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Food choice during the complementary feeding period – Variety in dietary practice and experimental optimisation using (LC-)PUFA rich foods

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“Tempora mutantur, nos et mutamur in illis.”

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PUBLICATIONS

This thesis aimed to examine the status quo of the used foods in products for complementary feeding (CF) on the German market and in dietary practice with respect to food variety, especially vegetable variety, the status quo of n-3 (LC-) PUFA rich food consumption (i.e. fish and rapeseed oil) and its determinants in German infants and their mothers and effects of fish or rapeseed oil consumption in CF on (LC-)PUFA supply of infants as well as cognitive and visual development in the second 6 months of life. It resulted in the following scientific papers:

Mesch C, Stimming M, Wagner A, Libuda L, Kersting M (2013): **Recruitment of mothers with infants in an intervention trial – initial findings from the PINGU-study. PINGU – Multimodal optimization of dietary supply of infants with polyunsaturated fatty acids in complimentary food: background and project structure.** Ernaehrungs Umschau international;60(7):110–115.

Mesch CM, Stimming M, Foterek K, Hilbig A, Alexy U, Kersting M, Libuda L (2014): **Food variety in commercial and homemade complementary meals for infants in Germany. Market survey and dietary practice.** Appetite;76:113-9.

Stimming M, **Mesch CM**, Kersting M, Libuda L (2015): **Fish and rapeseed oil consumption in infants and mothers: dietary habits and determinants in a nationwide sample in Germany.** Eur J Nutr;54(7):1069-80.

Libuda L, **Mesch CM**, Stimming M, Demmelmair H, Koletzko B, Warschburger P, Blanke K, Kalhoff H, Kersting M (2016): **Fatty acid supply with complementary foods and LC-PUFA status in healthy infants: results of a randomised controlled trial.** Eur J Nutr;55(4):1633-44.

Mesch CM, Stimming M, Israel A, Spitzer C, Beganovic L, Estella Perez R, Kalhoff H, Kersting M, Koletzko B, Warschburger P, Libuda L: **Effects of LC-PUFA supply via complementary food on infants' visual and cognitive development – Results of the randomised controlled trial PINGU.** (submitted)

SUMMARY

Background and Aim

The complementary feeding (CF) period is the first occasion for the infant to get familiar with solid foods that will be also part of the adult diet and thus features the opportunity to imprint healthy food preferences by using an appropriate food choice. A great food variety, especially a great vegetable variety, in this period was found to favour food acceptance in infancy and later in life. Regarding e.g. the low vegetable consumption in toddler-, childhood and adolescence, it seems reasonable to promote healthy eating habits early in life. Food variety does not only mean supporting infant's taste development but possibly also, with the integration of foods with specific nutrients, like fish with long-chain polyunsaturated fatty acids (LC-PUFA), especially docosahexaenoic acid (DHA), supporting infant's functional development. As the LC-PUFA intake decreases with the introduction of complementary food while the demand is still high for the rapid neural tissue development, strategies to optimise LC-PUFA supply in this period need to be developed. **The aim of the present thesis** was to evaluate the status quo of the used foods in products for CF on the German market and in CF meals used in dietary practice with respect to food variety, especially vegetable variety (**Q1a/b**), the status quo of n-3 (LC-)PUFA rich food consumption (i.e. fish and rapeseed oil) and its determinants in German infants and their mothers (**Q2**) and effects of fish (salmon) or rapeseed oil consumption in CF on LC-PUFA supply of infants as well as cognitive and visual development in the second 6 months of life (**Q3/4**). Databases for the 4 research questions (**Q1-4**) were the online complementary food database Nutrichild, the prospective study DONALD (DOrtmund Nutritional and Anthropometric Longitudinally Designed) and the double-blinded randomised controlled intervention trial PINGU (Polyunsaturated fatty acids in child nutrition – A German multimodal optimisation study).

Results

The evaluation of the market offer and the dietary practice in the CF period showed that vegetable variety is low in homemade as well as in commercial complementary meals in Germany (**Q1a/b**). At 12 months of age infants fed with commercial meals got a slightly higher vegetable variety compared to infants fed with homemade meals (**Q1b**). Compared to meat meals, the offer of fish meals is low (**Q1a/b**). This observation was also confirmed in the nationwide consumer survey of PINGU, revealing that only 1 in 4 German infants met the recommendation to eat fish at least once per week and about 1 in 3 infants never ate fish (**Q2**). In contrast, rapeseed oil was used by about 50% of the mothers in Germany for CF. While maternal eating behaviour was the main predictor of infant's fish consumption, infant's rapeseed oil consumption was also influenced by further factors like the social status of the mother. The partially replacement of meat by fish increased the erythrocyte (RBC) DHA levels (**Q3**) and slightly shortened the latencies of flash visual evoked potentials (FVEP) (**Q4**) compared to the control group (corn oil) after 4-6 months of feeding the study food. The replacement of corn oil by rapeseed oil increased the RBC and plasma eicosapentaenoic acid (EPA), but not the DHA levels (**Q3**). Rapeseed oil intake yielded slightly shorter FVEP latencies compared to the control group (**Q4**). Cognitive development was not affected by fish or rapeseed oil consumption.

Conclusions

The results of the present thesis showed a low vegetable variety and fish consumption but frequent rapeseed oil consumption in the CF period in Germany. While the optimisation of LC-PUFA supply via fish seems to be beneficial for the DHA status and potentially the visual development (also via rapeseed oil) in infancy, its long-term impact remains to be evaluated. Distinct and also practical recommendations in combination with an altered offer in commercial complementary meals – predominant in CF in Germany – could be an approach to promote variety and LC-PUFA supply.

ZUSAMMENFASSUNG

Hintergrund und Zielsetzung

Die Beikostphase (BKP) ist die erste Lebensphase, in der Säuglinge mit festen Lebensmitteln, die auch Teil der Erwachsenenernährung sein werden, vertraut gemacht werden. Diese Phase bietet bereits die Möglichkeit Lebensmittelpräferenzen durch eine entsprechende Lebensmittelauswahl zu prägen. Studien haben gezeigt, dass das Angebot einer Lebensmittelvielfalt, insbesondere einer Gemüsevielfalt, in dieser Phase die Lebensmittelakzeptanz im Säuglingsalter und darüber hinaus begünstigt. Hinsichtlich des beispielsweise niedrigen Gemüseverzehrs vom Kleinkind bis ins Erwachsenenalter scheint es sinnvoll früh die Basis für ein gesundes Ernährungsverhalten zu schaffen. Lebensmittelvielfalt bedeutet aber nicht nur die Geschmacksentwicklung, sondern möglicherweise auch, mit der Integration von Lebensmitteln mit spezifischen Nährstoffen, wie z.B. Fisch mit mehrfach ungesättigten Fettsäuren (LC-PUFA), insbesondere Docosahexaensäure (DHA), die funktionelle Entwicklung des Säuglings zu fördern. Da die LC-PUFA Aufnahme mit Einführung der Beikost sinkt, der Bedarf an diesen Fettsäuren jedoch aufgrund der schnellen Entwicklung neuraler Gewebe weiterhin hoch ist, ist es nötig Strategien für eine optimierte LC-PUFA Aufnahme in der BKP zu entwickeln. **Ziel dieser Arbeit** war es, den Status quo der verwendeten Lebensmittel in Beikostprodukten des deutschen Markts (**Q1a**) und in Beikostmahlzeiten, verwendet in der Ernährungspraxis, hinsichtlich Lebensmittelvielfalt, insbesondere Gemüsevielfalt (**Q1b**), zu bewerten, den Status quo des Fisch- und Rapsölverzehrs von deutschen Müttern und Säuglingen und dessen Determinanten (**Q2**) zu evaluieren und zu ermitteln, ob der Verzehr von Rapsöl und Fisch (Lachs) in der BKP Einfluss auf die LC-PUFA Versorgung des Säuglings (**Q3**) und die kognitive und visuelle Entwicklung (**Q4**) in der zweiten Hälfte des ersten Lebensjahres hat. Datengrundlage für die 4 Fragestellungen (**Q1-4**) waren Nutrichild, die prospektive Dortmund Nutritional and Anthropometric Longitudinally Designed Study (DONALD Studie) und die doppelblinde, randomisierte, kontrollierte Interventionsstudie PINGU (Polyunsaturated fatty acids in child nutrition – A German multimodal optimisation study).

Ergebnisse

Die Untersuchung des Marktangebots und der Ernährungspraxis in der BKP zeigte, dass die Gemüsevielfalt in selbsthergestellten und kommerziellen Beikostmahlzeiten in Deutschland niedrig ist (**Q1a/b**). Im Alter von 12 Monaten konnte eine etwas höhere Gemüsevielfalt bei Gabe von kommerziellen im Vergleich zu selbsthergestellten Mahlzeiten festgestellt werden. Im Vergleich zum Angebot an Fleischmahlzeiten ist das Angebot an Fisch niedrig. Diese Beobachtung wurde auch bei der nationalen Verbraucherbefragung der PINGU Studie bestätigt, die zeigte, dass nur 1 von 4 deutschen Säuglingen die Empfehlung mindestens einmal pro Woche Fisch zu essen erfüllte und 1 von 3 Säuglingen nie Fisch gegessen hat (**Q2**). Dagegen verwendeten ca. 50% der Mütter Rapsöl für die Beikost. Während das mütterliche Ernährungsverhalten wichtigster Einflussfaktor auf den Fischkonsum des Kindes war, wurde der Rapsölverzehr durch weitere Faktoren wie beispielsweise der soziale Status der Mutter beeinflusst. Das teilweise Ersetzen von

Fleisch durch Fisch in der Beikost führte zu einer Erhöhung der Erythrozyten (RBC) und Plasma DHA Spiegel (**Q3**) und verkürzte leicht die Latenzen der visuell evozierten Potenziale (FVEP) (**Q4**) im Vergleich zur Kontrollgruppe (Maiskeimöl) nach 4-6 monatigem Füttern der Studiennahrung. Das Ersetzen von Maiskeimöl durch Rapsöl erhöhte die RBC und Plasma Eicosapentaensäure (EPA), aber nicht die DHA Spiegel (**Q3**). Der Verzehr von Rapsöl führte zu einer leichten Verkürzung der FVEP Latenzen im Vergleich zur Kontrollgruppe (**Q4**). Es konnten keine Effekte auf die kognitive Entwicklung nachgewiesen werden.

Schlussfolgerung

Die Ergebnisse dieser Arbeit zeigen, dass die Gemüsevielfalt und der Fischkonsum in der BKP in Deutschland niedrig, der Rapsölkonsum üblich ist. Während die Optimierung der LC-PUFA Zufuhr mittels Fisch den DHA Status und möglicherweise auch die visuelle Entwicklung (auch mittels Rapsöl) in der BKP positiv zu beeinflussen scheint, muss der langfristige Einfluss noch untersucht werden. Klare und praktische Empfehlungen in Verbindung mit einem veränderten Angebot kommerzieller Beikostprodukte – vorherrschend in der BKF in Deutschland – könnten ein Ansatz sein die Lebensmittelvielfalt und LC-PUFA Versorgung zu verbessern.

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ABBREVIATIONS

AI	Acceptable intake
ALA	Alpha linolenic acid
ARA	Arachidonic acid
BSID	Bayley Scales of Infant Development
CCM	Commercial complementary meal
CF	Complementary feeding
CG	Control group
CHD	Coronary heart disease
DINO	Dortmund Intervention Trial for Optimization of Infant Nutrition
DGE	German Society of Nutrition
DHA	Docosahexaenoic acid
DONALD study	DOrtmund Nutritional and Anthropometric Longitudinally Designed study
DPA	Docosapentaenoic acid
EFSA	European Food Safety Authority
EPA	Eicosapentaenoic acid
FA	Fatty acid(s)
FADS	Fatty acid desaturase
FKE	Research Institute of Child Nutrition
FVEP	Flash visual evoked potential(s)
GP	Glycerophospholipids
HCM	Homemade complementary meal
IG-F	Intervention group fish
IG-R	Intervention group rapeseed oil
LA	Linolenic acid
LC-PUFA	Long chain polyunsaturated fatty acid
MeHg	Methyl mercury
MDI	Mental Developmental Index
n-6	Omega 6
n-3	Omega 3
PDI	Psychomotor Developmental Index
PINGU	Polyunsaturated fatty acids in child nutrition – A German multimodal optimisation study
PUFA	Polyunsaturated fatty acid(s)

RBC	Red blood cell(s)
SES	Socioeconomic status
VegVS	Vegetable variety score
VEP	Visual evoked potentials
%E	Percentage of energy

1 Background and introduction

In early life the child passes tremendous steps of development that are influenced by several factors. Nutrition is one of the main factors to support the rapid growth and an optimal development (Black et al. 2013). The appropriate early life nutrition might not only have an impact on health outcomes, but also on the development of dietary behaviour later in life. Therefore the time frame which already starts at the prenatal period, proceeding with the exclusive milk feeding, the complementary feeding (CF) period and finally the introduction of family food is suggested to provide a unique opportunity to already imprint food preferences influencing later dietary behaviour (Devine et al. 1998; Skinner et al. 2002b; Hetherington et al. 2011; Schwartz et al. 2011; Wild de et al. 2013). While research on the development of food preferences has mainly focused on the prenatal and the exclusive milk feeding period so far, the CF period has been less examined. However, the CF period as the first period of life where the infant gets familiar with new food textures and solid foods that will be also part of the adult diet features the opportunity to offer an appropriate variety of foods already right from the beginning. Food variety does not only mean supporting the infant's taste development but at the same time, with the integration of foods with specific nutrients, like fish with long-chain polyunsaturated fatty acids (LC-PUFA), in particular docosahexaenoic acid (DHA), supporting infant's functional development (Agostoni 2008; Koletzko et al. 2008). Thus, food choice during the CF period could also have subtle favourable effects on infant's development with long-term impact.

The following chapters deal with the importance of food variety in the CF period and (LC-) PUFA intake during CF.

1.1 Food variety in the CF period

Almost all national and global food-based dietary guidelines recommend eating a variety of foods (Clausen et al. 2005), i.e. the integration of a daily variety of different food items or food groups (e.g. vegetables) in the common diet (Clausen et al. 2005). Varied food choices are the key for meeting the macro- and micronutrient needs (Nicklaus 2009) and are an indicator for (better) dietary quality (Krebs-Smith et al. 1987). In addition to the nutritional aspect also the pleasure of eating is linked to food variety (Rolls et al. 2000). Food variety within and in-between meals leads to flavour differences provided by the same or different food groups in the diet (Nicklaus 2009) and supports alternation.

1.1.1 The continuum of opportunities for early exposure to food variety

Liking the taste of foods is one important factor for children to eat and accept them (Domel et al. 1996; Rasmussen et al. 2006; Krolner et al. 2011). The more often a food is consumed, it tends to be liked more (Scaglioni et al. 2008) and thus be accepted easier. Considering this so called mere exposure effect (Scaglioni et al. 2008), it seems obvious therefore to get children in touch with a variety of healthy foods such as vegetables and fruits in order to get familiar to the taste of these foods as early as possible. Figure 1 summarises the early sources of different flavours contributing to the experience of food variety in the pre- and postnatal period / in infancy.

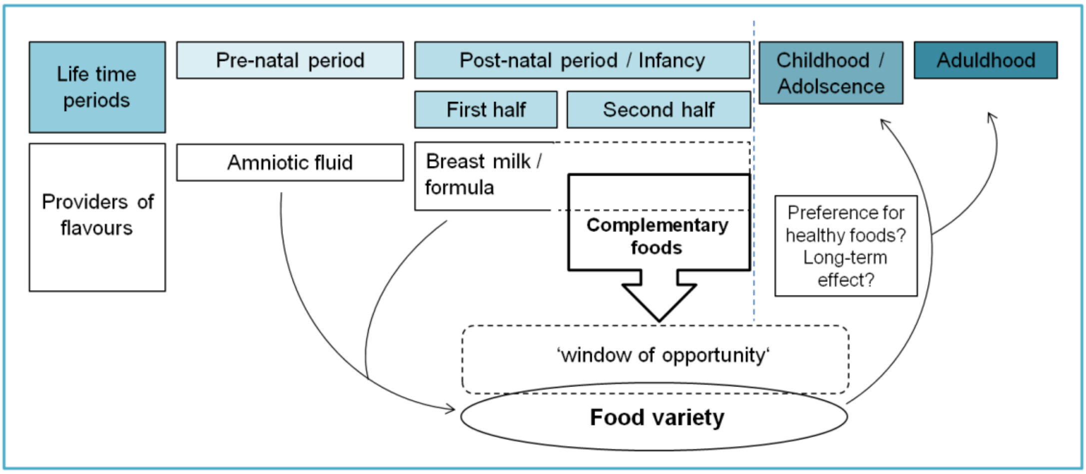

Fig. 1: Sources of food flavours
during the pre- and postnatal period / in infant's diet and the significance of a varied food choice in CF

The child's first available source of flavour variety is the amniotic fluid in the prenatal period (Mennella et al. 1995; Cook, Fildes 2011). The foetus swallows the amniotic fluid and therefore is suggested to receive the flavours of the foods consumed by the mother (Cooke, Fildes 2011). For instance, flavours like garlic (Hepper 1995; Mennella, Beauchamp 1993) and anise (Schaal et al. 2000) were detectable in the amniotic fluid.

Studies have shown evidence for an intrauterine flavour imprinting that might influence not only flavour experiences, food acceptance and preferences directly after birth but also later in life (Beauchamp, Mennella 2009; Beauchamp, Mennella 2011; Lipchock et al. 2011; Cooke, Fildes 2011). One study showed e.g. that infants liked more the cereals flavoured with carrot (less negative facial responses; p= 0.01) when their mothers drank carrot juice during the last trimester of pregnancy than those whose mothers didn't drink carrot juice or eat carrots (Mennella et al. 2001).

During the postnatal period and early infancy, the preferable food choice for an infant is breast milk (Kramer, Kakuma 2004). Within this period, flavour learning continues as flavours like garlic, vanilla as well as carrot have been shown to be transmitted through breast milk (Cooke, Fildes 2011). Hence, breast milk may vary from day to day depending on the mother's diet (Hausner et al. 2010). It is discussed that the early experience of varied flavours via breast milk promotes the infant's acceptance of complementary foods and is also associated with a reduced appearance of pickiness and a greater willingness to try new foods in childhood (Beauchamp, Mennella 2011; Mennella, Trabulsi 2012).
In contrast to breast-fed children, formula-fed children do not experience a great variety of flavours during the exclusive milk-feeding period because of the uniform flavour of formula. However, the taste of the different types of formula (milk-based, protein-hydrolysate) might also influence food acceptance and food preferences later in life (Hausner et al. 2009; Beauchamp, Mennella 2011). Mennella et al. found that infants who were fed extensively hydrolysed protein formula (ePHF), described to be bitter, sour and savoury, accepted more the savoury compared to the plain broth ($p < 0.01$) after 3 and 8 months of consumption (Mennella et al. 2011). Four to five years old children fed PHFs in infancy preferred more sour-flavoured juices than children fed milk-based formula ($p = 0.01$) (Mennella, Beauchamp 2002).

There is not only evidence for the imprinting of food preferences during the milk feeding period resulting from a varied diet of the mother (Cooke, Fildes 2011), but also during the period of CF. This period is suggested to be a 'window of opportunity' (HABEAT) where the taste development of a child may be advanced with the offer of varied solid foods. Complementary food is generally recommended to be introduced between the age of 17 and 26 weeks of life (Agostoni, Decsi et al. 2008; Koletzko et al. 2010) starting in Germany with a vegetable-potato-meat meal (fig. 2), which offers a high potential of flavour variety within and in-between this meal type.

The (repeated) exposure of a variety of foods during this period seems to increase the acceptance of novel (Maier et al. 2008; Coulthard et al. 2010) or disliked (Wardle et al. 2003; Forestell, Mennella 2007) foods and might contribute to a long-term preference effect (Nicklaus 2009; Schwartz et al. 2011; Caton et al. 2011; Mennella, Trabulsi 2012).

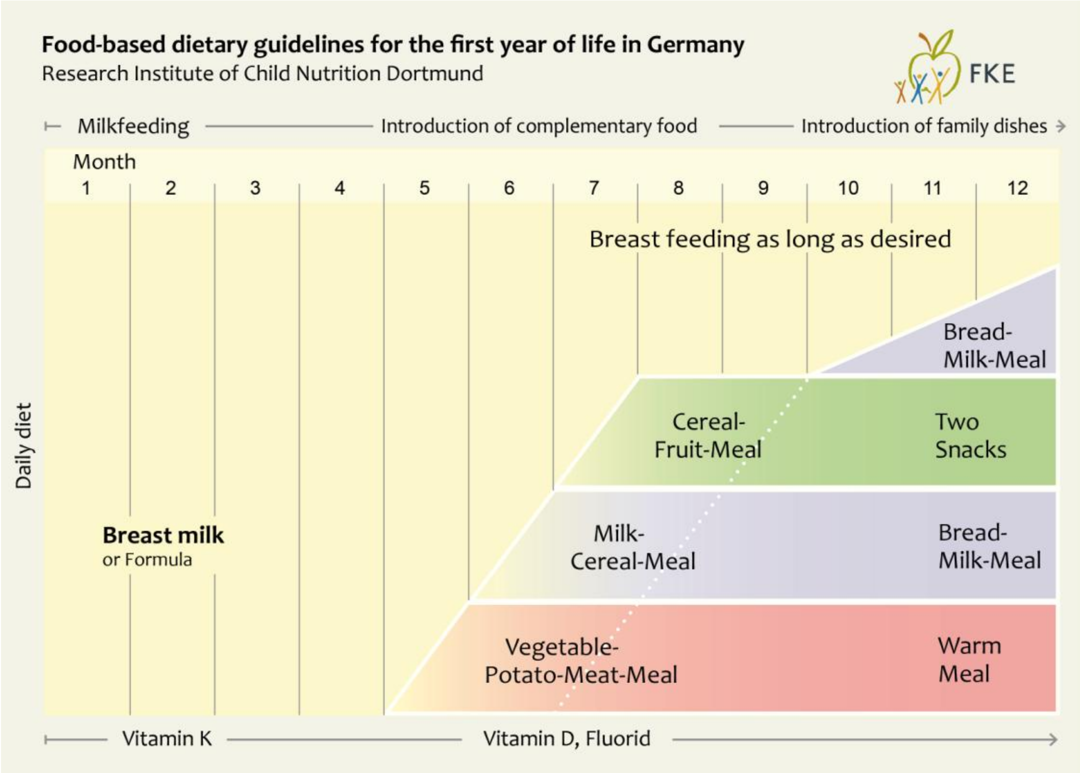

Fig. 2: Schedule of the food-based dietary guidelines for the first year of life in Germany
(Forschungsinstitut für Kinderernährung 2013)

1.1.2 Assessing and evaluating food variety

Assessing and evaluating dietary variety requires taking dietary guidelines as basis and scoring the presence of specific foods or food groups that are recommended (Cox et al. 1997) with more distinct results when including the portion size (Daniels et al. 2009). A well-known index which also considers food variety is the Healthy Eating Index (HEI) to assess the dietary quality of American adults and children based on the US dietary guidelines (Kennedy 2004). Children’s dietary variety was assessed in several studies by using dietary diversity or food variety scores (Cox et al. 1997; Arimond, Ruel 2004; Steyn et al. 2006; Daniels et al. 2009; Scott et al. 2012). In contrast, up to now only a few studies investigated infants’ dietary variety in CF by using a score (Dewey et al. 2006; Foterek et al. 2015). Other studies like a US study which examined the food variety in commercial vs. non-commercial baby foods, assessed the variety by counting the number of different types of fruits and vegetables consumed in the previous 24 hours (Hurley, Black 2010). Ahern et al. assessed the vegetable variety in infants in three European countries with a specific ‘vegetable questionnaire’ considering the frequency and liking of vegetables (Ahern et al. 2013).

