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The effect of lowering the ω-6/ω-3 long-chain polyunsaturated fatty acid ratio in the diet of pregnant and lactating women on fatty acid levels and body composition of the women and their newborns

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Abbreviations

AA	Arachidonic acid (C20:4n-6)
ALA	α-linolenic acid (C18:3n-3)
Apgar score	Score to assess the condition of a newborn after birth (1-10)
AT	Adipose tissue
BF	Body fat
BM	Breast milk
BMI	Body mass index
BW	Birth weight
cAMP	Cyclic adenosine monophosphate
CG	Control group
CNS	Central nervous system
DHA	Docosahexaenoic acid (C22:6n-3)
DHyLA	Dihomo gamma linolenic acid (C20:3n-6)
DPA	Docosapentaenoic acid (C22:5n-3)
EDTA	Ethylene diamine tetraacetic acid
EFA	Essential fatty acids
EPA	Eicosapentaenoic acid (C20:5n-3)
FA	Fatty acid
FABP	Fatty acid binding protein
FAME	Fatty acid methyl esters
FAT	Fatty acid translocase
FATP	Fatty acid transport protein
FFA	Free fatty acids
FFM	Fat free mass
FM	Fat mass
GLC	Gas liquid chromatography
GWG	Gestational weight gain
HDL	High density lipoprotein
IG	Intervention group
IGF-1	Insulin-like growth factor 1
INR	International normalized ratio
IOM	Insitute of Medicine (USA)

LA	Linoleic acid (C18:2n-6)
LCP	Long-chain polyunsaturated fatty acid
LC-PUFA	Long-chain polyunsaturated fatty acid
LDL	Low density lipoprotein
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular/cellular hemoglobin concentration
MCV	Mean corpuscular volume
MUFA	Monounsaturated fatty acid
MRI	Magnetic resonance imaging
ns	Non-significant
PC	Prostacyclin = PGI_2
p-$FABP_{pm}$	Placental plasma membrane fatty acid binding protein
PGI_2	Prostacyclin = PC
pL	Pico Liter
PL	Phospholipid
pp	Post partum
PPAR	Peroxisome proliferator-activated receptor
PTT	Partial thromboplastin time
PUFA	Polyunsaturated fatty acid
RBC	Red blood cells
RCT	Randomized controlled trial
RDW	Red blood cell distribution width
SD	Standard deviation
SFA	Saturated fatty acid
SFT	Skinfold thickness
TFA	Trans fatty acid
TAG	Triacylglycerid
VLDL	Very low densitiy lipoprotein
WMD	Weighted mean difference
wk	Week

1 Introduction

Childhood obesity continues to be a serious public health problem. Children who are overweight are at risk of health conditions that include dyslipidemia, hypertension, and type 2 diabetes. Childhood obesity is also a risk factor for adult obesity and its associated diseases. In a recent survey of German children and adolescents, it was shown, that of children between the ages of 3 and 17, 15 % are overweight and 6.3 % suffer from obesity. The prevalence of obesity in Germany is 2.9 % for 3-6-year-olds, 6.4 % for 7-10-year-olds and 8.5 % for children aged 14-17, respectively (KURTH AND SCHAFFRATH ROSARIO 2007). The development of overweight and obesity seems to be the result of complex interactions between environmental, genetic, and psychological variables that occur throughout the life span of an individual. The earliest influences may already occur within the intrauterine development and the early postnatal period (LUCAS 1991). The notion that health and disease may be programmed in early life has been demonstrated in research examining intrauterine exposures such as maternal smoking and gestational diabetes and their influence on growth (GILLMAN ET AL. 2003). Recent evidence from animal and human studies favor the possibility that changes in the balance of essential polyunsaturated fatty acids can influence the early stages of adipose tissue development, particularly during fetal life and infancy, periods showing a high adaptability and vulnerability to external influences. In cultured cells and rodent models, eicosanoids derived from arachidonic acid, a long-chain polyunsaturated fatty acid of the omega-6-family, appear to modify adipogenesis, thereby providing a molecular link between fatty acid uptake and fat cell development. These fatty acids stimulate the differentiation of preadipocytes into mature adipocytes and play a crucial role in the hyperplastic growth of adipose tissue. Eicosanoids derived from eicosapentaenoic and docosapentaenoic acid, two long-chain polyunsaturated fatty acids of the omega-3-family, in contrast, have the opposite effect on adipogenesis, both inhibiting fat cell differentiation and development (GAILLARD ET AL. 1989; AMRI ET AL. 1994; GREGOIRE ET AL. 1998). This led to the hypothesis that the composition of fatty acids and in particular the ratio of omega-6- to omega-3-long-chain polyunsaturated fatty acids in the maternal diet during pregnancy and lactation may play a role in early adipogenesis and affect adipose tissue development (AILHAUD AND GUESNET 2004). The purpose of the

present study was to examine if a diet with a low ratio of omega-6- to omega-3-long-chain, polyunsaturated fatty acids during pregnancy and lactation will lead to a less expansive development of adipose tissue in the infants. Supplementation of pregnant women with long-chain omega-3-fatty acids together with a reduction of arachidonic acid intake may thereby represent a novel strategy for the primary prevention of childhood obesity.

By understanding the early determinants of obesity, it may be possible to influence the course of excessive weight gain and help curtail the obesity epidemic.

2 Survey of existing literature

2.1 Long-chain polyunsaturated fatty acid physiology

The human organism is able to synthesize fatty acids from dietary precursors (acetyl-CoA-molecules) by endogenous biosynthesis and to introduce double bonds to the molecules. However, humans lack the enzyme system for introducing double bonds between the methyl terminus and carbon atom (C) number nine. Long-chain polyunsaturated fatty acids (LC-PUFAs) with double bonds after the ninth C-atom have to be provided by the diet and are therefore called essential fatty acids. There are two essential fatty acids (EFA) for humans, called linoleic acid (LA; C18:2 n-6) and alpha-linolenic acid (ALA; C18:3 n-3). The distinction between n-6 and n-3 is based on the location of the first double bond counting from the methyl terminal carbon atom of the fatty acid (FA) molecule. Through alternate elongation and desaturation steps in the endoplasmatic reticulum (and for DHA: following partial ß-oxidation in the peroxisomes) both LA and ALA are converted into their respective long-chain, more unsaturated metabolites which are - depending on their respective precursor - classified in one of the two families of FAs. Once consumed in the diet, LA can be converted via gamma-linolenic acid (C18:3 n-6), dihomo-gamma-linolenic acid (DHyLA; C20:3 n-6), arachidonic acid (AA; C20:4 n-6), and adrenic acid (C22:4 n-6) into osbond acid (C22:5 n-6) by the pathway outlined in figure 1. Using the same enzymes, the n-3 FA ALA can be converted via stearidonic acid (C18:4 n-3), eicosatetraenoic acid (C20:4 n-3), eicosapentaenoic acid (EPA; C20:5 n-3) and docosapentaenoic acid (DPA; C22:5 n-3) into docosahexaenoic acid (DHA; C22:6 n-3) (Fig. 1). Also, because mammalian tissues do not contain the Δ15-desaturase they cannot interconvert n-6 and n-3 FAs. EFA, can in part, be replaced in the diet through supply of their long-chain homologues.

Both families compete for the same enzymes whereas the enzymatic affinity to the FAs decreases in the order of n-3, n-6, and n-9. Even *trans*-FA (TFA) compete for the same binding sites of the enzymes and may thus increase the requirement of EFA or handicap the conversion of EFAs into their long-chain homologues (COOK AND EMKEN 1990; KOLETZKO AND DECSI 1997; STEINHART 1997). The conversion rate of the EFAs to their longer chain metabolites depends, among other factors, on the FA composition of the diet and accounts for a maximum of 10 % in the human organism

(GERSTER 1998). Previous studies have shown conversion of ALA to EPA of 6 – 21 % or lower values (0.1 – 0.2 %). Conversion of ALA to DHA is even lower and ranges from 4 – 9 % to almost 0 % (EMKEN ET AL. 1994; PAWLOSKY ET AL. 2001; BURDGE ET AL. 2002; BURDGE AND WOOTTON 2002; PAWLOSKY ET AL. 2003; HUSSEIN ET AL. 2005). Hence, particularly in pregnancy and lactation, a sufficient dietary intake of preformed n-3 LC-PUFAs is essential.

