affect the results, probably due to unequal distribution of home examinations (n=9) versus examinations at Department at Human Nutrition (n=51).

Data from the physical activity level (PAL) was not available in the present study, and there might be the possibility that PAL could have influenced blood pressure in that it contributes to a healthier lifestyle. However, the lack of PAL data did not make it possible to analyze if PAL would have been an explaining factor in blood pressure.

The OO-group had a significantly higher energy intake compared to the FO-group, however no association was found between diet and blood pressure in the two randomized groups, and when including EI and P/C-ratio in the statistical models it did not affect the results. In fact, when including EI in the statistical model the explanatory factor (R^2) remarkably decreased for primarily

every parameter for body composition and blood pressure. In general, it is complicated to adjust for unhealthy lifestyle patterns that involve factors such as PAL and E%.

Strengths and limitations

The present randomized controlled trial is a 13-year follow-up study wherein I conducted statistical analyzes in parameters for body composition and blood pressure in order to detect for any long-term effects of n-3 LCPUFA induced in the first four months of lactation. Follow-up data were collected from a total of sixty children, from which the group allocation were HF=21, OO = 19, FO =20, which resulted in a follow-up rate of 32,8 % (HF), 31,7 % (OO) and 32.3 % (FO) respectively, which represents a relatively high drop out rate which makes it difficult to draw any decisive conclusions on results from body composition and blood pressure. In addition, which must be emphasized in this present study, the small sample size further made it difficult to perform substantial statistical analysis, and a risk of making type II errors was considerable high and the obtained results therefore might be inaccurate.

Sample size

Limitation of the present study was the small sample size. The number of children in the two randomized groups was 39 in total, which makes the sample size for the experimental groups relatively small and without enough power to obtain any statistical accurate results. As it is emphasized in this thesis, it was of interest to detect for any sex-related differences in outcomes on blood pressure and body composition, as discussed previously. However, it should be emphasized that doing stratification for sex among the children in the randomized groups may have resulted in very small sample sizes, which would cause a loss of statistical power.

Study design

A strength quality in the present study is the fact that only one person carried out all the examinations on every child. The decision to include two children examined by a nutrition student was made to increase the sample size. Furthermore, the main researcher performed fifty-eight examinations and was also attended the two examinations that was carried out by the nutrition student, thereby ensuring that the examinations were performed equally.

It would have been of great interest to have the present RBC-DHA status of the children, to see if the fatty acid status in plasma reflected the intervention. Blood pressure in adolescents have been linked to n-3 LCPUFA in plasma (O'Sullivan, Bremner et al. 2012), and it would have been very interesting to see if any outcome in blood pressure from this study was reflected in the RBC-DHA status of the child. However, due to ethical approval and a time limit, it was not possible to collect any blood samples from the children.

The dietary data in this thesis was collected from an electronic FFQ, which the child filled out on a computer in its own home. The child was allowed to get help from a parent if any of the questions were difficult to answer. In the FFQ the child were asked to fill out what he or she's eating habit had been in the previous 12 months, thus it could have been expected that the child might lose focus when filling out the FFQ. However, at age 13 the child might have several meals outside the home, making it difficult for the parent to know the exactly foods consumed by the child, which is one of the reasons a self reported FFQ by the child was used in this study. Furthermore, in terms of dietary interactions, it would also have been favorable to have access to the fatty composition of the dietary intake of the children as well as the type of carbohydrate due to the dietary interactions, which might influence both body composition parameters (Madsen, Pedersen et al. 2008) and blood pressure (Gopinath, Flood et al. 2012). However, it was decided that specific distribution of macronutrients obtained from the FFQ might be too inaccurate to include in the dietary analysis.

Control group

It is uncertain whether the use of olive oil is a neutral choice of control group. Due to relatively low content of the n-6 PUFA linoelic acid (LA) in olive oil (>10%), it is not certain that a potential effect of n-6 PUFA in the control group would influence any long-term outcomes in body composition. However, in terms of blood pressure it have been documented that a diet rich in monounsaturated fatty acids resulted in a lower blood pressure compared to a diet rich in PUFA ((Ferrara, Raimondi et al. 2000). Still, a potential effect of olive oil as control group might be present, but if this might induce any potential long-term effect on body composition or blood pressure have to my knowledge not been reported.

Health implications

A number key finding should be emphasized from this present 13-year follow-up study. First of all a significant group*sex interactions in DBP and MAP was found indicating that the effect of the intervention differs between boys and girls. Other indicia of a long-term programming effect on both body composition and blood pressure were found that deserve to be taken into account: 1) DBP and MAP in boys was explained by a significant association with maternal RBC-DHA and MAP was also explained by a significant association with DHA in maternal breast milk, and 2) a significant association between 2.5/13-BMI ratio and DHA in maternal breast milk was found in girls from the two randomized groups. These results found in the dose-response analysis, further indicate that a sex-specific effect might be present. So far only few studies have reported a sex-specific difference in outcome in studies supplementing with n-3 LCPUFA in early life (Decsi and Kennedy 2011), however this present 13-year follow up study adds to the evidence that LC-PUFA supplementation in early life may exert different programming effects between sexes.

Supplementation with n-3 LCPUFA has become popular worldwide as a nutritional supplement with various intended health benefits. However, the possibility for nutritional toxicity is possible, and from studies in rodents it has actually been demonstrated that nutritional toxicity from overconsumption of n-3 LCPUFA had more adverse long-term health effects than n-3 LPUFA deficiency during pregnancy and lactation (Jen, Church et al. 2009). In the 7-year follow up study it was speculated that the significant higher blood pressure in boys from the FO-group might have adverse long-term effects, however the data from this 13-year follow up study showed that the significant difference no longer was evident. However, as already stated these results must be interpreted with caution due to the small sample size.

It has been reported that high dietary levels of n-6 PUFAs promote obesity in animals (Massiera, Saint-Marc et al. 2003) and that an increase in LA in breast milk have been observed to reflect the higher consumption of n-6 PUFAs in the Western diet. Together, it has been speculated that this might be a factor for childhood obesity (Ailhaud, Massiera et al. 2006). It has also been hypothesized that lowering the n-6:n-3-ratio during pregnancy and /or lactation might be beneficial for the prevention of childhood obesity (Ailhaud, Massiera et al. 2006) and a number of recent studies have raised the possibility that reducing the intake of n-6 PUFA intake during pregnancy and lactation might be more effective in reducing fat mass in the offspring than supplementing with n-3 LCPUFA (Moon, Harvey et al. 2013) (Hauner, Much et al. 2012). It has been demonstrated that maternal n-6 PUFA plasma concentration is positively associated with offspring fat mass (Moon, Harvey et al. 2013) and from animal studies it has been demonstrated that high n-6:n-3 ratio during

perinatal life promotes early fat mass growth in offspring (Massiera, Saint-Marc et al. 2003), (Korotkova, Gabrielsson et al. 2002). Mothers from this present follow-up study did not receive any dietary advice to reduce or control the n-6:n-3:ratio during the intervention, and since the n-6:n-3-ratio could influence the results of the outcome on body composition, it would be of interest to conduct studies in the future where this ratio is experimentally controlled.

Several human and animal studies have investigated if dietary interventions with n-3 LCPUFA in maternal diet during pregnancy and/or lactation have any implication in the long-term health outcomes. However, the results are contradicting and follow-up studies beyond the age of 7 are sparse, which limit the long-term knowledge on several health outcomes. Follow-up studies investigating the possible effect of n-3 LCPUFA on blood pressure and body composition have in general failed to show any long-term effect in offspring. However, new insights in relation to the long-term intervention have emerged, emphasizing that n-3 LCPUFA supplementation in pregnancy and/or lactation might be influenced by sex-specific molecular mechanisms that could influence the outcome (Decsi and Kennedy 2011) (Sardinha, Fernandes et al. 2013). This is indeed consistent with the findings of this present study, that indicated a significant group*sex interaction in MAP and DBP. Thus, this highlights the importance for performing subgroup analysis by sex in trials investigating the long-term effect of supplementation with n-3 LCPUFA during pregnancy and/or lactation. Furthermore, because sex hormones might modulate possible long-term effects, follow-up studies on offspring administrated to a dietary intervention during pregnancy and/or lactation it would be wise to conduct another follow-up after puberty has settled and the offspring has fully sexual matured (Katz, Hediger et al. 1980).

Conclusion

The significant difference in blood pressure observed between the boys from the two randomized groups at 7-year was no longer evident at 13-year. The difference in body composition observed at 2.5 years, was at 7 years diminished, and I observed no significant difference in body composition at 13 years. This 13-year follow up study shows a clear significant group*sex interactions in MAP and DBP in the randomized groups, indicating that an effect of the intervention differs between boys and girls. Furthermore, in boys from the randomized groups a significant association between maternal RBC-DHA and MAP was found, as was a significant association between DBP and DHA

in maternal breast milk. The 2.5/13-BMI ratio was positively significant associated with DHA in maternal breast milk for the girls in the randomized groups. Therefore, this present 13-year follow up study add to the evidence favoring that LCPUFA supplementation in early life may exert different programming effects between sexes. Outcomes in blood pressure and BMI could have been influenced the presence of sex hormones in the 13-year old children, and it would be favorable to perform another follow-up study to identify possible programming effects on blood pressure and body composition after end of puberty when sexual maturity has settled to investigate if the current findings still is present. It should be emphasized that due to a small sample size the occurrence of a direct programming effect may be hard to identify.

Perspectives

A sufficient supply of LCPUFA, especially DHA, has important physiological roles for optimal development of the nervous system during the perinatal period (Koletzko, Lien et al. 2008). Based on this well-known fact, many clinical studies have investigated the long-term effect of supplementation with n-3 LCPUFA in early life on different health aspects. However, we still do not know when the dietary intervention might have the biggest effect, in other words when the critical window might appear? Inconsistency in outcomes from different studies could be due to the period of time the dietary intervention applied. Based on the fact that n-3 LCPUFA accumulates in the brain from the third trimester through the first year of life, it would be expected that the most significant effect is during this period. Still, insufficient amount of studies have investigated the potential programming effect at different developmental stages (in utero, peri/postnaltal period) and therefore it is difficult to draw any specific conclusion on maternal n-3 LCPUFA supplementation on the metabolic health in the offspring. Clearly, further studies are required to clarify the mechanism of n-3 LCPUFA on fat depots and blood pressure during gestation and/or lactation.

The present 13-year follow-up study found a significant sex*group interaction in DBP and MAP in the two randomized groups that could contribute with new insights in a sex-specific the long-term effect of n-3 LCPUFA in lactation on blood pressure. Furthermore, the possibility that a long-term effect is present due to the significant associations that were found between dose-response parameters and 2.5/13-BMI ratio and blood pressure should also be emphasized. Thus, it was clearly a fact from this study that these significant associations were affected by gender, and

highlight the importance of that future follow-up studies from this intervention are strongly advised to perform analysis for sex*group interaction in every outcome.

References

Addo, O. Y. and J. H. Himes (2010). "Reference curves for triceps and subscapular skinfold thicknesses in US children and adolescents." Am J Clin Nutr **91**(3): 635-642.

Ailhaud, G. (1992). "Some new aspects on adipose tissue development." Diabetes Metab Rev **8**(1): 3-7.

Ailhaud, G. and P. Guesnet (2004). "Fatty acid composition of fats is an early determinant of childhood obesity: a short review and an opinion." Obes Rev **5**(1): 21-26.

Ailhaud, G., F. Massiera, et al. (2006). "Temporal changes in dietary fats: role of n-6 polyunsaturated fatty acids in excessive adipose tissue development and relationship to obesity." Prog Lipid Res **45**(3): 203-236.

Alvheim, A. R., M. K. Malde, et al. (2012). "Dietary linoleic acid elevates endogenous 2-AG and anandamide and induces obesity." Obesity (Silver Spring) **20**(10): 1984-1994.

Andersen, A. D., K. F. Michaelsen, et al. (2011). "A randomized controlled intervention with fish oil versus sunflower oil from 9 to 18 months of age: exploring changes in growth and skinfold thicknesses." Pediatr Res **70**(4): 368-374.