1.1.3 Recommendations on food variety in CF and current dietary behaviour

In Germany, a great variety of foods in CF, in particular with regard to vegetables or fish, was discouraged over many years for reasons of caution toward intolerances or allergy prevention. However, as there appeared the first scientific hints that supported the advantages of a great variety of foods, German guidelines changed in 2009 (Forschungsinstitut für Kinderernährung 2009). Now, the use of a wide range of different vegetables and the partly replacement of meat by fish in CF is recommended (compare 1.2.3) (Bührer et al. 2014; Hilbig et al. 2012). Latest findings of a European multicentre study for identifying strategies to promote healthy eating habits in infants also support the offer of a great vegetable variety in CF (HABEAT). Two studies found that in contrast to expose infants only to a single vegetable, infants eat more of a new vegetable if they got offered a range of vegetables before (Gerrish, Mennella 2001; Maier et al. 2008). In addition, it is supposed that also the frequency of exposing vegetables is crucial in determining later food preference (Coulthard et al. 2010) and new vegetable acceptance (Lange et al. 2013). Regarding the favourable age of vegetable introduction in CF, results have not been distinctive yet. A cohort study conducted in 4 European countries showed no significant associations between the age of vegetable introduction in the CF period (i.e. early vs. late introduction) and later vegetable intake (2-4 y old children) (Lauzon-Guillain et al. 2013). In general, promoting vegetable intake in children seems to be particularly challenging (Anderson et al. 1998; Sahyoun et al. 2004; Te Velde et al. 2008) probably caused by their sensory characteristics (e.g. bitter taste) (Drewnowski, Gomez-Carneros 2000; Nicklaus et al. 2005b; Krolner et al. 2011). In addition, they have a relatively low energy content compared to other foods which is suggested to have an impact on acceptance as well (Gibson; Wardle 2003). Studies have shown that green vegetables are more difficult to integrate into infant’s diet than orange vegetables (Mennella et al. 2008; Forestell, Mennella 2007).

Given the fact that vegetables are least preferred by children as compared to other food groups (Skinner et al. 1999; Skinner et al. 2002a; Cooke, Wardle 2005; Nicklaus et al. 2005b) it is not surprising that children and adolescents across

Europe do not eat the recommended amounts of vegetables (Yngve et al. 2005; Diethelm et al. 2012). In Germany, only 6-7% in the 6-11 years and 18-29% in the 12-17 years aged group met the age-specific vegetable recommendation in the period from 2003 to 2006 (Mensink et al. 2007). Similarly, the cross national survey Health Behaviour in School-aged Children, conducted every 4 years in almost 40 countries since 1982 to assess health and behaviour of 11, 13 and 15 years old children, revealed that on average only 32% of the German girls and 19% of the German boys consumed vegetables at least once per day in 2009/2010, also illustrating the gender diversity in vegetable consumption (Currie et al. 2012). German infants and toddlers (10-36 months of age) consumed on average only 70% of the recommended vegetable amounts in 2008 (Hilbig et al. 2011).

Studies showed that not only vegetable consumption, but also vegetable variety is low in infancy. For instance, Maier et al. showed that German mothers introduce only 3 different vegetables and made 3 changes from day-to-day within the first month of vegetable feeding indicating that vegetable variety is low, at least at the beginning of CF (Maier et al. 2007). In contrast, French mothers offered 6 different vegetables and made 18.5 changes from day-to-day within this period (Maier et al. 2007). Further detailed data on vegetable variety or the general food variety in CF in Germany is currently not available. As commercial complementary meals are more frequently offered than homemade complementary meals in Germany (59.3% commercial vs. 21.1% homemade) (Foterek et al. 2014), a low vegetable variety in CF might result from a lower vegetable variety in commercial vs. homemade complementary meals. However, currently neither the variety in commercial nor in homemade complementary meals is known and thus it remains unclear whether there is a difference in food variety in the diet of infants fed with commercial complementary or homemade meals.

1.2 (LC-)PUFA – functions and food sources in CF

1.2.1 Relevance of (LC-)PUFA intake in early life

In the last decades (LC-)PUFA achieved increasing scientific attention as they have a major impact on human health (Lattka et al. 2010) and have been associated with early visual, cognitive and motor development as well as several diseases like the metabolic syndrome, cardiovascular or atopic diseases (Lattka et al. 2010). The n-6 LC-PUFA arachidonic acid (ARA; 20:4n-6) and the n-3 LC-PUFA docosahexaenoic acid (DHA; 22:6n-3) are assumed to have a key role in those aspects (Lattka et al.

2010). The main functional tasks of LC-PUFA are (Kinsella et al. 1990; Grossfield et al. 2006; Chalon 2006; Innis 2007; Lattka et al. 2010):

- Part of cell membranes (membrane phospholipids), especially of cells of brain, neural system, retina: contribution to membrane integrity and fluidity, important for protection from oxidative stress, support of functions of e.g. membrane-bound receptors or transporters
- Involvement in signal transduction (second messenger) and neurotransmission
- Precursors for eicosanoids as mediators for e.g. vasoconstriction and -dilatation, blood pressure regulation, gene expression, inflammatory processes

During pregnancy and the first year of life an adequate supply with (LC-)PUFA is of special importance according to the potential association with cognitive and visual development. During the third trimester of pregnancy, the postnatal period and infancy the brain 'growth spurt' results in a 10-fold increase of the brain weight (Dobbing, Sands 1973). In these periods ARA and DHA accumulate rapidly in the brain (Clandinin et al. 1980; Martinez 1992; Lauritzen et al. 2001; Wainwright 2002) due to their high proportion of lipids in the grey matter of the brain (Wainwright 2002). 60% of the dry brain matter consists of phospholipids (Kurlak, Stephenson 1999) with the highest content of DHA in ethanolamine and serine glycerophospholipids (Crawford et al. 1999). In addition, DHA is highly concentrated in vesicles of the synapses (Breckenridge et al. 1973) and may also regulate myelination (Martinez, Mougan 1998). DHA does not only considerably accumulate in the brain, but also in the retina during growth spurt (Martinez, Mougan 1998). In the retina, 50% of the fatty acids (FA) of rod and cone outer segments consist of DHA (Stillwell, Wassall 2003).

1.2.2 Endogenous synthesis of LC-PUFA

Saturated and monounsaturated FA are, in contrast to n-3 and n-6 PUFA, synthesised by humans (Calder et al. 2010). Therefore, the n-6 FA LA and the n-3 FA ALA have to be provided alimentary. LA and ALA can endogenously be converted by delta-6 desaturation, elongation and delta-5 desaturation to ARA and eicosapentaenoic acid (EPA) respectively (Innis, Uauy 2003) that are initial substrates for the eicosanoid synthesis (fig. 3). The delta-5 and delta-6 desaturase are encoded by the genes FADS 1 and FADS 2, respectively (Nakamura, Nara

2004). After two further elongation steps, delta-6 desaturation and β-oxidation lead to DPA and DHA. All of these steps occur in the endoplasmatic reticulum (ER), except the final step, the β-oxidation, occurring in the peroxisome (Arterburn et al. 2006).

As the n-6 and n-3 FA share the same elongation and desaturation enzymes, the substrates compete for the use of them (Calder et al. 2010). In particular, the delta-6 desaturase is supposed to take a key role in the pathway as it is used twice in the conversion of ARA to docosapentaenoic acid (DPA) and ALA to DHA respectively (Gibson et al. 2011). If LA and ALA are present at high levels in the diet at the same time, the intermediate substrate 24:5n-3 has less or no access to the delta-6 desaturase, necessary for conversion and shared with the initial PUFA (Gibson et al. 2011). This may lead to lower or no DHA synthesis. Conversely, a diet low in both PUFA may increase DHA levels given that ALA is available in sufficient amounts (Gibson et al. 2011). Taken together, a high ratio of LA to ALA together with a high total PUFA content inhibits the elongation and desaturation and leads to lower LC-PUFA supply (Calder et al. 2010; Gibson et al. 2011).

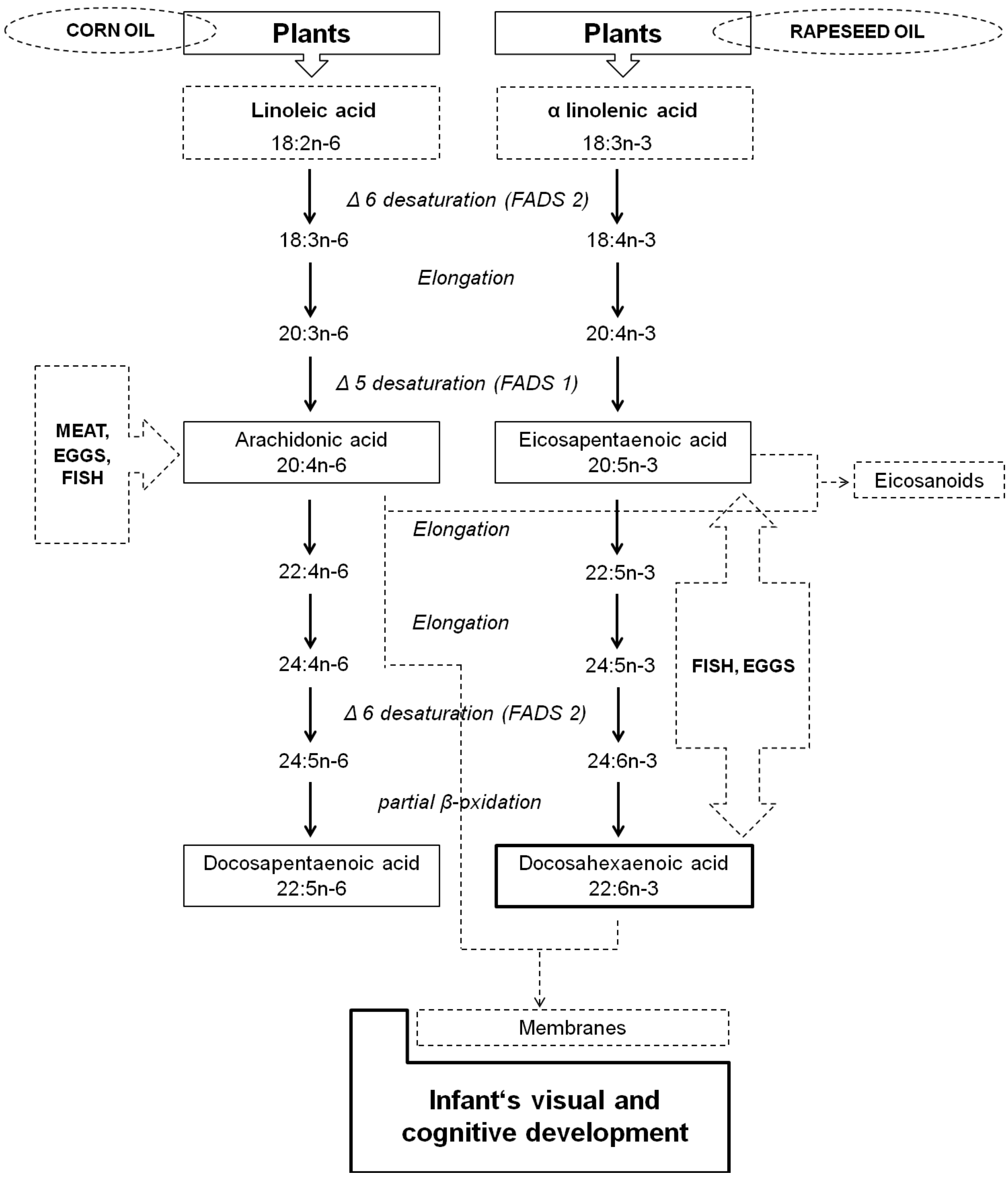

Fig. 3: Scheme of the metabolic pathway of n-6 and n-3 long-chain PUFA in human including their food sources (with examples), most important tasks and impacts

Preterm (Calder et al. 2010), as well as term infants (Demmelmair et al. 1995; Salem et al. 1996; Sauerwald et al. 1996; Sauerwald et al. 1997; Szitanyi et al. 1999) are able to convert PUFA into LC-PUFA from their precursors. However, the conversion, in particular of ALA, is quite limited (Hanebutt et al. 2008) and influenced by genetic single-nucleotide polymorphisms in FADS1 and 2 (Lattka et al. 2010), gender and the amount of the precursors available from the diet (Hoffman et al. 2000). As the DHA content in the brain increases simultaneously to the brain

weight (Lauritzen et al. 2001) and is relatively highest during foetal development and early infancy (Innis 2007), deficiency of DHA may have short- as well as long-term consequences for later brain function (Wainwright 2002; Muskiet et al. 2006; Innis 2007). In addition, impaired vision (Muskiet et al. 2006) may result from low DHA intake. Thus, DHA is considered to be at least ‘conditionally essential’ in early development (Muskiet et al. 2006; Calder et al. 2010).
In addition to the brain and retina, DHA accumulates also in foetal liver and adipose tissue during pregnancy (Lauritzen et al. 2001). Therefore, it is supposed that these tissues act as DHA-storages for the rapid brain and retina development in the postnatal period and infancy (Lauritzen et al. 2001) depleting during these periods (Soderberg et al. 1991).

The term ‘bioavailability’ is more and more used in nutritional science (Ghasemifard et al. 2014) although bioavailability is derived from pharmacology as it defines ‘the rate and extent to which a drug reaches the systematic circulation’ (Ghasemifard et al. 2014). Bioavailability of n-3 LC-PUFA might depend on whether they are ingested as ethyl esters, phospholipids or triacylglycerols, with the latter as the most abundant component in the human diet (Flachs et al. 2009). These are well absorbed by the intestine (> 95%) (Flachs et al. 2009). In addition, the n-3 LC-PUFA phospholipid forms enhance LC-PUFA levels in brain and other organs (Wijendran et al. 2002). Incorporation of dietary LC-PUFA in total plasma lipids lasts around 1 month, whereas slower kinetics were measured for red blood cells (RBC) (Kopecky et al. 2009).

1.2.3 Recommendations on (LC)-PUFA intake and food sources in the CF period

The German Society of Nutrition (DGE) recommends the proportion of LA and ALA of total energy intake without specifying ARA and DHA supply in infancy (tab. 1) (DGE, ÖGE, SGE 2015). In contrast, the European Food Safety Authority (EFSA) recommends exact amounts of an adequate DHA supply for the first 12 months and for ARA for the first 6 months, based on the varied composition of breast-milk in different countries and the effects of DHA-enriched formula or complementary foods on FA status (EFSA 2013). A recalculation of an adequate ARA:DHA ratio is only possible for the first 6 months of life and yields a ratio of 1.4:1 (EFSA) which is nearly in line with recommendations of experts who advise that ARA and DHA should be at equal levels in the diet of infants (Koletzko et al. 2008).

Tab. 1: Recommendations for FA-supply in the first year of life
made by the German Nutrition Society (DGE) and the European Food Safety Authority (EFSA)

	DGE		EFSA	
	0 - < 6 months	**6 - < 12 months**	**0 - < 6 months**	**6 - < 12 months**
Total fat (E%)	45-50	35-45	50-55	40
LA (E%)	4	3.5	4	4
ALA (E%)	0.5	0.5	0.5	0.5
LA:ALA*	8:1	7:1	8:1	8:1
ARA (mg/d)	n.s.	n.s.	140	n.s.
DHA (mg/d)	n.s.	n.s.	100	100
ARA:DHA*	n.s.	n.s.	1.4:1	n.s.

*recalculated; n.s.: not specified, ALA: alpha-linolenic acid, LA: linolenic acid, ARA: arachidonic acid, DHA: docosahexaenoic acid
[modified according to DGE, ÖGE, SGE 2015; EFSA 2013]

Human milk and/or LC-PUFA enriched formula are the only sources of LC-PUFA during the first 4-6 months of life. The DHA content of human milk varies not only among, but also within populations, depending on dietary habits, with values between < 0.1% and > 1.0% of total FA (Innis 2008). The higher amounts might result from higher fish and seafood intake in populations like Asians (Innis 2008). With the stepwise introduction of complementary food the total fat and LC-PUFA intake decreases (Niinikoski et al. 2007; Koletzko et al. 2008; Schwartz et al. 2010). Between 6 and 12 months, studies have reported an intake of 28-47 mg DHA/ d, compared to 57 mg DHA/ d below the age of six months (Lagstrom et al. 1997; Boer de, Hulshof ter Doest 2006; Fantino, Gourmet 2008; Schwartz et al. 2010). The intake of LA and ALA also decreases, although more slightly than the DHA intake (Schwartz et al. 2010). At the end of the first year of life, human milk and/or formula are still the predominant sources of ALA and LC-PUFA in German infants, whereas for other nutrients, including LA, nearly 50% are obtained by complementary food (Schwartz et al. 2010). Thus, a prudent selection of foods in CF to enhance dietary (LC-)PUFA intake should be pursued.

A simple and feasible approach to provide PUFA in CF, especially LA and ALA, is the use of vegetable oils with a favourable LA:ALA ratio, added e.g. to the vegetable-potato-meat meal. Table 2 shows that rapeseed oil provides a favourable balance of LA:ALA (2:1). Other oils, like linseed or walnut oil, also provide high amounts of ALA, but with a less favourable ratio of PUFA as rapeseed oil. In addition, they are not as cheap as rapeseed oil nowadays and have a dominant taste which might disguise the taste of other components in the meal, like vegetables. Some fruits and vegetables provide slight amounts of ALA, like strawberries (105 mg/100g) or kale (355 mg/100g) but due to the low amount may not substantially contribute to a well-balanced PUFA supply in CF.

Tab. 2: LA and ALA contents and ratios of vegetable oils
relevant for PUFA supply in CF in Germany [BLS]

	LA	ALA	LA : ALA*	n-6 : n-3*
	g/100g			
Corn oil	55.53	0.96	58:1	58:1
Linseed oil	14.30	52.80	1:4	1:4
Olive oil	8.29	0.86	10:1	10:1
Rapeseed oil	14.95	8.58	2:1	2:1
Sunflower oil	50.18	0.18	279:1	282:1
Walnut oil	52.40	12.20	4:1	4:1

*recalculated
LA: linolenic aid, ALA: alpha-linolenic acid

Meat (ARA), fish (DHA+EPA) and egg yolk (DHA+EPA+ARA) are commonly available sources of LC-PUFA. In contrast to meat and fish, egg yolk is not common or explicitly recommended at present in CF in Germany, i.a. due to hygienic aspects during homemade preparation of complementary meals. Table 3 shows fish types with their FA patterns possibly considered for the supply of preformed LC-PUFA in CF in Germany. The FA pattern varies considerably between the fish species with DHA values from e.g. < 0.5 g/100g in pollock to almost 1.5 g/100g in salmon.

The use of rapeseed oil and later on fish are recommended in CF in Germany since a few years (Forschungsinstitut für Kinderernährung 2009). For the standard recipe of vegetable-potato-meat/fish meals, the addition of 8-10g rapeseed oil per meal (190g-250g) is recommended (Forschungsinstitut für Kinderernährung 2013). A vegetable-potato-fish meal is recommended once or twice a week including 20-30g of fatty fish (Hilbig et al. 2012; Forschungsinstitut für Kinderernährung 2013; Bührer et al. 2014). Salmon is especially recommended due to the high content of DHA (tab. 3).

Currently, little is known about the actual use of fish or rapeseed oil in CF in Germany. The representative VELS study (Verzehrsstudie zur Ermittlung der Lebensmittelaufnahme von Säuglingen und Kleinkindern), conducted in 2001/02, showed that at most 10% of the 6-12 months aged infants receive fish (Kersting, Clausen 2003).

Tab. 3: Fatty acid pattern of fish species
relevant for the supply of preformed LC-PUFA in CF in Germany (BLS)

	Total fat	**ARA**	**EPA**	**DHA**
			(g/100g)	
Cod	0.80	0.02	0.08	0.21
Herring	10.39	0.06	0.69	1.09
Mackerel	13.43	0.19	0.70	1.24
Pollock	2.75	0.03	0.31	0.46
Salmon	12.20	0.06	0.92	1.47
Trout	3.19	0.03	0.16	0.58

ARA: arachidonic acid, EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid

The low fish consumption tracks into toddlerhood as mean fish consumption was still far below the recommended amounts in the Optimized Mixed Diet (1-3 years old children: < 60% of the recommended amount) in the nationwide GRETA Study in 2011 (Hilbig et al. 2011). In contrast to fish consumption, there is no representative data on rapeseed oil usage in infant nutrition in Germany. The DOrtmund Nutritional and Anthropometric Longitudinally Designed (DONALD) study, an ongoing, open-cohort study, yielded that the use of rapeseed oil compared to corn oil in homemade vegetable-potato-meat-meals increased since the 1990ies (infants: 9-12 months of age; from 1999 to 2006, proportion of rapeseed oil of total dietary fat increased to ~ 40% vs. corn oil, with a constant proportion of ~ 15% of total dietary fat) (Grube 2009) and indicates that rapeseed oil has become common in homemade CF in Germany. Currently there is also no quantitative data available on the use of rapeseed oil in commercial complementary meals in Germany. Commercial vegetable-potato-meat-meals are considerably more often used than homemade meals in the diet of 6-12 month old infants (60% of the infants) (Foterek 2013, not published).

1.2.4 Impact of (LC-)PUFA supply via complementary food on biomarker of fatty acid intake and infant's development

In contrast to the prenatal and exclusive milk feeding period, only a few studies examined (an optimised) (LC-)PUFA supply during the CF period, but not on a food-based approach. The Dortmund Intervention Trial for Optimization of Infant Nutrition (DINO) was the first study which investigated the effects of rapeseed oil vs. corn oil in commercial complementary food on PUFA and LC-PUFA plasma status in 4-10 months old infants. In the rapeseed oil group, significantly higher concentrations in plasma DHA were found, compared to the control group receiving corn oil (1.9% vs. 1.7% of total FA at 10 months of age, $p = 0.02$) (Schwartz et al. 2009). DHA-enriched egg-yolk consumption led to higher RBC DHA concentrations in comparison to the control group (no additional DHA supply) in infants during the second 6 months of life (5.5% vs. 3.0% of total FA at 12 months of age, $p < 0.002$, Hoffman et al. 2004). In breastfed infants the inclusion of 4 n-3 enriched eggs/wk in CF for 6 months yielded DHA concentrations of 6.7% vs. 4.8% (control group) of RBC total FA at 12 months of age, in formula fed infants 4.1% vs. 3.0% (control group) of total RBC FA ($p < 0.05$, Makrides et al. 2002).

Up to now, functional effects of LC-PUFA supply on infant's development in the CF period were only evaluated in one study. After consuming DHA-enriched egg yolk with complementary food for 6 months an improved visual acuity was observed in infants 9 and 12 months of age (0.14 vs. 0.16 logMAR, $p = 0.0002$) (Hoffman et al. 2004). Infants with higher levels of RBC DHA had better visual acuity (r -0.50; $p = 0.0002$). However, no study examined the effects of a solely food-based LC-PUFA supply (without enrichment) on functional development in infancy (and beyond).