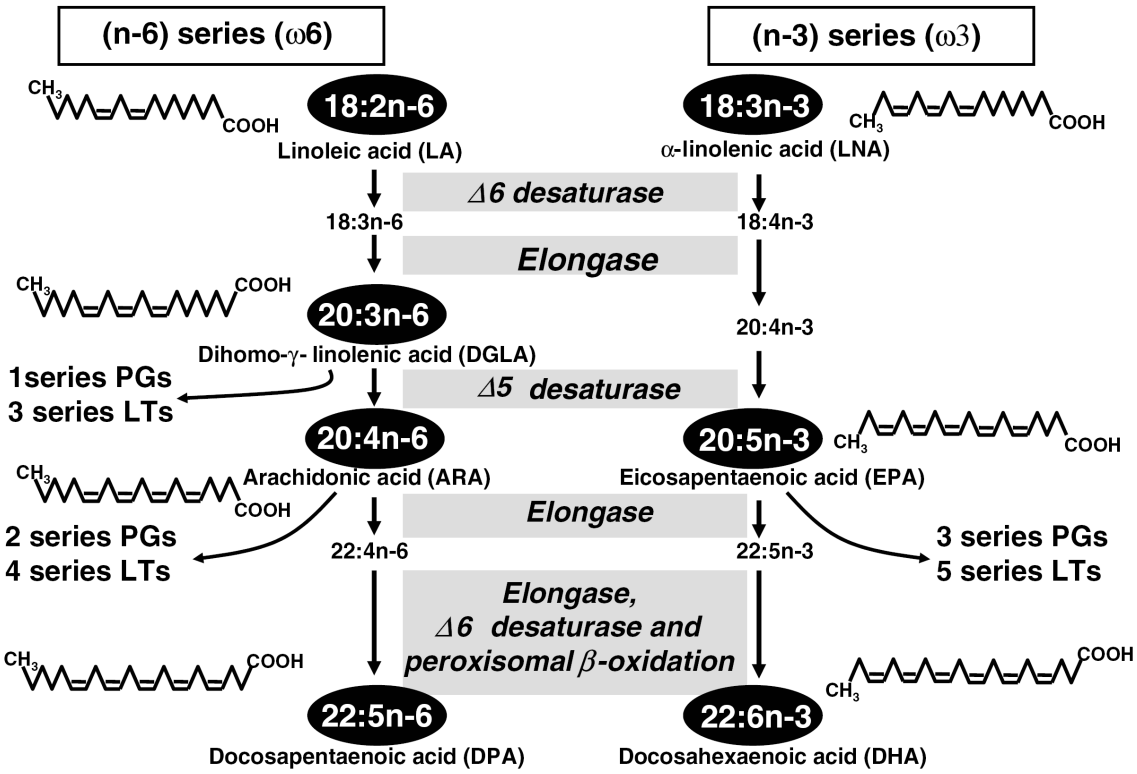

Figure 1 Fatty acid desaturation / elongation (AILHAUD 2006)

A ratio of LA/ALA of 5:1 or a daily intake of 2.5 % LA and 0.5 % ALA of total energy intake, respectively corresponds to the dietary reference intake recommendations of the German society of nutrition. Although EFA deficiency in Germany is rare, it does occur. n-6 FA deficiency augments the concentration of mead acid (C20:3 n-9) in plasma and tissue as oleic acid uses the enzymes of the n-6 biosynthesis pathway – otherwise hardly ever found long-chain n-9 homologues are formed. With n-3 deficiency the organism starts biosynthesis of the n-6 homologues similar to the n-3 FA; therefore, the concentration of osbond acid (C22:5 n-6) in plasma and tissue increases (DACH 2000).

LA and ALA are found in all plant fats and oils whereas AA and DHA are only found in animal derived products, specifically, DHA is mainly found in fatty sea fish like

herring, mackerel or sardine. A typical western diet contains approximately 100 – 200 mg EPA + DHA and ~ 130 – 150 mg AA per day (KRIS-ETHERTON ET AL. 2000; MEYER ET AL. 2003). In Germany, the mean dietary DHA intake is 140 mg/d for women, with much lower intakes (~ 85 mg/d) observed among young women (BAUCH ET AL. 2006). The mean ratio of n-6/n-3 PUFAs in the German diet is 7:1 as reported in the "ERNÄHRUNGSBERICHT 2004" and the "NATIONALE VERZEHRSTUDIE 2008" (DGE 2004; MAX-RUBNER-INSTITUT 2008).

FAs are an important source of energy for the human organism, are irreplaceable structural components of all cell membranes and AA, EPA and DHyLA are all precursors of short-lived, highly potent, regulatory hormones collectively named eicosanoids. They are of great importance for growth, development, and cell differentiation and influence numerous metabolic functions like cardiovascular and immune functions, insulin action, neuronal development and visual function. They are also involved in the regulation of plasma lipid levels and the regulation of gene expression (JUMP 2002).

The FA composition of the cell membrane influences its physio-chemical properties and has an impact on receptor function, ion transport and signal transduction (STORLIEN ET AL. 1998). Over 5000 scientific papers and studies dealing with possible functions in, and the impact of, LC-PUFAs on human metabolism were published in the past 2 decades (ARBEITSKREIS-OMEGA-3 2002).

DHA and AA are of particular interest because they are important structural components in the highly specialized membrane lipids of the human central nervous system (CNS) and therefore highly important for visual function and neurological development of infants. They occupy a central place in the structure of fluid, excitable, metabolically active membranes such as photoreceptor outer segments in the human retina and synaptic membranes. DHA accounts for about 50 % of total fatty acids in the structural phospholipids of photoreceptor outer segment disk membranes, a concentration higher than in any other tissue. High concentrations of DHA in the phospholipids of the brains grey matter (in particular in the synaptic membranes) are primarily found in phosphatidylethanolamin and phosphatidylserin and high concentrations of AA are found in phosphatidylinositol. Their importance is demonstrated by the fact that a depletion of DHA in the brains of monkeys during fetal and early postnatal development results in permanent deficits of visual function

despite later biochemical repletion of brain and retinal DHA (FLIESLER AND ANDERSON 1983; SASTRY 1985; NEURINGER AND CONNOR 1986).

Large amounts of AA and DHA are needed for the synthesis of structural lipids for the CNS, muscle and other organs during fetal and postnatal development. Thus, an adequate, sufficient pre- and postnatal supply of EFA and LC-PUFAs is essential for fetal and postnatal growth, eye and CNS function and development, and later learning and behavior of the newborns and infants (UAUY ET AL. 1996; HORNSTRA 2000).

2.2 Fatty acids as biomarkers of fatty acid intake

The FAs in chylomicrons reflect the FA intake until some hours after food intake. FAs in TAGs reflect the food intake over the last few hours, FAs in PLs and CEs reflect food intake over the last few days (KOHLMEIER 1995). Plasma lipid profile is, according to DOUGHERTY ET AL. (1987), KATAN ET AL. (1997) and MANSOUR ET AL. (2001) a sensible indicator for changes in dietary LC-PUFA intake. Red blood cell (RBC) lipids are suitable biomarkers for long-term dietary fat intake; they have a lifespan of ~ 120 days and thereby reflect dietary lipid intake over the last 3 – 4 months (THEWS ET AL. 1999; ARAB 2003).

Adipose tissue (AT) is considered the ideal biomarker of long-term dietary fat intake although changes of the lipid intake only become noticeable after quite some time (ARAB 2003, KATAN 1997).

Quantifying the dietary intake of saturated FAs (SFA) and monounsaturated FAs (MUFA) on the basis of blood levels is quite difficult because these FAs are synthesized on a large scale by the human body itself and influence the results. Even the EFA content in blood lipids does not exactly reflect dietary EFA intake because of numerous complex metabolic processes like desaturation, elongation, acetylation and transport between body compartments in the human body (MA ET AL. 1995).

2.3 Adipose tissue

In recent years it became more and more clear that adipose tissue represents not only a passive tissue which merely responds to nutritional challenges, but that it is rather an organ actively involved in energy homeostasis and regulation of important metabolic functions. Furthermore, it was recognized that AT is not a homogeneous tissue: different anatomical depots display different metabolic properties and are

subject to different endocrine and neural regulation. One distinguishes between subcutaneous, visceral, retrorbital, and mammary white adipose tissue which serves as an energy reservoir, thermal and mechanical insulation, and endocrine organ, brown adipose tissue mainly responsible for thermogenesis and bone marrow fat which also acts amongst others as an energy reservoir. Normal ranges of relative body fat mass are 12 – 20 % and 20 – 30 % in normal weight men and women, respectively, with the larger portion present in subcutaneous depots. Depending on the anatomical location, adipose depots display considerable differences in metabolic activity which means that not fat mass alone but also body fat distribution can have profound effects on metabolic complications associated with obesity (KLAUSS 2001).
Adipocytes represent roughly one half of the total number of cells in adipose tissue. The remaining stromal-vascular fraction consists of blood and endothelial cells, macrophages, pericytes, fibroblasts and AT precursor cells like adipoblasts and preadipocytes. Methods for determining adipocyte number and size are unfortunately not very accurate and only count cells with lipid vacuoles, i.e. preadipocytes which do not yet contain lipids are not counted. Preadipocytes are still able – in contrast to mature adipocytes - to divide, and therefore represent an important factor of adipose tissue development hard to evaluate (SPALDING ET AL. 2008).

2.3.1 Human adipose tissue development

Humans differ from most mammals by depositing significant quantities of body fat in utero. The human newborn has a body fat content of about 15 %, mainly in subcutaneous regions (WIDDOWSON 1950). Although humans are already born relatively plump, they still increase body fat in early infancy reaching an adiposity peak of approximately 25 % at 6 – 9 month of age which afterwards declines to leaner childhood values at 5 – 6 years of age of about 13 % and 16 % fat for boys and girls, respectively. About 40 – 65 % of body weight gain during the first 6 months is accounted for by body fat deposition (FOMON ET AL. 1982; MCLAREN 1987). This involves hyperplasia (formation of new adipocytes from precursor cells) and hypertrophy (increase in adipocyte size) of adipocytes.
First adipocytes in the human fetus can be found between week 14 and 16 of gestation whereas fat cell numbers vary from one body site to another and some fat deposits appear to grow primarily through hypertrophy while others grow mainly by hyperplasia (POISSONNET ET AL. 1983; BURDI ET AL. 1985). More than 90 % of the fetal

fat deposition occurs in the last 10 weeks of pregnancy and reaches 7 g/d at term (HAGGARTY 2002). Both, adipocyte number and adipocyte size are determinants of body fat mass (SPALDING ET AL. 2008). The relative contribution of hyperplasia or hypertrophy to adipose tissue development is very controversial. On the one hand it is reported that the number of adipocytes increases during the first 2 years of life (KNITTLE ET AL. 1977; BAUM ET AL. 1986; HAUNER 1989; PRINS AND O'RAHILLY 1997) while other authors observed a cell enlargement during the first year (HAGER ET AL. 1977; BOULTON ET AL. 1978), followed by cell multiplication up to the age of 8 years.
Studies based on animal experiments in rats let to the “adipocyte-number hypothesis” which postulates that the number of adipocytes is fixed in early life and predestines an individual to be lean or obese depending on changes in the number of adipocytes (ROCHE 1981). Even if this theory was highly controversial some recent studies on the correlation of weight gain in the first weeks and months of life and the prevalence of overweight in later life seem to point in the same direction (STETTLER ET AL. 2002; DEHEEGER AND ROLLAND-CACHERA 2004; STETTLER ET AL. 2005; MACE ET AL. 2006).