Appel, L. J., E. R. Miller, 3rd, et al. (1993). "Does supplementation of diet with 'fish oil' reduce blood pressure? A meta-analysis of controlled clinical trials." Arch Intern Med **153**(12): 1429-1438.

Armitage, J. A., A. D. Pearce, et al. (2003). "Increased blood pressure later in life may be associated with perinatal n-3 fatty acid deficiency." Lipids **38**(4): 459-464.

Asserhoj, M., S. Nehammer, et al. (2009). "Maternal fish oil supplementation during lactation may adversely affect long-term blood pressure, energy intake, and physical activity of 7-year-old boys." J Nutr **139**(2): 298-304.

Ayer, J. G., J. A. Harmer, et al. (2009). "Dietary supplementation with n-3 polyunsaturated fatty acids in early childhood: effects on blood pressure and arterial structure and function at age 8 y." Am J Clin Nutr **90**(2): 438-446.

Begg, D. P., A. J. Sinclair, et al. (2010). "Hypertension induced by omega-3 polyunsaturated fatty acid deficiency is alleviated by alpha-linolenic acid regardless of dietary source." Hypertens Res **33**(8): 808-813.

Berger, J. and D. E. Moller (2002). "The mechanisms of action of PPARs." Annu Rev Med **53**: 409-435.

Bergmann, R. L., K. E. Bergmann, et al. (2012). "Does docosahexaenoic acid (DHA) status in pregnancy have any impact on postnatal growth? Six-year follow-up of a prospective randomized double-blind monocenter study on low-dose DHA supplements." J Perinat Med.

Beyerlein, A. and R. von Kries (2011). "Breastfeeding and body composition in children: will there ever be conclusive empirical evidence for a protective effect against overweight?" Am J Clin Nutr **94**(6 Suppl): 1772S-1775S.

Bhatia, H. S., R. Agrawal, et al. (2011). "Omega-3 fatty acid deficiency during brain maturation reduces neuronal and behavioral plasticity in adulthood." PLoS One **6**(12): e28451.

Bonaa, K. H., K. S. Bjerve, et al. (1990). "Effect of eicosapentaenoic and docosahexaenoic acids on blood pressure in hypertension. A population-based intervention trial from the Tromso study." N Engl J Med **322**(12): 795-801.

Bordini, B. and R. L. Rosenfield (2011). "Normal pubertal development: Part I: The endocrine basis of puberty." Pediatr Rev **32**(6): 223-229.

Buckley, J. D. and P. R. Howe (2010). "Long-chain omega-3 polyunsaturated fatty acids may be beneficial for reducing obesity-a review." Nutrients **2**(12): 1212-1230.

Burdge, G. C. (2006). "Metabolism of alpha-linolenic acid in humans." Prostaglandins Leukot Essent Fatty Acids **75**(3): 161-168.
Burdge, G. C. and S. A. Wootton (2002). "Conversion of alpha-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women." Br J Nutr **88**(4): 411-420.
Cade, J. E., V. J. Burley, et al. (2004). "Food-frequency questionnaires: a review of their design, validation and utilisation." Nutr Res Rev **17**(1): 5-22.
Calder, P. C. (2012). "Mechanisms of action of (n-3) fatty acids." J Nutr **142**(3): 592S-599S.
Cole, T. J., M. C. Bellizzi, et al. (2000). "Establishing a standard definition for child overweight and obesity worldwide: international survey." BMJ **320**(7244): 1240-1243.
Cripps, R. L., M. S. Martin-Gronert, et al. (2005). "Fetal and perinatal programming of appetite." Clin Sci (Lond) **109**(1): 1-11.
Dahl, L., T. Bjorkkjaer, et al. (2006). "[Fish--more than just omega 3]." Tidsskr Nor Laegeforen **126**(3): 309-311.
Darimont, C., G. Vassaux, et al. (1994). "Differentiation of preadipose cells: paracrine role of prostacyclin upon stimulation of adipose cells by angiotensin-II." Endocrinology **135**(5): 2030-2036.
Das, U. N. (2001). "Can perinatal supplementation of long-chain polyunsaturated fatty acids prevent hypertension in adult life?" Hypertension **38**(3): E6-8.
Deckelbaum, R. J. and C. L. Williams (2001). "Childhood obesity: the health issue." Obes Res **9 Suppl 4**: 239S-243S.
Decsi, T. and K. Kennedy (2011). "Sex-specific differences in essential fatty acid metabolism." Am J Clin Nutr **94**(6 Suppl): 1914S-1919S.
DeFilippis, A. P. and L. S. Sperling (2006). "Understanding omega-3's." Am Heart J **151**(3): 564-570.
Dietz, W. H. (1994). "Critical periods in childhood for the development of obesity." Am J Clin Nutr **59**(5): 955-959.
Dobbing, J. and J. Sands (1979). "Comparative aspects of the brain growth spurt." Early Hum Dev **3**(1): 79-83.
Drevon, C. A. (1992). "Marine oils and their effects." Nutr Rev **50**(4 (Pt 2)): 38-45.
Due, M. R. o. P. (2011). "Skolebørnsundersøgelsen 2010." © 2011, Forskningsprogrammet for Børn og Unges Sundhed (FoBUS)
Statens Institut for Folkesundhed, Syddansk Universitet.
Dziedzic, B., J. Szemraj, et al. (2007). "Various dietary fats differentially change the gene expression of neuropeptides involved in body weight regulation in rats." J Neuroendocrinol **19**(5): 364-373.
Eckert, G. P., C. Franke, et al. (2010). "Plant derived omega-3-fatty acids protect mitochondrial function in the brain." Pharmacol Res **61**(3): 234-241.
Ellis, J. R., R. B. McDonald, et al. (1990). "A diet high in fat stimulates adipocyte proliferation in older (22 month) rats." Exp Gerontol **25**(2): 141-148.
Farnsworth, E., N. D. Luscombe, et al. (2003). "Effect of a high-protein, energy-restricted diet on body composition, glycemic control, and lipid concentrations in overweight and obese hyperinsulinemic men and women." Am J Clin Nutr **78**(1): 31-39.
Ferrara, L. A., A. S. Raimondi, et al. (2000). "Olive oil and reduced need for antihypertensive medications." Arch Intern Med **160**(6): 837-842.
Fomon, S. J., F. Haschke, et al. (1982). "Body composition of reference children from birth to age 10 years." Am J Clin Nutr **35**(5 Suppl): 1169-1175.
Forsyth, J. S., P. Willatts, et al. (2003). "Long chain polyunsaturated fatty acid supplementation in infant formula and blood pressure in later childhood: follow up of a randomised controlled trial." BMJ **326**(7396): 953.

Freedman, D. S. and B. Sherry (2009). "The validity of BMI as an indicator of body fatness and risk among children." Pediatrics **124 Suppl 1**: S23-34.
Fujimori, K. (2012). "Prostaglandins as PPARgamma Modulators in Adipogenesis." PPAR Res **2012**: 527607.
Gaillard, D., R. Negrel, et al. (1989). "Requirement and role of arachidonic acid in the differentiation of pre-adipose cells." Biochem J **257**(2): 389-397.
Gebauer, S. K., T. L. Psota, et al. (2006). "n-3 fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits." Am J Clin Nutr **83**(6 Suppl): 1526S-1535S.
Geleijnse, J. M., A. Hofman, et al. (1997). "Long-term effects of neonatal sodium restriction on blood pressure." Hypertension **29**(4): 913-917.
Gopinath, B., V. M. Flood, et al. (2012). "Influence of high glycemic index and glycemic load diets on blood pressure during adolescence." Hypertension **59**(6): 1272-1277.
Greisen, G. and K. F. Michaelsen (1989). "[Perinatal growth. A practical perinatal growth curve]." Ugeskr Laeger **151**(28): 1813-1815.
Hao, Q., H. H. Lillefosse, et al. (2012). "High-glycemic index carbohydrates abrogate the antiobesity effect of fish oil in mice." Am J Physiol Endocrinol Metab **302**(9): E1097-1112.
Harris, W. S. (1996). "n-3 fatty acids and lipoproteins: comparison of results from human and animal studies." Lipids **31**(3): 243-252.
Hauner, H., D. Much, et al. (2012). "Effect of reducing the n-6:n-3 long-chain PUFA ratio during pregnancy and lactation on infant adipose tissue growth within the first year of life: an open-label randomized controlled trial." Am J Clin Nutr **95**(2): 383-394.
Helland, I. B., L. Smith, et al. (2008). "Effect of supplementing pregnant and lactating mothers with n-3 very-long-chain fatty acids on children's IQ and body mass index at 7 years of age." Pediatrics **122**(2): e472-479.
Hornell, A., H. Lagstrom, et al. (2013). "Breastfeeding, introduction of other foods and effects on health: a systematic literature review for the 5th Nordic Nutrition Recommendations." Food Nutr Res **57**.
Howell, C. C., M. H. Rice, et al. (2007). "The relationships among anxiety, anger, and blood pressure in children." Appl Nurs Res **20**(1): 17-23.
Innis, S. M. (1991). "Essential fatty acids in growth and development." Prog Lipid Res **30**(1): 39-103.
Innis, S. M. (1992). "n-3 fatty acid requirements of the newborn." Lipids **27**(11): 879-885.
Innis, S. M. (1994). "The 1993 Borden Award Lecture. Fatty acid requirements of the newborn." Can J Physiol Pharmacol **72**(12): 1483-1492.
Innis, S. M. (2011). "Metabolic programming of long-term outcomes due to fatty acid nutrition in early life." Matern Child Nutr **7 Suppl 2**: 112-123.
Jen, K. L., M. W. Church, et al. (2009). "Perinatal n-3 fatty acid imbalance affects fatty acid composition in rat offspring." Physiol Behav **98**(1-2): 17-24.
Jo, J., O. Gavrilova, et al. (2009). "Hypertrophy and/or Hyperplasia: Dynamics of Adipose Tissue Growth." PLoS Comput Biol **5**(3): e1000324.
Katz, S. H., M. L. Hediger, et al. (1980). "Blood pressure, growth and maturation from childhood through adolescence. Mixed longitudinal analyses of the Philadelphia Blood Pressure Project." Hypertension **2**(4 Pt 2): 55-69.
Kaufman, L. N., M. M. Peterson, et al. (1994). "Hypertensive effect of polyunsaturated dietary fat." Metabolism **43**(1): 1-3.
Kennedy, K., S. Ross, et al. (2010). "The 10-year follow-up of a randomised trial of long-chain polyunsaturated fatty acid supplementation in preterm infants: effects on growth and blood pressure." Arch Dis Child **95**(8): 588-595.