2 Objectives and research questions

The 4 research questions (Q1 - Q4) of the present thesis are deduced from the two main research topics (T):

- T1: the food variety in CF
- T2: n-3 (LC-)PUFA-rich foods in CF

and the 3 key aims of the thesis (A) (fig. 4):

- A1: Evaluating the status quo of the used foods in CF in Germany regarding food variety, with a main focus on vegetables (related to T1)
- A2: Evaluating the status quo of fish and rapeseed oil consumption and its determinants in German infants and their mothers (related to T2)
- A3: Evaluating whether rapeseed oil or fish might be able to optimise LC-PUFA supply and positively affect infant's development in the second 6 months of life (related to T2)

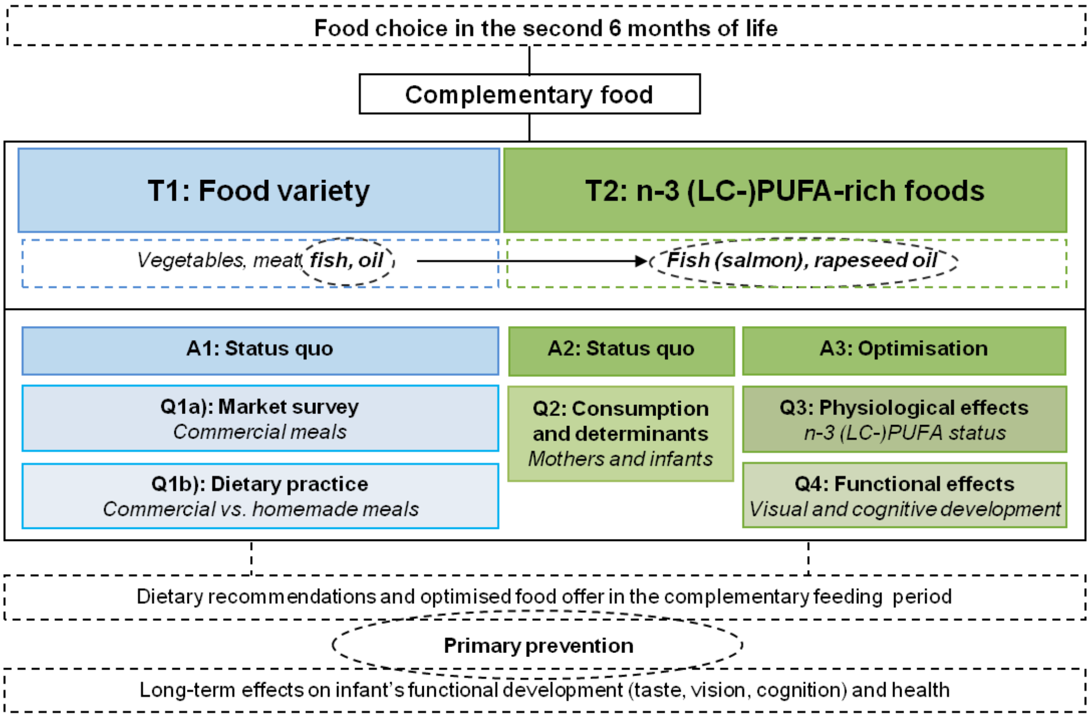

Fig. 4: Research topics (T), aims (A) and questions (Q)
embedded into the general research context

Research topic 1 (T1): Food variety in CF

Background: In Germany, the use of commercial complementary food is more common than homemade complementary food. Little is known about food, especially vegetable variety in commercial complementary meals and if it differs from homemade complementary meals.

Question Q1: Market survey (a) and dietary practice (b)

- a) How frequently are specific food groups used in the commercial vegetable-potato-meat meal on the German baby food market in 2012?
- b) Is there a difference in vegetable variety between commercial and homemade vegetable-potato-meat meals?

Research topic 2 (T2): n-3 (LC-)PUFA-rich foods in CF

Background: With the introduction of complementary food, LC-PUFA supply usually decreases in the second 6 months of life. However, the physiological need for LC-PUFA is still high for infant's rapid brain and visual development. The addition of rapeseed oil, with a favourable ratio of n-6 LA:n-3 ALA PUFA (2:1) to enforce the endogenous synthesis of DHA (Schwartz et al. 2009; Gibson et al. 2011), and oily fish, like salmon, containing preformed DHA, might be feasible approaches to contribute to an adequate LC-PUFA supply.

Data on current consumption of rapeseed oil and fish in CF in Germany and its determinants is scarce. In addition, no data is available on functional effects of an optimised n-3 (LC-)PUFA supply via 'natural' food sources in CF on infant's development in the second 6 months of life.

Question Q2: Consumption of fish and rapeseed oil and its determinants examined in mothers and infants

- How is the supply with fish and rapeseed oil in German mothers and infants?
- Which determinants predict infant's and mother's fish and rapeseed oil consumption?

Question Q3: Physiological effects of an optimised n-3 (LC-)PUFA supply in infants

- Does the replacement of corn oil by rapeseed oil in vegetable-potato-meat meals significantly enhance the RBC (and plasma) DHA status of infants?
- Does the partial replacement of meat by fatty fish (i.e. salmon) in vegetable-potato-meat meals significantly enhance the RBC (and plasma) DHA status of infants?

Question Q4: Functional effects of an optimised n-3 (LC-)PUFA supply in infants

- Is the replacement of corn oil by rapeseed oil in vegetable-potato-meat meals beneficial for cognitive and visual development of infants?
- Is the partial replacement of meat by fatty fish (i.e. salmon) in vegetable-potato-meat meals beneficial for cognitive and visual development of infants?

3 Research projects, methods and subjects

The topics and research questions of this thesis were examined with data of different sources and research projects (see also tab. 5):

- **Q1a**: The online data base **'Nutrichild'** served to conduct the market survey
- **Q1b**: The **DONALD study** provided the data to examine dietary practice
- **Q2**: Data for assessing the consumption of fish and rapeseed oil and its determinants resulted from the pre-study, a nationwide online survey, of the intervention trial **PINGU** (Polyunsaturated fatty acids in child nutrition – A German multimodal optimisation study)
- **Q3, Q4**: The core-study of **PINGU** provided data for the physiological and functional assessments

3.1 Food variety: market survey (Q1a) and dietary practice (Q1b)

Definitions: For investigating research question 1a) and b) complementary food was defined as all semi-solid, strained or mashed foods fed with a spoon during the CF period. The meals investigated are summarised under the term 'vegetable-potato-meat meal' in the German dietary schedule for the first year of life (compare fig. 2) which includes: pure-vegetable, blend of meat, vegetable-potato/pasta/rice, potato/pasta/rice-meat and vegetable-potato/pasta/rice/meat/-fish meals. Commercial complementary meals were defined as all industrially processed, pre-packaged complementary meals in jars or pots.

All home-prepared complementary meals were defined as homemade meals if at least two out of the three components, i.e. meat, vegetables or the starchy component were fresh or frozen and unprocessed. In case of homemade pure vegetable meals or meals with only two components (e.g. vegetables and potatoes), both components had to be fresh or frozen and unprocessed.

Nutrichild (Verbraucherfenster Hessen 2013) was used as data basis for examining research question 1a). It is an online data base for complementary food available on the German market that was initialised in the 1990s by the Research Institute of Child Nutrition Dortmund (FKE) and the University of Gießen to support parents and nutrition professionals searching for commercial complementary food and assessing the adequacy of the products. The data base is updated annually by the FKE, based on detailed product information provided directly by food companies to the FKE.

Response rate is almost complete (> 95%). The website presents information derived from product labelling and offers the opportunity to compare all labelled ingredients and nutrients within commercial products. In addition, it enables the identification of products and their ingredients, e.g. meat, vegetables or pasta, by the meal type, product brand and the labelled age of child the product is intended for.

The investigation of food variety in commercial complementary meals was based on the market survey for Nutrichild in 2012 and considered products of 14 food companies (15 brands) offered for infants between 5 and 12 months of age.

For investigating research question 1b) the DONALD study was used as data basis. The **DONALD study** started in 1985 as an ongoing, open cohort study at the FKE in Dortmund, Germany. The aim of the study is to get detailed information on the relationship of nutrition, development, metabolism and hormonal status from infancy to adulthood. The study is observational and non-invasive till the age of 18 when blood samples of the participants are collected. It was approved by the Ethics Committee of the Rheinische Friedrich-Wilhelm-Universität Bonn, Germany.

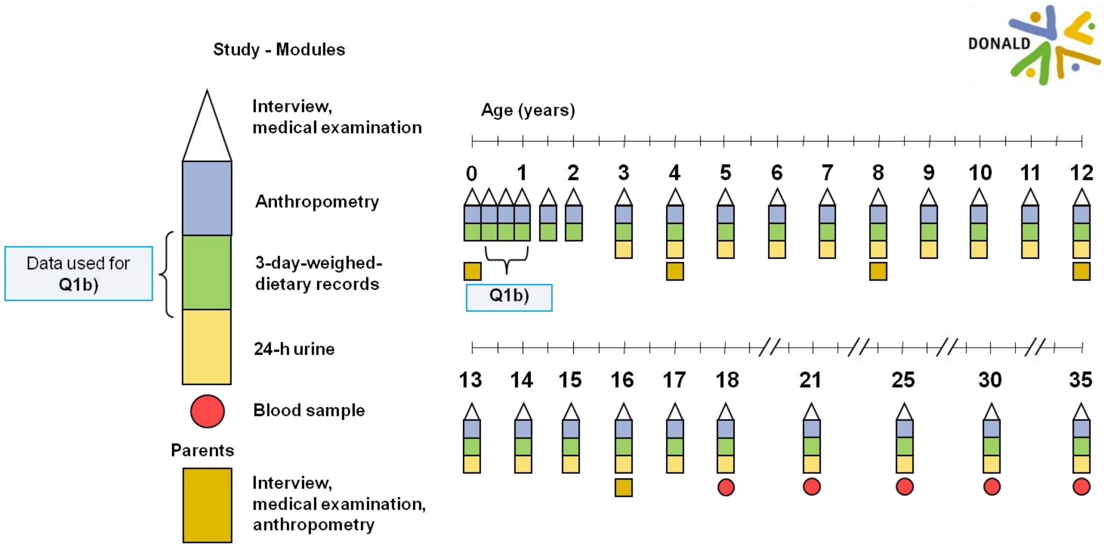

Fig. 5: Schedule of the DONALD study and marked souces of data for this thesis
[modified according to Kroke et al. 2004]

Recruitment of healthy infants takes place in Dortmund and surrounding communities. Every year approximately 40 infants are newly recruited. Parents have to agree participating in a longitudinally study and one of the parents has to be able to speak sufficient German.

After parents' written consent, participants are first examined at the age of three months, followed by regular examinations until adulthood at the DONALD study centre in Dortmund. In the first year examinations take place every three months, twice a year in the second year of life and once a year from the age of two onwards (fig. 5). In addition to anthropometric, medical examinations, interviews on lifestyle and socio-demographic issues, the regular assessment includes a 3-day weighed dietary record. All consumed foods ever recorded are documented in the in-house food and nutrient database LEBTAB, which is continuously updated by new recorded foods. LEBTAB provides information on ingredients, energy and nutrient contents, based on product labels, standard nutrient tables and recipe simulation for composite foods using labelled nutrient contents and ingredients.

For research question 1b) 3-day weighed dietary records were used collected between 2008 and 2012 (n= 477 records) from participants 6, 9 and 12 months of age (n= 222 participants, 48.7% females). For dietary recording parents weighted all foods and beverages before consumption of the infant, as well as any leftovers, to nearly 1 g with the help of a regularly calibrated electronic food scale on 3 consecutive days (mean individual number of 3-day weighed dietary records per subject = 2.1). If recipes for homemade meals were used, all ingredients were recorded separately. Parents chose the first day of dietary recording within 8 weeks after visiting the study centre. Initially they were instructed by a trained dietician. A written example should support the parents in recording. The records were controlled for completeness and plausibility by the dietician when visiting the parents.

For this research question the 3-day weighed dietary records were classified into 2 groups: the commercial meal group and the homemade meal group. In the commercial meal group, pure vegetable meals or meals with only two components (e.g. vegetables and potatoes) consisted exclusively of commercial complementary food components and the 'vegetable-potato-meat meals' contained at least two commercial complementary food components. In the homemade meal group, pure vegetable meals or meals with only two components (e.g. vegetables and potatoes) were exclusively made with complementary components (fresh or frozen and unprocessed) and the 'vegetable-potato-meat meals' contained at least two fresh or frozen and unprocessed ingredients.

A vegetable variety score (VegVS) was calculated as the sum of the individual number of different vegetables consumed with 'vegetable-potato-meat meals' within the 3 recorded days. All vegetables above a cut-off of 10 g (Daniels et al. 2009) per meal were considered for score calculation as some vegetables (e.g. eggplant) were consumed only in very small amounts and might therefore hardly contribute to the taste of a meal.

3.2 n-3 (LC-)PUFA rich foods: consumption, determinants, physiological and functional effects (Q2, Q3, Q4)

The data for investigating research questions Q2-Q4 derived from the randomised controlled trial (RCT) PINGU.

3.2.1 Study design and aims

PINGU is a double-blinded randomised control intervention trial conducted from April 2011 to August 2013 in Dortmund, Germany. It was carried out as a multimodal, interdisciplinary joint research project with partners from nutrition science, paediatrics and psychology and consisted of pre-, core and sub-studies (fig. 6). The study was approved by the Ethics Committee of the Rheinische Friedrich-Wilhelm-Universität Bonn, Germany. All assessments were performed with parents' written informed consent. The study was funded by the Federal Ministry of Education and Research (BMBF, study code 01EA1335).

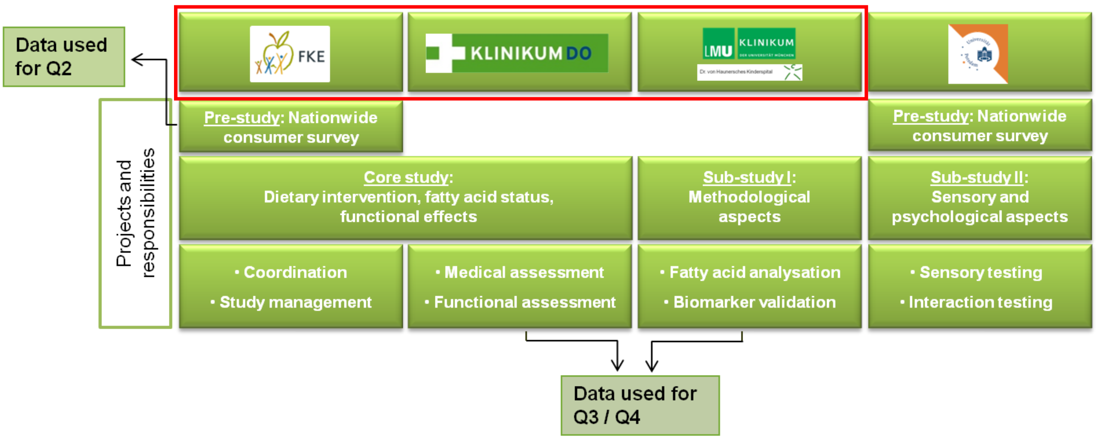

Fig. 6: Joint research structure of collaborating institutes in the PINGU project with marked sources of data for this thesis. Additional involved partners: TU Dortmund, Department of Economics and Social Statistics; Helmholtz Zentrum Munich, Department of Epidemiology – Biological Samples – Genomics; HiPP GmbH & Co. Vertrieb KG; Nestlé PTC Singen; Kantar Health, Munich; Chrestos Concept GmbH & Co. KG; DIfE – German Institute of Human Nutrition, Department of Molecular Genetics; Friedrich Schiller University Jena, Institute of Nutrition, Research Group Bioactive Plant Products.
[Modified according to Mesch et al. 2013]

The aim of the pre-study was to assess the knowledge, habits and attitudes towards n-3 (LC-)PUFA rich foods, in particular rapeseed oil and fish, in young families in

Germany. For the **nationwide consumer survey (Q2)**, conducted in November / December 2010, mothers with children aged 5-36 months were selected from the existing panel data base of the market research company KantarHealth GmbH, Munich, Germany which also conducted the National Nutrition Survey II. Mothers had to answer 48 questions applying the youngest child of the family and their own nutritional behaviour (e.g. frequency of rapeseed oil and fish consumption). Socio-economic and socio-demographic data was also available from the panel.
The survey retrospectively assessed feeding practices during infancy. Mothers were eligible to participate in the online interview, if they had already started with complementary feeding. Of 1,013 mothers, 28 had to be excluded due to implausible or conflicting answers. Finally 985 interviews were analysed.

As described by Stimming, Mesch et al. 2015 outcome variables were defined as follows.

- ‘Rapeseed oil consumption’:
  Infant nutrition: rapeseed oil was used for homemade and/or commercial complementary meals
  Maternal nutrition: rapeseed oil was used for cooking and/or for salads
- ‘High fish consumption’: eating fish at least once per week

Potential determinants of fish and rapeseed oil consumption:

- mother’s age (median cut; ≤ 32 or > 32)
- number of children (firstborn vs. not firstborn)
- residence in the New or the Old Federal German states (former GDR)
- residence in Northern (coastal region: Lower Saxony, Schleswig-Holstein, Mecklenburg Western Pomerania, Hamburg, Bremen) or Southern Germany; size of residence (small: ≤ 2,000 citizens, medium: 2,001-100,000 citizens, high: > 100,000 citizens)
- social class (low (1), medium (2), high (3); calculated by maternal level of school education, maternal level of professional education and household income)
- ‘breastfeeding initiation’ (child had been breastfed to some extent); ‘≥ 4 months exclusive breastfeeding’ (child received only breast milk, no complementary food or formula)
- ‘offers’ of the vegetable-potato-meat meal (compare fig. 2): ‘mainly commercial complementary meals’, ‘mainly homemade complementary meals’, use of ‘both balanced’.

For a rough classification of mothers' levels of knowledge concerning n-3 PUFA a 'knowledge score' was defined. The score based on 5 food and health related questions on n-3 FA. A 'low n-3 knowledge' was defined as: none of the 5 questions correctly answered or negation of the question whether the mother had ever heard the term 'n-3 fatty acids' (question A); a 'medium n-3 knowledge': 1-3 questions were answered correctly (+ question A); a 'high n-3 knowledge': all answers were correct. (Stimming et al. 2015)

The main objectives of the core-study of PINGU were to analyse the effects of two approaches of an improved n-3 (LC-)PUFA supply via complementary food on the **endogenous FA status** (in particular the DHA status) **(Q3)** and the **functional development (Q4)** in the second 6 months of life: using
- a) n-3 ALA-rich rapeseed oil (intervention group IG-R) in complementary food to increase the endogenous DHA synthesis or
- b) preformed DHA via fish in complementary food (intervention group IG-F)
and n-6 LA-rich corn oil instead of rapeseed oil (control group (CG)).

3.2.2 Study sample

A priori power analysis was conducted for the primary outcome 'DHA concentration in erythrocyte phospholipids': assumptions (standard deviation σ= 0.7 (derived from the DINO study), power 1-β= 0.8, significance level α= 0.05, one-sided t-test)) revealed a group size of n= 63 (including 10% drop-outs) to detect a difference of 0.33% DHA concentration between the IG-R and the CG. Assuming a similar effect size in the IG-F (IG-F vs. CG), a total sample of n= 189, also for the secondary outcomes, was aimed.

Women after delivery with their neonates were recruited in maternity hospitals in the region of Dortmund, Germany (April 2011 to October 2012). Inclusion criteria were: healthy term newborn (gestational age ≥ 37 weeks, birth weight ≥ 2500 g); mother German speaking, of full age, intention to breast-feed, willing to use the study food according to the study protocol. Recruitment ended when the sample size was approximately reached (n= 214). The entire study ended after the last participant had finished the study program (August 2013).

Study participation started with the mother's written informed consent that must have been given within eight weeks after birth. Immediately after providing written informed consent the study staff enrolled and randomly assigned mother-child-dyads to one of the 3 study groups (by using computer generated random number lists; (statistical software R Version 2.12.2); provided by an external statistical company (Chrestos Concept GmbH & Co.KG, Ratingen, Germany); using a block wise randomisation (block= 6), stratified by delivery hospitals as recruiting field (n= 9) and sex.

In total, 214 parents provided written informed consent before the first examination (T0). Of the 214 enrolled and randomised participants, 165 completed the study (fig. 7). Complete data of RBC FA status before and after the intervention (T1 and T2) was available from 158 participants and from 155 subjects for plasma FA.
Complete data before and after the intervention (T1 and T2) for investigating visual development was available from 134 participants and for cognitive development from 124 participants.

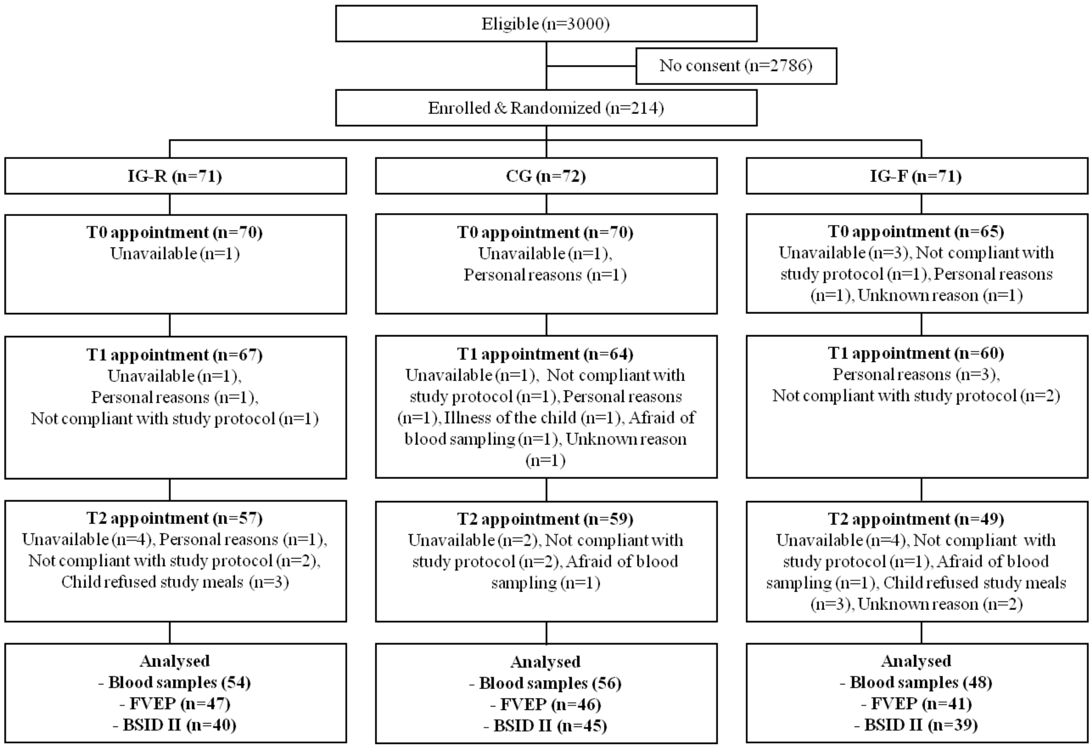

Fig. 7: Flow chart for enrolment and progress through the PINGU study
BSID II: Bayley Scales of Infant Development II; FVEP: flash visual evoked potentials

3.2.3 Study program and dietary intervention

A special consultation had been set up for the PINGU-study in the paediatric clinic Dortmund, Germany. At the infant's 8th week of life the first appointment at the clinic took place. Mother's characteristics and sociodemographic data were recorded (T0). In addition a breast milk and a blood sample of the mother were collected. At the end of the 4^{th} (T1) and 10^{th} (T2) month paediatric examinations, including non-fasting blood sample collection and flash visual evoked potential (FVEP) measurements were conducted at the paediatric and neurological clinic Dortmund, resp., assessments of cognitive development via Bayley Scales of Infant Development II (BSID II) at the FKE, Dortmund, Germany. Figure 8 presents the study program of the infant with the data and parameters recorded at the end of the 4^{th} (T1) and 10^{th} (T2) month. Data used for this thesis are highlighted in red. The maternal study program and the recruitment process are described in Mesch et al. 2013.