Compared to normal weight patients, the number of adipocytes and to a lesser extent the size of the adipocytes of obese patients were reported to be higher and depended on the age of obesity onset. Thus, the difference in adipocyte number between lean and obese subjects is established during childhood (KNITTLE ET AL. 1979) and the total number of adipocytes for different weight categories stays constant during adulthood (SPALDING ET AL. 2008). The ability of clones of precursor cells to differentiate and proliferate in rats and humans is highest very early in life (DJIAN ET AL. 1983; HAUNER 1989; KIRKLAND ET AL. 1990). Taken together this indicates that early life may be a very sensitive period of AT development although the proliferation of precursor cells is undetectable at that time. Precursor cells are found to remain present lifelong in AT and the ability of proliferation and differentiation decreases with age but does not cease (KIRKLAND ET AL. 1990; AILHAUD 2004). The size and self-renewal of the adipose precursor pools as a function of age or in response to different diets is presently unknown in humans, owing to the absence of specific markers of these cells although they represent the true potential of adipose tissue development (AILHAUD AND GUESNET 2004). SPALDING ET AL. demonstrated that although the adipocyte number is static in adults, there is a remarkable turnover within this population of cells, indicating that the adipocyte number is tightly controlled and not influenced by energy balance. Altogether these observations emphasize the

fact that adipocyte formation is an irreversible process and that prevention of this phenomenon should represent a key issue from a health perspective (AILHAUD ET AL. 2006).

2.3.2 The role of LC-PUFAs in adipocyte differentiation

Regarding the molecular level of adipose tissue development, both in rodents and humans, LC-PUFAs act at the preadipocyte stage and trigger the formation of adipocytes. Fatty acids as well as eicosanoids, i.e. prostaglandins and leukotriens emanating from AA, behave as activators/ligands of PPARβ/δ and PPARγ which are in turn critically required for adipogenesis (AMRI ET AL. 1994; WU ET AL. 1995). Adipogenesis is a sequential process in which glucocorticoids, insulin and IGF-1 have been identified as the major adipogenic hormones (WABITSCH 2000; AILHAUD 2004). The first line of evidence that FAs are involved was obtained after purification of the main adipogenic component of fetal bovine serum which was characterized as AA. *In vitro*, AA is very adipogenic and plays in preadipocytes the role as precursor of prostaglandins (PG), a family of compounds including PGI_2, PGJ_2, PGE_2 and $PGF_{2\alpha}$, which play an important role in cellular processes. Especially prostacyclin (PC = PGI_2) is known, to promote adipogenesis *in vitro* (VASSAUX ET AL. 1992; REGINATO ET AL. 1998). In contrast, the two major metabolites of the ALA pathway (EPA and DHA), which are not metabolized to PC, are less potent promoters of adipogenesis than AA. The adipogenic effect of AA is partially blocked by COX inhibitors and anti-PC antibodies added externally, and is mimicked by carbacyclin, a stable analogue of PC (GAILLARD ET AL. 1989; NEGREL ET AL. 1989). This strongly suggests an adipogenic role of PC through the cell surface PC receptor IP as an autocrine / paracrine mechanism (Fig. 2). Among all natural FAs, only AA triggers cAMP production and activates, through the IP/PC system the protein kinase A pathway. EPA and to a lesser extend DHA, while being inactive as cAMP-elevating agents, inhibit the stimulatory effect of AA on cAMP production (VASSAUX ET AL. 1992; MASSIERA ET AL. 2003). The second line of evidence that FA are important regulators of adipogenesis was obtained when it was shown that most of the long-chain FAs could act as transcriptional regulators of some lipid-related genes. The intracellular sensors of long-chain FAs have been identified as nuclear receptors of the family of PPARs. AA and some of its metabolites generated through COX and LOX are implicated in adipogenesis and behave as activators/ligands of PPARs (Fig. 2). First, AA up-

regulates the expression of CCAAT / enhancer binding protein β (C/EBPβ) and C/EBPδ via the PC/IP receptor system and the protein kinase A pathway. C/EBPβ and C/EBPδ are trans-acting factors, which up-regulate the expression of PPARγ. Secondly, AA may also act through PC as activator / ligand of PPARβ/δ which in turn, up-regulate the expression of PPARγ. In *in vitro* experiments with PPAR agonists (MASSIERA ET AL. 2003), it was shown that in contrast to AA, saturated, monounsaturated and n-3 LC-PUFAs (EPA and DHA) are not more adipogenic than a specific PPARβ/δ agonist, emphasizing the unique adipogenic and early role of AA in AT development. EPA and to a lesser extent DHA, while being inactive as cAMP-elevating agents, inhibit the stimulatory effect of AA on cAMP production. Thus long-chain FAs are not equipotent in promoting adipogenesis and AA appears as a remarkable adipogenic "booster" (AILHAUD ET AL. 2006).

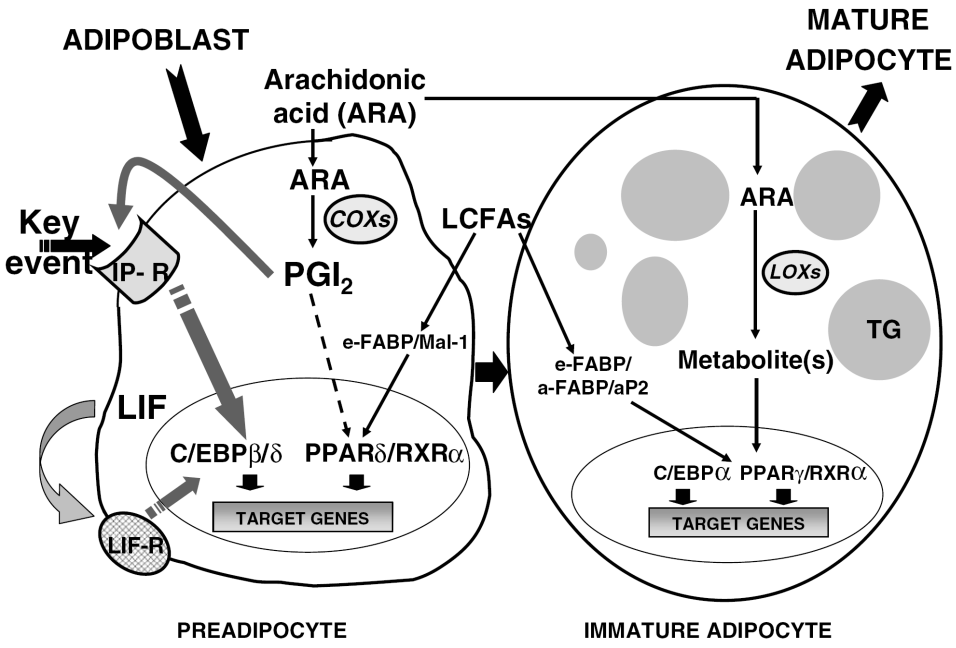

Figure 2 Pathways and LC-PUFAs implicated in adipogenesis (AILHAUD 2006)

AA, EPA and DHA are formed through a series of common desaturation and elongation steps as mentioned above. As the same enzymes are used, metabolic competition exists between LA and ALA. Therefore, manipulation of dietary LA/ALA ratio has a strong impact on the proportion of both AA and EPA/DHA accretion in tissues. To find out whether dietary intake of high levels of n-6 FAs could promote AT development *in vivo*, MASSIERA ET AL. carried out nutritional interventions in wild-type (wt) and prostacyclin-receptor knockout (ip-r-/-) mice (MASSIERA ET AL. 2003). Wild-type mother mice were fed before mating and during the gestation and suckling

period with either a high-fat diet rich in LA, a precursor of AA, or the same isocaloric diet enriched in LA and ALA. The ratios of n-6 PUFAs vs. n-3 PUFAs were 59:1 and 2:1. From weaning onwards body weight, fat mass, epididymal fat pad weight and adipocyte size at 8 weeks age were higher in the offspring of dams fed the diet with the high ratio of LA to ALA (59:1). Inclusion of ALA seems to prevent the enhancement of fat mass. The importance of the gestation/suckling period is demonstrated by the fact that when mother mice are fed a standard diet, i.e. a high-carbohydrate, low-fat diet, the body weight of pups fed after weaning only with the ALA low diet remains similar to that of the pups fed the ALA rich diet. In contrast to wild-type mice, ip-r-/- mice showed no additional increase of body weight. The authors concluded that the consumption of LA as a precursor of AA in large amount during gestation and the suckling period may enhance adipose tissue development and later obesity through PC signaling. A study of JAVADI ET AL. seems to support this conclusion by demonstrating in 5 week old mice fed with five different high-fat diets, that the animals fed a diet rich in LA had the highest proportion of body fat while the animals fed a diet rich in ALA had the lowest (JAVADI ET AL. 2004). Nevertheless, other animal studies investigating the ratio of n-6 to n-3 FAs in the maternal diet could not confirm the adipogenic effect of LA (HAUSMAN ET AL. 1991; KOROTKOVA ET AL. 2002). In the study of HAUSMAN ET AL. the offspring of dams fed a high-fat diet before mating, during pregnancy and lactation, with a LA/ALA ratio of 41:1 or 9:1, respectively, had a similar body weight and fat mass at weaning. KOROTKOVA ET AL. showed similar results, comparing high-fat diets with a LA/ALA ratio of about 215 and 9, respectively.