Kim, H. K., M. Della-Fera, et al. (2006). "Docosahexaenoic acid inhibits adipocyte differentiation and induces apoptosis in 3T3-L1 preadipocytes." J Nutr **136**(12): 2965-2969.
Kim, J. B., H. M. Wright, et al. (1998). "ADD1/SREBP1 activates PPARgamma through the production of endogenous ligand." Proc Natl Acad Sci U S A **95**(8): 4333-4337.
Knorr, D., F. Bidlingmaier, et al. (1974). "Plasma testosterone in male puberty. I. Physiology of plasma testosterone." Acta Endocrinol (Copenh) **75**(1): 181-194.
Koletzko, B., B. Brands, et al. (2012). "Early nutrition programming of long-term health." Proc Nutr Soc **71**(3): 371-378.
Koletzko, B., E. Lien, et al. (2008). "The roles of long-chain polyunsaturated fatty acids in pregnancy, lactation and infancy: review of current knowledge and consensus recommendations." J Perinat Med **36**(1): 5-14.
Korotkova, M., B. Gabrielsson, et al. (2002). "Leptin levels in rat offspring are modified by the ratio of linoleic to alpha-linolenic acid in the maternal diet." J Lipid Res **43**(10): 1743-1749.
Korotkova, M., B. G. Gabrielsson, et al. (2005). "Gender-related long-term effects in adult rats by perinatal dietary ratio of n-6/n-3 fatty acids." Am J Physiol Regul Integr Comp Physiol **288**(3): R575-579.
Kotchen, J. M., H. E. McKean, et al. (1982). "Blood pressure trends with aging." Hypertension **4**(5 Pt 2): III128-134.
Krue, S. and J. Coolidge (2010). "The prevalence of overweight and obesity among Danish school children." Obes Rev **11**(7): 489-491.
Lagstrom, H., M. Hakanen, et al. (2008). "Growth patterns and obesity development in overweight or normal-weight 13-year-old adolescents: the STRIP study." Pediatrics **122**(4): e876-883.
Lane, D. A. and P. Gill (2004). "Ethnicity and tracking blood pressure in children." J Hum Hypertens **18**(4): 223-228.
Larnkjaer, A., J. H. Christensen, et al. (2006). "Maternal fish oil supplementation during lactation does not affect blood pressure, pulse wave velocity, or heart rate variability in 2.5-y-old children." J Nutr **136**(6): 1539-1544.
Lauritzen, L., H. S. Hansen, et al. (2001). "The essentiality of long chain n-3 fatty acids in relation to development and function of the brain and retina." Prog Lipid Res **40**(1-2): 1-94.
Lauritzen, L., C. Hoppe, et al. (2005). "Maternal fish oil supplementation in lactation and growth during the first 2.5 years of life." Pediatr Res **58**(2): 235-242.
Lauritzen, L., M. H. Jorgensen, et al. (2004). "Maternal fish oil supplementation in lactation: effect on visual acuity and n-3 fatty acid content of infant erythrocytes." Lipids **39**(3): 195-206.
Li, J. and Y. Wang (2008). "Tracking of dietary intake patterns is associated with baseline characteristics of urban low-income African-American adolescents." J Nutr **138**(1): 94-100.
Liu, Y. X., F. Jalil, et al. (1998). "Risk factors for impaired length growth in early life viewed in terms of the infancy-childhood-puberty (ICP) growth model." Acta Paediatr **87**(3): 237-243.
Liu, Y. X., K. A. Wikland, et al. (2000). "New reference for the age at childhood onset of growth and secular trend in the timing of puberty in Swedish." Acta Paediatr **89**(6): 637-643.
Lucas, A. (1991). "Programming by early nutrition in man." Ciba Found Symp **156**: 38-50; discussion 50-35.
Lucas, A. (1998). "Programming by early nutrition: an experimental approach." J Nutr **128**(2 Suppl): 401S-406S.
Lucas, A., D. L. Sarson, et al. (1980). "Breast vs bottle: endocrine responses are different with formula feeding." Lancet **1**(8181): 1267-1269.
MacDougald, O. A. and S. Mandrup (2002). "Adipogenesis: forces that tip the scales." Trends Endocrinol Metab **13**(1): 5-11.
Madsen, L., L. M. Pedersen, et al. (2008). "cAMP-dependent signaling regulates the adipogenic effect of n-6 polyunsaturated fatty acids." J Biol Chem **283**(11): 7196-7205.

Madsen, L., R. K. Petersen, et al. (2005). "Regulation of adipocyte differentiation and function by polyunsaturated fatty acids." Biochim Biophys Acta **1740**(2): 266-286.
Martin, R. M., D. Gunnell, et al. (2005). "Breastfeeding in infancy and blood pressure in later life: systematic review and meta-analysis." Am J Epidemiol **161**(1): 15-26.
Martin, R. M., A. R. Ness, et al. (2004). "Does breast-feeding in infancy lower blood pressure in childhood? The Avon Longitudinal Study of Parents and Children (ALSPAC)." Circulation **109**(10): 1259-1266.
Massiera, F., P. Guesnet, et al. (2006). "The crucial role of dietary n-6 polyunsaturated fatty acids in excessive adipose tissue development: relationship to childhood obesity." Nestle Nutr Workshop Ser Pediatr Program **57**: 235-242; discussion 243-235.
Massiera, F., P. Saint-Marc, et al. (2003). "Arachidonic acid and prostacyclin signaling promote adipose tissue development: a human health concern?" J Lipid Res **44**(2): 271-279.
Mathai, M. L., M. Soueid, et al. (2004). "Does perinatal omega-3 polyunsaturated fatty acid deficiency increase appetite signaling?" Obes Res **12**(11): 1886-1894.
Miall, W. E. and P. D. Oldham (1963). "The hereditary factor in arterial blood-pressure." Br Med J **1**(5323): 75-80.
Michaelsen, K. F. (1997). "Nutrition and growth during infancy. The Copenhagen Cohort Study." Acta Paediatr Suppl **420**: 1-36.
Moon, R. J., N. C. Harvey, et al. (2013). "Maternal plasma polyunsaturated fatty acid status in late pregnancy is associated with offspring body composition in childhood." J Clin Endocrinol Metab **98**(1): 299-307.
Much, D., S. Brunner, et al. (2013). "Effect of dietary intervention to reduce the n-6/n-3 fatty acid ratio on maternal and fetal fatty acid profile and its relation to offspring growth and body composition at 1 year of age." Eur J Clin Nutr **67**(3): 282-288.
Muhlhausler, B. S., R. Cook-Johnson, et al. (2010). "Opposing effects of omega-3 and omega-6 long chain polyunsaturated Fatty acids on the expression of lipogenic genes in omental and retroperitoneal adipose depots in the rat." J Nutr Metab **2010**.
Muhlhausler, B. S., D. Miljkovic, et al. (2011). "Maternal omega-3 supplementation increases fat mass in male and female rat offspring." Front Genet **2**: 48.
Muntner, P., J. He, et al. (2004). "Trends in blood pressure among children and adolescents." JAMA **291**(17): 2107-2113.
Nakamura, M. T. and T. Y. Nara (2003). "Essential fatty acid synthesis and its regulation in mammals." Prostaglandins Leukot Essent Fatty Acids **68**(2): 145-150.
Nestel, P., H. Shige, et al. (2002). "The n-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid increase systemic arterial compliance in humans." Am J Clin Nutr **76**(2): 326-330.
Neuringer, M. and W. E. Connor (1986). "n-3 fatty acids in the brain and retina: evidence for their essentiality." Nutr Rev **44**(9): 285-294.
Niinikoski, H., A. Jula, et al. (2009). "Blood pressure is lower in children and adolescents with a low-saturated-fat diet since infancy: the special turku coronary risk factor intervention project." Hypertension **53**(6): 918-924.
NNR prjocet gropup, 2004. Nordic nutrition reccomendations 2004, integrating nutrition and physical activity. 4th ed., pp. 13-424. Norden Copenhagen
Noh, H. Y., Y. J. Song, et al. (2011). "Dietary patterns are associated with physical growth among school girls aged 9-11 years." Nutr Res Pract **5**(6): 569-577.
Nysom, K., C. Molgaard, et al. (2001). "Body mass index of 0 to 45-y-old Danes: reference values and comparison with published European reference values." Int J Obes Relat Metab Disord **25**(2): 177-184.
O'Sullivan, T. A., A. P. Bremner, et al. (2012). "Polyunsaturated fatty acid intake and blood pressure in adolescents." J Hum Hypertens **26**(3): 178-187.

O'Tierney, P. F., D. J. Barker, et al. (2009). "Duration of breast-feeding and adiposity in adult life." J Nutr **139**(2): 422S-425S.
Obarzanek, E., P. A. Velletri, et al. (1996). "Dietary protein and blood pressure." JAMA **275**(20): 1598-1603.
Ogden, C. L., Y. Li, et al. (2011). "Smoothed percentage body fat percentiles for U.S. children and adolescents, 1999-2004." Natl Health Stat Report(43): 1-7.
Ohlsson, C., M. Lorentzon, et al. (2012). "Age at adiposity rebound is associated with fat mass in young adult males-the GOOD study." PLoS One **7**(11): e49404.
Okuno, M., K. Kajiwara, et al. (1997). "Perilla oil prevents the excessive growth of visceral adipose tissue in rats by down-regulating adipocyte differentiation." J Nutr **127**(9): 1752-1757.
Olsen, J., M. Melbye, et al. (2001). "The Danish National Birth Cohort--its background, structure and aim." Scand J Public Health **29**(4): 300-307.
Orshal, J. M. and R. A. Khalil (2004). "Gender, sex hormones, and vascular tone." Am J Physiol Regul Integr Comp Physiol **286**(2): R233-249.
Owen, C. G., R. M. Martin, et al. (2005). "Effect of infant feeding on the risk of obesity across the life course: a quantitative review of published evidence." Pediatrics **115**(5): 1367-1377.
Owen, C. G., P. H. Whincup, et al. (2003). "Effect of breast feeding in infancy on blood pressure in later life: systematic review and meta-analysis." BMJ **327**(7425): 1189-1195.
Park, M. K., S. W. Menard, et al. (2005). "Oscillometric blood pressure standards for children." Pediatr Cardiol **26**(5): 601-607.
Patterson, E., R. Wall, et al. (2012). "Health implications of high dietary omega-6 polyunsaturated Fatty acids." J Nutr Metab **2012**: 539426.
Pearson, S., L. W. Olsen, et al. (2005). "[Increase in overweight and obesity amongst Copenhagen schoolchildren, 1947-2003]." Ugeskr Laeger **167**(2): 158-162.
Pedersen, L., L. Lauritzen, et al. (2012). "Polyunsaturated fatty acid content of mother's milk is associated with childhood body composition." Pediatr Res **72**(6): 631-636.
Pouteau, E., O. Aprikian, et al. (2010). "A low alpha-linolenic intake during early life increases adiposity in the adult guinea pig." Nutr Metab (Lond) **7**: 8.
Raclot, T., R. Groscolas, et al. (1997). "Site-specific regulation of gene expression by n-3 polyunsaturated fatty acids in rat white adipose tissues." J Lipid Res **38**(10): 1963-1972.
Rapkin, A. J., J. C. Tsao, et al. (2006). "Relationships among self-rated tanner staging, hormones, and psychosocial factors in healthy female adolescents." J Pediatr Adolesc Gynecol **19**(3): 181-187.
Rasmussen, B. M., B. Vessby, et al. (2006). "Effects of dietary saturated, monounsaturated, and n-3 fatty acids on blood pressure in healthy subjects." Am J Clin Nutr **83**(2): 221-226.
Rebholz, C. M., E. E. Friedman, et al. (2012). "Dietary protein intake and blood pressure: a meta-analysis of randomized controlled trials." Am J Epidemiol **176 Suppl 7**: S27-43.
Reckelhoff, J. F. (2001). "Gender differences in the regulation of blood pressure." Hypertension **37**(5): 1199-1208.
Rimm, E. B., E. L. Giovannucci, et al. (1992). "Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health professionals." Am J Epidemiol **135**(10): 1114-1126; discussion 1127-1136.
Ritz, E., K. Amann, et al. (2011). "Prenatal programming-effects on blood pressure and renal function." Nat Rev Nephrol **7**(3): 137-144.
Rogol, A. D., P. A. Clark, et al. (2000). "Growth and pubertal development in children and adolescents: effects of diet and physical activity." Am J Clin Nutr **72**(2 Suppl): 521S-528S.
Rolland-Cachera, M. F., T. J. Cole, et al. (1991). "Body Mass Index variations: centiles from birth to 87 years." Eur J Clin Nutr **45**(1): 13-21.
Rolland-Cachera, M. F., M. Deheeger, et al. (1984). "Adiposity rebound in children: a simple indicator for predicting obesity." Am J Clin Nutr **39**(1): 129-135.