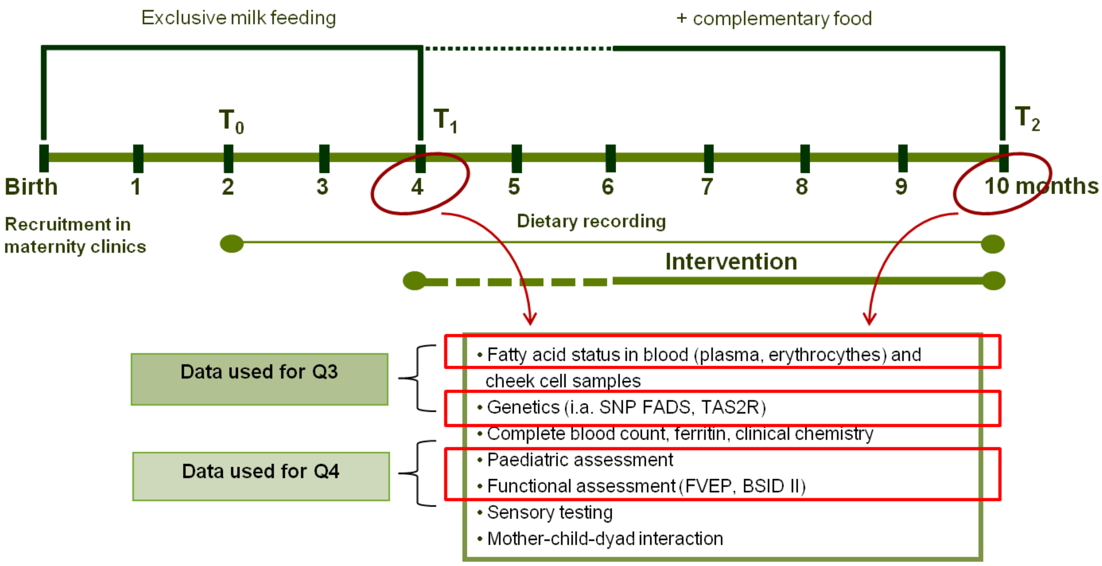

Fig. 8: Study program child (T1; T2)
and marked souces of data for this thesis. BSID II: Bayley Scales of Infant Development II; FVEP: flash visual evoked potentials; FADS: fatty acid desaturase; TAS2R: taste 2 receptor (bitter taste receptor); T0: mother's appointment at hospital; T1, T2: child's appointments at hospital and FKE
[Modified according to Mesch et al. 2013]

As fish can be easily identified by smell, only affiliation in the IG-R and CG was blinded to subjects and investigators.

Dietary intervention was embedded in the German recommendations for infant nutrition (Koletzko et al. 2010; Hilbig et al. 2012) and covered the period of CF, starting at the beginning of the 5^{th} to 7^{th} month and ending at the age of 10 months, resulting in an individual intervention period between 4 and 6 months. Dietary records were used to assess compliance and the exact age at introduction of study food and individual duration of intervention. Intervention focused on the vegetable-potato-meat (-fish) meal which is commonly the first complementary meal introduced in Germany (compare fig. 2). Study foods were commercial meals in jars with vegetables, potatoes, and meat (mean meat content: 8.1 g/100 g) or fish (mean fish content: 13.0 g/100 g) (HiPP GmbH & Co. Vertrieb KG, Pfaffenhofen, Germany) with portion sizes as usual in Germany, i.e. 'baby' jars (190 g) intended for the age of 4-7 months and 'junior' jars (220 g) for the age of 8-12 months. The FA composition of the study food was externally analysed (SGS Institut Fesenius GmbH). In PINGU the IG-F received the same menus as the CG, but two fish menus per week (salmon, rich in DHA) replaced two meat menus. The IG-R and the CG only differed with respect to the sort of the added vegetable oil. Mean rapeseed oil content of the study food in IG-R was 1.4 g per 100 g. All groups received 4 different baby and junior meals respectively to provide sufficient taste variety. Mothers were requested to use the study food at least 5 times per week (IG-F, 2 times fish per week). The study staff delivered the study food and advised the parents generally on infant nutrition (Koletzko et al. 2010; Hilbig et al. 2012).

3.2.4 Blood sample preparation and analysis of fatty acid status in plasma and red blood cells (RBC)

Paediatric examinations included monitoring of the general development of the infant. For assessing the **FA status (Q3)** non-fasting blood samples (plasma, erythrocytes) were collected at T1 and T2. Plasma phospholipids were taken as short-term and sensitive biomarkers for revealing effects of (LC-)PUFA intake on (LC-)PUFA status (Dougherty et al. 1987; Corrocher et al. 1992) and RBC, with a half-life of about 120 days, to reflect the long-term intake (Arab 2003). In both blood compartments glycerophospholipids, specifically the content of phosphatidylethanolamine and phosphatidylcholine was determined (Winkler et al. 2008).

As described by Libuda, Mesch et al. 2016 venous blood samples (2.0 mL) were collected in EDTA coated sampling tubes at T1 and T2, placed on ice and centrifuged within 2 hours after collection. Plasma, RBC and buffy coat were separated by centrifugation (1500 *X* g, 4°C, 10 min). Plasma and buffy coat (for analyses of fatty acid desaturase (FADS) polymorphisms at the Helmholtz centre

Munich) were stored at -80°C. RBC were washed with NaCl (0.9%) 3 times. For RBC haemolysis, 100 µL dest. water was added to 100 µL washed RBC. Subsequently, 260 µl methanol (+ BHT) was added and the samples were vortexed and stored at -80°C.The FA status in RBC and plasma was analysed at the Dr. von Hauner Children's Hospital, Univ. of Munich Medical Centre, Germany.

The FA analysis in plasma glycerophospholipids (GP) was performed as described by Glaser et al. 2010. Briefly, 100 µl of plasma, internal standard (1,2 dipentadecanoyl-sn-glycero-3-phosphocholine) and 0.6 ml methanol were combined in glass tubes and mixed. The precipitated proteins were eliminated by centrifugation, the methanolic supernatant containing mainly polar lipids was transferred into another glass tube, and sodium methoxide was added for base catalysed transesterification. The selective synthesis of methyl esters from GP FAs proceeded at room temperature until the reaction was stopped by adding methanolic HCl. FA methyl esters (FAMEs) were extracted into hexane (containing 0.2 g/l BHT) for gas chromatographic (GC) analysis.

For the analysis of RBC GP FA the method described for plasma GP was adapted as described by Klem et al. 2012, introducing a 5 min ultrasound (40 kHz, 120 W) treatment to ensure dissolution of GP.

FAME of plasma and RBC were quantified by GC (Agilent 7890, Waldbronn, Germany) with a programmable temperature vaporizer (Gerstel, Mühlheim, Germany) and a flame ionisation detector using BPX-70 column (60 m, 0.25 mm ID, SGE, Weiterstadt, Germany). Chromatographic data were evaluated with Chemsation (Rev. B.03, Agilent, Waldbronn, Germany). (Libuda et al. 2016)

3.2.5 FADS genotyping

In addition to the (LC-)PUFA status, also several **single nucleotide polymorphisms (SNPs)** were examined in the blood samples as SNPs in the desaturase encoding genes (FADS 1 and 2) are associated with LC-PUFA levels in plasma and RBC membrane (Malerba et al. 2008; Xie, Innis 2008; Rzehak et al. 2009). Carriers of the minor alleles of FADS SNP show lower LC-PUFA contents in RBC compared to major allele carriers (Schaeffer et al. 2006; Koletzko et al. 2011). The FADS genotype seems to have an impact on DHA status in adults as well as in infants (Harslof et al. 2013). Thus, determination of the FADS genotype is reasonable when interpreting the effects of LC-PUFA supply in infancy. In PINGU, the buffy coat from the collected blood samples was used to determine the genotype.

5 SNPs from the FADS1, FADS2 and FADS3 gene cluster which are known to have a substantial effect on DHA status were considered as potential covariates or effect modifiers in statistical analyses: rs3834458, rs174448, rs174574, rs174575, rs174548 (Koletzko et al. 2011; Steer et al. 2012; Harslof et al. 2013) . For statistical analyses each SNP was categorised in 3 classes: homozygous for the major allele, heterozygous, or homozygous for the minor allele.

For analyses of genetic polymorphism genomic DNA was extracted from buffy coats using a standard precipitation procedure modified according to Miller et al. 1988. Samples were quantified by Nanodrop measurements (ThermoScientific, Wilmington, DE, USA) and quality-controlled by gel electrophoresis. The samples were genotyped with the MassARRAY system using iPLEX chemistry as suggested by the manufacturer (Sequenom, San Diego, CA, USA). Briefly, 5 ng of genomic DNA was amplified by polymerase chain reaction (PCR) using Hot-StarTaq DNA Polymerase (Qiagen GmbH, Hilden, Germany). After PCR, a shrimp alkaline phosphatase and primer extension reaction was carried out according to the iPLEX reaction protocol (Sequenom). The final base extension products were treated with SpectroCLEAN resin (Sequenom) and 30 nl of reaction solution was dispensed onto a 384 format SpectroCHIP (Sequenom). Data acquisition was done with a matrix-assisted laser desorption ionisation-time-of-flight (MALDI-TOF) mass spectrometer (Sequenom). The resulting mass spectra were analysed automatically for allele identification using the SpectroTYPER RT 4.0 software (Sequenom). (Libuda et al. 2016)

3.2.6 Cognitive assessment: Bayley Scales of Infant Development (BSID II)

The Bayley Scales of Infant Development II (BSID II) are generally considered to be the ‘gold standard’ in terms of assessing infant’s **cognitive development** (Heird, Lapillonne 2005). They are well established (Gagnon, Nagle 2000) and used to assess mental and psychomotor development from 1 to 42 months of age (Reuner et al. 2008).

The test battery consists of 3 parts: the mental scale which assesses e.g. memory, learning and problem solving, the psychomotor scale (e.g. body control) and the behaviour rating scale (e.g. motivation and interest) (Herndon 2006). Each age level has a specific number of items. Scoring the assessed items results in the Mental Developmental Index (MDI) and the Psychomotor Developmental Index (PDI)

(Herndon 2006). MDI and PDI standard scores have a mean of 100 with a standard deviation of 15 (Colombo et al. 2013).
Most studies examining the effects of LC-PUFA supply in preterm and term infants used the BSID II test (Gould et al. 2013). In PINGU, the **BSID II** test (MDI, PDI) served to assess the **cognitive development** of the infants **(Q4)**. The application of the T1 appointment for conducting the BSID II was exactly timed close to individual time at introduction of CF within the recommended period. The time slot of the conduction ranged from infant's 5th to 7th month.

The BSID II test was administered by three certified assessors under standardised conditions. The BSID III test was not available in German at the time of the study period. The first examination was conducted immediately before the mother introduced complementary food (T1), the second examination after the intervention (T2 ± 14 days). The cognitive assessments started later in the study period as the qualified study personnel has not yet been available at the beginning. Therefore T1 values were only available from 124 participants.

3.2.7 Visual assessment: Flash visual evoked potentials (FVEP)

For examining the **visual development** in infants, visual evoked potentials (VEP) are generally used in electrophysiologically based tests (Heird, Lapillonne 2005). In principle, a visual stimulus evokes electrophysiological responses (American Clinical Neurophysiology Society 2008), the VEP, showing that the visual information was exactly processed by the retina and transmitted via the geniculostraite pathway to the visual cortex (Heird, Lapillonne 2005). VEP are detected by scalp electrons (Neuringer, Jeffrey 2003) overlying the visual cortex (Creel 2012). The electro-encephalogram (EEG) extracts the VEP waveforms (Creel 2012). The amplitude of the VEP waveform is smaller than the one of the EEG and is more difficult to distinguish (Stöhr, 1996). Thus, the visual stimulus is given repeatedly several times and detected after defined time intervals by the EEG (Stöhr, 1996). In the end, all time intervals are averaged which results in mapping the distinctive stimulus response of the VEP (Stöhr, 1996). Patterned VEP are stimulated by contrast-reversing stimuli like checkerboards (Heird, Lapillonne 2005). In contrast, unpatterned VEP evoke responses to flash stimuli (strobe flash, flashing light-emitting diodes (LED)) (American Clinical Neurophysiology Society 2008; Creel 2012). Most of the studies examining effects of LC-PUFA supply on visual development in infancy and childhood assessed the visual acuity (Simmer et al.

2011 ; Gould et al. 2013; Qawasmi et al. 2013). Visual acuity mirrors the ability of the eye to detect fine distinctions in the environment (Walker et al. 1990; Kolb et al. 1995).
In PINGU, the **FVEP** method (flash LED) was used to examine **visual development (Q4)**. The method is valid to measure the maturation of optic pathways and the visual cortex (Sokol, Moskowitz 1985; Faldella et al. 1996), i.e. the myelination of neural tracts. It is independent of infant’s cooperation (Taylor, McCulloch 1992). The measured outcomes are the peak latency, i.e. the time (msec) from stimulus to peak detection (the ‘processing time’ of the stimulus) (Khedr et al. 2004), and / or the amplitude (peak to trough) (Shepherd et al. 1999) of the FVEP waveforms. In healthy infants, the prominent waveforms are: negative N1, N2 and positive P1 (Haan 2012). Peak latencies of the waveforms change with age e.g. the maturing P1 peak latency shortens from 200 msec at 5 weeks of age in full term infants to 110 msec at about 4-5 years (Creel 2012). Compared to peak latencies, amplitudes seem to vary more interindividually (Harden 1982).

FVEP measurements (T1, T2; ± 14 days) were conducted in a darkened room, while the infant was sitting on the mother’s lap. The LED goggles (Nihon Kohden, LS-102J) were placed directly in front of the infant’s eyes. No mydriatic or sedative was used. The infant was usually awake. Silver-plated EEG-electrodes were placed at midline occipital (active electrode) according to the international 10–20 electrode placement system (10–20 System), left and right retroauricular interconnected (reference electrodes). Each eye was examined separately, usually starting with the left eye. The filter frequency was 1-100 Hz and the analysis time 300 ms. Up to 100 single stimuli were averaged; stable FVEP were mostly registered between 30 and 50 stimuli. The rate of stimulation was 0.9 Hz. In order to assure reproductive measurements, two assessments per eye were conducted. The P100 latencies (msec) were analysed as they were observed to be most reliable (American Clinical Neurophysiology Society 2008).
Table 4 summarises the data and methods used for this thesis.

Tab. 4: Methods and data used for this thesis

Research question	**Q1**		**Q2**	**Q3**[a]	**Q4**[a]
	a	**b**			
Methods	Market survey	Dietary assessment (3-day weighed dietary records)	Online survey	Double-blinded randomised control trial (RCT)	
Data source	Online database Nutrichild for complementary food on the German market	DONALD; food and nutrient database LEBTAB	Online panel consisting of German households provided by Kantar Health GmbH	Blood samples (plasma, erythrocytes, buffy coat) before and after the intervention period (T1; T2)	BSID II and FVEP before and after the intervention period (T1; T2)
Sample	328 products of 14 food companies (15 brands) offered for infants between 5 and 12 months of age	Dietary records collected between 2008 and 2012 (n= 477) from participants 6, 9 and 12 months of age (n= 222, 48.7% females)	985 interviews of mothers with children between 5 and 36 months of age	Plasma: 155 participants (n= 56 CG, n= 52 IG-R, n= 47 IG-F); erythrocytes: 158 participants (n= 56 CG, n= 54 IG-R, n= 48 IG-F)	BSID II: 124 participants (n= 45 CG, n= 40 IG-R, n= 39 IG-F); FVEP: 134 participants (n= 46 CG, n= 47 IG-R, n= 41 IG-F)
Outcomes	Frequency of used food categories in products (vegetables, meat, fish, potato, rice, pasta, oil, spices/herbs), frequency of used vegetable types in products	Differences in VegVS between the commercial and the homemade CF group; frequency of food categories (vegetables, meat, fish)	Fish and rapeseed oil consumption of mothers and infants and its determinants	Fatty acid composition in RBC membrane and plasma	Bayley's MDI and PDI; FVEP latencies right and left eye, mean of both eyes

[a]T1 BSID II: dependent on individual introduction of complementary food: between the 5th and 7th month of age, FVEP: 4 months of age ± 2 weeks; T2 BSID II and FVEP: 10 months of age ± 2 weeks

CF: complementary feeding; CG: control group, IG-F: intervention group fish, IG-R: intervention group rapeseed oil, MDI: Mental Developmental Index, PDI: Psychomotor Developmental Index, VegVS: vegetable variety score, RBC: red blood cells

3.3 Statistics

SAS procedures (version 9.2; SAS Inc., Cary, NC) were used for all data analyses ($p < 0.05$ was considered as significant).

3.3.1 Food variety: market survey (Q1a) and dietary practice (Q1b)

For the descriptive market survey and the DONALD analysis frequencies were investigated separately. For the market survey (Q1a), food categories were vegetables, meat, fish, potato, rice, pasta, oil and spices/herbs. For the analysis of dietary practice (Q1b), food categories were vegetables, meat and fish. Ingredients of commercial meals were evaluated by means of the labelled ingredients sequence, ingredients of homemade meals by means of their labelled sequence of consumed amounts.

Differences in the vegetable variety score (VegVS) between the commercial and the homemade meal group in DONALD were analysed using Kruskal-Wallis test. Analyses were stratified by age group in order to take potential effects of infants' age into account.

3.3.2 n-3 (LC-)PUFA rich foods: consumption, determinants, physiological and functional effects (Q2, Q3, Q4)

Q2: As the consumer survey was conducted via internet which excluded mothers without internet access, data was weighed for mothers' age, maternal level of school and professional education, household incomes, number of persons in the households, size of residences and federal states to approach a representative sample of German mothers.

For the description of mothers' and infants' fish consumption as well as the use of different oil types and the unifactorial analysis of determinants by chi-square tests or Cochran-Armitage trend tests, the procedure FREQ was used. Logistic models (PROC Logistic) considered all potential determinants for multifactorial analyses. 2 statistical models were created for each, infant and mother.

Analysis infants: Model 1 considered mother's age, social class, part of Germany, knowledge, breastfeeding behaviour, number of children and convenience degree of the meals. Model 2 additionally included maternal fish and rapeseed oil consumption as the maternal nutritional habits could be associated with these infant variables.

Analysis mother: Model 1 contained mother's age, social class, residential part of Germany and knowledge. Model 2 further included breastfeeding.

Q3, Q4: All statistical tests were defined *a priori* in a statistical analysis plan (statistical support: Chrestos Concept GmbH & Co.KG, Ratingen, Germany). ANOVA, Kruskal-Wallis (Q3, Q4) or chi-q test (Q4) were used for assessing group differences in study sample characteristics. In general, subjects with complete data (T1, T2) were examined within their randomised group. All analyses were conducted stratified for the comparison of IG-R vs. CG and IG-F vs. CG.

Physiological effects

As described in Libuda, Mesch et al. 2016 primary outcome of the confirmatory analysis was RBC-DHA (compare *a priori* power analysis). Further explorative analyses considered PUFA in RBC or plasma as outcome variables. The intervention effects were analysed using ANCOVA. The basic models included the group affiliation and the basic value of the respective outcome at T1 as principal exposition variables. Sex, age at T2, breastfed at birth, breastfed at T0, birth weight, gestational age, birth complication, number of children and socioeconomic status (SES) were tested as potential covariates. In addition, the exact age of introduction of complementary feeding and length of the intervention were tested as covariates. In a separate step FADS SNPs were considered as potential covariates. Only those variables that were significantly associated with DHA status in the basic models of the confirmatory analyses (model 1 for SNPs) were finally included into model 1 (model 2 for SNPs).

Functional effects

The intervention effects were analysed using ANCOVA. The P100 latency of the left and the right eye, both at T2, were used as separate outcome variables. Mean values of both eye latencies were used to describe the individual general visual development. Separate models included the MDI and the PDI at T2 as outcome variables. Only covariates which were significantly associated with the respective outcome variable were included into the final models.

4 Results

4.1 Research topic 1 (T1): Food variety in CF – market survey (Q1a) and dietary practice (Q1b)

4.1.1 Summary

Research topic 1 evaluated the status quo of the used foods in commercial complementary vegetable-potato-meat/fish meals available on the German baby food market in 2012 regarding food variety (Q1a) and examined differences in vegetable variety (Q1b) in commercial vs. homemade complementary meals in Germany. It is the first detailed assessment of the food offer and variety in commercial and homemade complementary meals in Germany.

For the description of the food offer on the market, products of 14 food companies (15 brands) offered for infants between 5 and 12 months of age provided by the online data base 'Nutrichild' were available. 3-day weighed dietary records from the German DONALD study, collected between 2008 and 2012 (n= 477 records) from participants 6, 9 and 12 months of age (n= 222 participants, 48.7%) were used to assess vegetable variety in dietary practice by using a vegetable variety score (VegVS) which was calculated as the sum of the individual number of different vegetables consumed with 'vegetable-potato-meat meals' within the 3 recorded days.

The market survey showed that mainly carrot was used in the meals offered in 2012. The meal types were mainly offered as vegetable-potato/rice/pasta meals (vegetarian or meat meal). No pure vegetable meals were offered in junior age and there were no pure fish meals on the market. In general, the offer of fish meals was rare. The DONALD data showed that vegetable variety was low in both, homemade as well as commercial meals. Carrot was mainly used in both types of meals. Differences between infants fed homemade or commercial meal were only observed at the age of 12 months with those fed commercial meals getting a higher vegetable variety compared to infants fed with homemade meals (3.7 vs. 2.7 within 3 days, $p = 0.0001$). In dietary practice fish meals were also rarely used.

4.1.2 Results

Market survey

On the German market 328 commercial complementary meals of the 'vegetable-potato-meat' type from 14 companies (15 brands) were offered in 2012. Almost all companies offered organic produced meals (organic: 11 companies, 287 meals (87.5%)). The meals were mainly sold in jars (92.4%, 7.6% plastic pots).

In Germany the complementary meals can be classified into two age categories according to the food texture:

1) Strained 'baby' meals without solid pieces offered for the beginning of the CF period (in general from the 5th month of age; portion size 190 g)

2) Lumpy 'junior' meals offered for infants 8-12 months of age (portion size 220 g in general).

The market was dominated by baby meals (62.2%) and vegetable-potato/rice/pasta and vegetable-potato/rice/pasta-meat meals (71% baby, 94% junior) (tab. 5). Mean number of ingredients was 7.3 ± 3.6 (mean ± SD) per meal.

Tab. 5: Commercial complementary 'vegetable-potato-meat'-meals on the German baby food market in 2012 (n= 328)

	Commercial meals [%]	
	Age	
Meal type	Baby[a] (n= 204)	Junior[a] (n= 124)
Vegetable-containing		
Pure vegetable	19.1	0.0
Vegetable-Potato/rice/pasta	35.3	24.2
Vegetable-Potato/rice/pasta-Meat	35.3	70.2
Vegetable-Potato/rice/pasta-Fish	1.0	4.8
Vegetable-Meat	0.5	0.0
Vegetable-Potato/rice/pasta-Meat-Fish	0.0	0.8
Non-vegetable-containing		
Blend of meat	4.9	0.0
Potato/rice/pasta-Meat	3.9	0.0

[a]baby: from the 5th month of age, portion size 190 g in general; junior: from 8 to 12 months of age, portion size 220 g in general

Vegetable-containing products consisted of 2.2 ± 1.3 (mean ± SD) different vegetables, with a maximum of 6 different vegetables per product (n= 3; 2 brands). In total 16 different vegetables were identified: Carrot in 69% of commercial meals, onions (24.7%), tomato (24.4%), parsnip (17.1%), peas (10.7%), pumpkin (9.8%), spinach (8.5%), zucchini (8.2%), leek (8.2%), corn (7%), broccoli (6.7%), cauliflower (3.4%), fennel (3.4%), celery (2.7%), pepper (2.4%), eggplant (0.6%).