The impact of the n-6 to n-3 LC-PUFA-ratio of maternal nutrition during pregnancy and lactation on infant AT development in humans has not yet been investigated.

In two recent reviews of SIMMER ET AL. on LC-PUFA supplementation in infants born at term and in preterm infants (SIMMER ET AL. 2008; SIMMER ET AL. 2008), no consistent effect of LC-PUFA supplementation on infant growth was found. This was confirmed in an IPD (individual patient data) meta-analysis of LC-PUFA supplementation on infant growth at 18 month (ROSENFELD ET AL. 2009). As discussed in chapter 2.10 so far there is also limited information on the impact of n-3 LC-PUFA supplementation during pregnancy on infant body composition and adipose tissue development. It remains to be elucidated whether a decrease in the ratio of n-6/n-3 LC-PUFAs affects early AT development in humans.

2.4 Transport of lipids across the placenta

The nidation of the blastocyst (differentiated into embryoblast and trophoblast) into the endometrium takes place at day 6 - 7 after fertilization. The trophoblast infiltrates the endometrium and the cells confluate to the syncytiotrophoblast. The remaining trophoblast cells are then called cytotrophoblasts. The nutrition of the embryo occurs exclusively through the proteolytic activity of the syncytiotrophoblast, which resorbs the released nutrients. This is called the histotrophic phase of embryonic nutrition. Maternal blood moves in and out of the eroded maternal tissue and lacunar networks, thus establishing the uteroplacental circulation between the 8th and 10th wk of gestation which marks the beginning of the haemotrophic phase of embryonic nutrition. The uteroplacental unit is composed of both fetal tissue (chorionic plate) and maternal tissue (basal plate). In between these tissues is the intervillous space, which contains the branched villous structures containing fetal blood vessels. Circulating maternal blood enters this space via spiral endometrial arteries, bathes the villi and drains back through endometrial veins. Oxygen-deficient fetal blood passes via two umbilical arteries and the chorionic arteries to the villi and returns well-oxygenated to the fetus via the chorionic veins and the single umbilical vein (Fig. 3). Fetal blood is separated from maternal blood by the placental membrane (barrier), which is composed of 4 layers: the syncytiotrophoblast, the cytotrophoblast, villous stroma and the endothelium of the fetal capillaries. Around the 20th wk of gestation, the cytotrophoblast cell layer becomes attenuated and disappears facilitating the exchange of metabolites (DREWS 1993; THEWS ET AL. 1999; BÜHLING AND FRIEDMANN 2004; GUDE ET AL. 2004; SCHNEIDER ET AL. 2004; HANEBUTT ET AL. 2008).

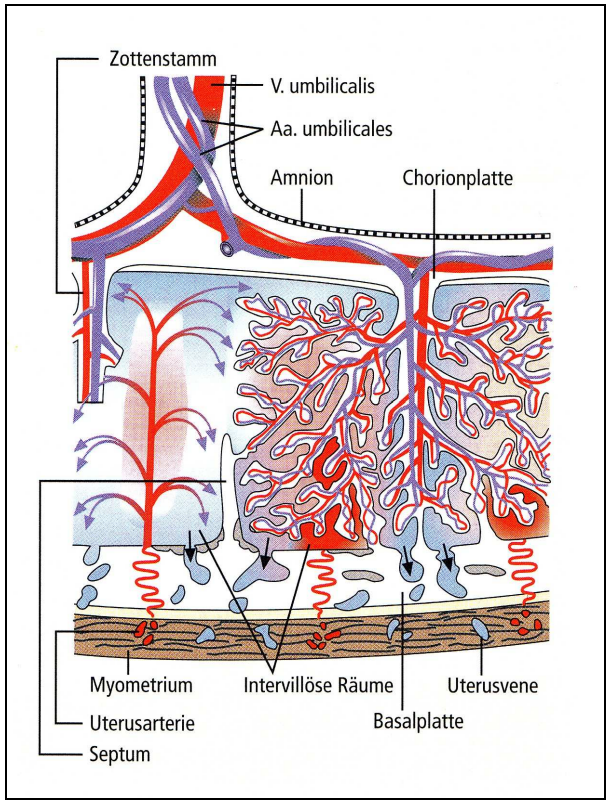

Figure 3 Placenta, longitudinal cut, schematic (THEWS ET AL. 1999)

The term human placenta is a discoidal organ with a diameter of 18 – 20 cm, a thickness of 2 – 3 cm and a weight between 400 – 700 g.

The placenta acts to provide oxygen, water, carbohydrates, amino acids, lipids, vitamins, minerals, and other nutrients to the fetus whilst removing carbon dioxide and other waste products. It metabolizes a number of substances and can release metabolic products into maternal or fetal circulations. It also releases hormones (e.g. progesterone, estrogen, human placental lactogen (hPL), and human chorionic gonadotropin (hCG)) into both the maternal and fetal circulations affecting pregnancy, fetal growth and metabolism.

Fatty acids cross the villi barrier only as non-esterified-FAs derived from the pool of free fatty acids (FFA) bound to albumin in maternal plasma or from TAG transported via LPs in maternal plasma (primarily form TAG rich LPs, i.e. post-hepatic LDL and VLDL). Lipoprotein-receptors, lipoproteinlipase, phospholipase A_2 and intracellular lipase activity of the placenta (located at the maternal surface) permit the release and supply of the FFA for the fetus (NAOUM ET AL. 1987; RICE ET AL. 1998; HERRERA 2002; HAGGARTY 2004; DUTTAROY 2009). Most likely will occur the release of FFA from placental lipid depots through a placental lipase (WATERMAN ET AL. 1998; DUTTAROY 2004). FFA can then cross the membranes of the syncytiotrophoblast by simple

diffusion or via the action of membrane-bound and cytosolic FA binding proteins like the FA translocase (FAT/CD36), FA- transport protein (FATP), plasma membrane FA binding protein (p-$FABP_{pm}$), and other FA binding proteins (FABPs) of the cytoplasma like H-FABP (heart-FABP), and L-FABP (liver-FABP) (Fig. 4). Lipid transport depends on - and is driven by - the concentration gradient of non esterified FAs between the mother and the fetus and the placental plasma membrane binding sites. The non esterified FA concentration gradient increases steadily during pregnancy and, in maternal blood, is three times that of the fetal blood. In contrast, the concentration of albumin, the transport protein, is higher in fetal plasma when compared to maternal plasma (BENASSAYAG ET AL. 1997; BENASSAYAG ET AL. 1999).

FAT and FATP are present in both placental membranes, microvillous and basal membranes, p-$FABP_{pm}$ only in the microvillous membrane facing fetal circulation (CAMPBELL AND DUTTA-ROY 1995; CAMPBELL ET AL. 1998). In contrast to the two other FA binding proteins, p-$FABP_{pm}$ shows a higher affinity and a higher binding capacity for AA and DHA compared to LA and oleic acid, indicating that it is involved in preferential uptake of LC-PUFAs by these cells and may allow a selective transport of FAs form the maternal to the fetal circulation (CAMPBELL ET AL. 1998).

The preferential uptake of LC-PUFAs by placental membranes and Be Wo cells (human choriocarcinoma cells) was supported by a observation using a human placental perfusion system (HAGGARTY ET AL. 1999). They reported a selective, preferential transport of DHA > AA > ALA > LA in human placenta. Further evidence was provided by LARQUÉ ET AL. who showed in a study with radioactive labeled FAs a preferential uptake of DHA in placental tissue (LARQUE ET AL. 2003).

TFAs originating from the maternal diet can also – like the other FAs – cross the placenta (KOLETZKO 1992; BERGHAUS ET AL. 1998).