Rolland-Cachera, M. F., M. Deheeger, et al. (2006). "Early adiposity rebound: causes and consequences for obesity in children and adults." Int J Obes (Lond) **30 Suppl 4**: S11-17.
Ruzickova, J., M. Rossmeisl, et al. (2004). "Omega-3 PUFA of marine origin limit diet-induced obesity in mice by reducing cellularity of adipose tissue." Lipids **39**(12): 1177-1185.
Rytter, D., B. H. Bech, et al. (2011). "Intake of fish oil during pregnancy and adiposity in 19-y-old offspring: follow-up on a randomized controlled trial." Am J Clin Nutr **94**(3): 701-708.
Rytter, D., J. H. Christensen, et al. (2012). "The effect of maternal fish oil supplementation during the last trimester of pregnancy on blood pressure, heart rate and heart rate variability in the 19-year-old offspring." Br J Nutr **108**(8): 1475-1483.
Sardinha, F. L., F. S. Fernandes, et al. (2013). "Sex-dependent nutritional programming: fish oil intake during early pregnancy in rats reduces age-dependent insulin resistance in male, but not female, offspring." Am J Physiol Regul Integr Comp Physiol **304**(4): R313-320.
Savva, S. C., M. Tornaritis, et al. (2000). "Waist circumference and waist-to-height ratio are better predictors of cardiovascular disease risk factors in children than body mass index." Int J Obes Relat Metab Disord **24**(11): 1453-1458.
Shankar, R. R., G. J. Eckert, et al. (2005). "The change in blood pressure during pubertal growth." J Clin Endocrinol Metab **90**(1): 163-167.
Shils (2006). "Modern nutrition in health and disease."
Shomaker, L. B., M. Tanofsky-Kraff, et al. (2010). "Puberty and observed energy intake: boy, can they eat!" Am J Clin Nutr **92**(1): 123-129.
Simopoulos, A. P. (2000). "Human requirement for N-3 polyunsaturated fatty acids." Poult Sci **79**(7): 961-970.
Simopoulos, A. P. (2002). "The importance of the ratio of omega-6/omega-3 essential fatty acids." Biomed Pharmacother **56**(8): 365-379.
Soenen, S., A. G. Bonomi, et al. (2012). "Relatively high-protein or 'low-carb' energy-restricted diets for body weight loss and body weight maintenance?" Physiol Behav **107**(3): 374-380.
Soriguer Escofet, F. J., I. Esteva de Antonio, et al. (1996). "Adipose tissue fatty acids and size and number of fat cells from birth to 9 years of age--a cross-sectional study in 96 boys." Metabolism **45**(11): 1395-1401.
Srinivasan, M. and M. S. Patel (2008). "Metabolic programming in the immediate postnatal period." Trends Endocrinol Metab **19**(4): 146-152.
Stanfield, C. (2008). Human Physiology.
Sundhedsstyrelsen (2009). "Anbefalinger for den danske institutionskost."
Tai, C. C. and S. T. Ding (2010). "N-3 polyunsaturated fatty acids regulate lipid metabolism through several inflammation mediators: mechanisms and implications for obesity prevention." J Nutr Biochem **21**(5): 357-363.
Taittonen, L., M. Nuutinen, et al. (1996). "Prenatal and postnatal factors in predicting later blood pressure among children: cardiovascular risk in young Finns." Pediatr Res **40**(4): 627-632.
Tamashiro, K. L. and T. H. Moran (2010). "Perinatal environment and its influences on metabolic programming of offspring." Physiol Behav **100**(5): 560-566.
Tanner JM: Growth at adolescence, 2nd ed. Oxford, Blackwell Scientific Publishers, 1962
Taylor, C. B. H. (2010). Human Growth and Development.
Taylor, R. W., A. Goulding, et al. (2004). "Rate of fat gain is faster in girls undergoing early adiposity rebound." Obes Res **12**(8): 1228-1230.
Touwslager, R. N., M. Gielen, et al. (2011). "Changes in genetic and environmental effects on growth during infancy." Am J Clin Nutr **94**(6): 1568-1574.
Trumbo, P., S. Schlicker, et al. (2002). "Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids." J Am Diet Assoc **102**(11): 1621-1630.
Ulbak, J., L. Lauritzen, et al. (2004). "Diet and blood pressure in 2.5-y-old Danish children." Am J Clin Nutr **79**(6): 1095-1102.

Valdez, R., K. J. Greenlund, et al. (1996). "Use of weight-for-height indices in children to predict adult overweight: the Bogalusa Heart Study." Int J Obes Relat Metab Disord **20**(8): 715-721.
van Rossem, L., A. H. Wijga, et al. (2012). "Blood pressure in 12-year-old children is associated with fatty acid composition of human milk: the prevention and incidence of asthma and mite allergy birth cohort." Hypertension **60**(4): 1055-1060.
Vassaux, G., D. Gaillard, et al. (1992). "Prostacyclin is a specific effector of adipose cell differentiation. Its dual role as a cAMP- and Ca(2+)-elevating agent." J Biol Chem **267**(16): 11092-11097.
Vassaux, G., D. Gaillard, et al. (1992). "Differential response of preadipocytes and adipocytes to prostacyclin and prostaglandin E2: physiological implications." Endocrinology **131**(5): 2393-2398.
Veldhuis, J. D., J. N. Roemmich, et al. (2005). "Endocrine control of body composition in infancy, childhood, and puberty." Endocr Rev **26**(1): 114-146.
Voors, A. W., L. S. Webber, et al. (1978). "Blood pressure of children, ages 2 1/2-5 1/2 years, in a total community--the Bogalusa heart study." Am J Epidemiol **107**(5): 403-411.
Wang, H., L. H. Storlien, et al. (2002). "Effects of dietary fat types on body fatness, leptin, and ARC leptin receptor, NPY, and AgRP mRNA expression." Am J Physiol Endocrinol Metab **282**(6): E1352-1359.
Weisinger, H. S., J. A. Armitage, et al. (2001). "Perinatal omega-3 fatty acid deficiency affects blood pressure later in life." Nat Med **7**(3): 258-259.
Weisinger, R. S., J. A. Armitage, et al. (2010). "Sodium appetite in adult rats following omega-3 polyunsaturated fatty acid deficiency in early development." Appetite **55**(3): 393-397.
Weisinger, R. S., D. A. Denton, et al. (1988). "The effect of captopril or enalaprilic acid on the Na appetite of Na-deplete rats." Clin Exp Pharmacol Physiol **15**(1): 55-65.
Williams, C. M. and G. Burdge (2006). "Long-chain n-3 PUFA: plant v. marine sources." Proc Nutr Soc **65**(1): 42-50.
Williams, S. M. and A. Goulding (2009). "Patterns of growth associated with the timing of adiposity rebound." Obesity (Silver Spring) **17**(2): 335-341.
Wilson, A. C., J. S. Forsyth, et al. (1998). "Relation of infant diet to childhood health: seven year follow up of cohort of children in Dundee infant feeding study." BMJ **316**(7124): 21-25.
Wyrwoll, C. S., P. J. Mark, et al. (2006). "Prevention of programmed hyperleptinemia and hypertension by postnatal dietary omega-3 fatty acids." Endocrinology **147**(1): 599-606.
Xu, J., M. T. Nakamura, et al. (1999). "Sterol regulatory element binding protein-1 expression is suppressed by dietary polyunsaturated fatty acids. A mechanism for the coordinate suppression of lipogenic genes by polyunsaturated fats." J Biol Chem **274**(33): 23577-23583.
Yin, J., S. Quinn, et al. (2012). "Maternal diet, breastfeeding and adolescent body composition: a 16-year prospective study." Eur J Clin Nutr **66**(12): 1329-1334.

Appendix

Appendix 1	Information letter to parents and child
Appendix 2	Brochure about the rights of the participant as a test participant
Appendix 3	Statement of consent
Appendix 4	Data table of measurements from examinations
Appendix 5	Tanner Scales
Appendix 6	Food frequency questionnaire

Appendix 1

Tillægsprotokol til KF01-300/98
Protokolresumé og deltagerinformation
Version. 1.0/sep 2012.

DELTAGERINFORMATION Oktober 2012

Kære _____

Vi skriver til jer, fordi I tidligere har deltaget i projektet "Betydningen af dokosahexaensyre (DHA) i modermælk for børns vækst- og mentaludvikling" (KF01-300/98) på Institut for Human Ernæring. Sidst vi så på en del af jer var ved 7 års-undersøgelserne. Her fandt vi, at børnene i fiskeoliegruppen havde et højere blodtryk end dem i olivenoliegruppen, og at den forskel vi så i krops-sammensætning hos børnene, da de var 2½ år, var forsvundet. Vi fandt også at der tilsyneladende var forskel i børnenes adfærd med hensyn til fysisk aktivitet, kost og sociale kontakter.

Vi vil gerne se om disse forskelle er reelle og vedvarende og vil derfor gerne undersøge børnene nu ved 12-13 års-alderen. Vi håber derfor at I har lyst til at deltage igen, uanset om I deltog i 7-års undersøgelsen eller ej.

Formålet med dette opfølgningsstudie er at undersøge om modermælkens indhold af langkædede flerumættede fedtsyrer har nogen effekt på barnets kropssammensætning, blodtryk, sundhed, velvære og adfærd på længere sigt.

På de næste sider vil vi beskrive, hvad undersøgelsen indebærer. Deltagelse i undersøgelsen er naturligvis fuldstændig **frivillig** og I kan til hver en tid trække jeres samtykke tilbage og træde ud af forsøget uden yderligere forklaring eller konsekvens. Dette gælder også selvom I har underskrevet samtykkeblanketten. I kan læse mere om forsøgspersoners rettigheder i pjecen "Forsøgspersonens rettigheder i et sundhedsvidenskabeligt forskningsprojekt", som er vedlagt dette brev.

Det er vigtigt at I forældre giver jer god tid til at læse alle papirerne, og forklarer materialet til jeres barn, før I beslutter om I vil være med i undersøgelsen. *Deltagelse kræver at alle der har forældremyndighed over barnet skriver under på samtykkeblanketten.*

Har I spørgsmål er I velkomne til at ringe til Sara Eriksen på tlf: 42702008 mandag-fredag kl 9-15.

I kan også lægge en besked på telefonsvareren, så ringer vi jer op.

Hvis I, efter at have læst vedlagte deltagerinformation, overvejer at deltage, vil vi bede jer ringe til os. Hvis ikke vi hører fra jer, vil vi tillade os at kontakte jer om en uges tid. Ønsker I ikke at deltage meddeler I blot det, og så vil vi ikke ulejlige jer yderligere. Imodsat fald vil vi invitere jer til et mundtligt informationsmøde.

Med venlig hilsen,

Lotte Lauritzen
Lektor, lic. scient

Sara Elisabeth Eriksen
Specialestuderende i Human Ernæring

Institut for Human Ernæring,
Det Natur- og Biovidenskabelige Fakultet, Københavns Universitet
Rolighedsvej 30, 1958 Frederiksberg C

Information til forældre om projektet

"Betydningen af langkædede n-3 fedtsyrer i modermælk for kropssammensætning og blodtryk, samt mentalt velvære og adfærd hos børn i 12-13 års alderen".

Formålet med undersøgelsen er at se om moderens indtag af n-3 fedtsyrer fra fisk via modermælken kan påvirke kropssammensætning og blodtryk hos barnet, der nu er nået 12-13 års alderen, og om en sådan effekt evt. er afhængig af barnets egen kost – specielt indtaget af kulhydrat og protein. Vi vil derudover også undersøge om moderens fiskeindtag påvirker barnets mentale velvære og adfærd, og om der en sammenhæng med barnets blodtryk.
For at kunne deltage i undersøgelsen skal følgende kriterier være opfyldt;
Inklusionskriterier

- 12-13-årige børn hvis mødre deltog i det oprindelige interventionsstudie (KF01-300/98)

I kan udelukkes fra deltagelse hvis;

Eksklusionskriterier

- Barnets aktuelle bopæl er beliggende i så stor afstand fra Institut for Human Ernæring at det ikke er muligt at foretage undersøgelsesbesøg.
- Barnet lider af kroniske sygdomme eller fysiske handicap, der gør det umuligt at udføre undersøgelsen.

Hvad indebærer undersøgelsen?

! Et undersøgelsesbesøg på Institut for Human Ernæring eller i jeres eget hjem
! Udfyldning af spørgeskema om jeres barns kost
! 7 dages måling af barnets fysiske aktivitet, ved hjælp af en lille aktivitetsmåler, der sættes på barnets hofte
! Udfyldning af spørgeskemaer vedrørende barnets helbred, trivsel og udvikling, samt mentale velvære og adfærd.