Carrot topped the list of the first vegetable declared in the list of ingredients (60% of the vegetable-containing meals), followed by tomato (15%), pumpkin (9.0%), parsnip (8.0%), spinach (4.0%) and other vegetables (each < 4.0%). Most of the pure vegetable meals (87.2%, n= 39) contained only 1 vegetable, 7.6% and 5.1% contained 2 and 3 vegetables respectively per meal.

When meat was part of a meal mainly poultry or beef and less often pork was used (tab. 6). Only 9 fish meals (4 brands) were available, mainly as junior meals (tab. 6).

Tab. 6: Types of meat and fish used in commercial and homemade complementary meals

	Commercial meals market survey[a]	Dietary practice in the DONALD study	
		Homemade meal group	Commercial meal group
	Frequency [%]	Frequency [%]	
Meat type	n= 179	n= 387[c]	n= 714[c]
Poultry	48.6	26.0	36.8
Beef	34.6	65.6	44.0
Pork	11.7	3.7	9.7
Calf, lamb	5.1	4.7	9.7
Fish type	n= 9[b]	n= 6[c]	n= 45[c]
Salmon	55.6	50.0	26.7
Sea fish	22.2		24.4
Alaska-coalfish	22.2		11.1
Shark catfish	33.3		4.4
Codfish		50.0	33.3

[a]includes baby and junior meals; baby: from the 5[th] month of age, portion size 190 g in general; junior: from 8 to 12 months of age, portion size 220 g in general; [b]3 meals contained 2 types of fish; [c]total number of meals with meat or fish within the 3 recorded days
Blank cells: fish type was not used for meals

As a starchy component, the commercial meals most often contained potatoes (44.8%), followed by rice (28.4%) and pasta (19.5%). 12.5% of the meals contained spices and herbs, mainly parsley. Although spices and herbs were also added to baby meals, more junior meals contained spices and herbs (77.9%). On average 2.1 ± 1.1 (mean ± SD) different types of spices/herbs, with a maximum of 5 different spices/herbs used in the same product (9 products) were offered. 246 meals contained oil (75%), mainly rapeseed oil (89%). Other oils were sunflower, corn, olive or non-specified oil. Mean oil content of the meals was 1.0 ± 0.7 g/100g (mean ± SD).

Dietary practice

Vegetable-containing commercial meals contained on average more different vegetables per meal (2.2 ± 1.4 (mean ± SD); $p < 0.0001$) than homemade meals (1.6 ± 1.3 (mean ± SD)), with maximum numbers of 10 different types per homemade and 9 per commercial meal. For homemade meals overall 26 different vegetables were used. In contrast, in all examined commercial meals 17 different vegetables were found. The total number of vegetables increased from 6 to 9 months of age especially in homemade meals and decreased slightly afterwards at 12 months of age in the homemade meal group.

Carrot was mainly used in commercial and homemade meals (each 34.9%) after pooling data of the 3 age categories (6, 9, 12 months of age), followed by zucchini (11.8%), pumpkin (8.7%), parsnip (8.3%), broccoli (6.2%) and cauliflower (5.5%) in homemade and tomato (14.2%), onions (13.1%), parsnip (7.7%), corn (5.3%) and zucchini (4.5%) in commercial meals (tab. 7).

Analysis of variance showed a significant effect of age group on the VegVS with infants 12 months of age. At this age infants fed with commercial meals got a greater vegetable variety than those fed with homemade meals. This effect was not significant at 6 and 9 months of age (tab. 8).

Meat-containing commercial and homemade meals mainly contained beef or poultry (tab. 6). Fish was rarely used in the homemade and commercial meal group, although it was fed more often in the latter (tab. 6).

Tab. 7: Vegetables used in commercial and homemade complementary meals at 6, 9 and 12 months of age in dietary practice in the DONALD study

	Commercial vegetable-containing meals [%]			**Homemade vegetable-containing meals [%]**		
	Age (months)			Age (months)		
Vegetable type[a]	6 (n=380[b])	9 (n=767[b])	12 (n=715[b])	6 (n=185[b])	9 (n=302[b])	12 (n=261[b])
Asparagus					0.7	0.4
Beet					1.0	0.4
Broccoli	2.4	3.4	3.7	2.2	6.0	9.2
Brussels sprouts					0.3	1.2
Carrot	72.9	33.9	27.7	27.1	32.5	35.3
Cauliflower	2.9	0.8	7.7	4.9	4.0	1.7
Celery		2.6	3.9		0.7	2.7
Chard					0.3	0.4
Corn	2.6	7.3	4.6		1.0	0.4
Cucumber				0.5	0.7	
Eggplant		0.3	0.3		0.7	1.2
Fennel	2.1	1.4	0.8	6.5	3.3	2.3
Green beans		0.1	0.4		0.3	1.2
Green cabbage						0.4
Leek		1.0	3.9		1.2	2.7
Onions	5.5	13.7	17.2		1.0	5.8
Parsnip	14.2	7.0	4.9	12.4	10.3	3.1
Peas	2.6	3.7	3.1		0.3	3.5
Pepper		0.5	0.8		1.3	5.7
Pumpkin	6.3	2.9	2.1	13.0	10.6	3.5
Spinach	1.3	0.9	2.2		0.7	1.5
Swede				0.5	0.7	
Tomato	5.5	16.7	16.2		2.7	2.7
Turnip cabbage				3.8	6.6	3.9
White cabbage				1.6	1.3	
Zucchini	4.2	4.1	5.0	16.2	12.3	8.1

[a]listed in alphabetic order; [b]n: total number of meals with vegetables within the 3 recorded days
Blank cells: Vegetable was not used for meals in this age

Tab. 8: Vegetable variety indicated by the vegetable variety score (VegVS) at 6, 9 and 12 months of age in the homemade vs. commercial meal group in the DONALD study

	Homemade vs. commercial meal group		
	VegVS[a] [mean ± SD (max)]		
Age (months)	Homemade	Commercial	p[b]
6	1.4 ± 0.6 (4)	1.7 ± 1.0 (5)	0.18
9	2.4 ± 1.2 (7)	2.7 ± 1.3 (7)	0.25
12	2.7 ± 1.4 (7)	3.7 ± 1.5 (8)	0.0001

[a]VegVS: number of different vegetables consumed with a 'vegetable-potato-meat meal' within the 3 recorded days. All vegetables above a cut-off of 10 g (Daniels 2007) per meal were considered for score calculation; [b]tested by Kruskal-Wallis test

4.2 Research topic 2 (T2): n-3 (LC-)PUFA-rich foods in CF – consumption, determinants, physiological and functional effects (Q2, Q3, Q4)

4.2.1 Summary

Research topic 2 examined the consumption of rapeseed oil and fish in German mother-child-dyads as well as determinants that predict the consumption of these n-3 (LC-)PUFA-rich foods by mothers and their infants. Further, effects of an optimised n-3 (LC-)PUFA supply of infants via rapeseed oil and fish on RBC (plasma) DHA status and cognitive and visual development were investigated.

All investigations were part of the double-blinded randomised controlled intervention trial PINGU conducted from April 2011 to August 2013 in Dortmund, Germany. Mother-child-dyads were recruited between April 2011 and October 2012 in delivery hospitals in the region of Dortmund and at 2 months of infant's age randomly assigned to one of the 3 study groups. During the intervention period (5^{th}-10^{th} month of age) intervention group IG-R received jars of baby food with rapeseed oil (n= 54), IG-F jars of baby food with fish twice a week (n= 48) and the control group (CG) the same jars as IG-F with corn oil instead of rapeseed oil (n= 58).

The pre-study of PINGU was an online nationwide consumer survey conducted in November/ December 2010 for investigating the consumption and determinants. Mothers with children aged 5-36 months had to answer 48 questions covering amongst others the topics:

- dietary behaviour in terms of fish, rapeseed oil consumption
- maternal nutritional knowledge with respect to n-3 (LC-)PUFA.

985 mother-child-dyads were included into the final analysis.

The core study of PINGU started in April 2011. Blood sampling for the evaluation of FA status including paediatric assessment, monitoring of infants´ physical development, FVEP, Bayley's MDI and PDI were assessed immediately before and after the intervention period (T1: 4 months of age ± 14 days, T2: 10 months of age ± 14 days) at the Paediatric Clinic Dortmund. Of the 214 enrolled and randomised participants, 165 completed the study. Complete data from 158 (155) infants were available for the assessment of the FA status in RBC (plasma), for the visual evaluation from 134 and for the cognitive evaluation from 124 participants.

The survey revealed that rapeseed oil usage was more frequent than the use of fish. In contrast, Mothers' rapeseed oil usage for their own diet was less frequent than the fish consumption. Mothers met more often the recommendations of eating fish at

least once per week than their infants. While maternal eating behaviour was the main predictor of infant's fish consumption, infant's rapeseed oil consumption was as well influenced by further factors like the social status of the mother and 'omega-3' knowledge, which were also key determinants of mothers' own fish and rapeseed oil consumption.

RBC and plasma levels at T2 were almost identical. In IG-R significantly higher levels of ALA, the ratio of ALA to LA and EPA were determined. In contrast, the DHA status did not differ between IG-R and CG. RBC concentrations of EPA, DHA, and total n-3 LC-PUFA were significantly higher in IG-F than CG. At 10 months of age, the mean latency of both eyes in IG-R and the left eye latency in IG-F were slightly but significantly shorter than in CG. There were no differences in cognitive development between the IGs and the CG.

4.2.2 Results

4.2.2.1 Consumption and determinants of n-3 (LC-)PUFA-rich foods in CF

Sample characteristics and consumption habits

As presented in Stimming, Mesch et al. sample characteristics in the pre-study were similar in terms of children's age strata (tab. 9) (Stimming et al., 2015). Most mothers had a medium socioeconomic status, came from the Old Federal States and habited in the southern part of Germany. Most children were exclusively breastfed ≥ 4 months of age.

Tab. 9: Baseline characteristics of mother-child-dyads (n= 985)

Variable			
Mothers's age [ys; median (range)]	32 (18-50) P25; P75: 29; 36		
Number of children [median (range)]	2 (1-7) P25; P75: 1; 2		
First child[a] [n (%)]	Yes: 465 (47.2)	No: 520 (52.8)	
Age of youngest child [mo; median (range)][b]	18 (5-36) P25; P75: 11; 27		
Age strata of youngest child [n (%)]	5-12 mo: 305 (31)	13-24 mo (34)	25-36 mo: 341 (35)
		n (%)	
Socioeconomic status[c]	Low: 295 (29.9)	Medium: 387 (39.3)	High: 303 (30.8)
New / Old Federal States	New: 205 (20.8)	Old: 780 (79.2)	
Northern / Southern part of Germany	Northern: 186 (18.9)	Southern: 799 (81.1)	
Breastfeeding initiation	Yes: 771 (78.3)	No: 214 (21.8)	
≥ 4 months exclusive breastfeeding	Yes: 547 (55.6)	No: 438 (44.4)	
Offer of vegetable-potato-meat meals[d]	Mainly CCM: 401 (40.7)	Mainly HCM: 405 (40.2)	Both balanced: 178 (18.1)
Knowledge[e]	Low (1): 194 (19.7)	Medium(2): 534 (54.2)	High (3): 257 (26.6)

CCM: commercial complementary meals; HCM: homemade complementary meals
[a]no older siblings; [b]≥ 5 months and complementary food already induced; [c]calculated by maternal level of school education, maternal level of professional education and household income; [d]1 sample pair missing, n= 984; [e]score of five food and health-related questions
[Modified according to Stimming et al. 2015]

Infants' and mothers' consumption of rapeseed oil and fish

For homemade complementary meals mothers mainly used rapeseed oil (49%), followed by sunflower (17%), olive (10%) and corn oil (3%). 16% added no oil or used other oils (5%) (data not shown, see Stimming et al. 2015). 40% of the mothers who fed commercial vegetable-potato-meat meals did not remember the labelled oil type. Most of the mothers who could remember the oil type stated that the meals contained rapeseed oil (38%), followed by sunflower (8%), olive or corn oil (5% respectively; 4% other oil types).

In contrast to infant's nutrition, mothers mainly used sunflower oil (37%) for cooking in their own dietary practice, followed by rapeseed oil (30%) and olive oil (23%) (data not shown, see Stimming et al. 2015). Olive oil was the most favoured oil used for salads, followed by sunflower (24%) and rapeseed oil (18%).
Mothers and their children both consumed fish to some extent (fig. 9). More infants (31%) never ate fish than meeting the recommendation to eat fish at least once per week (25%). However, 41% of mothers stated that they ate fish at least once per week or more frequently. Only 3% of the mothers never ate fish.

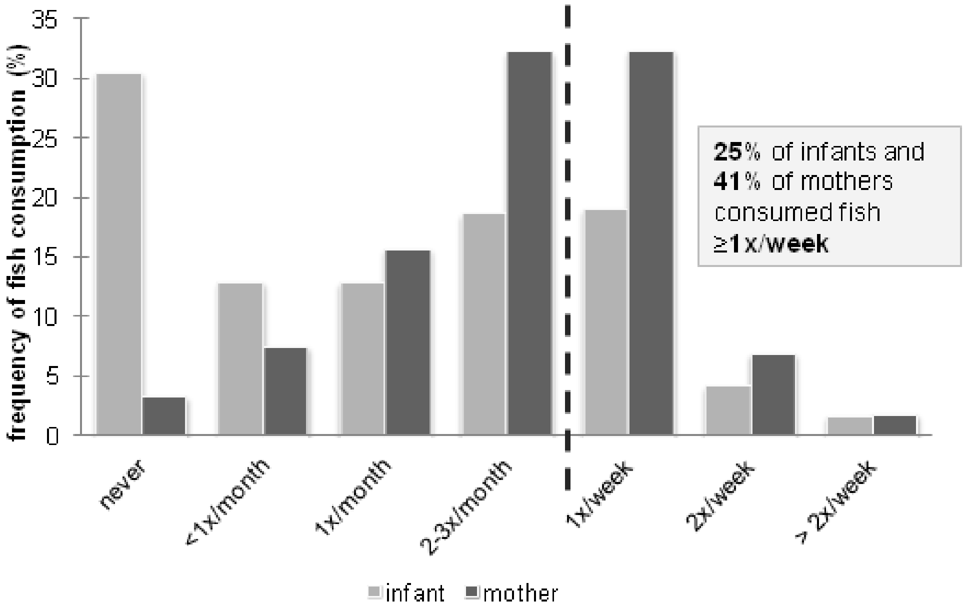

Fig. 9: Frequencies of fish consumption (in general) of infants and their mothers (n= 985)
(Stimming et al. 2015)

Determinants of infants' and mothers' rapeseed oil and fish consumption

Unifactorial analyses showed that **infants' rapeseed oil consumption** was more common when they were firstborn, from higher social classes and lived in the Old Federal States (data not shown, see Stimming et. al 2015). The higher the maternal knowledge about n-3 FA, the more frequent was the infants' rapeseed oil consumption. In addition, mother's own usage of rapeseed oil was strongly associated with the usage of this oil type for the vegetable-potato-meat meals. Multifactorial analyses showed amongst others, that infants' rapeseed oil consumption was significantly associated with social class or maternal knowledge (tab. 10, model 1). Considering maternal dietary habits led to a strong association with infants' rapeseed oil consumption (tab. 10, model 2). In addition, nutritional knowledge and social class were also key determinants of infants' rapeseed oil consumption.

In both, unifactorial (data not shown, see Stimming et al. 2015) and multifactorial analyses (tab. 11), results showed that maternal nutritional knowledge of n-3 FA was the only significant determinant regarding **mothers' rapeseed oil consumption**.

Tab. 10: Multifactorial analysis of potential determinants of infant's fish and rapeseed oil consumption (n= 985)

Variable		**High fish consumption[a]**		**Rapeseed oil consumption**	
		OR	95% CI	OR	95% CI
Model 1					
Mother's age [ys]	> 32 vs. ≤ 32	0.80	0.58–1.10	0.77	0.57–1.03
Social class[b]	1 vs 3	1.07	0.72–1.58	**0.38**	**0.26–0.55**
	2 vs. 3	0.76	0.53–1.09	**0.50**	**0.36–0.70**
Part of Germany	OFS vs. NFS	0.95	0.65–1.37	**1.62**	**1.14–2.29**
	Southern vs. northern part	1.24	0.84–1.84	1.21	0.85–1.73
Knowledge[c]	Low vs. high	0.70	0.45–1.10	**0.16**	**0.10–0.24**
	Middle vs. high	**0.64**	**0.46–0.90**	**0.32**	**0.23–0.45**
Breastfeeding	No vs. yes	**1.58**	**1.00–2.47**	0.67	0.44–1.02
	< 4 vs. ≥ 4 mo	0.82	0.56–1.22	0.74	0.52–1.05
First child	No vs. yes	1.31	0.97–1.79	**0.74**	**0.55–0.98**
Offer	Mainly CCM vs. mainly HCM	**1.39**	**1.00–1.93**	1.01	0.74–1.37
	Both balanced vs. mainly HCM	1.01	0.66–1.54	0.85	0.57–1.26
Model 2					
Mother's age [ys]	> 32 vs. ≤ 32	**0.69**	**0.49–0.96**	**0.73**	**0.54–1.00**
Social class[b]	1 vs. 3	1.14	0.74–1.74	**0.35**	**0.24–0.53**
	2 vs. 3	0.73	0.49–1.07	**0.47**	**0.33–0.67**
Part of Germany	OFS vs. NFS	1.03	0.69–1.54	**1.57**	**1.09–2.27**
	Southern vs. northern part	1.07	0.70–1.63	1.24	0.85–1.81
Knowledge[c]	Low vs. high	0.86	0.53–1.39	**0.16**	**0.10–0.26**
	Middle vs. high	0.79	0.54–1.15	**0.34**	**0.24–0.49**
Breastfeeding	No vs. yes	1.46	0.90–2.38	**0.64**	**0.41–0.99**
	< 4 vs. ≥ 4 mo	1.08	0.71–1.64	0.78	0.54–1.13
First child	No versus yes	1.34	0.96–1.86	0.76	0.57–1.03
Offer	Mainly CCM vs. mainly HCM	1.26	0.88–1.80	1.04	0.75–1.45
	Both balanced vs. mainly HCM	0.94	0.59–1.48	0.88	0.58–1.32
Mother's rapeseed oil consumption	No vs. yes	0.82	0.59–1.14	**0.24**	**0.18–0.33**
Mother's fish consumption	No vs. yes	**0.16**	**0.12–0.23**	1.06	0.79–1.43

CCM: commercial complementary meals; HCM: homemade complementary meals; OFS: Old Federal States; NFS: New Federal States; [a]at least once per week fish; [b]calculated by maternal level of school education, maternal level of professional education and household income; [c]calculated by a score of five food and health-related questions. Bold values indicate 95% CI not overlapping the value
[Modified according to Stimming et al. 2015]

Tab. 11: Multifactorial analysis of potential determinants of maternal fish and rapeseed oil consumption (n= 985)

Variable		High fish consumption[a]		Rapeseed oil consumption	
		OR	95% CI	OR	95% CI
Model 1					
Mother's age [ys]	> 32 vs. ≤ 32	**1.35**	**1.03–1.76**	1.05	0.80–1.38
Social class[b]	1 vs. 3	0.80	0.57–1.13	0.85	0.59–1.21
	2 vs. 3	0.97	0.71–1.32	1.02	0.74–1.41
Part of Germany	OFS vs. NFS	0.79	0.57–1.09	1.16	0.83–1.63
	Southern vs. northern part	**1.46**	**1.04–2.06**	1.06	0.75–1.49
Knowledge[c]	Low vs. high	**0.59**	**0.40–0.87**	**0.49**	**0.43–0.79**
	Middle vs. high	**0.59**	**0.44–0.80**	**0.58**	**0.43–0.79**
Model 2					
Mother's age [ys]	> 32 vs. ≤ 32	**1.31**	**1.00–1.71**	1.03	0.79–1.36
Social class[b]	1 vs. 3	0.91	0.64–1.30	0.91	0.63–1.31
	2 vs. 3	1.00	0.74–1.37	1.04	0.76–1.43
Part of Germany	OFS vs. NFS	0.79	0.57–1.10	1.17	0.83–1.65
	Southern vs. northern part	**1.46**	**1.04–2.06**	1.06	0.75–1.50
Knowledge[c]	Low vs. high	**0.62**	**0.42–0.92**	**0.50**	**0.34–0.76**
	Middle vs. high	**0.60**	**0.44–0.82**	**0.59**	**0.43–0.80**
Breastfeeding	No vs. yes	1.28	0.86–1.92	1.07	0.71–1.63
	< 4 vs. ≥ 4 mo	**0.55**	**0.39–0.77**	0.75	0.53–1.05

OFS: Old Federal States; NFS: New Federal States
[a]At least once per week fish; [b]calculated by maternal level of school education, maternal level of professional education and household income; [c]calculated by a score of five food and health-related questions. Bold values indicate 95% CI not overlapping the value
[Modified according to Stimming et al. 2015]

Unifactorial analysis only showed that mother's fish consumption was strongly associated with **infant's fish consumption** (data not shown, see Stimming et al. 2015): In 44% of cases infants of those mothers who ate fish at least once per week were high fish consumers. In contrast, 11% of those infants whose mothers ate fish less frequently were high fish consumers. The multifactorial analyses supported the observation that maternal fish consumption is a major determinant of a high fish consumption in infants (tab. 10). In contrast to model 1, model 2 only revealed that mother's age determined infants' fish consumption (the younger the mother (≤ 32 years), the more frequent was infant's fish consumption).

The unifactorial (data not shown, see Stimming et al. 2015) as well as the multifactorial analyses (tab. 11) showed that mothers' age, the place of residence (the South) and a higher n-3 knowledge was associated with high **maternal fish consumption** (tab. 11, model 1). Model 2 further revealed that exclusively breastfeeding for more than 4 months also determined the high maternal fish consumption (tab. 11, model 2).

4.2.2.2 Effects of n-3 (LC-)PUFA-rich foods in CF on physiological and functional development

Sample characteristics

In the samples of Q3 and Q4 characteristics of mothers and their infants did not differ between the 3 study groups (tab. 12 and 13). A high social status dominated in all 3 groups and sex of infants was equally distributed. Most infants were breastfed at birth. In both samples the rate of breastfeeding decreased to about 71% at the age of 2 months (T0). At both times breastfeeding rates did not differ between study groups.

Dietary fatty acid intake

Taking analysed FA values and assuming the feeding practices as set in the study protocol, the IG-F received about 42 mg (190 g jars) or 52 mg (220 g jars) more DHA per day and the IG-R about 140 mg more ALA per day (190 g, 220 g jars) than the CG (tab. 14). Compliance rate, i.e. feeding according to study protocol, was around 70% and 90% (1 week at month 6 and 9 were analysed. Availability of dietary records: 120 participants at 6 months, 108 participants at 9 months). There were no significant differences in compliance between the groups (data not shown).
There was only one food-related adverse event reported in the IG-F. However, the fish allergy was diagnosed when the infant had already finished the study.