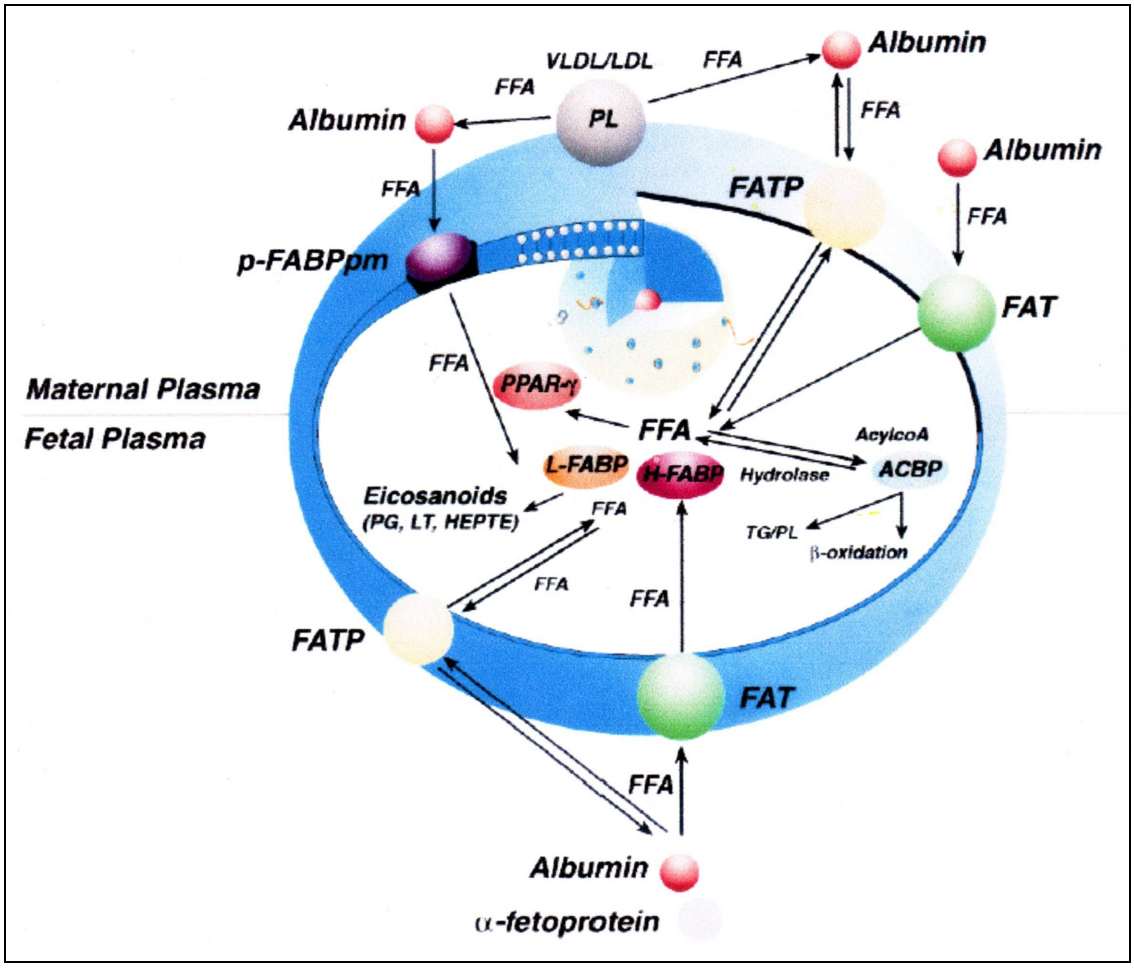

Figure 4 Placental fatty acid uptake and metabolism (CAMPBELL ET AL. 1998)

The presence of FAT and FATP in both membranes permits an exchange of FAs from the maternal into the fetal circulation and vice versa. (HAGGARTY 2002). The cytosolic FABPs bind the FFA and are possibly involved in effective FA transport and metabolism in the placenta (synthesis of storage and structural lipids, ß-oxidation, eicosanoids, PPAR activation) (DUTTAROY 2004; DUTTAROY 2009).

The placenta itself also regulates the substrate supply with FFA through different mechanisms: on the one hand through the activity of the lipoproteinlipase (dramatically increased during the last trimester of pregnancy) and the number of lipoprotein-binding sites, on the other hand through the formation of leptin, released into maternal and fetal circulation, stimulating lipolysis (WATERMAN ET AL. 1998; HOGGARD ET AL. 2001; HAGGARTY 2002; HERRERA ET AL. 2006).

2.5 Fetal and infant lipid accretion

Glucose is quantitatively the most important nutrient crossing the placenta, followed by amino acids, FA and glycerol (HAY 1994). The fetus is able to synthesize SFA and MUFA from glucose and keton bodies but is dependent on maternal EFA transfer (KIMURA 1989; KOLETZKO ET AL. 2008). Liver microsomes of human fetus and neonates demonstrate significant delta-6- and delta-5-desaturase activities (POISSON ET AL. 1993; RODRIGUEZ ET AL. 1998) and stable isotope technology has provided evidence that both term and preterm infants are able to convert LA to AA and ALA to DHA (CLARK ET AL. 1992; DEMMELMAIR ET AL. 1995; JENSEN ET AL. 1996; SALEM ET AL. 1996; SAUERWALD ET AL. 1996; SAUERWALD ET AL. 1997; SZITANYI ET AL. 1999; BRENNA ET AL. 2009). However, whether the capacity of these metabolic systems proceeds at a sufficient rate to meet tissue demands is at present controversial and is influenced by genetics, gender, and the amount of precursor fatty acids available in the diet (CARNIELLI ET AL. 1996; HOFFMAN ET AL. 2000; UAUY ET AL. 2000; CARNIELLI ET AL. 2007). LC-PUFAs have therefore to be regarded as essential for human infants.

There is very little data on the accretion of LC-PUFA in the developing human brain. Available data indicates that accretion starts slowly early in fetal life (PERCY ET AL. 1996). During the last trimester of pregnancy however, the lipid deposition of the fetus is vast. Intrauterine requirements for n-6 and n-3 FA of the human fetus have been estimated to be 400 mg/kg/d or 50 mg/kg/d, respectively. Autopsy analysis has estimated that the fetus accumulates approximately 30 – 70 mg/d DHA during the last trimester of gestation mainly deposited in white adipose tissue while AA accretion mainly occurs postnatally (CLANDININ ET AL. 1980; CLANDININ ET AL. 1981; MARTINEZ AND MOUGAN 1998; FLEITH AND CLANDININ 2005). CLANDININ and coworkers have also measured that the fetal brain incorporates PUFAs with an n-6/n-3 ratio of approximately 8:1, compared with a typical North American maternal diet with a ratio of > 10:1.

Several studies on the FA composition of neonatal and maternal plasma at term delivery have been published. Whether the level of DHA is higher in the neonate than in the mother depends on which lipid pool is examined. The absolute concentration of all plasma lipids is higher in the mother than in the infant (FRIEDMAN ET AL. 1978; BERGHAUS ET AL. 1998). Therefore, the absolute concentration of DHA is higher in maternal than in fetal blood, in contrast to the percentage of DHA in total FAs in circulating PLs, which seems to be consistently higher in the neonate (AL ET AL. 1990;

RUYLE ET AL. 1990; BERGHAUS ET AL. 1998; ELIAS AND INNIS 2001; LAURITZEN ET AL. 2001; HERRERA ET AL. 2004; INNIS 2005). This is also the case for AA and other LC-PUFAs. There seem to be an even more pronounced difference in the relative contribution of DHA in the pools of plasma TAGs and CEs (VAN DER SCHOUW ET AL. 1991; BERGHAUS ET AL. 1998), although this is not consistent with the results from the entire plasma lipid pool (YOUNG ET AL. 1997). The DHA content in percent of total FA in RBCs does not seem to differ between the mother and the infant. Before birth, FFAs in fetal blood are transported bound to albumin and to a lesser extent also bound to α-fetoprotein. They are returned to the fetal liver, are reesterified and released into the fetal blood circulation (BENASSAYAG ET AL. 1997).

After delivery and during the first weeks and months of life, the high percentage of AA and DHA content of infant blood decreases, in contrast to an increasing content of LA. Additionally, the content of TAG rich LPs in infant plasma increases as the FA supplied by breast milk or formula after birth are mainly transported via this pathway (INNIS 2005).

2.6 Metabolism in pregnancy and lactation

2.6.1 Anthropometry

In pregnancy, extra energy and nutrients are needed to cover the costs of maternal and fetal tissue accretion, and the rise in energy expenditure attributable to basal metabolism and physical activity. Because of uncertainties regarding desirable gestational weight gain (GWG), maternal fat deposition, putative reductions in physical activity and energetic adaptations to pregnancy, controversy remains regarding energy requirements during pregnancy. GWG is a major determinant of the incremental energy needs during pregnancy, since it determines not only energy deposition, but also the increase in basal metabolic rate and total energy expenditure due to the energy cost of moving a larger body mass. GWG comprises the increase of fetal and maternal tissues (e. g. placenta, amniotic fluid, uterus, breasts, blood), and the increases in maternal fat stores. In the early seventies, data from more than 3800 primigravidae who were eating without restriction were analyzed to establish the physiologic norm for weight gain in pregnancy and the weight gain associated with the best reproductive performance (HYTTEN AND LEITCH 1971). Two decades later, the WHO Collaborative Study on Maternal Anthropometry and Pregnancy

Outcomes (WHO 1995) reviewed information on 110.000 births from 20 different countries in order to define those anthropometric indicators which are most predictive of optimal fetal and maternal outcomes. The study was used to define desirable birth weights and maternal weight gains associated with lower risk of fetal and maternal complications, i.e. low birth weight, intrauterine growth restriction (IUGR), preterm birth, preeclampsia, postpartum haemorrhage and assisted delivery. Birth weights between 3.1 and 3.6 kg (mean, 3.3 kg) were associated with the optimal ratio of maternal and fetal outcomes. The range of GWGs associated with birth weights greater than 3 kg was 10 – 14 kg (mean, 12 kg). Because of the interaction between the pre-pregnancy body mass index (BMI) and GWG on birth weight (BW), the Institute of Medicine (IOM) took into account the nutritional status of a woman before conception and recommends different ranges of GWG for women with low BMI (< 18.5 kg/m^2: 12.5 – 18 kg), normal BMI (18.5 – 24.9 kg/m^2: 11.5 – 16 kg) and high BMI (overweight: BMI > 24.9 – 29.9 kg/m^2: 7 – 11.5 kg or obese: BMI > 29.9 kg/m^2: 5 - 9 kg) (BUTTE 2005; IOM 2009). A review of ABRAMS ET AL. showed that GWG within the range recommended from the IOM was in fact associated with the best fetal and maternal outcomes (ABRAMS ET AL. 2000). The mean gain in adipose tissue mass associated with the mean GWG of 12 kg observed in the WHO Study was 3.7 (range: 3.1 – 4.4) kg. LEDERMAN ET AL. and BUTTE ET AL. showed that GWG is positively correlated with fat gain and that excessive weight gain was primarily attributed to fat mass gain, not fat free mass (LEDERMAN ET AL. 1997; BUTTE ET AL. 2003). Body fat as a percent of total body weight of normal weight women, gaining weight as recommended by the IOM was 17.9 ± 5.4 % at week 14 and 21.7 ± 5.8 % at week 37 of pregnancy, respectively. Of the 3.7 kg fat mass gained on average according to a total GWG of about 12 to 13 kg, approximately 400 – 450 g of fat is accumulated by the fetus and 2.5 – 3 kg by the mother. Of the remaining 9 kg, ~ 1 kg accounts for the deposition of protein and approximately 8 kg for water retention (PRENTICE ET AL. 1996).