Ved **undersøgelsesbesøget** vil vi:

- Måle og veje barnet.
- Måle blodtryk og puls

- Tage en lille blodprøve på barnet med en priktest i fingerspidsen
- Instruere jer i brug af fysiske aktivitetsmålere.
- Bede jer udfylde spørgeskemaer om barnets trivsel og udvikling
- Informere jer om udfyldelse af kostregistreringsskema
- Bede barnet udfylde et spørgeskema om pubertet

Flere oplysninger

Måling af vægt, højde, hofteomkreds og hovedomfang. Barnets vægt måles med en gulvvægt og højden måles med en højdemåler. Talje, hofte samt hovedomfang måles med et almindeligt målebånd.

Blod. Der tages nogle få dråber blod fra barnets fingerspids med et lille udstyr kaldet en "finger prick", hvor en lille steril nål vil prikke hul på spidsen af fingeren. Bloddråberne opsamles på et filterpapir. Bloddråberne skal bruges til indholdet af langkædede flerumættede n-3 fedtsyrer. Udover eventuel let rødme omkring fingerspidsen og den umiddelbare fornemmelse af prikket har proceduren ingen kendte bivirkninger. Vi udtager en blodmængde på ca. 0,1 ml, som destrueres umiddelbart efter analyse.

Måling af kropssammensætning. Hudfoldstykkelsen på overarmen og under skulderbladet måles med en hudfoldstang. Kropssammensætningen måles ved brug af bioimpedans-måler, som barnet holder i hånden i ca. 10 sekunder. Princippet i impedans-måleren, svarer til det der anvendes i lignede vægtlignende impedansmålere som er opsat i mange fitnesscentre og svømmehaller. Der sendes en meget svag strøm gennem kroppen og derudfra kan man beregne fedt og muskelandelen.

Blodtryk og puls. Barnets blodtryk og puls måles ved hjælp af et automatiseret måleudstyr, der registrerer blodtrykket i overarmen under oppustningen af en manchet. Blodtryksmålingen tager kun få minutter.

Pubertetsudvikling. Barnets pubertetsudvikling vurderes ved brug af skema med tegninger og pubertetsspørgsmål kaldet Tanner-skalaer, som barnet selv udfylder, evt. sammen med en forælder. Skemaet præsenteres of udfyldes ved undersøgelsen, så eventuelle spørgsmål kan blive besvaret.

Mentalt velvære og adfærd. Spørgeskemaer om barnets trivsel og sundhed samt mentale velvære udfyldes. Information omkring spørgeskemaer vil foregå ved undersøgelsen, så eventuelle spørgsmål kan blive besvaret.

Kostregistrering. Barnets kost i den forgangne måned registreres via et kostregistreringsspørgeskema.

Fysisk aktivitet. Ved undersøgelsen vil der også blive udleveret aktivitetsmålere og der vil blive givet instruktion om hvordan den påsættes og anvendes. Måleren skal sidde på barnets hofte fra barnet står op til det går i seng (undtagen ved svømning og bad) i 7 dage, men er ikke til gene for bevægelsesfriheden. Der skal desuden udfyldes et lille aktivitetsskema sammen med måleren. Udfyldte spørgeskemaer indsamles ved undersøgelsen, eller bedes indsendt sammen med fysisk aktivitetsmålere i den svarkuvert, som udleveres ved undersøgelsen.

Honorar og økonomiske forhold.

Jeres barn vil modtage et skattepligtigt vederlag på 200 kr som udbetales til bank-konto hurtigst muligt efter forsøgets afslutning og efter at I har returneret aktivitetsmåleren. Undersøgelsen er finansieret af Københavns Universitet.

Bivirkninger, risici, komplikationer og ulemper

I forbindelse med at der tages nogle få dråber blod fra barnets fingerspids kan barnet kan opleve en kortvarig let smertefornemmelse. Der er ingen væsentlig risiko ved blodprøven, men i meget sjældne tilfælde kan der forekomme misfarvning omkring stedet for blodprøvetagningen eller ekstremt sjældent opstå en infektion. Erfaringer med aktivitetsmåleren viser at børnene ikke føler sig væsentligt generet af denne.

Udelukkelse fra og afbrydelse af forsøg

Vi forventer ikke at der vil være grund til udelukke eller afbryde din deltagelse i forsøget, men såfremt vi mod forventning vurderer at dit barn ikke kan forsætte i undersøgelsen vil I blive informeret herom. Såfremt dit barn vælger trække sig fra studiet før afslutning, og I ikke ønsker at de allerede opsamlede data bruges, skal I gøre opmærksom på dette. I modsat fald vil de opsamlede data blive brugt i forsøgets dataanalyse.

Forsikring

I er dækket af "Lov om arbejdsskadesikring" i henhold til gældende forsikringsforhold ved Det Biovidenskabelige fakultet, Københavns Universitet. Endvidere er alle forsøgspersoner omfattet af "Lov om klage- og erstatningsadgang inden for sundhedsvæsnet" (jf. lov nr. 547 24/06/2005 wwww.retsinformation.dk)

Nytte ved forsøget

Du og dit barn vil ikke umiddelbart drage nytte af forsøget. Forsøget har en samfundsmæssig nyttefunktion som opvejer det tidsforbrug og eventuelle gener som dit barn oplever.

Offentliggørelse af studiets resultater

Såvel positive som negative resultater fra forsøget vil blive offentliggjort, uden oplysninger om barnets identitet.

Finansiering

Der er ingen kommercielle eller personlige økonomisk interesse forbundet med dette studie. Projektmidlerne er indsat på en separat konto, der er under offentlig revision, og finansieret af Københavns Universitet

Praktiske oplysninger:

- Undersøgelserne foregår i på Institut for Human Ernæring på Rolighedsvej 30, 2. sal, Frederiksberg eller i jeres eget hjem
- Undersøgelsen tager ca. 1½ time
- Tidspunkt for undersøgelserne vil blive tilrettelagt efter aftale med jer, så det passer ind i jeres øvrige aktiviteter
- Undersøgelserne bliver udført af specialestuderende i Human Ernæring, Sara Eriksen.
- Jeres kontaktperson er Sara Eriksen, tlf. **42702008**, eller e-mail; sara.elisabeth.eriksen@gmail.com. Lektor Lotte Lauritzen vil desuden kunne kontaktes angående overordnede spørgsmål vedrørende projektet på tlf. 35332508 eller e-mail: ll@life.ku.dk
- Opfølgningsprojektet er finansieret af Københavns Universitet og basisprojektet blev finansieret af det Fødevareteknologiske Forsknings- og Udviklingsprogram (FØTEK II).
- Undersøgelsen er godkendt af De Videnskabsetiske Komitéer for Københavns og Frederiksbergs kommuner (J.Nr. .....).
- Oplysningerne vedrørende dit barn som forsøgsperson er beskyttet efter "Persondataloven" og "Sundhedsloven"
- Studiet er igangsat på initiativ af Lotte Lauritzen, lektor ved Institut for Human Ernæring.
- Vi forventer at lave en lignende undersøgelse, når dit barn er 17-18 år. Til den tid vil vi tillade os at kontakte dig eller dit barn, hvis han/hun til den tid er blevet myndig. Det er helt frivilligt om I til den tid vil deltage eller ej.

DET VIDENSKABSETISKE KOMITÉSYSTEM

Forsøgspersoners rettigheder i et sundhedsvidenskabeligt forskningsprojekt.

Som deltager i et sundhedsvidenskabeligt forskningsprojekt skal du vide at:

- Din deltagelse i forskningsprojektet er helt frivillig og kun kan ske efter, at du har fået både skriftlig og mundtlig information om forskningsprojektet og underskrevet samtykkeerklæringen
- Du til enhver tid mundtligt, skriftligt eller ved anden klar tilkendegivelse kan trække dit samtykke til deltagelse tilbage og udtræde af forskningsprojektet. Såfremt du trækker dit samtykke tilbage påvirker dette ikke din ret til nuværende eller fremtidig behandling eller andre rettigheder, som du måtte have
- Du har ret til at tage et familiemedlem, en ven eller en bekendt med til informationssamtalen
- Du har ret til betænkningstid, før du underskriver samtykkeerklæringen
- Oplysninger om dine helbredsforhold, øvrige rent private forhold og andre fortrolige oplysninger om dig, som fremkommer i forbindelse med forskningsprojektet, er omfattet af tavshedspligt
- Opbevaring af oplysninger om dig, herunder oplysninger i dine blodprøver og væv, sker efter reglerne i lov om behandling af personoplysninger og sundhedsloven
- Der er mulighed for at få aktindsigt i forsøgsprotokoller efter offentlighedslovens bestemmelser. Det vil sige, at du kan få adgang til at se alle papirer vedrørende din deltagelse i forsøget, bortset fra de dele, som indeholder forretningshemmeligheder eller fortrolige oplysninger om andre
- Der er mulighed for at klage og få erstatning efter reglerne i lov om klage- og erstatningsadgang inden for sundhedsvæsenet

(S5)

Samtykke fra forældremyndighedens indehaver til deres barns deltagelse i et sundhedsvidenskabeligt forskningsprojekt.

Forskningsprojektets titel:

Erklæring fra indehaveren af forældremyndigheden:

Jeg/vi har fået skriftlig og mundtlig information og jeg/vi ved nok om formål, metode, fordele og ulemper til at give mit/vores samtykke.

Jeg/vi ved, at det er frivilligt at deltage, og at jeg/vi altid kan trække mit/vores samtykke tilbage uden, at min/vores datter/søn mister sine nuværende eller fremtidige rettigheder til behandling.

Jeg/vi giver samtykke til, at _____(barnets navn) deltager i forskningsprojektet. Jeg/vi har fået en kopi af dette samtykkeark samt en kopi af den skriftlige information om projektet til eget brug.

Navnet eller navnene på forældremyndighedens indehaver(e):

_____ _____

Dato: _____ Underskrift: _____

Dato: _____ Underskrift: _____

Ønsker du/I at blive informeret om forskningsprojektets resultat samt eventuelle konsekvenser for Dit/jeres barn?:

Ja____(sæt x) Nej_____(sæt x)

Erklæring fra den, der afgiver information:

Jeg erklærer, at forældrene/barnet har modtaget mundtlig og skriftlig information om forsøget.

Efter min overbevisning er der givet tilstrækkelig information til, at forældrene kan træffe beslutning om barnets deltagelse i forsøget.

Navnet på den, der har afgivet information:

Dato: _____ Underskrift: _____

Projektidentifikation: (Fx komiteens Projekt-ID, EudraCT nr., versions nr./dato eller lign.)

Appendix 4

Dataark for _____Forsøgspersonsnummer_____

	1. måling	2.måling	3.måling	Gennemsnit	Eventuelle kommentarer
Blodtryk (sys/dia)					
Højde (cm)					
Vægt (kg)					
Hudfold- skulder (mm)					
Hudfold- triceps (mm)					
Taljeomkreds (cm)					
Hofteomkreds (cm)					
Hovedomkreds (cm)					
Bio-impedans (fedtprocent)					

Personnummer_____

Kontonummer_____

Tanner Scales
Spørgeskema om pubertetsudvikling

Til piger

Til registrering. Udfyldes af interviewer.

ID-nr.

A

Blev spørgeskemaet udfyldt ?

Ja ☐ 1 Nej ☐ 0

→

B

Hvis nej, hvorfor?

_____

C

Var der en person, som hjalp barnet med at udfylde spørgeskemaet?

Ja ☐ 1 Nej ☐ 0

→

D

Hvis ja, hvem?

Mor..... ☐ 0

Far ..... ☐ 1

Anden ..... ☐ 2

DET BIOVIDENSKABELIGE FAKULTET

I puberteten ændres blandt andet højde og vægt. For at vi bedst muligt kan vurdere resultaterne, spørger vi derfor i det følgende til din pubertetsudvikling.
Vi beder dig om, så godt du kan, og eventuelt i samarbejde med din mor eller far, at besvare nedenstående spørgsmål:

1. Har du haft din første menstruation?

Ja..…… ☐ 1

Nej……...…….. ☐ 0 } Gå til spørgsmål 2

Ved ikke……...…….. ☐ 8

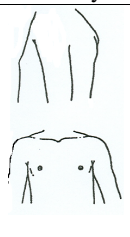
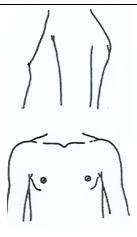
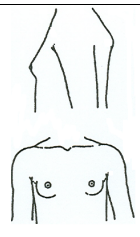
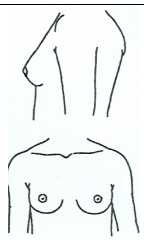
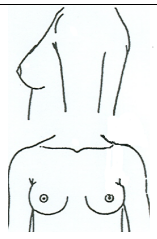
Hvis ja:

a Hvornår fik du din første menstruation?