Tab. 12: Q3 Baseline characteristics of mother-child-dyads in the rapeseed oil (IG-R), fish (IG-F) and control (CG) group (means ± SD or P50 (P25;P75))

Variable	IG-R (n=54)	IG-F (n=48)	CG (n=56)	p
Infant				
Sex, male [%]	50.9	48.9	50.0	0.98
Gestational age [wk]	39.5 (38.0;41.0) (n=53)	40.0 (39.0;41.0)	40.0 (39.0;41.0)	0.92
Birth weight [kg]	3443.1 ± 393.6	3420.6 ± 472.7	3456.6 ± 447.4	0.76
Birth length [cm]	52.0 (51.0;53.0)	52.0 (50.0;54.0)	52.0 (51.0;53.0)	0.46
First born [%][1]	61.8	55.3	51.8	0.56
Breastfed at birth [%]	96.4	91.3	91.1	0.79
Breastfed at T0 [%]	74.6	78.7	69.7	0.89
Age at T2 [d]	312.0 (307.0;319.0)	309.0 (305.0;315.0)	311.0 (307.0;316.5)	0.13
Body weight at T2 [g]	9347.6 ± 1200.3	9360.6 ± 1032.2	9564.9 ± 1332.7 (n=55)	0.58
Body length at T2 [cm]	73.0 (72.0;75.5) (n=52)	73.0 (71.5;75.0)	73.5 (71.0;75.0) (n=55)	0.90
Length of intervention [d][2]	150.5 (128.5; 160.0)	153.0 (135.0; 159.0)	144.0 (133.0; 158.0)	0.54
Mother				
Age at T2 [ys]	33.5 (4.8)	32.9 (4.8)	34.0 (4.9)	0.45
Social status [%][3]	(n=53)	(n=46)	(n=53)	
Low	5.6	4.6	5.6	0.88
Medium	27.8	25.0	40.7	
High	66.7	70.5	53.7	

Baseline characteristics did not differ between study groups (continuous variables: ANOVA or Kruskal-Wallis, categorical variables: chi-square test (fisher's exact test));
[1]Biological child; [2]Derived from dietary records; [3]Defined by calculating a score of mother's graduation, education and parental income before pregnancy
[Modified according to Libuda et al. 2016]

Tab. 13: Q4 Baseline characteristics of mother-child-dyads in the rapeseed oil (IG-R), fish (IG-F) and control (CG) group (means ±SD)

Variable	IG-R (n=54)	IG-F (n=48)	CG (n=58)	p
Infant				
Sex, male [%]	50	52	50	0.97
Gestational age [wk]	39.6 ± 1.5 (n=53)	39.9 ± 1.2	39.8 ± 1.2	0.64
Birth weight [g]	3446.4 ± 399.0	3412.9 ± 470.4	3464.9 ± 441.9	0.83
Birth length [cm]	52.2 ± 1.9	52.2 ± 5.6	52.0 ± 2.0	0.63
Caesarian [%]	43.0	31.0	33.0	0.39
First born [%][1]	59.0	58.0	53.0	0.80
Breastfed at birth [%]	94.0	88.0	91.0	0.46
Breastfed at T0 [%]	72.0	77.0	71.0	0.75
Body weight at T2 [g]	9348.9 ± 1200.0	9386.6 ± 1065.5 (n=47)	9546.8 ± 1333.2 (n=57)	0.66
Body length at T2 [cm]	73.0 ± 4.7 (n=52)	72.8 ± 4.9 (n=47)	73.3 ± 3.1 (n=57)	0.93
Head circumference at T2 [cm]	46.3 ± 1.6 (n=52)	45.9 ± 1.1 (n=45)	46.2 ± 1.5 (n=57)	0.47
Introduction of study food [d][2]	162.0 ± 22.9 (n=45)	159.4 ± 21.7 (n=30)	161.2 ± 15.7 (n=36)	0.88
Length of intervention [d][2]	146.9 ± 23.4 (n=45)	146.3 ± 22.0 (n=30)	147.1 ± 19.5 (n=36)	0.80
Mother				
Age T2 [ys]	33.5 ± 4.5 (n=40)	32.6 ± 5.1 (n=39)	34.0 ± 4.9 (n=45)	0.39
Social status [%][3]	(n=53)	(n=45)	(n=56)	
Low	3.8	4.0	5.0	0.56
Medium	30.0	24.0	39.0	
High	66.0	71.0	55.0	

Baseline characteristics did not differ between study groups (Chi-square test, Kruskal-Wallis test, ANOVA); [1]Biological child; [2]Derived from dietary records; [3]Defined by calculating a score of mother's graduation, education and parental income before pregnancy

Tab. 14: DHA, ARA, ALA and LA intakes from study food (estimated) in infants in the rapeseed oil (IG-R), fish (IG-F) and control (CG) group per week from 5 months to 10 months of age[1]

Estimated intake per week [mg]	IG-R	IG-F	CG
Baby jars[2]			
DHA	3.2	297.1	3.2
ARA	44.2	44.7	34.8
ALA	1454.6	866.2	467.5
LA	6171.6	10723.8	11677.8
Junior jars[2]			
DHA	3.4	370.3	4.0
ARA	36.5	49.3	37.0
ALA	1521.2	860.2	488.8
LA	6030.7	10950.8	10723.8

[1]Baby jars: 190g/per jar (5th to 7th month); junior jars: 220g/jar (8th to 10th month)
[2]Estimated ARA:DHA ratios in the groups: IG-R: 14:1, IG-F: 1:7, CG: 11:1 (baby jars), IG-R:11:1, IG-F: 1:8, CG 9:1 (junior jars)
ALA: alpha-linolenic acid; ARA: arachidonic acid; LA: linolenic acid

Intervention effects of fish and rapeseed oil consumption on physiological and functional outcomes

At T1 FA composition in RBC (plasma) did not differ between groups (data not shown, compare Libuda et al. 2016). No differences in outcome variables of FVEP and BSID II were found between the groups at T1 (tab. 15).

As results of plasma FA levels were almost identical to those of RBC levels, only results of the latter are presented. Details on plasma results see Libuda, Mesch et al. 2016. Rapeseed oil consumption yielded higher RBC ALA and EPA, but not RBC DHA levels (% wt./wt.) in the IG-R vs. CG at T2 (tab. 16). Higher EPA levels and higher ratios of EPA:AA in RBC in IG-R indicate a higher endogenous n-3 LC-PUFA synthesis. At T2 infants in the IG-F had higher RBC DHA and EPA levels (% wt. /wt.) than the CG. The sum of all analysed n-3 (LC-)PUFA was substantially higher than in CG. AA levels were slightly lower in IG-F. RBC-concentrations of LA were slightly lower in both IG-F and IG-R than in CG. The results presented in tab. 16 (model 1) were not substantially influenced by FADS SNPs as potential covariates which was tested in additional models (see Libuda et al. 2016).

Tab. 15: Descriptive outcome variables of FVEP and BSID II in the rapeseed oil (IG-R), fish (IG-F) and control (CG) group at T1[1] and T2[1]

	IG-R	IG-F	CG	
FVEP (mean ± SD)	**(n=47)**	**(n=41)**	**(n=46)**	**p[2]**
T1				
Age [d]	128.0 ± 8.9	127.0 ± 8.7	127.4 ± 8.6	0.96
		Latency (msec)		
Mean left + right eye	123.8 ± 18.2	128.4 ± 17.9	127.7 ± 18.1	0.30
Left eye	123.0 ± 18.4 (n=46)	128.3 ± 18.1	128.2±19.4	0.35
Right eye	124.1 ± 18.5	128.6 ± 19.2	127.2±18.8 (n=45)	0.28
T2				
Age [d]	314.0 ± 8.3	309.3 ± 7.6	312.3 ± 8.5	0.04
		Latency (msec)		
Mean left + right eye	111.5 ± 13.0	111.9 ± 12.6	117.9 ± 18.1	0.07
Left eye	112.3 ± 15.7	111.1 ± 12.6	118.7 ± 18.7 (n=45)	0.03
Right eye	111.3 ± 11.0 (n=44)	113.2 ± 13.0 (n=40)	117.6 ± 18.2 (n=45)	0.14
BSID II (mean ± SD)	**(n=40)**	**(n=39)**	**(n=45)**	**p[2]**
T1				
Age [mo]	171.2 ± 32.9	173.3 ± 29.5	170.8 ± 27.8	0.75
MDI	97.7 ± 7.9	94.8 ± 11.2 (n=37)	98.2 ± 8.5	0.33
PDI	93.2 ± 10.0 (n=37)	89.9 ± 10.4 (n=37)	94.4 ± 9.4 (n=41)	0.12
T2				
Age [mo]	315.9 ± 10.6	320.6 ± 13.6	320.2 ± 19.6	0.19
MDI	99.1 ± 9.3	98.7 ± 10.9	96.8 ± 8.8	0.78
PDI	100.4 ± 7.9	99.8 ± 9.2	100.2 ± 9.3	0.83

[1]T1 BSID II = dependent on introduction of complementary food: between the 5th and 7th month of age, FVEP = 4 months of age ± 2 weeks; T2 BSID II and FVEP = 10 months of age ± 2 weeks. P< 0.05 was considered significant.
[2] Kruskal-Wallis test
BSID II: Bayley Scales of Infant Development II; FVEP: flash visual evoked potentials; MDI: Mental Developmental Index; PDI: Psychomotor Developmental Index

Tab. 16: Intervention effects on fatty acid composition (erythrocyte glycerophospholipids, % wt./wt. (n= 158 infants))

	IG-R vs. CG		**IG-F vs. CG**	
	Erythrocytes phospholipids (RBC)		**Erythrocytes phospholipids[1](RBC)**	
	LS-Means	p	LS-Means	p
DHA	4.5 vs. 4.5	0.85	**6.2 vs. 4.6**	**0.0001**
EPA	**0.33 vs. 0.27**	**0.0001**	**0.64 vs. 0.29**	**0.0001**
AA	15.6 vs. 15.4	0.348	**14.5 vs. 15.3**	**0.007**
ALA	**0.14 vs. 0.11**	**0.0001**	0.11 vs. 0.11	0.564
LA	**12.7 vs. 13.4**	**0.002**	**13.0 vs. 13.5**	**0.027**
∑n-3 LC-PUFA	6.8 vs. 6.5	0.239	**9.1 vs. 6.7**	**0.0001**
∑n-3 PUFA	7.0 vs. 6.6	0.177	**9.2 vs. 6.8**	**0.0001**
Ratios				
∑n-3LC-PUFA: ∑n-6 LC-PUFA	0.31 vs. 0.30	0.267	**0.47 vs. 0.32**	**0.0001**
∑n-3 PUFA: ∑n-6 PUFA	0.20 vs. 0.19	0.057	**0.28 vs. 0.19**	**0.0001**
ALA:LA	**0.011 vs. 0.008**	**0.0001**	0.009 vs. 0.008	0.158
EPA:AA	**0.12 vs. 0.11**	**0.0004**	**0.15 vs. 0.11**	**0.0001**

Differences were tested by ANCOVA generally including group affiliation and the baseline value of the respective outcome variable as exposition variables; [1]Models further included breastfeeding at birth as additional exposition variable (information on breastfeeding at birth was not available for one subject); interactions of study group affiliation with sex, SES, number of children, and FADS SNPs were tested on the primary outcome variable RBC-DHA in the basic model; No interactions were observed accept for effects of the fish intervention in subjects with high SES (p= 0.002) and in first born children (p= 0.02).
LS-Means: Least square means; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; AA: arachidonic acid; ALA: alpha-linolenic acid; LA: linolenic acid, LC-PUFA: long-chain polyunsaturated fatty acids
[Modified according to Libuda et al. 2016]

At T2, a shorter latency for the mean of both eyes was found for the IG-R compared to CG ($p < 0.05$) (final model, tab. 17). In the IG-F the latency was significantly shorter for the left eye ($p = 0.03$) compared to CG (final model, tab. 17). No differences in Bayley's MDI or PDI values were found between the groups at T2 (final models, tab. 17). Consideration of exact age at T2 did not substantially change results of former models (final model, tab. 17).

Tab. 17: Intervention effects on FVEP and BSID II outcomes in the rapeseed (IG-R) vs. control (CG) group and fish group (IG-F) vs. CG[1] at T2, adjusted for T1

		IG-R vs. CG			IG-F vs. CG		
	Model	**LS-Means (95% CI)**	**Difference between means (95% CI)**	**p[2]**	**LS-Means (95% CI)***	**Difference between means (95% CI)**	**p[2]**
FVEP latency[3,4] [msec]							
Mean left + right eye	A	111.5 (106.9-116.0) vs. 118.0 (113.3-122.6) (n=93)	-6.5 (-12.9-0.0) (n=93)	0.050	111.9 (107.0-116.8) vs. 117.9 (113.3-122.6) (n=87)	-6.0 (-12.8-0.7) (n=87)	0.078
	B	111.4 (106.8-116.0) vs. 118.0 (113.4-122.6) (n=93)	-6.6 (-13.1- -0.02) (n=93)	0.049	111.9 (107.0-116.8) vs. 117.9 (113.3-122.6) (n=87)	-6.0 (-12.8-0.7) (n=87)	0.080
	C						
Left eye	A	112.3 (107.3-117.3) vs. 118.7 (113.6-123.8) (n=92)	-6.4 (-13.5-0.8) (n=92)	0.080	111.1 (106.1-116.1) vs. 118.7 (113.9-123.5) (n=86)	-7.6 (-14.5- -0.7) (n=86)	0.032
	B	111.8 (106.7-116.9) vs. 118.7 (113.6-129.9) (n=91)	-7.0 (-14.2-0.3) (n=91)	0.061	111.1 (106.1-116.1) vs. 118.7 (113.9-123.5) (n=86)	-7.6 (-14.5- -0.6) (n=86)	0.033
	C	112.0 (106.9-117.1) vs. 118.9 (113.9-124.0) (n=89)	-7.0 (-14.2-0.02) (n=89)	0.060			
Right eye	A	111.3 (106.7-115.8) vs. 117.6 (113.1-122.0) (n=89)	-6.3 (-12.7-0.1) (n=89)	0.052	113.2 (108.1-118.2) vs. 117.6 (112.8-122.3) (n=85)	-4.4 (-11.3-2.5) (n=85)	0.208
	B	111.2 (106.6-115.8) vs. 117.6 (113.1-122.2) (n=88)	-6.5 (-12.9-0.02) (n=88)	0.051	113.2 (108.1-118.3) vs. 117.5 (112.7-122.4) (n=84)	-4.3 (-11.3-2.7) (n=84)	0.225
	C						

		IG-R vs. CG			IG-F vs. CG		
BSID II[3,5]	**Model**	**LS-Means (95% CI)**	**Difference between means (95% CI)**	**p**[2]	**LS-Means (95% CI)**	**Difference between means (95% CI)**	**p**[2]
MDI	A	99.1 (96.2-101.9) vs. 96.8 (94.2-99.5) (n=85)	2.2 (-6.1-1.7) (n=85)	0.260	98.7 (95.5-101.8) vs. 96.8 (93.9-99.8) (n=84)	1.8 (-6.1-2.4) (n=84)	0.400
	B	99.1 (96.3-101.9) vs. 96.8 (94.1-99.5) (n=85)	2.3 (-6.2-1.6) (n=85)	0.247	99.3 (96.1-102.5) vs. 96.7 (93.8-99.6) (n=82)	2.6 (-7.0-1.8) (n=82)	0.241
	C						
PDI	A	100.4 (97.6-103.1) vs. 100.2 (97.6-102.8) (n=85)	0.2 (-3.4-3.6) (n=85)	0.936	99.8 (96.9-102.8) vs. 100.2 (97.5-102.9) (n=84)	-0.4 (-4.4-3.6) (n=84)	0.936
	B	100.3 (97.4-103.1) vs. 99.9 (97.2-102.6) (n=78)	0.3 (-4.3-3.6) (n=78)	0.860	100.1 (97.0-103.2) vs. 99.7 (96.7-102.6) (n=78)	0.4 (-4.8-3.9) (n=78)	0.850
	C	98.6 (95.7-101.5) vs. 99.8 (97.1-102.5) (n=78)	-1.2 (-5.0-2.7) (n=78)	0.552	98.9 (95.7-102.2) vs. 98.9 (95.9-101.9) (n=78)	-0.03 (-4.3-4.3) (n=78)	0.991

Model A included the group affiliation as exposition variable; Model B included group affiliation and the baseline value (T1) of the respective outcome variable as exposition variables; Model C included group affiliation, the baseline value (T1) of the respective outcome variable plus covariates as exposition variables. Tested covariates (Model C) were: FVEP: sex, head circumference at T2, vigilance (awake, sleepy), exact age at T2, exact age at introduction of study food, length of intervention, breastfeeding at 2 months; MDI, PDI: sex, gestational age, birth weight, head circumference at T2, birth length, caesarian, maternal social status, breastfeeding at 2 months, exact age at T2, exact age at introduction of study food, length of intervention, number of children (first born vs. not-first born). Model C was only calculated if at least one of the tested covariates was significantly associated with the respective outcome.

[1]T1 BSID II = dependent on introduction of complementary food: between the 5^{th} and 7^{th} month of age, FVEP = 4 months of age ± 2 weeks; T2 BSID II and FVEP = 10 months of age ± 2 weeks. P< 0.05 was considered significant.[2]Tested by ANCOVA; [3]Interactions (p< 0.05 considered significant) with the group affiliation tested for sex (FVEP, BSID II), maternal social status (BSID II), breastfeeding at 2 months (FVEP, BSID II) and number of children (first born) (BSID II). No significant interactions between the group comparisons were observed for the tested variables.[4]The significant covariate head circumference at T2 was included into the final model of IG-R vs. CG, left eye. No significant influence of the tested covariates in comparison of the IG-R vs. CG, right eye, mean left+right eye and IG-F vs. CG, all outcome variables, was found. IG-R vs. CG left eye: n= 2 no value head circumference, n= 1 no baseline value (T1), n= 1 no T2 value; right eye: n= 1 no baseline value (T1), n= 4 no T2 value. IG-F vs. CG left eye: n= 1 no T2 value; right eye: n= 1 no baseline value (T1), n= 2 no T2 value. [5]MDI: No significant influence of the tested covariates in comparison of the IG-R or IG-F vs. CG; PDI: significant covariates (breastfed at 2 months, exact age at T2) were included into the final model of IG-R vs. CG; the significant covariate breastfed at 2 months was included into the final model of IG-F vs. CG. IG-R vs. CG PDI: n= 7 no baseline value (T1). IG-F vs. CG MDI: n= 2 no baseline value (T1); PDI: n= 6 no baseline value (T1).

LS-means: Least-square means; BSID II: Bayley Scales of Infant Development II; FVEP: flash visual evoked potentials; MDI: Mental Developmental Index; PDI: Psychomotor Developmental Index

5 Discussion

5.1 Synopsis of research results

The key aims of this thesis were tripartite

- to evaluate the status quo of the used foods in products for CF available on the German market and in CF meals used in dietary practice with respect to food variety, in particular vegetable variety
- to evaluate the status quo of fish and rapeseed oil consumption and its determinants in German infants and their mothers
- to evaluate whether the (LC-)PUFA supply in the CF period can be optimised by 2 feeding approaches, rapeseed oil (rich in ALA) and fish (rich in DHA), and whether these approaches affect the LC-PUFA status and cognitive and visual development in the second 6 months of life.

The market survey showed that mainly carrot was used in commercial complementary vegetable-potato-meat/fish meals offered in 2012 in Germany. Compared to meat-containing meals, the offer of fish-containing meals was low. No pure vegetable meals were offered in junior age and there were no pure fish meals on the market. (Q1a) Regarding dietary practice, results revealed that vegetable variety in commercial as well as homemade complementary meals is low in Germany (Q1b). Differences between infants fed homemade or commercial meals were only observed at the age of 12 months with those fed commercial meals getting a higher vegetable variety compared to infants fed with homemade meals. In addition, fish was rarely offered in commercial and homemade meals in dietary practice in infancy. This observation was also confirmed in the nationwide consumer survey, revealing that only 1 in 4 German infants met the recommendation to eat fish in 2010 (Q2). In contrast, rapeseed oil was used by about 50% of the mothers in Germany for CF. While maternal eating behaviour was the main predictor of infant’s fish consumption, infant’s rapeseed oil consumption was as well influenced by further factors like the social status of the mother.

The intervention trial PINGU revealed that the replacement of corn oil by rapeseed oil increased the RBC EPA, but not the DHA levels. The replacement of meat by fish (salmon) increased the RBC DHA levels (Q3) compared to the control group (corn oil) after 4-6 months of feeding. Similar effects were found for plasma levels. Both, rapeseed oil and fish consumption yielded slightly shorter FVEP latencies compared to the control group, but no effects on cognitive development (Q4).

5.2 Strengths and weaknesses of the data

5.2.1 Food variety in CF

The data base **Nutrichild** provides the opportunity to compare all labelled ingredients and nutrients within the total of commercial baby products on the German market. However, there is no information on exact food amounts in a meal, i.e. no exact vegetable amounts (Q1a). This disguises the most prominent vegetable in a meal which biases the exact assessment of variety.

The repeated collection of 3-day weighed dietary records in the **DONALD** study provides a direct and prospective assessment of the dietary habits from infancy to adulthood. The longitudinal structure is a further strength of the DONALD study as it enables to use existing and ongoing dietary data (from more than 25 years) for assessing nutritional intake and consumption patterns of foods and food groups. For investigating the vegetable variety in the second six months of life, the regular and close collection of the dietary records at 6, 9 and 12 months facilitated the assessment of age-specific vegetable consumption. Compared to e.g. a 24-hours dietary record, the 3-day dietary records allowed generating a vegetable variety score to assess variety offer over time. A limitation of the 3-day weighed dietary records is that consumption of specific foods like fish might be underestimated as such foods are likely to be less often consumed than meat or potatoes in this age group (Hilbig 2011). In general, data validity in the DONALD study proved to be high as e.g. the protein intake in preschool children calculated from a 1-day dietary record agreed well with nitrogen excretion of the 24-hours urine sample (Bokhof et al. 2010). A limitation of the DONALD study is the high socioeconomic status of the sample which hampers a reasonable comparison to the general German population (Kroke et al. 2004). It was shown that adults in higher-income households have higher varied diets than those in low income households (Worsley et al. 2003). Thus the observed low food variety in this thesis may be even overestimated.

A limitation of the VegVS is that meals with more than 1 vegetable with meaningful amounts increased the score by more than 1 point (above the 10 g cut-off) as it was impossible to objectively select the taste dominating vegetable in a homemade or commercial meal. Thus, intra-meal variety was also included in the VegVS although vegetable variety is recommended between meals (Q1b).

Taken together and despite some limitations, Nutrichild and the DONALD study provide comprehensive valuable data base to assess both, the German market offer and food choice in infancy by parents.

5.2.2 Optimisation of (LC-)PUFA intake in CF

The **PINGU** study is the first true-to-life study of food-based interventions conducted in the CF period that allowed assessing both, the effects on (LC-)PUFA - particularly DHA - status and on cognitive and visual development. Due to the incorporation of two intervention approaches, it was possible on the one hand to verify the results of the previous study DINO (PUFA supply via rapeseed oil) and on the other hand to examine the effects of preformed LC-PUFA via fish. Fish and rapeseed oil can easily be implemented into dietary practice in infancy via commercial as well as homemade complementary meals. Rapeseed oil is, in contrast to fish, already common in CF in Germany (compare results of Q1, Q2; Grube 2009). Although the meals used in the study were already on the market and both intervention strategies were integrated in typical current infant feeding practice in Germany under real life conditions, transfer of the results to the general population is limited. Limitations are the high maternal social status and the regional focus of the study.

As part of the PINGU-study, the nationwide consumer survey revealed consumption habits of fish and rapeseed oil and their determinants in almost 1,000 German mother-child dyads. The online-based conduction simplified and shortened the data collection as e.g. no study personnel were needed. However mothers without internet access could not be included. A limitation of online questionnaires is the uncontrollable issue that the questionnaire might not have been filled in by the target person. This risk, however, has been reduced by falling back on an existing panel regularly contacted to participate in consumer surveys. In addition, there might have been a recall bias in participants with a longer recall period. Only maternal characteristics were considered as they are the main caretakers of their infants (Lawrence et al. 2009). A further limitation of this survey is the cross-sectional design (Stimming et al. 2015). Hence the identification of causalities between potential determinants and dietary behaviour was not possible. However, the study design enabled identifying vulnerable groups with low fish and rapeseed oil consumption in infancy.