2.6.2 Lipid metabolism

Changes in hepatic and adipose tissue metabolism during pregnancy alter circulating concentrations of triacylglycerols (TAG), free fatty acids, cholesterol, cholesterolester and PLs. After an initial decrease in the first 8 wk of pregnancy, there is a steady increase in TAGs, FAs, cholesterol, cholesterolesters, lipoproteins and PLs in blood

plasma. Changes in lipid metabolism promote the accumulation of maternal fat stores in early and mid pregnancy and enhance fat mobilization in late pregnancy, coinciding with the time of maximal fetal growth. During the first and second trimenon of pregnancy, increased estrogen, progesterone, and insulin concentrations favor lipid deposition and inhibit lipolysis. In late pregnancy, maternal metabolism shifts from an anabolic to a catabolic state and promotes the use of lipids as a maternal energy source while preserving glucose and amino acids for the fetus. Decreasing lipid uptake into adipose tissue through physiological relative insulin resistance and reduced lipoprotein lipase activity, and increased lipolysis and mobilization of lipid stores results in a constant increase of TAG, non esterified FA, cholesterol, phospholipid (PL) and lipoprotein concentrations in blood plasma until delivery and during the last trimester of pregnancy, respectively (KNOPP ET AL. 1986; LESSER AND CARPENTER 1994; ALVAREZ ET AL. 1996; BENASSAYAG ET AL. 1997; BUTTE 2000; PRENTICE AND GOLDBERG 2000). After the first 10 weeks of pregnancy, the serum TAG concentration in pregnant women is 20 % higher than in nonpregnant women; reaching a value of approximately 3 times that of nonpregnant women by term (DESOYE ET AL. 1987; LAIN AND CATALANO 2007; BASARAN 2009). Other serum lipids also increase during pregnancy, but the net change is less than that of TAG (KING 2000).

Not only lipid concentration in blood plasma changes during pregnancy but also the fatty acid pattern of the blood lipids. There is a progressive increase in the proportion of total saturated fatty acids in maternal plasma from the first trimester through to delivery. LA and oleic acid remain stable throughout pregnancy but the proportion of DHyLA, AA, EPA and DHA decreases (HERRERA ET AL. 2004). In RBCs a decrease of LA, expressed as percentage of total FAs, in contrast to a significant increase of ALA and DHA was observed during gestation (STEWART ET AL. 2007).

Due to the increase of blood volume and the development of maternal hyperlipidemia, the absolute amounts of EFA and LC-PUFA in blood lipids and RBCs of pregnant women are higher than in nonpregnant women.

The „Food and Agriculture Organization of the World Health Organization“ notes that approximately 2.2 g n-6- + n-3-FAs / d are deposited in maternal and fetal tissue during pregnancy (WHO 1993). This value may underestimate the dietary requirement in that FA turnover in eicosanoid synthesis, ß-oxidation, and membrane turnover is not considered (INNIS AND ELIAS 2003).

2.7 Programming - impact on birth weight and body composition

The term “programming” was introduced over 30 years ago by G. DÖRNER, head of the Institute of Experimental Endocrinology, Charité, Berlin (DÖRNER 1975). He stated that “the concentrations of hormones, metabolites, and neurotransmitters during critical early periods of development are capable of pre-programming brain development, functional disturbances, and diseases as well as syndromes or reproduction and metabolism in human adulthood.” He also proposed a gene-environment-interaction during early development to determine later function in adult life.

There are many examples of this process observable within the natural world. One that is frequently cited describes the mechanisms that determine sex in crocodilians (MILNES ET AL. 2002), where the temperature of the eggs determines the expression of genes encoding aromatases that are responsible for synthesis of the sex steroids.

“Nutritional programming” was introduced by A. LUCAS (LUCAS 1991) and is the process through which variation in the quality of nutrients consumed during pregnancy or early life or in more general terms: during a critical period of development - exerts permanent effects upon the developing fetus (LANGLEY-EVANS 2008).

Although the fetal genome determines the growth potential in utero, the weight of the evidence suggests that it plays a subordinate role in determining the growth that is actually achieved (SNOW 1989). Rather, it seems that the dominant determinant of fetal growth is the nutritional and hormonal milieu in which the fetus develops, and in particular the nutrient and oxygen supply. Whereas maternal diet during pregnancy appears to exert relatively modest effect on offspring BW, in contrast to the impact of maternal pregnancy metabolism (WELLS ET AL. 2007).

Evidence supporting a long-term effect of *absolute levels* of maternal nutrient intake during pregnancy were shown in follow-up studies following the Dutch famine of 1944 – 1945 which found that maternal energy restriction at different stages of pregnancy was variously associated with obesity, dyslipidaemia, and insulin resistance in the offspring (RAVELLI ET AL. 1998; ROSEBOOM ET AL. 1999; ROSEBOOM ET AL. 2000; ROSEBOOM ET AL. 2000; GODFREY AND BARKER 2003).

In Western communities, randomized controlled trials (RCTs) of maternal macronutrient supplementation have had relatively small effects on birth weight (KRAMER 1993; KRAMER AND KAKUMA 2003; LAGIOU ET AL. 2004). Observational studies

point into the same direction (MATHEWS ET AL. 1999; MATHEWS ET AL. 2004). This suggests that mechanisms in the maternal and placental systems act to ensure that human fetal growth and development is little influenced by normal variations in maternal macronutrient intake and that there is a simple relationship between a women's body composition and the growth of her fetus.

In contrast, some studies indicate that *the balance* of macronutrients in the mother's diet can have important short- and long-term effects on the offspring as shown in experimental studies in rats (LANGLEY-EVANS ET AL. 1994) and humans (CAMPBELL ET AL. 1996). These have found that maternal diets with a low ratio of protein to carbohydrates were associated with an increased risk of low birth weight and an increased risk of raised blood pressure in the offspring in adult life.

In humans, few studies have examined the possibility of maternal nutrition during pregnancy having *tissue-specific* effects in the fetus, leading to greater alterations in neonatal proportions than in birth weight (GODFREY AND BARKER 2003). GODFREY ET AL. found that women with low diary protein intakes in late pregnancy tended to have babies that were thinner at birth but that maternal diary protein intakes were not, however, related to birth weight (GODFREY ET AL. 1996; GODFREY ET AL. 1997).

In this context, the role of LC-PUFAs and their possible influence/impact on adipocyte differentiation and the development of adipose tissue in the infant seem to be of major interest, as postulated by AILHAUD and coworkers (AILHAUD AND GUESNET 2004), already mentioned before. They could demonstrate in a rodent model that the ratio of n-6 to n-3 LC-PUFAs in the maternal diet during pregnancy and lactation seems to influence AT development and later body weight of the pups. However, up to now, the adipogenic effect of the n-6 FAs has not been clearly established *in vivo* and human data supporting a causal link between high n-6 LC-PUFA intakes and increased AT development, childhood obesity or elevated birth weight is lacking.

Various factors other than nutrition such as genetic and environmental parameters are known to influence the size of newborns. Numerous studies have shown the significant effect of pre-pregnancy weight or BMI, GWG, height, maternal smoking (OKEN ET AL. 2008), age and parity on fetal and newborn anthropometry. These relationships are most likely modified by additional factors or interfere with each other. It has been explicitly recognized since at least 1990, when the first IOM recommendations for GWG were published, that both pre-pregnancy BMI and GWG are associated with the outcome of pregnancy (IOM 1990). A higher *pre-pregnancy*

weight or *BMI* was associated with increased infant BW in a number of studies (ABRAMS AND LAROS 1986; ROSSNER AND OHLIN 1990; KIRCHENGAST AND HARTMANN 1998; THAME ET AL. 2004). Apart from this well known association, SEWELL ET AL. showed that the increase in birth weight seems to be attributable primarily to an increase in fat mass and not in LBM of the infant (SEWELL ET AL. 2006). The strong association between increasing *GWG* and increasing birth weight was reported in several studies (IOM 1990; ZHOU AND OLSEN 1997; CNATTINGIUS ET AL. 1998; LEDERMAN ET AL. 1999; BUTTE ET AL. 2003; CEDERGREN 2006; FORSUM ET AL. 2006; STOTLAND ET AL. 2006; DEVADER ET AL. 2007; KIEL ET AL. 2007; VISWANATHAN ET AL. 2008). BUTTE ET AL. (2003) and LEDERMAN ET AL. (1999) showed that it was rather the gain in fat free mass (FFM) of the mother which was associated with the BW of the infant and not the gain in maternal fat mass. FORSUM ET AL. (2006) instead demonstrated a correlation of maternal fat mass with the overall BW of the infants, that was not correlated with the AT mass of the infant.