Jan.	**Feb.**	**Marts**	**Apr.**	**Maj**	**Juni**	**Juli**	**Aug.**	**Sep.**	**Okt.**	**Nov.**	**Dec.**
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Årstal | | | | |

2. Hvor vil du placere dig på nedenstående skala vedrørende dine brysters udvikling?
Afkryds nedenfor

				
Brystvorten er ikke begyndt at vokse	Brystvorten er lige begyndt at vokse	Selve brystet er ved at vokse	Brystet er *næsten* som en ung, voksen kvindes	Brystet er vokset som hos en ung, voksen kvinde
1☐	2☐	3☐	4☐	5☐

Tanner Scales

Spørgeskema om pubertetsudvikling

Til drenge

Til registrering. Udfyldes af interviewer.

A
Blev spørgeskemaet ud yldt ?

Ja ☐ 1 Nej ☐ 0

→

B
Hvis nej, hvorfor?

_____

C
Var der en person, som hjalp barnet med at udfylde spørgeskemaet?

Ja ☐ 1 Nej ☐ 0

→

D
Hvis ja, hvem?

Mor ..... ☐ 0

Far ..... ☐ 1

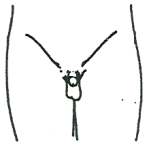
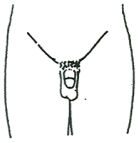
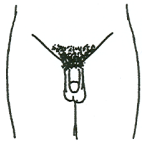
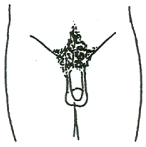
Anden ..... ☐ 2

I puberteten ændres blandt andet højde og vægt. For at vi bedst muligt kan vurdere resultaterne, spørger vi derfor i det følgende til din pubertetsudvikling.

3. Hvor vil du placere dig på nedenstående skala vedrørende din kønsbehåring?

Afkryds nedenfor

Vi beder dig om, så godt du kan, og eventuelt i samarbejde med din mor eller far, at vurdere hvor på skalaen du er:

1	2	3	4	5
Der er ikke kønsbehåring	Der er næsten ikke/ eller ganske lidt kønsbehåring	Kønsbehåringen er blevet lidt mørkere og fylder mere	Kønsbehåringen er *næsten* som hos en ung, voksen mand	Kønsbehåringen er som hos en ung, voksen mand
				
☐	☐	☐	☐	☐

Spørgsmåls index

1 Eksempler
2 Data om dig
3 Drikkevarer
4 Mejeriprodukter
5 Morgenmadsprodukter og brød
6 Pålæg
7 Kolde og varme retter
8 Tilbehør til kolde og varme retter
9 Frugt og grøntsager
10 Snacks og desserter
11 Måltidsvaner
12 Aktivitet
13 Hjælp til at udfylde spørgeskemaet

Eksempel 1: **"Hvor ofte drak du mælk"?**

1. Lad os forestille os, at du oftest drikker minimælk. Hvis du får mælk på din morgenmad, får 1 glas mælk til frokost og 2 glas mælk til aftensmaden hver dag, drikker du ca. 4 glas mælk om dagen, og du skal vælge svaret: "4 eller flere gange om dagen."

* Hvor ofte drak du minimælk de seneste 12 måneder?

	Mindre end 1 gang om måneden	1-3 gange om måneden	2-4 gange om ugen	5-6 gange om ugen	1 gang om dagen	2-3 gange om dagen	4 eller flere gange om dagen
	○	○	○	○	○	○	◉

Eksempel 2: **"Hvor ofte spiste du pålægschokolade?"**
1. Hvis du får pålægschokolade én gang hver weekend, skal du vælge svaret: "1 gang om ugen".

* Hvor ofte spiste du nedenstående pålægsprodukter?

	Spiste ikke sidste år	Mindre end 1 gang om måneden	1-3 gange om måneden	1 gang om ugen	2-4 gange om ugen	5-6 gange om ugen	1 gang om dagen	2 eller flere gange om dagen
Pålægschokolade eller Nutella	○	○	○	◉	○	○	○	○

Eksempel 3: **"Hvor ofte spiste du popcorn?"**
1. Lad os forestille os, at du får popcorn hver anden fredag til fredagsslik. Hvis du også har været i biografen en gang den sidste måned og fået popcorn, skal du vælge svaret: "1-3 gange sidste måned".

* Hvor ofte spiste du nedenstående snacks og desserter?

	Spiste ikke sidste år	Mindre end 1 gang om måneden	1-3 gange om måneden	1 gang om ugen	2-4 gange om ugen	5-6 gange om ugen	1 gang om dagen	2 eller flere gange om dagen
Popcorn	○	○	◉	○	○	○	○	○

Data om dig

* **Hvor høj er du?**

Angiv i cm.
Rund op til nærmeste hele cm.

171 cm

* **Hvor meget vejer du?**

Angiv i kg.
Rund op til nærmeste hele kg.

53 kg

Drikkevarer

Tænk 12 måneder tilbage – alle dagens måltider og mellemmåltider både hverdag og weekend.

* **Hvilken type mælk drikker du oftest?**

Vælg én af følgende svarmuligheder

- ○ Sødmælk
- ○ Letmælk
- ○ Minimælk
- ◉ Skummetmælk
- ○ Sojamælk/Sojadrik
- ○ Jeg ved ikke
- ○ Jeg drikker ikke mælk

* **Hvor ofte drak du skummetmælk de seneste 12 måneder?**

	Mindre end 1 gang om måneden	1-3 gange om måneden	2-4 gange om ugen	5-6 gange om ugen	1 gang om dagen	2-3 gange om dagen	4 eller flere gange om dagen
	○	◉	○	○	○	○	○

- **Hvor ofte drak du nedenstående drikkevarer?**

	Drak ikke sidste år	Mindre end 1 glas om måneden	1-3 glas om måneden	1 glas om ugen	2-4 glas om ugen	5-6 glas om ugen	1 glas om dagen	2-3 glas om dagen	4 eller flere glas om dagen
Saft, almindelig	◉	○	○	○	○	○	○	○	○
Saft, light	◉	○	○	○	○	○	○	○	○
Appelsinjuice	○	○	◉	○	○	○	○	○	○
Æblejuice	○	◉	○	○	○	○	○	○	○
Kakaomælk	○	○	◉	○	○	○	○	○	○
Vand, postevand, mineralvand eller danskvand	○	○	○	○	○	○	○	○	◉
Smoothie	○	○	◉	○	○	○	○	○	○

- **Hvor ofte drak du nedenstående drikkevarer?**

	Drak ikke sidste år	Mindre end 1 flaske om måneden	1-3 flasker om måneden	1 flaske om ugen	2-4 flasker om ugen	5-6 flasker om ugen	1 flaske om dagen	2-3 flasker om dagen	4 eller flere flasker om dagen
Sodavand, almindelig	○	◉	○	○	○	○	○	○	○
Sodvand, light	○	◉	○	○	○	○	○	○	○
Sportsdrik (f.eks. Powerade)	◉	○	○	○	○	○	○	○	○
Energidrik (f.eks. Red Bull, Pure Rush, Cult, Burn)	◉	○	○	○	○	○	○	○	○
Øl	○	○	○	○	◉	○	○	○	○
Cider (f.eks. Somersby, Tempt)	○	○	○	○	◉	○	○	○	○
Alkohol (f.eks. Bacardi Breezer)	○	○	○	◉	○	○	○	○	○

* **Hvor ofte drak du nedenstående drikkevarer?**

	Drak ikke sidste år	Mindre end 1 kop om måneden	1-3 koppper om måneden	1 kop om ugen	2-4 koppper om ugen	5-6 koppper om ugen	1 kop om dagen	2-3 koppper om dagen	4 eller flere koppper om dagen
Kaffe, sort	◉	○	○	○	○	○	○	○	○
Kaffe med mælk (f.eks. Cafe Latte, Cappuccino)	◉	○	○	○	○	○	○	○	○
Te	○	○	○	◉	○	○	○	○	○

Morgenmadsprodukter og brød

Tænk 12 måneder tilbage – alle dagens måltider både hverdag og weekend.

* **Hvor ofte spiste du nedenstående morgenmadsprodukter?**

	Spiste ikke sidste år	Mindre end 1 gang om måneden	1-3 gange om måneden	1 gang om ugen	2-4 gange om ugen	5-6 gange om ugen	1 gang om dagen	2 eller flere gange om dagen
Cornflakes, Havrefras, Special-K eller lign. (skål)	◉	○	○	○	○	○	○	○
Guldkorn, Frostflakes, Choco Pops eller lign. (skål)	◉	○	○	○	○	○	○	○
Havregryn (skål)	○	○	○	○	○	○	◉	○
Mysli (skål)	○	◉	○	○	○	○	○	○
Grød (f.eks. havregrød, øllebrød, risengrød) (skål)	○	○	○	○	◉	○	○	○

* **Hvor ofte kom du en smørklat i grøden?**

Vælg én af følgende svarmuligheder

- ○ Altid
- ○ Nogen gange
- ◉ Aldrig

Mejeriprodukter

Tænk 12 måneder tilbage – alle dagens måltider og mellemmåltider både hverdag og weekend.

*** Hvilken slags mejeriprodukter spiste du oftest?**

Vælg én af følgende svarmuligheder

- (•) Fedtfattige mejeriprodukter (f.eks. Cheasy yoghurt, Cheasy ost)
- ( ) Fede mejeriprodukter (f.eks. sødmælksyoghurt, Havarti ost)
- ( ) Jeg spiser lige meget af fedtfattige og fede mejeriprodukter
- ( ) Jeg ved ikke
- ( ) Jeg spiser ikke mejeriprodukter

*** Hvor ofte spiste eller brugte du følgende mejeriprodukter?**

Tænk 12 måneder tilbage - alle dagens måltider og mellemmåltider både på hverdag og weekend.

	Spiste ikke sidste år	Mindre end 1 gang om måneden	1-3 gange om måneden	1 gang om ugen	2-4 gange om ugen	5-6 gange om ugen	1 gang om dagen	2-3 gange om dagen	4 eller flere gange om dagen
Ost som tilbehør (f.eks. revet, som drys, salattern)	○	○	(•)	○	○	○	○	○	○
Ostehaps (stk.)	(•)	○	○	○	○	○	○	○	○
Ost, skære- og smøreost (på brød)	○	○	(•)	○	○	○	○	○	○
Yoghurt naturel (f.eks. A38) (skål)	○	○	○	○	○	○	(•)	○	○
Frugtyoghurt (f.eks. jordbær, pære/banan) (skål)	(•)	○	○	○	○	○	○	○	○
Drikkeyoghurt (f.eks. Cultura, Yoggi) (flaske)	(•)	○	○	○	○	○	○	○	○
Koldskål (skål)	○	(•)	○	○	○	○	○	○	○
Fløde, flødeskum, creme fraiche (f.eks. til is, kage)	○	○	(•)	○	○	○	○	○	○

* **Hvor ofte spiste du nedenstående brødtyper?**
Tænk 12 måneder tilbage - alle dagens måltider og mellemmåltider både på hverdag og weekend.