A strength of assessing the **FA status** was the measurement of the FA pattern in both, plasma and RBC, which reflects the short-term as well as the longer-term supply and the endogenous status (Katan et al. 1997; Sun et al. 2007). In addition, it was possible to adjust for FADS genotype that may explain differences in infants' FA status in the groups.

Due to the higher as expected drop-out-rate of around 20% the aimed sample size (n= 171) for the primary outcome DHA was not reached. However, significant differences in the FA status were determined between the groups (Q3). In general, recruitment of mother-child-dyads was very difficult. Compared to the preceding study DINO in which 1 in 7 addressed women in childbed agreed to take part, twice as many women had to be contacted for the PINGU-study (Mesch et al. 2013). The main reason for drop-outs was the unavailability of the participants in all study groups. Therefore it can be assumed that drop-out may not have biased the findings. As the parents were not able to track the effects of fish and rapeseed oil consumption in CF on the **visual and cognitive assessment**, it is unlikely that those who did not complete the study stopped study participation due to the absence of beneficial effects. In addition, mother-child-dyads were randomly assigned and in the IG-R and CG blinded to their group affiliation.

A limitation of the study is that the evaluation of **functional outcomes** was part of an exploratory data analysis (no *a priori* power calculation) (Q4). *Post hoc* power calculation showed that the sample size was too small for a sufficient statistical power for the functional outcomes (power of visual variables ranged from 0.23 to 0.67; for cognitive variables from 0.23 to 0.82 (α= 0.05, f^2= calculated accordingly; G*Power)). Higher sample sizes might have yielded more distinct results for the **FVEP** outcomes and probably any beneficial effects on cognitive development (Q4) (Meldrum et al. 2011; Gould et al. 2013). Nevertheless plausible and favourable tendencies were found.

The instrument used for assessing cognitive development in the study may have not been sensitive enough to measure benefits of (LC-)PUFA supply in infancy (Taylor, McCulloch 1992; Gould et al. 2013). Although the **BSID** test is an established instrument for cognitive assessment (Colombo 2001) and was used in many studies examining LC-PUFA supplementation effects (Bouwstra et al. 2003; Heird, Lapillonne 2005), the instrument provides only global indicators of cognitive functioning (Cheatham et al. 2006; Colombo, Carlson 2012) and measures no specific area of cognition, like attention or information processing. Hence, it seems unlikely to determine dietary effects with this instrument (Taylor, McCulloch 1992). In addition to the BSID test, several other instruments were used in previous studies (McCann, Ames 2005), mostly the Fagan test of infant intelligence (Bouwstra et al. 2003), which assesses infant's interest in novelty, i.e. information processing (Fagan III 1983). Other researchers used non-standardised laboratory methods like the free-play paradigm (Harbild et al. 2013) which provides an opportunity to measure the development of attention shown to correlate positively with vocabulary memory in childhood (Kannass, Oakes 2008). More distinct results might be achieved by the combination of psychophysiological and electrophysiological measurements (Colombo 2001) by using e.g. brain imaging techniques (Thomas 2003).

Besides the **FVEP** method, which measures the maturation of the visual pathways (Faldella et al. 1996), previous studies mainly investigated visual acuity (Heird, Lapillonne 2005), which might be a more distinct method to assess visual development. However, it was stressed that also this instrument is not proper enough to measure clinically meaningful outcomes (Chambers et al. 2013). Thus, there is currently still no ‘gold standard’ for investigating (LC-)PUFA effects on visual as well as on cognitive development in infancy although almost 10 years ago Cheatham et al. (2006) already claimed essential comprehensive research on adequate instruments to measure such effects in infancy and beyond.

It is not only the issue to choose the adequate test methods, but also to decide at which age dietary effects on neurodevelopment should be examined. Some investigators doubt that differences between LC-PUFA supplemented and unsupplemented groups of term infants are detectable before 24 months of age (Jong et al. 2010). Measuring e.g. effects on visual acuity might even be most relevant only after 7 years of age (Chambers et al. 2013). And even if differences were found in infancy, the issue remains of how clinically meaningful they are and whether they are predictive of later cognitive or visual function (Chambers et al. 2013). Thus, long-term follow-ups are necessary for assessing effects.

During the first year of life, medical, environmental and social factors play an important role in development (Largo et al. 1990; Dietrich et al. 2005). The PINGU study did not record factors like the (continuous) cognitive stimulation provided by parents (Yarrow et al. 1972), other caretakers or environment (e.g. day-care facilities), which might have biased results. However, randomisation should have prevented biased results.

There might be other nutrients beside from LC-PUFA such as iodine or iron which could have an impact on infants’ development but potential differences should have been minimised by the randomisation per se. In the study food of IG-R and CG the content of nutrients which do not derive from the vegetable oil was identical.

5.3 Consequences for dietary recommendations on food variety in CF

5.3.1 The CF period as a 'window of opportunity'

Poor food choices in adulthood that contribute to emergence of chronic diseases like obesity or diabetes may be associated with little food and flavour experiences early in life (Mennella 2014). Emerging research indicates that food choices, e.g. the offer of a range of different foods with different sensory properties, made by parents and caregivers in early childhood (Nicklaus et al. 2005b; Mennella 2014) in combination with genetic factors (Scaglioni et al. 2011) can influence shaping preferences and thus food habits later in life (Singer et al. 1995; Skinner et al. 2002b; Nicklaus et al. 2004; Nicklaus et al. 2005a; Nicklaus 2009; Lioret et al. 2013; Lange et al. 2013; Mennella 2014). It is suggested that after the age of 3-4 years these habits remain almost stable (Singer et al. 1995; Mennella 2014) which underscores the assumption that the early life period is crucial for promoting healthy eating. As food neophobia (rejection of unfamiliar foods) and "picky eating" appear mainly around 19-24 months of age (Carruth et al. 2004), increase afterwards (Carruth et al. 1998) and as infants accept new foods more readily than children (Nicklaus 2011), the CF period might especially be a favourable period of time to establish healthy eating patterns (Sullivan, Birch 1994; Birch et al. 1998; Dovey et al. 2008; Maier et al. 2008; Nicklaus 2009). After exclusive milk-feeding in the postnatal period, the CF period is the first stage of life where the infant gets to know novel food textures and flavours that will be also part of the adult diet. Thus, offering a great variety of foods in CF may be a measure to establish long-term preferences.

Implementing a variety of foods into the diet might not only contribute to healthy eating patterns and prevent diseases like obesity but may at the same time result in the contrary as a high food variety within a meal was in general shown to enhance food intake (Rolls et al. 1981; Rolls et al. 1984; Stubbs et al. 2001), probably due to higher pleasure of eating (Nicklaus 2009). However, obesity was primarily linked to a high variety in energy-dense foods like sweets or snacks, but not to vegetables (McCrory et al. 1999; Nicklaus 2009). Overall only few studies investigated the impact of a high varied diet on the development of obesity (Nicklaus 2009). Infants who had higher weight-for-height percentile values were less often picky eaters and thus showed more likely a varied diet (Carruth et al. 2004). No relationship was shown between food variety and body mass index in children aged 2-3 years who could choose foods without restriction (Nicklaus et al. 2005a). Thus, offering a variety of healthy foods in infancy may contribute to the preference for these foods later in life.

Up to now, studies examining the impact of food variety in total in CF on later dietary behaviour have not been conducted yet. Recent studies focused more on the acceptance of single new foods introduced into the diet than on the impact of high food variety in CF on dietary variety later in life (Maier et al. 2008; Mennella et al. 2008; Lange et al. 2013). Lange et al. found that the total number of new foods introduced in the first 2 months of CF influences the acceptance of vegetables ($R= 0.22$, $p= 0.0002$), fruits ($R= 0.15$, $p= 0.04$) and meat ($R= 0.16$, $p= 0.02$) till 15 months of age (Lange et al. 2013). Investigating long-term consequences of free food choices at 2 to 3 years showed an association with food variety (vegetables, dairy, meat products (at 16 years of age)) in the diet of 22 years old adults (Nicklaus et al. 2005a). These results may underscore the importance of setting the foundation for healthy eating habits early in life.

As vegetables are least preferred by children (Nicklaus et al. 2005b; Lange et al. 2013) and consumption is still low in childhood (Yngve et al. 2005; Diethelm et al. 2012), studies of the last decades focused especially on this food type and the impact of early vegetable variety on later food acceptance (Mennella et al. 2001; Maier et al. 2008; Mennella et al. 2008; Coulthard et al. 2010). Compared to the offer of a small selection of vegetables, the exposure to a great vegetable variety especially in the beginning of CF seems to increase acceptance of new vegetables until 15 months of age (Lange et al. 2013). High vegetable variety (3 different vegetables, changed daily over a period of 9 days) at around 5 months of age was shown to improve acceptance of new foods up to 2 months after the exposure ($p< 0.0001$) (Maier et al. 2008).

In 1993 Harris first hypothesised that there might be a ‘sensitive period’ in infancy (between 4 to 6 months) for accepting and willing to eat a wide range of foods that tracks into adulthood (Harris 1993). Vegetables were shown to be accepted easily in the beginning of weaning (Nicklaus 2011), with especially no clear rejection of sour or bitter tasting foods (Schwartz et al. 2011). However, there might not only be one sensitive period for imprinting flavour preferences (Mennella et al. 2011), but rather a timeframe up to the age when picky eating and food neophobia increase (Nicklaus 2011). Picky eating (Carruth et al. 2004) and food neophobia (Cashdan 1994) involve decreasing of food variety (Falciglia et al. 2000; Carruth et al. 2004; Nicklaus et al. 2005c). Studies have shown that vegetable variety scores remain low (Cox et al. 1997; Skinner et al. 1999; Nicklaus et al. 2005b), maybe due to the fact that mothers offer vegetables less often after the age of 12 months (Ahern et al. 2013), maybe also due to appearance of neophobia and picky eating.

5.3.2 Current recommendations on food variety in CF and implementation of vegetable variety in dietary practice

Introducing complementary food is often described as a stressful period by parents and is related to 'fear' or 'concern' (Schwartz et al. 2013). Parents often seem to be frustrated and confused by inconsistencies in recommendations from multiple information sources (e.g. health professionals, family members, internet) (Synnott et al. 2007; Caton et al. 2011; Moore et al. 2012; Libuda et al. 2014). They are faced with a huge offer of complementary products on the market and feel to be pushed to implement authoritative recommendations into daily dietary practice.

After years of discouraging variety of foods in CF, in particular with regard to vegetables, latest German dietary guidelines recommend a high variety in the diet of infants in the second six months of life (Hilbig et al. 2012; Bührer et al. 2014). In the past, guidelines on food choice in CF focused more on nutritional quality (nutrients) and on allergy prevention[1] than on imprinting of preferences. Now, the recommendation also includes the use of fish, mainly fatty fish like salmon as a potential supplier of (LC-)PUFA during a period of decreasing LC-PUFA intake (Schwartz et al. 2009). However, taking a closer look at the current German recommendations, a precise definition of food variety is not fixed (Bührer et al. 2014; Forschungsinstitut für Kinderernährung 2013; Hilbig et al. 2012) which allows scope for interpretation. It is only recommended that complementary food should be varied to support favourable taste imprinting and acceptance of new foods (Bührer et al. 2014; Forschungsinstitut für Kinderernährung 2013; Hilbig et al. 2012). Vegetable variety is described as alternately offering vegetables, e.g. carrot, cauliflower, broccoli, in the recommendations (Hilbig et al. 2012).

As vegetable variety in infancy has not been recommended for many years in Germany it is not surprising that German infants currently do not get a great vegetable variety (Q1b). This thesis focused on vegetable variety in commercial and homemade complementary foods for German infants aged 6-12 months (Q1b) as vegetable consumption in toddler-age (Hilbig et al. 2011) and childhood (Mensink et al. 2007; Currie et al. 2012) is far below the recommended amounts in Germany.

[1] Former assumption: high dietary variety leads to an allergy

Commercial (CCM) and homemade (HCM) complementary meals are both recommended in CF in Germany (Forschungsinstitut für Kinderernährung 2013) and choice is left to mothers who may ponder advantages and disadvantages of each of them by their personal preferences.

It is suggested that HCM provide a broader range of different flavours and textures than CCM (Koletzko et al. 2013). European mothers stated that HCM are more ‘tasty’, ‘natural’ and easier to digest than CCF (Synnott et al. 2007; Schwartz et al. 2013). In contrast, CCM was appraised as ‘non-authentic’ and depicted to convey a false, uniform and bland taste (Coulthard et al. 2010; Schwartz et al. 2013) and to hardly vary in fruits or vegetables in particular (Coulthard et al. 2010). In contrast to French mothers, German mothers do not seem to first focus on taste when taking decisions on infant’s diet but rather on caution (allergy) (Maier et al. 2008; Schwartz et al. 2013). As CCM are more often used than HCM in Germany (59.3% commercial vs. 21.1% homemade) (Foterek et al. 2014), research should not only focus on the impact of vegetable variety itself, but also on the preparation method in CF. Coulthard et al. showed that infants fed homemade fruit or vegetable meals ate more fruits and vegetables with a greater variety at 7 years compared to infants fed CCM ($p< 0.001$; fruit: adjusted $R^2= 0.03$, $\beta= 0.09$; vegetable: adjusted $R^2= 0.04$, $\beta= 0.14$) (Coulthard et al. 2010). Recently, a prospective study revealed that German boys with a high proportion of CCM in their diet at 6-12 months have a lower vegetable intake in preschool age (3-4 years, $p= 0.036$) (Foterek et al. 2015). In addition, a high proportion of CCM predicted a low vegetable variety in school age (6-7 years, $p= 0.029$) (Foterek et al. 2015).

5.3.3 Green and bitter tasting vegetables in CF

Green vegetables were scarcely used in HCM as well as CCM (Q1a, b) which automatically reduces the abundance of different flavours. Carrot and tomato were the predominant vegetables in both, HCM and CCM (Q1a, b). Rarely offering green vegetables might be linked to the fact that orange vegetables (Gerrish, Mennella 2001) seem to be easier implemented into infant’s diet (Mennella et al. 2008). This might not only be related to the mothers’ suggestion that infants are already colour sensitive and do not like green vegetables (Schwartz et al. 2013), but also to mother’s own preferences for vegetables that predict vegetable variety in children (Fisher et al. 2002; Skinner et al. 2002b; Cooke et al. 2004; Hart et al. 2010; McGowan et al. 2012). Mothers hesitate to introduce vegetables that they do not like themselves (Schwartz et al. 2013). In addition, they offer those vegetables more often, thought to be more readily accepted by their children (Wardle et al. 2001; Cooke und Wardle 2005). Likewise, there seems to be a low consumer acceptance of CCM in green colours as product colour is linked to the expected flavour in the minds of consumers and might affect the product perception and acceptance (Garber et al. 2000; Ares und Deliza 2010). The high frequency of using carrot is obviously also linked to the sweet taste of the vegetable, which is preferred by humans (Birch 1999).

In this thesis, total vegetable variety at a first glance seemed lower in CCM than in HCM (16 vs. 26 different vegetables offered). However, in dietary practice the vegetable variety score even revealed a higher variety in CCM than in HCM at 12 months of age (Q1b). In contrast, in an American study fruit and vegetable variety was higher in CCM than in HCM for the entire CF period (6-12 months, Hurley, 2010). This might be caused by the fact that CCM consisted on average of more than 2 different vegetables per meal (Q1b). However, variety of vegetables should be offered across meals, i.e. a single vegetable per meal, and not by mixing vegetables in one meal as the particular taste of a single vegetable might be masked by another one (Caton et al. 2011). As a bitter taste is innately rejected by infants (Mennella, 2008), it might be especially advantageous to offer bitter tasting vegetables like Brassica alone in one meal with a familiar flavour (Hausner et al. 2012) and by repeated exposure (8-10 exposures) (Birch et al. 1987; Sullivan, Birch 1994; Maier et al. 2007; Lakkakula et al. 2010; Anzman-Frasca et al. 2012; Caton et al. 2013) to get infants familiar with these flavours. In general, all types of Brassica, like cabbage, broccoli or cauliflower, are suggested to cause gastro-intestinal problems in infants by mothers (Schwartz et al. 2013) which results in avoiding these vegetables. For instance, cauliflower was not even used in 10% of the commercial or homemade meals in Germany (Q1b). However, German dietary recommendations for the first year of life do not support the exclusion of such vegetables as there is not enough evidence that confirms a relation between consumption and gastro-intestinal problems (Scott et al. 2012). On the contrary, Brassica vegetables should be part of infant’s diet as they possess strong anti-inflammatory and antioxidative abilities (Scott et al. 2012). However, most mothers give up offering an initially disliked vegetable too early (Maier et al. 2007; Vereijken et al. 2011; Schwartz et al. 2013), especially when trying to implement bitter-tasting foods (Forestell, Mennella 2007).

Taken together, food variety, especially vegetable variety, in CF and the long-term effects on dietary habits is a complex framework which rests upon current recommendations and their implementation in dietary practice, the product offer on the market, parents' behaviour (e.g. time of introduction of complementary food, feeding style, preparation method), environmental influences (e.g. health professionals, media) and the preferences of parents and infants (genetically or environmentally driven). A long-term favourable impact of early food variety in CF on later dietary patterns is currently not fully understood as prudent follow-up studies are scarce. However, it seems reasonable setting the foundation of food preferences and habits early in life as they tend to be fixed before 3 years of age and might track into adulthood (Nicklaus et al. 2005a; Caton et al. 2014).

5.3.4 Suggestions for achieving food variety in CF

Currently, German recommendations on food variety in CF are presented in a vague manner. Thus, health professionals may give non-specific and inconsistent advices which may lead to confused parents who decide to act on instinct (Synnott et al. 2007; Vereijken et al. 2011; Schwartz et al. 2013). Therefore, distinct recommendations on variety and their implementation into dietary practice are necessary. However, changing behaviour of parents / caregivers will take time as it remains always the general problem of providing dietary advice (Kannan et al. 1999; Sellen 2001; Foote, Marriott 2003).

Table 18 summarises suggestions for achieving food variety in the CF period with respect to the current situation in Germany.

In addition to these suggestions, other European mothers like French mothers could be a role model for offering vegetable variety. In contrast to mothers in Germany, French mothers offer a quite high variety to their infants (e.g. on average 38 different vegetables until 15 months of age) (Lange et al. 2013). For French mothers infant's taste experience and development is primarily important, whereas German mothers currently focus more on safety (allergy prevention, suggested to be related to low food variety) (Maier et al. 2007; Schwartz et al. 2013).

Tab. 18: Suggestions to achieve food variety in infancy

1. Contents of prospective dietary recommendations
• **Defining** the term **'food variety'**, especially 'vegetable variety', in CF with a focus on **healthy foods** (i.e. nutrient-dense, low-energy, foods like fruits and vegetables), considering the current knowledge base of importance for (later) dietary habits **('sensitive period')**
• **Explaining** of how to **achieve variety**: repeated exposure (for disliked or rejected foods), changing food offer, within and between meals, introducing a **'rainbow' of vegetables**, offering a single vegetable in a meal with an already familiar flavour / food
• **Promoting** of at least sometimes **homemade complementary meals** with practical advice (e.g. preparation of more unfamiliar foods like fish)
• **Discussing alternatives** like ready-to-use frozen or cook & chill meals that are available on niche markets which might provide a more 'original' taste than sterilised meals in jars
2. Transfer of guidelines to health professionals
• Continuously updated to **communicate new recommendations**, prevent confusion of parents, explain how to implement recommendations in a **practical way**
• Explain the different developmental ('sensitive') periods of acceptance of foods and the **'specific world' of flavours in CF**
• Give **practical recommendations for implementation** of a great variety (e.g. if a vegetable is disliked, offering an already familiar flavour / food in a meal with the disliked vegetable)
• Explain **advantages and limitations** of preparation methods: homemade vs. commercial meals
• Support a potential **long-term effect**: including foods as part of the family's diet
3. Baby food companies
• Offer a greater vegetable variety between meals; **green and Brassica type vegetables**, especially for the introduction period of CF

5.4 Optimisation of (LC-)PUFA intake in CF

The EFSA recommends a daily Adequate Intake (AI) of 100 mg DHA from 0 to 12 months of age (EFSA 2013). However, the authority notes that a final evident recommendation on LC-PUFA supply in infancy is currently not possible as beneficial effects of LC-PUFA supplementation early in life on development are not distinctly shown (SanGiovanni et al. 2000; Lauritzen et al. 2001; Simmer 2001; McCann, Ames 2005; Eilander et al. 2007; Agostoni 2008; Hoffman et al. 2009; Beyerlein et al. 2010; Campoy et al. 2012; Qawasmi et al. 2012; Gould et al. 2013; Kuratko et al. 2013; Qawasmi et al. 2013; Janssen, Kiliaan 2014). While some reviews and meta-analyses concluded that LC-PUFA supplementation via formula positively affects visual development of infants (Eilander et al. 2007; Hoffman et al. 2009; Qawasmi et al. 2013), others (SanGiovanni et al. 2000) as well as Cochrane-analyses (Simmer 2001; Simmer et al. 2011) did not support these results. Regarding cognitive development, reviews, meta- and Cochrane-analyses mostly revealed no impact of LC-PUFA supplementation in infancy (Qawasmi et al. 2012; Beyerlein et al. 2010; Simmer et al. 2011; Hoffman et al. 2009; Gould et al. 2013). Authors explained that assessing existing studies is difficult as there are methodological inconsistencies between studies with differences e.g. in LC-PUFA sources, dose of FA, FA ratio, study sample sizes, intervention periods, time of examinations and assessment instruments (Eilander et al. 2007; Campoy et al. 2012; Qawasmi et al. 2012; Gould et al. 2013). The currently still incomplete and partly divergent recommendations of authorities and institutions like the EFSA or DGE[2] show that there is a need for tailored research on LC-PUFA intake and supply in term infants, especially for the single FA per se as well as their ratios in the diet. In total, the indistinct data situation mirrors the basic question which specific developmental functions LC-PUFA might be able to modulate and when manifested benefits are reasonably detectable (Meldrum et al. 2011).

The intake of DHA, in particular important during brain ‘growth spurt’, decreases in the second 6 months of life from on average of 34 mg at 6 to 19 mg DHA/d at 9 months of age in the diet of German infants (Schwartz et al. 2010) which is far below the recommended daily intake of 100 mg DHA. Breast milk or supplemented formula are the main suppliers of DHA throughout the first year of life (Schwartz et al. 2009). However, these are partly replaced by complementary food which usually is low in

[2]DGE: no recommendation on intake of ARA or DHA for infants

DHA (Schwartz et al. 2009). Thus, two different approaches to optimise (LC-)PUFA supply during the CF period (via rapeseed oil and fish) were investigated in this thesis.

5.4.1 Impact of optimised ALA intake via rapeseed oil on DHA status

Rapeseed oil in complementary food may be a potential source to optimise LC-PUFA supply in infants as it has a favourable balance of LA and ALA (Gibson et al. 2011) and is common in dietary practice in Germany. LA and ALA can be converted to LC-PUFA endogenously, even though not in large amounts (Poisson et al. 1993; Salem et al. 1996). In adults, fractional conversion rates of ALA to DHA are estimated to be between < 0.05% (Burdge et al. 2003) and 4% (Emken et al. 1994), dependent e.g. on the composition of the meal used as carrier for the labelled ALA tracer, the gender mix in the study sample or the age (Burdge, Calder 2005). It is suggested that the LA and ALA conversion rate is not sufficient to achieve normal or optimal LC-PUFA levels in tissues (Clandinin et al. 1981; Salem et al. 1996). However, the conversion rate in infants may be more efficient than in adults. Some studies have shown that addition of ALA to infant formula with a constant LA level and without addition of LC-PUFA increased RBC DHA levels in infants compared to the control group (Jensen et al. 1996). The rapid development during infancy may contribute to a higher rate of precursor utilisation (Rise et al. 2013).