Often, pre-pregnancy BMI and GWG have a combined effect on pregnancy outcome as analyzed in more detail in some of the studies (CEDERGREN 2006; CEDERGREN 2007; DEVADER ET AL. 2007; KIEL ET AL. 2007; NOHR ET AL. 2008; CRANE ET AL. 2009).
As mentioned before, BW also increases with maternal age up to 35 y of age and parity (MACLEOD AND KIELY 1988; COGSWELL AND YIP 1995; DHALL AND BAGGA 1995).
The factors influencing fat storage measured by neonatal skinfold thickness (SFT) were found to be others than influencing the more common newborn dimensions like BW, birth length and head circumference. GUIHARD-COSTA ET AL. showed that subscapular SFT depends only on the nutritional status of the mother characterized by maternal pre-pregnancy BMI and GWG and is far less influenced by height for example (GUIHARD-COSTA ET AL. 2004). Another point of discussion is whether infant body fat or infant lean body mass or the relation of body fat/lean body mass increases with increasing BW.
However, the two most notable maternal conditions that seem to predispose offspring to increased adiposity at birth are maternal obesity and maternal diabetes.
Whereas maternal glucose is freely transferred to the fetus, maternal insulin does not cross the placenta. The developing fetal pancreas responds to a glucose load by producing insulin, which also acts as a fetal growth hormone (FREINKEL 1980). LGA infants of mothers with gestational diabetes mellitus appear to have increased fat mass and decreased lean mass in anthropometric measurements when compared to

LGA infants born to non-diabetic mothers (CATALANO ET AL. 1992; DURNWALD ET AL. 2004; SCHAEFER-GRAF ET AL. 2005; HILLIER ET AL. 2007).

Parental adiposity is directly associated with offspring BW, with stronger associations for the mother than for the father (GUILLAUME ET AL. 1995; OKUN ET AL. 1997; EHRENBERG ET AL. 2004; WHITAKER 2004; CATALANO AND EHRENBERG 2006; CATALANO ET AL. 2009).

2.8 Birth weight - association with later body composition and obesity

As mentioned in chapter 2.7 BW and AT mass seem to be influenced by numerous parameters – among others maternal nutrition and metabolism. But is BW or adiposity at birth associated with later obesity? Numerous studies have addressed the association between birth weight and later attained BMI, traditionally interpreted as greater fatness or obesity. Almost all of the studies have found direct associations, i.e., that higher birth weight is associated with higher attained BMI later in life (FALL ET AL. 1995; RICH-EDWARDS ET AL. 1997; SORENSEN ET AL. 1997; RASMUSSEN AND JOHANSSON 1998; PARSONS ET AL. 1999; GALE ET AL. 2001; PARSONS ET AL. 2001; OKEN AND GILLMAN 2003; SCHAEFER-GRAF ET AL. 2005; APFELBACHER ET AL. 2008). Unfortunately, most of the studies include incomplete data on possible confounding factors like gestational age or parental body size. In the U. S. Growing Up Today Study, a 1 kg increment in BW among full term infants was associated with an increase in the risk of being overweight at ages 9 to 14 years of approximately 50 % (GILLMAN ET AL. 2003; OKEN AND GILLMAN 2003). Cigarette smoking may complicate the relationship between BW and later body size because mothers who smoke have infants with lower BW (HARRISON ET AL. 1983; ZAREN ET AL. 1996). In a recent review and meta-analysis it was shown, that prenatal exposure to smoking appeared to increase rates of overweight in childhood (OKEN ET AL. 2008). Similarly, social and economic factors may confound the association between BW and later adiposity. Furthermore, these studies do not necessarily imply a linear association between BW and later fatness across the entire range of BW. Some studies suggest a “U-shaped” association, implying a high prevalence of obesity in those of low or high BW (ROGERS 2003). Although a higher BMI is traditionally interpreted as “greater fatness”, it also reflects LBM or FFM and is therefore a somewhat poor indicator as an index of adiposity (WELLS ET AL. 2007).

More recently, several studies explored the relation of BW and later body composition in more detail, as summarized by CHOMTHO ET AL. (CHOMTHO ET AL. 2008). The cited studies differ in the age range, body composition measurement techniques and statistical approaches. However, some of them have shown a positive association between BW and later FFM (GALE ET AL. 2001; LOOS ET AL. 2001; LOOS ET AL. 2002; LI ET AL. 2003; SINGHAL ET AL. 2003; SAYER ET AL. 2004; KENSARA ET AL. 2005; SACHDEV ET AL. 2005; WELLS ET AL. 2005; LABAYEN ET AL. 2006; MURPHY ET AL. 2006; ROGERS ET AL. 2006; ELIA ET AL. 2007). In contrast, the results have been less consistent for fat mass and fat distribution, reporting negative, positive and nonsignificant associations. COMTHO ET AL. investigated the associations between BW and later fat mass (FM), FFM and fat distribution using the 4-component model, which has the best precision of all techniques and is the most robust technique for detecting interindividual variability in the composition of FFM (BRAY ET AL. 2002). In this study, birth weight was significantly positively associated with height in boys and girls and significantly positively associated with FFM in boys. In contrast, BW was not related to percentage body fat or FFM in both sexes and the evidence for fetal programming of later FM or central adiposity was weak.

Moreover, a growing body of literature suggests that not only birth weight, but also the first few weeks to months of life are a particularly sensitive period for the development of obesity. In 2005, BAIRD ET AL. published a systematic review of 10 studies that assessed the relation of infant weight gain after birth with subsequent obesity (BAIRD ET AL. 2005). Relative risks of later obesity ranged from 1.17 to 5.70 among infants with more rapid weight gain in the first year of life. In 2008, GILLMAN summarized the latest results of several studies concerning this topic, also showing strong evidence for a positive association between rapid infancy weight gain and later obesity (SACHDEV ET AL. 2005; STETTLER ET AL. 2005; DENNISON ET AL. 2006; BELFORT ET AL. 2007; EKELUND ET AL. 2007; GILLMAN 2008; TAVERAS ET AL. 2009). In accordance with these results, many other studies, summarized in systematic reviews or very recently published, concluded, that rapid weight gain in infancy is a significant risk indicator for later adiposity (TOSCHKE ET AL. 2002; MONTEIRO AND VICTORA 2005; ONG AND LOOS 2006; TAVERAS ET AL. 2009). However, the lack of experimental evidence in humans limits the ability to make causal inferences, especially in a context where unmeasured or unknown confounding factors could explain much of the observed associations without a real cause–effect relationship.

In addition, it is also plausible that prenatal factors may influence the association of BW and later obesity, such as maternal smoking, parity, gestational age, GWG, alterations in glucose-insulin homeostasis, or other nutrient-hormonal adaptions in the maternal-placental-fetal unit (FISCH ET AL. 1975; ONG AND DUNGER 2004; WHITAKER 2004; REILLY ET AL. 2005; MOREIRA ET AL. 2007; GILLMAN ET AL. 2008; WROTNIAK ET AL. 2008). In particular, GWG was directly associated with later BMI and risk of obesity in adolescence, as shown in a recent analysis of the U. S. Growing Up Today Study (OKEN ET AL. 2008).

2.9 Lipids in human milk

During the first days after delivery, the mammary gland secretes colostrum (1 to 5 days post partum (pp)), breast milk (BM) especially rich in protein, immunglobulines and leukocytes. Over the course of lactation, the composition of breast milk is changing. The protein and mineral content of breast milk decreases, in contrast to an increasing fat and lactose concentration. The concentration of PL and cholesterol decreases with the course of lactation, as well (BITMAN ET AL. 1984; BOERSMA ET AL. 1991; IDOTA ET AL. 1991).

Mature breast milk is secreted from the 2^{nd} / 3^{rd} wk after delivery onwards. Breast milk composition also changes during one infant feeding with a pronounced, 2- to 3-fold increase of the fat content by the end, without a change in the percentage of each fatty acid (HARZER ET AL. 1983; JENSEN 1999). The average fat content of BM is ~ 4 %. In general it is assumed that the total lipid content of BM is not influenced by the diet. New studies seem to confirm the assumption that a high dietary intake of TFAs may reduce the total lipid content of BM from lean women (ANDERSON ET AL. 2005). Furthermore, the lipid content of BM oscillates diurnally in undernourished women with a low fat and carbohydrate intake (JENSEN 1999). The fatty acid composition of breast milk lipids depends on nutrition, length of gestation, time of lactation, diseases of the mother like diabetes mellitus and other individual and genetic factors. Nutrition seems to have the most important impact on BM fatty acid composition. Observed differences in various regions of the world are primarily due to different dietary habits (FIDLER AND KOLETZKO 2000). The most important difference between BM from women who follow a typical “western-style-diet” and women who follow a “non-western-diet” are a lower percentage of LA and LC-PUFA in BM from women with a typical western-style-diet and a lower level of TFA in BM of women

following a non-western-style-diet. There are evident differences in the fatty acid profile of breast milk from Japan (IDOTA ET AL. 1991), Hungary (GERE ET AL. 1983), Nigeria (OGUNLEYE ET AL. 1991), Israel (BUDOWSKI ET AL. 1994), Canada (CHEN ET AL. 1995), Iran (BAHRAMI AND RAHIMI 2005) and France (CHARDIGNY ET AL. 1995) as reviewed by JENSEN (1999).