	Spiste ikke sidste år	Mindre end 1 gang om måneden	1-3 gange om måneden	1 gang om ugen	2-4 gange om ugen	5-6 gange om ugen	1 gang om dagen	2-3 gange om dagen	4 eller flere gange om dagen
Franskbrød	○	○	◉	○	○	○	○	○	○
Bolle, flute	○	○	◉	○	○	○	○	○	○
Grovbolle, grovbrød	○	○	○	○	◉	○	○	○	○
Rugbrød	○	○	○	○	○	○	◉	○	○
Knækbrød	○	○	○	○	○	◉	○	○	○
Riskiks	○	◉	○	○	○	○	○	○	○

* **Når du spiste brød, hvor meget spiste du typisk ad gangen?**

Franskbrød	1-2 halve skiver
Bolle, flute	1-2 halve skiver
Grovbolle, grovbrød	1-2 halve skiver
Rugbrød	1-2 halve skiver
Knækbrød	1-2 halve skiver
Riskiks	1-2 halve skiver

* **Når du spiste brød, hvor meget spiste du typisk ad gangen?**

Franskbrød	1-2 halve skiver
Bolle, flute	1-2 halve skiver
Grovbolle, grovbrød	1-2 halve skiver
Rugbrød	1-2 halve skiver
Knækbrød	1-2 halve skiver
Riskiks	1-2 halve skiver

* **Hvor ofte kom du fedtstof på dit brød?**

F.eks. smør, Kærgården, Lätta

Vælg én af følgende svarmuligheder

- ○ Altid
- ◉ Nogen gange
- ○ Aldrig

* **Hvilken type fedtstof brugte du typisk på brødet?**

Vælg én af følgende svarmuligheder

- ○ Smør eller Kærgården
- ◉ Margarine/Minarine (f.eks Becel eller Lätta)

Pålæg

Tænk 12 måneder tilbage – alle dagens måltider og mellemtider både hverdag og weekend.

* **Hvor ofte spiste du nedenstående pålægsprodukter?**

	Spiste ikke sidste år	Mindre end 1 gang om måneden	1-3 gange om måneden	1 gang om ugen	2-4 gange om ugen	5-6 gange om ugen	1 gang om dagen	2 eller flere gange om dagen
Pålægschokolade eller Nutella	○	◉	○	○	○	○	○	○
Marmelade, syltetøj eller honning	○	○	◉	○	○	○	○	○
Hamburgerryg, skinke	◉	○	○	○	○	○	○	○
Flæskesteg, rullepølse	◉	○	○	○	○	○	○	○
Roastbeef	◉	○	○	○	○	○	○	○
Leverpostej, pate eller postej	◉	○	○	○	○	○	○	○
Spegepølse eller pepperoni	◉	○	○	○	○	○	○	○
Kylling- eller kalkunfilet	◉	○	○	○	○	○	○	○
Skinkesalat, hønsesalat eller sommersalat	◉	○	○	○	○	○	○	○
Makrel- eller tunsalat (færdigblandet f.eks. K-salat)	◉	○	○	○	○	○	○	○
Sild (f.eks. karrysild, dildsild)	○	○	◉	○	○	○	○	○

Skinkesalat, hønsesalat eller sommersalat	⦿	○	○	○	○	○	○	○
Makrel- eller tunsalat (færdigblandet f.eks. K-salat)	⦿	○	○	○	○	○	○	○
Sild (f.eks. karrysild, dildsild)	○	○	⦿	○	○	○	○	○
Fisk fra dåse (f.eks. tun eller makrelfilet)	○	○	○	○	⦿	○	○	○
Rejer	○	○	○	○	⦿	○	○	○
Frugtpålæg	○	○	○	○	⦿	○	○	○

Kolde og varme retter

Tænk 12 måneder tilbage – alle dagens måltider og mellemtider både hverdag og weekend.

* **Hvor ofte spiste du nedenstående retter?**

	Spiste ikke sidste år	Mindre end 1 gang om måneden	1-3 gange om måneden	1 gang om ugen	2-4 gange om ugen	5-6 gange om ugen	1 gang om dagen	2 eller flere gange om dagen
Oksekød eller kalvekød (f.eks. culottesteg, roastbeef)	⦿	○	○	○	○	○	○	○
Hakkebøf	⦿	○	○	○	○	○	○	○
Svinekød (f.eks. flæskesteg, kotelet, hamburgerryg)	⦿	○	○	○	○	○	○	○
Frikadeller	⦿	○	○	○	○	○	○	○
Kylling eller kalkun	⦿	○	○	○	○	○	○	○
Lammekød	⦿	○	○	○	○	○	○	○

Pastaret med kød og pasta/nudler/ris (f.eks. lasagne)	◉	○	○	○	○	○	○	○
Biksemad	◉	○	○	○	○	○	○	○
Tærte med kød eller grøntsager	○	◉	○	○	○	○	○	○
Retter med bønner, linser eller ærter (f.eks. falafel, hummus)	○	○	○	◉	○	○	○	○
Pølser uden brød	◉	○	○	○	○	○	○	○
Hotdog, fransk hotdog eller pølsehorn (pølser med brød)	◉	○	○	○	○	○	○	○
Pizza	○	○	◉	○	○	○	○	○
Burger	◉	○	○	○	○	○	○	○
Toast eller parisertoast	◉	○	○	○	○	○	○	○
Sandwich (f.eks. med pålæg og salat)	○	○	◉	○	○	○	○	○

Pita med fyld	○	◉	○	○	○	○	○	○
Æg (f.eks. kogt æg, spejlæg eller omelet)	◉	○	○	○	○	○	○	○
Sushi	○	○	◉	○	○	○	○	○
Fisk (f.eks. laks, torsk, rødspætte)	○	○	○	○	○	◉	○	○
Fiskeprodukt (f.eks. fiskefrikadelle, fiskepinde)	○	○	○	○	○	◉	○	○
Suppe (f.eks. kartoffelsuppe, tomatsuppe)	○	◉	○	○	○	○	○	○

* **Angiv venligst hvilken type suppe, du spiste oftest:**

Vælg én af følgende svarmuligheder

- ○ Suppe med kød (f.eks. med kylling eller kødboller)
- ◉ Suppe uden kød (f.eks. kartoffel-porre suppe)
- ○ Suppe med nudler/pasta
- ○ Ved ikke

Tilbehør til kolde og varme retter

Tænk 12 måneder tilbage – alle dagens måltider og mellemmåltider både hverdag og weekend.

* **Hvor ofte spiste du nedenstående tilbehør?**

	Spiste ikke sidste år	Mindre end 1 gang om måneden	1-3 gange om måneden	1 gang om ugen	2-4 gange om ugen	5-6 gange om ugen	1 gang om dagen	2-3 gange om dagen	4 eller flere gange om dagen
Kartofler: kogt, ovnstegt, mos	○	○	◉	○	○	○	○	○	○
Pommes frites	○	◉	○	○	○	○	○	○	○
Stegte eller brasede kartofler	○	○	◉	○	○	○	○	○	○
Kartoffelsalat eller kartoffelgratin	○	◉	○	○	○	○	○	○	○
Ris (f.eks. til gryderetter/wokretter)	○	○	◉	○	○	○	○	○	○
Nudler/pasta, bulgur (f.eks. til gryderetter/wokretter)	○	○	◉	○	○	○	○	○	○
Linser, kikærter eller lign. bælgfrugter	○	○	○	◉	○	○	○	○	○
Blandet salat uden kød	○	○	○	○	○	○	◉	○	○
Blandet salat med kød	◉	○	○	○	○	○	○	○	○
Varme grøntsager	○	○	○	○	○	◉	○	○	○

Fed sovs (f.eks. karrysovs, bearnaisesovs, brunsovs)	○	⊙	○	○	○	○	○	○	○
Let sovs (f.eks. tomatsovs, pizzasovs, pastasovs)	○	⊙	○	○	○	○	○	○	○
Dressing med creme fraiche (f.eks. Thousand Island, dip)	⊙	○	○	○	○	○	○	○	○
Dressing (f.eks. olie-eddike)	○	⊙	○	○	○	○	○	○	○
Mayonnaise eller remoulade	○	○	⊙	○	○	○	○	○	○
Ketchup eller sennep	○	⊙	○	○	○	○	○	○	○

* **Hvor ofte tilsatte du selv salt til din mad ved bordet?**

F.eks. på æg, pommes frites eller kød.

	Det gjorde jeg ikke sidste år	1-3 gange om måneden	1 gang om ugen	2-4 gange om ugen	5-6 gange om ugen	1 gang om dagen	2-3 gange om dagen	4 eller flere gange om dagen
Antal gange	○	⊙	○	○	○	○	○	○

* **Hvor ofte tilsatte du sukker til dine drikkevarer eller din mad (1 tsk./1 sukkerknald)?**

F.eks. på din morgenmad, på grød, i kaffe eller te - tænk på alle dagens måltider.

	Det gjorde jeg ikke sidste år	1-3 gange om måneden	1 gang om ugen	2-4 gange om ugen	5-6 gange om ugen	1 gang om dagen	2-3 gange om dagen	4 eller flere gange om dagen
Antal gange	⊙	○	○	○	○	○	○	○

Frugt og grøntsager

Tænk 12 måneder tilbage – alle dagens måltider og mellemmåltider både hverdag og weekend.

* **Hvor ofte spiste du nedenstående frugter?**

	Spiste ikke sidste år	Mindre end 1 gang om måneden	1-3 gange om måneden	1 gang om ugen	2-4 gange om ugen	5-6 gange om ugen	1 gang om dagen	2-3 gange om dagen	4 eller flere gange om dagen
Æble (1 stk)	○	○	○	○	○	○	○	◉	○
Pære (1 stk)	○	○	○	○	◉	○	○	○	○
Banan (1 stk)	○	○	◉	○	○	○	○	○	○
Appelsin, grapefrugt (½-1 stk)	○	○	◉	○	○	○	○	○	○
Nektarin, fersken, abrikos eller blomme (1 stk)	○	○	◉	○	○	○	○	○	○
Melon (1 skive)	○	○	○	◉	○	○	○	○	○
Kiwi (1 stk)	○	○	○	◉	○	○	○	○	○
Ananas (1 skive)	○	○	◉	○	○	○	○	○	○
Bær, friske eller frosne (1 håndfuld)	○	○	○	○	○	○	◉	○	○
Vindruer (1 håndfuld)	○	○	○	◉	○	○	○	○	○
Rosiner (½ håndfuld)	○	◉	○	○	○	○	○	○	○

* **Hvor ofte spiste du nedenstående grøntsager?**

	Spiste ikke sidste år	Mindre end 1 gang om måneden	1-3 gange om måneden	1 gang om ugen	2-4 gange om ugen	5-6 gange om ugen	1 gang om dagen	2-3 gange om dagen	4 eller flere gange om dagen	Ved ikke
Agurk (1 stk ca. 4-5 cm)	○	○	○	○	○	◉	○	○	○	○
Tomat (1 stk)	○	○	○	○	○	◉	○	○	○	○
Grønne bønner (1 håndfuld)	○	○	◉	○	○	○	○	○	○	○
Broccoli (1 håndfuld)	○	○	○	◉	○	○	○	○	○	○
Hvidkål eller blomkål (1 håndfuld)	○	○	◉	○	○	○	○	○	○	○
Løg eller porre (½ håndfuld)	○	○	○	◉	○	○	○	○	○	○
Avocado (½ stk)	○	○	○	◉	○	○	○	○	○	○
Majs (½ kolbe eller 1	○	○	◉	○	○	○	○	○	○	○

Svampe (f.eks. champignon) (1 håndfuld)	○	○	◉	○	○	○	○	○	○	○
Ærter (½ håndfuld)	○	○	◉	○	○	○	○	○	○	○
Salat, kun grønne blade (1 håndfuld)	○	○	○	○	○	○	○	◉	○	○
Spinat (1 håndfuld)	○	○	○	○	○	○	◉	○	○	○
Grøn, gul eller rød peberfrugt (1 håndfuld)	○	○	○	○	○	○	◉	○	○	○
Gulerødder (1 stk)	○	○	○	○	○	◉	○	○	○	○
Rødbede (1 håndfuld)	○	○	○	○	◉	○	○	○	○	○
Knoldselleri (1 håndfuld)	○	○	◉	○	○	○	○	○	○	○
Pastinak, persillerod eller lign. rodfrugter (1 håndfuld)	○	○	◉	○	○	○	○	○	○	○
Squash eller aubergine (1 stk ca. 4-5cm)	○	○	○	◉	○	○	○	○	○	○

Snacks og desserter

ænk 12 måneder tilbage – alle dagens måltider og mellemmåltider både hverdag og weekend