In PINGU the replacement of corn oil by rapeseed oil in vegetable-potato-meat meals significantly enhanced the levels of RBC (plasma) ALA and RBC EPA and reduced the RBC LA levels in IG-R compared to the CG, but did not alter DHA levels (Q3). This may be explained by the possible low rate of endogenous DHA formation due to the rate limiting step (DPA to DHA) in the conversion of ALA to DHA (Arterburn et al. 2006) and the high oxidation rate of ALA (Nettleton 1991). According to Libuda et al. only 0.06 mg plasma DHA may be endogenously synthesised, considering the estimated conversion rate from dietary ALA to plasma DHA of 0.04%, a total daily turnover of 16.7 mg plasma DHA in neonates (Lin et al. 2010) and the additional 150 mg/d of ALA provided in IG-R compared to CG (Libuda et al. 2016).

The results of the biomarker levels are in line with other studies in humans that showed higher RBC EPA, but not RBC DHA levels after increased ALA intake (Burdge et al. 2002; Wallace et al. 2003; Goyens et al. 2006; Harper et al. 2006; Zhao et al. 2007; Barcelo-Coblijn et al. 2008; Makrides et al. 2000a). In contrast, infants in the previous DINO study who also received complementary food with rapeseed oil had higher plasma DHA levels than infants receiving corn oil, although higher ALA intake did not cause higher ALA levels (Schwartz et al. 2009). Schwartz et al. hypothesised that the higher ALA amounts in the study food enhanced synthesis of n-3 LC-PUFA although the rapeseed oil amount and thus the ALA amount in the jars was even lower than in the jars used for IG-R in PINGU (0.8 g/100g vs. 1.4 g/100g). In addition, the rapeseed oil group in DINO received significantly higher amounts of meat with the study food which might have affected PUFA metabolism due to provision of meat-derived preformed LC-PUFA.

5.4.2 Impact of optimised DHA intake via fish on DHA status

In the PINGU study infants received twice a week fatty fish (salmon) as a good source of preformed DHA. The following scenario estimates the daily DHA intake of the study population via salmon compared to the other groups during the intervention period. As there is no representative data available on average daily breast milk or formula consumption of infants in Germany, data of the DONALD study was used which is the only German study that provides consumption details in infants.

<u>DHA intake via milk</u>:

a) Mean amount of consumed milk: breast milk or formula between 6 and 10 months: approx. 300 ml per day (Foterek, 2014, not published)

b) Mean content of DHA in breast milk or LC-PUFA enriched formula (FM+) in PINGU: approx. 6.3 mg per 100 ml breast milk and FM+ (Stimming et al. 2014)

Total DHA supply milk: 300ml/d * 6.3 mg DHA/100ml = approx. <u>20 mg DHA/d</u>

<u>Study food</u>:

a) Estimated daily dietary DHA intake <u>IG-F</u> (average of baby and junior meals) between 5 and 10 months of age: approx. <u>50 mg DHA/d</u>

b) Estimated daily dietary DHA intake <u>IG-R and CG</u> (average of baby and junior meals) between 5 and 10 months of age: approx. <u>0.4 mg DHA/d</u>

<u>In total</u>:

IG-F received per day:

milk (formula: supposed that all mothers used FM+ in the study), 20 mg DHA + study food, 50 mg DHA = approx. <u>70 mg DHA</u>.

This is an almost 3-fold increase in DHA intake compared to the IG-R and CG (approx. 20 mg/d). In the DINO trial the average DHA intake was also around 20-30 mg/d between 6 and 9 months of age (low DHA study food + milk) in the IG-R and CG (Schwartz et al. 2010). Thus, with the integration of 2 fish meals per week into the dietary schedule during the CF period, infants are able to achieve almost the AI of DHA. The higher intake of DHA was reflected in the RBC (plasma) DHA status which was significantly higher in the IG-F than in the CG (Q3). However, in contrast to the RBC DHA status of infants in the studies of Hoffman et al. and Makrides et al. (DHA-enriched egg-yolk in complementary food), the DHA status did not increase during the intervention period in PINGU, but maintained constant (Makrides et al. 2002; Hoffman et al. 2004). This might be due to the fact that infants in the study of Hoffman et al. received about twice as much DHA (133 mg DHA/d, study food + milk) between 6 and 9 months of age. Makrides et al. did not itemise the exact DHA intake derived from study food and milk consumption. It should be considered that RBC-DHA levels of infants in the CG of PINGU decreased by more than 20% between the age of 4 and 10 months (from 5.9% to 4.5%).

5.4.3 Effects of rapeseed oil (ALA) and fish (DHA) intake on functional outcomes

The results of PINGU are in line with the mixed results of pre- and postnatal LC-PUFA supplementation (McCann, Ames 2005; Hoffman et al. 2009; Simmer 2011; Qawasmi et al. 2012; Gould et al. 2013, Qawasmi et al. 2013), as well as follow-up studies in childhood (effects on visual development, no effects on cognitive development (Janssen, Kiliaan 2014)). In some studies the addition of rapeseed (Makrides et al. 2000a) or fish oil (Makrides et al. 1995) to formula led to better visual acuity in infancy while studies on cognitive development revealed no impact of these oils on cognitive performance (Auestad et al. 2001; Makrides et al. 2000b; Auestad et al. 2003; Bouwstra et al. 2005).

In PINGU rapeseed oil consumption led to slightly shorter FEVP latencies. Conversion of ALA is supposed to mainly take place in the liver, but also in the brain (Barcelo-Coblijn, Murphy 2009). It is suggested that the majority of dietary ALA (~80%) is transported via plasma to the brain (Barcelo-Coblijn, Murphy 2009) and that astrocytes can synthesise DHA from ALA (Moore et al. 1991) and provide neurons DHA (Moore et al. 1991; Moore 1993). About 70% of the myelin sheath lipids are composed of FA (Chang et al. 2009). With the FVEP method, the maturation of optic pathways and the visual cortex is reflected (Faldella et al. 1996) and hence the myelination of neural tracts. It is supposed that the myelination reflects the functional maturity of neurons, axons and oligodentrocytes that are rich in LC-PUFA (Heird, Lapillonne 2005). Thus, it can be hypothesised that the higher supply of ALA via rapeseed oil compared to corn oil may have favoured higher conversion rates of ALA to DHA in the brain which supported myelination and further enhanced the efficacy of providing information (Cheatham et al. 2006). However, this hypothesis would support doubts that biomarkers like plasma or RBC reflect the FA status in the brain. This thesis revealed that only RBC (plasma) ALA and EPA levels increased in the IG-R compared to CG, but not RBC DHA or plasma levels. No effects were found on cognitive development. In contrast, slightly shorter latencies were determined in the IG-R and IG-F.

Several studies have shown that RBC DHA levels correlate with visual acuity in infants (Makrides et al. 1993; Makrides et al. 1995; Birch et al. 2002; Hoffman et al. 2003; Uauy et al. 2003; Hoffman et al. 2004; Birch et al. 2005; Birch et al. 2010), i.e. the higher the RBC DHA levels, the more mature the visual acuity (Hoffman et al. 2003; Hoffman et al. 2004). In addition, some authors concluded that plasma phospholipid or RBC DHA concentrations can be used to mirror tissue DHA concentrations (Arterburn et al. 2006), i.e. also DHA concentration in brain tissue (Kuratko, Salem 2009) and neural performance (correlation between DHA status and VEP acuity; Birch et al. 1992; Carlson et al. 1993; Makrides et al. 1993; Uauy et al. 2003) while others are uncertain about the extent to which the LC-PUFA concentration in these biomarkers reflects the concentration of these FA in the brain of individuals (Heird, Lapillonne 2005). Animal studies showed that changes in dietary FA composition is reflected in changes in concentration of FA in the brain (Foot et al. 1982; Bourre et al. 1984; Barcelo-Coblijn et al. 2003).

Despite the lower DHA supply via fish in PINGU compared to the supply in the study of Hoffman et al. (Hoffman et al. 2004), small short-term beneficial effects on visual development were determined (although the difference of FVEP latencies between the IG-F and CG was marginal with only about 8 ms (Q4)). It must be scrutinised whether this effect is clinically meaningful. Thus, it is reasonable to examine whether the observed tendencies of shorter FVEP latencies track into childhood as it is supposed that effects at least on visual acuity can only be determined after 7 years of age (Chambers et al. 2013). At this age, clinically meaningful effects like effects on e.g. reading are of special interest. However, up to now, the few existing follow-up studies (infants fed LC-PUFA enriched formula in infancy) in toddlers (Birch et al.

2007; Jacques et al. 2011) and school children (Auestad et al. 2001) showed inconsistent results.

Currently, it is not known, whether a specific level or a threshold of (LC-)PUFA intake is necessary to be reached before a positive effect on cognitive outcomes can be detected (Ryan et al. 2010). It is also not known whether there is an upper level that limits effects of further higher supply of (LC-)PUFA. If the brain DHA concentration is linked to cognitive performance, slight differences in DHA may not be detected by global developmental assessments like the BSID (compare 5.2.2, limitations) which are probably not sensitive enough to determine even slight effects of LC-PUFA intake (Bradbury 2011).

Fatty acid ratios

Interestingly, adding rapeseed oil to formula with a final ratio of LA:ALA of 5:1 increased plasma and RBC DHA levels in infants compared to infants fed formula with a ratio of LA:ALA 10:1, but no difference in VEP acuity was found at 16 or 34 wk of life (Makrides et al. 2000a). Thus, the ratio of LA:ALA in formula or complementary food seems to be primarily crucial for improving the DHA status of infants. It is supposed that a ratio of < 6:1 in formula favours higher DHA levels in infants (Fleith, Clandinin 2005). According to the DGE and EFSA recommendations in the second 6 months of life, the LA:ALA ratio should be 7:1 and 8:1 respectively (compare tab. 1). In the PINGU study, the LA:ALA ratio of the study food was around 4:1 in the IG-R and 24:1 in the CG. The ratio of the total diet is not known. In DINO, the overall LA:ALA ratio in the diet was around 10:1. As the rapeseed oil amount in the jars used in DINO was lower than in those used in the PINGU study (0.8 g/100g vs. 1.4 g/100g), the ratio in PINGU should have been lower overall.

In addition to the assumption, that a higher DHA supply may have revealed more distinct results, also the ARA:DHA ratio could be relevant. Fatty fish, such as salmon, is a good source of preformed DHA, but not of ARA. Currently the optimal nutritional ARA:DHA ratio to support infant’s development is not clear, especially not in CF. A ratio of 1:1 (ARA:DHA) is discussed as sufficient for best visual outcomes in infancy (Qawasmi et al. 2013). For formula feeding, it is generally recommended that the content of ARA should be at least equal to DHA (Koletzko et al. 2008; Koletzko et al. 2014). In contrast to the EFSA recommendation of an AI of DHA for infants aged 0-6 months, no quantitative advice on ARA intake during CF has been provided due to lack of sufficient evidence and in consideration of provision of preformed ARA with usual complementary food (Koletzko et al. 2013; Koletzko et al. 2014). In PINGU, the ARA:DHA ratio in the study food of the IG-F was estimated to be around 1:7 (baby jars) and 1:8 (junior jars). The overall dietary ratio in the groups is not known.

In general, a more favourable ARA:DHA or LA:ALA ratio might have revealed more distinct results.

The results show the complexity and challenges of assessing dietary (LC-)PUFA intake and its effects on FA status and functional outcomes. A pool of factors influences the conversion of n-3 and n-6 precursors to their LC-PUFA, which includes e.g. the LA:ALA ratio, the FADS genotype or gender. In addition, effects of dietary preformed DHA on functional outcomes seem to rely on e.g. the ARA:DHA ratio and the amount of provided DHA with the diet.

Taken together, the results suggest that including fish in the diet of infants in the second six months of life is a promising strategy to maintain the DHA status in infants. If this is effective to improve visual development in infants and – maybe more important – in children is still unanswered and has to be further examined. Up to now follow-up studies on effects of dietary LC-PUFA supply via formula, fed in infancy, did not find differences in cognitive (e.g. IQ scores (at 39 months, Auestad et al. 2003; at age 4 y, Birch et al. 2007; at age 6 y, Willatts et al. 2013) or Hempel and Bayley scales (at 18 months, Bouwstra et al. 2005, Beyerlein et al. 2010; at 24 months, Makrides et al. 2000b) or visual outcomes (e.g. visual acuity (at 39 months, Auestad et al. 2003), compared to children fed formula without LC-PUFA enrichment, in childhood.

In addition, rapeseed oil consumption may also contribute to better visual development. As PINGU was the first study which used ‘natural’ food sources, further investigations have to verify the results.

5.4.4 Feasibility of integration of dietary n-3 (LC-)PUFA sources in infant’s diet

Compared to corn oil, using rapeseed oil in homemade vegetable-potato-meat-meals increased since the 1990ies (Grube 2009). Nowadays, it is – compared to other oils – the most frequently used oil in commercial and homemade complementary meals (Q1, Q2).

The addition of rapeseed oil led to higher RBC EPA, but not to higher DHA levels in infants. The high total level of PUFA (~ 30%) in rapeseed oil (Gibson et al. 2011) might impede the conversion of EPA to DHA as the delta-6 desaturase is also needed for the conversion of ALA to EPA. Blends of oils could be an alternative (Gibson et al. 2011), consisting of mixtures of different plant oils or mixtures of plant oils and DHA rich fish or alga oils, all with a focus on the balance of LA:ALA, ARA:DHA and total PUFA content. Attention has to be paid to the stability of oils with higher amounts of (LC-)PUFA as they are less stable at high temperatures and bear the risk of hydrolysis and oxidation products (Ganesan et al. 2014). The amount of added oil has to be taken into account as the fat content in commercial complementary food is limited by EU law (40 E%). Commercial meals currently contain no more than around 1-3 g oil per jar in Germany, probably resulting from the use of fatty meat. For homemade meals, the addition of 8-10 g oil per meal is recommended (Hilbig et al. 2014). The efficacy of this recommendation or higher oil amounts remains to be evaluated.

Even if early consumption of fish will not be proven to affect developmental outcomes, it supports maintaining DHA levels in the CF period which might be relevant for later health outcomes and protects against e.g. atopic diseases (Muche-Borowski et al. 2009). Currently 20-30 g fatty fish per week is recommended for homemade complementary meals in Germany, i.e. (at least) one fish meal per week (Hilbig et al. 2012; Bührer et al. 2014). In the PINGU study, infants consumed approx. 32 g fish per week with 2 fish meals. Regarding the favourable effects on DHA status, the recommendation should be changed to 2 fish meals per week[3]. However, also with the inclusion of 2 fish meals per week in CF (IG-F: 70 mg DHA/d, milk + study food) the EFSA recommendation of consuming 100 mg DHA/d is not met. As fish is currently not common in infant's diet (Q1, Q2) and consumption is primarily dependent on mother's own eating behaviour, strategies should be developed to encourage mothers to use also fish for the infant in CF. As low n-3 knowledge was associated with lower maternal fish consumption (Q2), extending nutritional knowledge regarding n-3 could be helpful to promote mothers' fish consumption and as a consequence the fish consumption of their infants.

As fish consumption in childhood (Mensink et al. 2007) and adulthood (Max Rubner-Institut 2008) is still lower than recommended in Germany, the early familiarisation of fish might sustainably support the consumption later in life.

A further opportunity to increase LC-PUFA intake in the CF period is the use of egg yolk. Studies investigating the effect of preformed DHA supply via complementary food used DHA-enriched egg yolk, 4 portions per week (Makrides et al. 2002;

[3]includes consideration of natural variation in DHA content of fish

Hoffman et al. 2004). The consumption enhanced the RBC DHA levels in the studies (Makrides et al. 2002; Hoffman et al. 2004) and in one study also improved visual acuity at 9 and 12 months of age (Hoffman et al. 2004). However, high egg yolk consumption might be difficult to recommend in Germany as the majority of the population may be still suspicious of eating too many eggs because of believing in enhanced blood cholesterol levels caused by egg consumption (Gray, Griffin 2009; Fernandez 2010). For homemade preparation of complementary meals, egg yolk is not explicitly recommended e.g. due to hygienic aspects. In addition, DHA-enriched eggs are rare on the German market and standardisation of DHA-enriched chicken feed may be difficult. Therefore the recommendation to feed fish twice a week and to use rapeseed oil is more feasible.

5.4.5 Constraints of rapeseed oil and fish promotion in CF

Peroxidation

Due to the susceptibility to peroxidation of the (LC-)PUFA carbon double bonds, an increased (LC-)PUFA supply may lead to increased oxidative damage of e.g. blood components (Stimming et al. 2014). Therefore, (LC-)PUFA rich foods are favourable when they simultaneously provide high levels of tocopherol, the major lipid-soluble antioxidant. Salmon and rapeseed oil have both high levels of tocopherol (Bragadóttir 2001; CODEX). In adults, fish or rapeseed oil did not yield higher plasma lipid peroxidation compared to controls (Higdon et al. 2000; Poornima et al. 2003). On the contrary, consumption of rapeseed oil in comparison to sunflower oil even led to a lower in vitro peroxidation sensitivity of the lipoproteins in male adults (Nielsen et al. 2002). However, possible long-term negative effects of increased fish or rapeseed oil in infancy on a long-term enhancement of LC-PUFA synthesis or LC-PUFA supply and tocopherol status have not yet been examined.

Contamination

Fish is the primary source of methyl mercury (MeHg) in the human diet (Clarkson 2002; Johnsson et al. 2005; Knobeloch et al. 2005). MeHg is a toxicant that affects the central nervous system (Castoldi et al. 2003) and thus might be in particular crucial during rapid brain development in infancy. As infants and children have higher consumption rates of foods per kg body weight, they have an increased relative health risk of exposure to food contaminants/ toxicants compared to adults (Nunes et al. 2014). Studies of low frequent MeHg exposure and its' effects on neurodevelopment in infants and children give inconsistent results (Jurewicz et al.

2013). These studies focused on the prenatal exposure via the maternal diet and assessed subsequent neurodevelopment with different tests (cognition, memory, IQ) at different ages (shortly after birth to 14 years of age). While some of them found inverse associations between mercury exposure in utero and test outcomes (Jurewicz et al. 2013), others could not establish any associations between the exposure and neurodevelopmental outcomes (Myers et al. 2003; Jurewicz et al. 2013). A recent Italian study did not find any associations between MeHg concentrations in breast milk and cognitive outcomes of children at 18 months of age (Valent et al. 2013). No studies are currently available on infant's exposure to dietary MeHg via their own fish consumption and the impacts on neurodevelopment. Comparing beneficial effects of fish consumption on nutrient intake, especially n-3 LC-PUFA, and health (EFSA 2014) with potential risks, fish can be recommended as part of infant's diet (EFSA 2013) although not every fish type is eligible (Nunes et al. 2014). From scenarios of fish consumption during childhood in Portugal (Andrade 2006) and MeHg exposure of fish species (Nunes et al. 2014) it was concluded that children should avoid eating some specific fish types but not salmon (Nunes et al. 2014).

Sustainability

Fish stocks are vulnerable to overfishing all over the world. Environmental and commercial organisations established several certification schemes to brand sustainable products or provide websites to check which fish stocks are under pressure[4]. Aquaculture or offshore fish-farming might be a future-oriented solution (Retail Forum for Sustainability 2012) although also negative aspects, e.g. intensive farming methods and environmental pollution must be considered (Retail Forum for Sustainability 2012). A meta-analysis of 39 studies did not find a difference in n-3 LC-PUFA content between farmed and wild fish (Sales 2010). Thus, farmed fish can be taken into account as n-3 LC-PUFA source in infant's diet.

[4]Greenpeace, International Council for Exploration of the Seas (ICES)

6 Conclusion and future prospects

This thesis provides for the first time detailed insights into 2 aspects of food choice in CF that may be related to later health, (1) food variety as an early promoter of a well-varied plant-based diet as recommended for prevention of hypertension, CHD and stroke in adulthood (Boeing, 2012), (2) (LC-)PUFA-rich foods which are discussed to be related to functional outcomes in infancy.

Results of this thesis indicate that especially vegetable variety is low in CF in Germany and PUFA-rich rapeseed oil is already common in infant's diet, whereas fish consumption is still rare and probably driven by maternal dietary behaviour as a strong predictor of infant's dietary behaviour. The optimisation of LC-PUFA supply via rapeseed oil and fish seems to be beneficial for infant's LC-PUFA status. In addition, the consumption of rapeseed oil and fish may be favourable for visual development. Further food-based studies with larger study populations are needed to confirm these results and to assess long-term effects on LC-PUFA status and development for establishing evidence-based dietary recommendations.

Distinct and also practical recommendations could facilitate the change from the present monotonous food choice to a greater variety, especially in vegetables in CF as food preferences and habits tend to track into adulthood. Adoption of such recommendations in product development of commercial complementary meals would reach large parts of infants as they are predominant in CF in Germany.

More research is needed on long-term effects of early food consumption, especially vegetable variety on food habits / food choices and patterns and related health parameters in childhood and – more important – beyond. Although difficult to realise in practice, longitudinal studies with large population samples should start in the 'sensitive periods of eating' before the age of 3 years, e.g. in the CF period, which would facilitate to assess how stable habits are. They should also consider the determinants, i.e. environmental and genetic factors that predict food variety in child- and adulthood:

Environmental factors, e.g.:

- Feeding practices (e.g. mother-child interaction)
- Preparation methods (homemade vs. commercial meals)
- Socio-cultural influences like eating habits, socio-economic status

- Timing of introducing specific foods like Brassica type vegetables (related to the subsequent acceptance of bitter tasting foods) or fish (to further examine the 'sensitive' period hypothesis)

Genetic factors, e.g.:

- Importance of innate flavour preferences (e.g. sweet vs. bitter) for accepting a variety of foods in CF
- Effects of genetic variation (e.g. SNPs) on implementing a varied diet

More research is needed on the generalisability of the variety effect from a given food category to the acceptance of new foods from another category (Lange et al. 2013) due to inconsistent data. While some studies showed e.g. a positive effect of vegetable variety on later acceptance of a new type of meat (Gerrish, Mennella 2001; Maier et al. 2008), other studies could not confirm this observation (Mennella et al. 2008; Nicklaus 2011).

Ideally, representative data on the current status quo of (LC-)PUFA intake of term infants (especially in CF) in Germany / Europe with respect to the essential PUFA ALA and LA and the LC-PUFA ARA, EPA and DHA may support assessing deficiency and facilitate to give well-founded recommendations of an AI of LC-PUFA in infants (especially in the CF period). In contrast to the invasive collection of blood samples, the more feasible, simpler and non-invasive method for measuring LC-PUFA status in infants might be the collection of cheek cells which also facilitates reaching high sample sizes in representative assessments to assess the supply in a population. In PINGU, FA status in infants' cheek cell phospholipids was shown to reflect the status in plasma and erythrocyte phospholipids (Demmelmair et al., not published). Research on optimal ratios of LA and ALA and ARA and DHA respectively in CF, considering genetic aspects and conducted in large sample sizes, is crucial. Studies should examine the food habits in different (European) countries comparing the intake of different 'natural' food sources of (LC-)PUFA like egg yolk, fish or oil mixtures as well as effects of different amounts of foods providing ALA and DHA on LC-PUFA status and functional development. In addition, the demand for long-term studies, more consistent in their study design (e.g. sample size, dose of FA) to enable comparison of the results in meta-analyses leading to consistent final evidence of (LC-)PUFA supply in infancy, has to be met. For assessing effects on cognitive and visual development in the future it is necessary to establish age appropriate sensitive and – ensuing – standardised methods to facilitate comparing studies in different ages from infancy to adolescence.

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