* **Hvor ofte spiste du nedenstående snacks og desserter?**

	Spiste ikke sidste år	Mindre end 1 gang om måneden	1-3 gange om måneden	1 gang om ugen	2-4 gange om ugen	5-6 gange om ugen	1 gang om dagen	2 eller flere gange om dagen
Kage (1 stk.)	○	○	◉	○	○	○	○	○
Blandet slik (f.eks. vingummi, bolcher, lakrids, karamel)	○	○	◉	○	○	○	○	○
Chokolade (f.eks. Toms, Marabou, Ritter sport)	○	○	◉	○	○	○	○	○
Chokoladebar (f.eks. Mars, Snickers)	○	◉	○	○	○	○	○	○
Chips (f.eks. franske kartofler, sour cream)	○	◉	○	○	○	○	○	○
Majs chips, Tortilla chips (f.eks. Doritos, Buggles)	○	◉	○	○	○	○	○	○

Popcorn	◉	○	○	○	○	○	○	○
Peanuts	○	◉	○	○	○	○	○	○
Andre nødder (ikke peanuts)	○	○	○	○	○	○	◉	○
Saltstænger	◉	○	○	○	○	○	○	○
Wienerbrød	○	○	◉	○	○	○	○	○
Tærte (f.eks. æbletærte)	○	○	◉	○	○	○	○	○
Pandekager eller æbleskiver	○	◉	○	○	○	○	○	○
Småkager	○	◉	○	○	○	○	○	○
Kiks	○	○	◉	○	○	○	○	○
Flødeboller	○	◉	○	○	○	○	○	○
Energi-bar	◉	○	○	○	○	○	○	○
Müslibar	○	○	◉	○	○	○	○	○
Frugtstang	○	○	○	◉	○	○	○	○
Sodavandsis	○	◉	○	○	○	○	○	○
Flødeis (f.eks. Magnum, Kæmpe Eskimo)	○	○	◉	○	○	○	○	○

Måltidsvaner

Disse spørgsmål handler om dine måltidsvaner for de seneste 12 måneder.

*** Hvor mange gange om ugen spiste du morgenmad?**

Hverdage og weekend

	Det gør jeg aldrig	1-3 gange om måneden	1-2 gange om ugen	3-4 gange om ugen	5-6 gange om ugen	Hver dag
Antal gange	○	○	○	○	○	◉

*** Hvor ofte sad du ned og spiste frokost eller aftensmad sammen med din familie på hverdage?**

	Det gør jeg aldrig	1-3 gange om måneden	1 gang om ugen	2-4 gange om ugen	5-6 gange om ugen	Hver dag
Frokost	○	◉	○	○	○	○
Aftensmad	○	○	○	○	◉	○

*** Hvor ofte sad du ned og spiste frokost eller aftensmad sammen med din familie i weekender?**

	Det gør jeg aldrig	1-3 gange om måneden	1 gang om ugen	2 gange om ugen
Frokost	○	○	○	◉
Aftensmad	○	○	○	◉

*** Hvor fik du typisk din frokost fra på skoledage?**

Vælg én af følgende svarmuligheder

- ◉ Tog madpakke med hjemmefra
- ○ Købte i skolen
- ○ Købte udenfor skolen
- ○ Spiste typisk ikke frokost på skoledage

*** Hvor ofte spiste du noget fra en fastfood eller take away restaurant?**

F.eks. McDonalds, Burger King, pizzeria, shawarma bar osv.

	Det gjorde jeg aldrig	Mindre end 1 gang om måneden	1-3 gange om måneden	1 gang om ugen	2-4 gange om ugen	5-6 gange om ugen	1 gang om dagen	2-3 gange om dagen
Fast food eller take away	○	◉	○	○	○	○	○	○

* **Hvor ofte tog du kosttilskud?**

	Spiste ikke sidste år	Mindre end 1 gang om ugen	1-2 gange om ugen	3-4 gange om ugen	5-6 gange om ugen	7 gange om ugen (hver dag)	Mere end 1 gang om dagen
Multivitaminpiller	◉	○	○	○	○	○	○
Calcium	◉	○	○	○	○	○	○
Fiskeolie	○	○	○	○	○	◉	○
Jern	○	◉	○	○	○	○	○
Vitamin C	◉	○	○	○	○	○	○
Vitamin D	○	○	○	○	○	◉	○
Andet	○	○	○	○	○	◉	○

Hvilket 'andet' kosttilskud tog du?

Vitamin B kompleks

* **Er der nogle typer mad, du ikke kan tåle (fødevareallergi)?**

○ Ja ◉ Nej

* **Er der noget, du undgår at spise?**

Af andre grunde end madallergi.

◉ Ja ○ Nej

* **Hvad undgår du at spise og hvorfor?**

Animalsk fedt – hovedsageligt kødprodukter.

Aktivitet

Disse spørgsmål handler om din aktivitet de seneste 12 måneder.
Vælg det svar, der bedst beskriver, hvor aktiv du var det seneste år.
Tænk både på hverdag og weekend.

* **Havde du idræt i skolen?**

(•) Ja ( ) Nej

* **Hvor mange timer om ugen?**

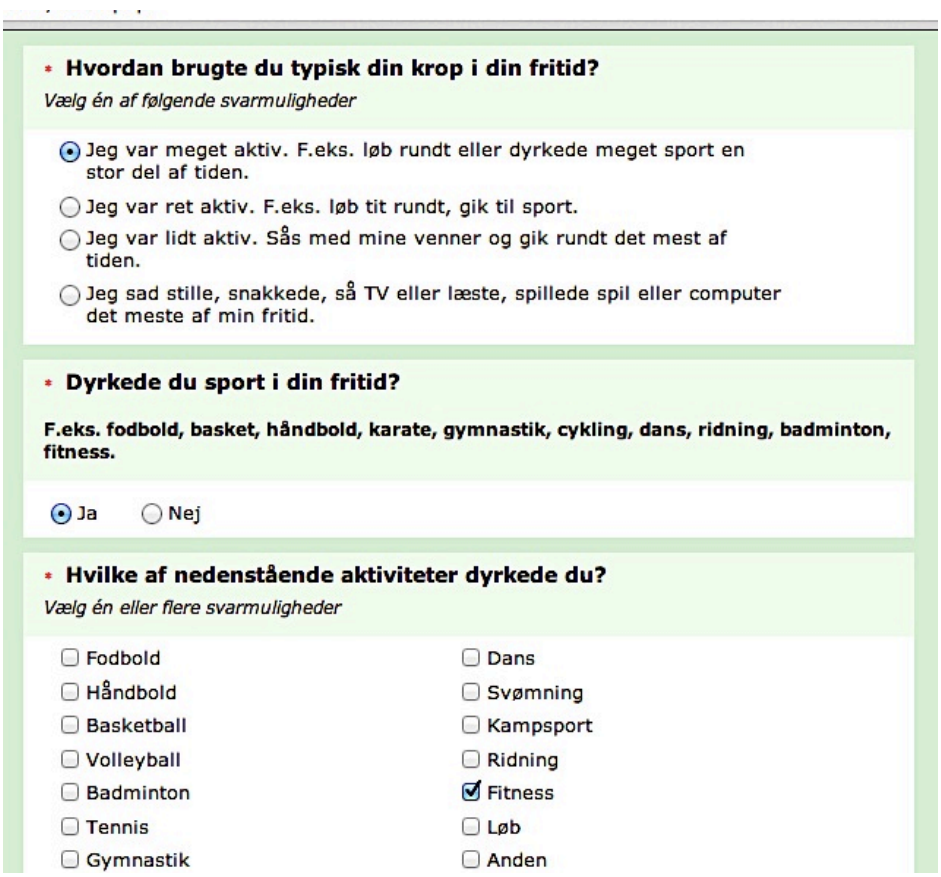
Vælg én af følgende svarmuligheder

( ) 1 time om ugen
( ) 2 timer om ugen
( ) 3 timer om ugen
( ) 4 timer om ugen
( ) 5 timer om ugen
(•) Mere end 5 timer om ugen

* **Hvordan brugte du typisk din krop i frikvartererne?**

Vælg én af følgende svarmuligheder

(•) Jeg var meget aktiv. F.eks. løb rundt eller spillede bold en stor del af tiden.
( ) Jeg var ret aktiv. F.eks. løb tit rundt eller spillede bold.
( ) Jeg gik rundt de fleste frikvarterer.
( ) Jeg sad stille, snakkede, læste eller spillede spil de fleste frikvarterer.



* **Hvordan brugte du typisk din krop i din fritid?**

Vælg én af følgende svarmuligheder

- (•) Jeg var meget aktiv. F.eks. løb rundt eller dyrkede meget sport en stor del af tiden.
- ( ) Jeg var ret aktiv. F.eks. løb tit rundt, gik til sport.
- ( ) Jeg var lidt aktiv. Sås med mine venner og gik rundt det mest af tiden.
- ( ) Jeg sad stille, snakkede, så TV eller læste, spillede spil eller computer det meste af min fritid.

* **Dyrkede du sport i din fritid?**

F.eks. fodbold, basket, håndbold, karate, gymnastik, cykling, dans, ridning, badminton, fitness.

(•) Ja ( ) Nej

* **Hvilke af nedenstående aktiviteter dyrkede du?**

Vælg én eller flere svarmuligheder

- [ ] Fodbold
- [ ] Håndbold
- [ ] Basketball
- [ ] Volleyball
- [ ] Badminton
- [ ] Tennis
- [ ] Gymnastik
- [ ] Dans
- [ ] Svømning
- [ ] Kampsport
- [ ] Ridning
- [x] Fitness
- [ ] Løb
- [ ] Anden

* **Hvornår står du op og går i seng i weekenden?**

	Klokkeslæt (timer)		Klokkeslæt (minutter)
Jeg står typisk op klokken	08		45
Jeg går typisk i seng klokken	01		45

* **Hvor mange timer om ugen plejer du at se TV i din fritid?**

(Tæl også video, DVD, Playstation, Xbox, biografture osv. med)

Angiv timer for alle 5 hverdage i alt og for hele weekenden.

	Ingen - ½ time om ugen	1-5½ time om ugen	6-10½ time om ugen	11-15½ time om ugen	16-20½ time om ugen	21-30½ time om ugen	Mere end 31 timer om ugen
5 hverdage	○	○	○	◉	○	○	○
Hele weekenden	○	◉	○	○	○	○	○

* **Hvor mange timer om ugen plejer du at bruge på computer i din fritid?**

(Tæl chatting, surfing på internettet, spil, e-mail, spil på mobilen, lektier osv. med)

Angiv timer for alle 5 hverdage i alt og for hele weekenden.

	Ingen - ½ time om ugen	1-5½ time om ugen	6-10½ time om ugen	11-15½ time om ugen	16-20½ time om ugen	21-30½ time om ugen	Mere end 31 timer om ugen
5 hverdage	○	◉	○	○	○	○	○
Hele weekenden	○	◉	○	○	○	○	○

* **Hvor mange timer om ugen gik du i gennemsnit til fitness?**

Afkryds for hver sæson:

	Ingen	Mindre end ½ time om ugen	½ - 3½ time om ugen	4-6½ time om ugen	7-9½ time om ugen	Mere end 10 timer om ugen
Efterår	○	○	○	◉	○	○
Vinter	○	○	○	◉	○	○
Forår	○	○	○	◉	○	○
Sommer	○	○	○	◉	○	○

* **Cykler eller går du til og fra skole og/eller fritidsaktiviteter**

Vælg én af følgende svarmuligheder

◉ Ja
○ Nej

* **Hvor mange timer i alt om ugen?**

Vælg én af følgende svarmuligheder

○ ½ time om ugen
○ 1 time om ugen
○ 2 timer om ugen
◉ 3 timer om ugen
○ 4 timer om ugen
○ Mere end 4 timer om ugen

Hjælp til at udfylde spørgeskemaet

* **Har du fået hjælp til at udfylde spørgeskemaet?**

Vælg én af følgende svarmuligheder

○ Ja, jeg har fået lidt hjælp fra en voksen
○ Ja, jeg har fået nogen hjælp fra en voksen
○ Ja, jeg har fået meget hjælp fra en voksen
◉ Nej, jeg har selv udfyldt spørgeskemaet

Godt gået! Du er nu færdig med at udfylde spørgeskemaet.
Husk at trykke send.