The fatty acids of human milk are either synthesized by the mammary gland or derived from the blood. Maternal diet, AT stores and hepatic metabolism also determine the FA composition of the blood. TAGs from chylomicrons and VLDL lipoproteins appear to supply most of the FAs for milk secretion (INSULL ET AL. 1959; MELLIES ET AL. 1979; VUORI ET AL. 1982; HACHEY ET AL. 1987; EMKEN ET AL. 1989; FRANCOIS ET AL. 1998). It is known from animal studies that TAGs are transported to the mammary gland and incorporated in chylomicrons, VLDLs and LDLs, where they are hydrolyzed through the lipoproteinlipase to free fatty acids and to 2-monoacylglycerides and are incorporated after 6 – 8 hours into BM, TAGs, PLs, and CEs and then released as lipid droplets (BARRY ET AL. 1963; MCBRIDE AND KORN 1964).

Probably 12 % of total lipids account for the saturated, medium-chain FAs, synthesized by the mammary gland and 30 % for the long-chain FAs directly from the diet. The remaining 60 % originate from tissue synthesis and lipid depots.

In a study with pregnant women and radioactive labeled LA, DEMMELMAIR ET AL. could also confirm that approx. 30 % of the LA in BM originates form the diet (DEMMELMAIR ET AL. 1998). These results indicate that each meal may influence the fatty acid composition in BM lipids. In contrast, the lipid stores of the body reflect the long-term average intake of dietary fatty acids and attenuate the variation of the FA composition in BM. A high proportion of carbohydrates and a low proportion of lipids in the diet stimulate the *de novo* synthesis of C8:0 – C14:0 FAs from glucose in the mammary gland. Their percentage can increase from 10 % to 20 % or more of total lipids in colostrum as demonstrated in a study from Nigeria (KOLETZKO ET AL. 1992).

Lipids in breast milk are emulsified lipid globules with a diameter of 2 to 4 µm, surrounded by milk lipid globule membranes. BM lipids consist of 98 % TAGs, 0.8 % PL, 0.5 % cholesterol and other minor components (JENSEN 1999). They provide 40 % to 50 % of the total energy intake of a breast-fed infant and also supply the infant with fat soluble vitamins, EFA, LC-PUFA, and cholesterol. BM also contains short-

chain FA (C8:0 – C14:0) which can be metabolized easily and are therefore an appropriate energy source for the infant.

Up to now, over 200 different FAs were identified in BM (JENSEN 1999). They are not evenly distributed overall theoretical possible positions of the glycerol molecule (MARTIN ET AL. 1993; WINTER ET AL. 1993) but the distribution determines the melting point of BM fat and thereby influences the digestibility (BRACCO 1994).

Milk lipids are composed of approx. 40 % SFA, between 40 % and 45 % MUFA and approx. 15 % PUFA. LC-PUFAs account for approx. 3 %, TFA for 3.5 %, the sum of n6-FA between 10 % and 12 %, and the sum of n3-FA for 1.5 % of total fatty acids (FIDLER & KOLETZKO 2000; PRECHT & MOLKENTIN 1999; GENZEL-BOROVICZENY ET AL. 1997; CHARDIGNY ET AL. 1995). The content of LC-PUFA is highest in colostrum and declines during the course of lactation (SALA-VILA ET AL. 2005; MINDA ET AL. 2004; Yu ET AL. 1998; GENZEL-BOROVICZENY ET AL. 1997; BOERSMA ET AL. 1991; HARZER ET AL. 1983). LA content alternates between 8 % and 14 %, ALA content between 0.4 % and 1 %. The main LC-PUFAs of the n-6-family are: AA, DHyLA and eicosadienoic acid (C20:2 n6) and the main LC-PUFAs of the n-3-familiy are: DHA, DPA und EPA (FIDLER & KOLETZKO 2000). In most western populations the mean level of DHA in BM is between 0.2 and 0.4 % of total FAs. In a recent meta-analysis, BRENNA ET AL. (BRENNA ET AL. 2007) reviewed the AA and DHA concentration in mature breast milk worldwide. The mean levels of DHA and AA in BM were 0.32 ± 0.22 % and 0.47 ± 0.13 %, respectively. Milk DHA content appears to be closely linked to maternal dietary DHA intake, with dose-dependent linear increases in BM concentrations of this nutrient with increased maternal intake (MAKRIDES ET AL. 1996). The response of milk AA levels to maternal dietary AA intake is less predictable than that of DHA and may be more sensitive to the profile of other maternal dietary FAs (SMIT ET AL. 2000). In other words, AA concentrations, on average, vary much less than do DHA concentrations indicating a tight control of the AA level in BM.

2.10 n-3 LC-PUFA supplementation in pregnancy and lactation

The effects of supplementing pregnant women with n-3 LC-PUFAs on pregnancy outcomes have been evaluated in a number of randomized controlled clinical trials, which provided daily DHA intakes ranging from 200 mg to 1200 mg DHA, and up to 2.7 g total n-3 LC-PUFAs. Most of the studies aimed to prevent pre-eclampsia, to prolong the duration of gestation, to prevent preterm birth and to improve fetal growth

by modifying the synthesis of the eicosanoids involved in pregnancy and delivery. Others aimed to assess the efficacy of n-3 LC-PUFA supplementation during pregnancy and lactation on infant visual and cognitive development. SZAJEWSKA ET AL. (SZAJEWSKA ET AL. 2006) summarized the results of 6 RCTs (OLSEN ET AL. 1992; HELLAND ET AL. 2001; MALCOLM ET AL. 2003; SMUTS ET AL. 2003; SMUTS ET AL. 2003; SANJURJO ET AL. 2004), which included 1278 participants, regarding the effect of n-3 LC-PUFA supplementation during pregnancy on pregnancy outcomes and infant growth measures at birth (Fig. 5). The duration, starting time of the supplemention and the sources and amounts of n-3 LC-PUFAs, EPA and DHA, varied between the trials. n-3 LC-PUFA supplementation was associated with a significantly longer duration of gestation (weighted mean difference (WMD): 1.57 d; 95 % CI: 0.35, 2.78 d) compared to control subjects. There was no significant difference, regarding preterm or cesarean delivery, rate of low birth weight, pre-eclampsia or eclampsia and other maternal outcome parameters between the supplemented and the control subjects.

Characteristics of included trials[1]

Study	Generation of randomization	Allocation	Blinding	ITT	Follow-up	Risk of bias[2]	Intervention	Control	Duration of intervention
Helland et al (14)[3]	Adequate (computer program)	Unclear (not reported)	Yes	No	341/590[4] (58%)	Medium	Cod liver oil (10 mL/d); 1183 mg DHA/10 mL, 803 mg EPA/10 mL, 117 μg vitamin A/mL, 1 μg vitamin D/mL 1.4 mg dl-α-tocopherol/mL	Corn oil (10 mL/d); 117 μg vitamin A/mL, 1 μg vitamin D/mL, 1.4 mg dl-α-tocopherol/mL	From week 18 of gestation to 3 mo after delivery
Malcolm et al (24)[5]	Unclear (not reported)	Unclear (not reported)	Yes	No	63/100 (63%); later, 60/100 (60%)	High	Fish oil (200 mg DHA/d; < 40 mg EPA/d)	323 mg Sunflower oil with high concentrations of oleic acid	From week 15 of gestation to delivery
Olsen et al (21)[6]	Adequate (sealed, opaque envelope)	Adequate	Yes	Yes	100%	Low	Fish oil (4 g/d); 920 mg DHA/d + 1280 mg EPA/d + 2 mg tocopherol/d, ≈2.7 g n–3 PUFA/d	Olive oil (4 g/d) or no oil supplement	From week 30 of gestation to delivery
Sanjurjo et al (25)[7]	Adequate (computer-generated number table)	Adequate	No	No	16/20 (80%)	Medium	Fat (2 g/d); 200 mg DHA/d + 40 mg EPA/d	Placebo	From weeks 27–28 of gestation to delivery
Smuts et al (19)[8]	Unclear (not reported)	Unclear (not reported)	Yes	No	37/52 (71%)	High	High-DHA eggs (135 mg DHA/egg; mean intake 206 ± 112 mg DHA/d)	Ordinary eggs (18 mg DHA/egg; mean intake 23 ± 9 mg DHA/ d) Low-egg-intake group (mean DHA intake 7 ± 9 mg/d)	Possibly 3rd trimester (no information provided); mean: ≈13 wk
Smuts et al (20)[9]	Adequate (computer-generated number table)	Unclear (not reported)	Yes	No	291/350 (83%)	Medium	High-DHA eggs (133 mg DHA/egg); mean intake 7.2 ± 3.4 eggs/wk = 137 ± 65 mg DHA/d	Ordinary eggs (33 mg DHA/egg); mean intake 7.3 ± 3.4 eggs/wk = 34 ± 16 mg DHA/d	From weeks 24–28 of gestation to delivery

[1] DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ITT, intention-to-treat analysis; PUFA, polyunsaturated fatty acid.

[2] Based on the number of criteria judged inadequate in each study: low risk of bias (≤ 1 inadequate criterion); medium risk of bias (≤ 3 inadequate criteria); high risk of bias (> 3 inadequate criteria).

[3] *n* followed = 175 and 166, and *n* randomly assigned = 301 and 289 for the intervention and control groups, respectively.

[4] *n* followed/*n* initially randomly assigned (all such values).

[5] *n* followed = 31 and 29, and *n* randomly assigned = 50 and 50 for the intervention and control groups, respectively.

[6] *n* followed = 266 for the intervention group and 136 (olive oil) and 131 (no oil supplement) for the control group.

[7] *n* followed = 8, and *n* randomly assigned = 10 for the intervention group.

[8] *n* followed = 18 and 19, and *n* randomly assigned = 27 and 25 for the intervention and control (ordinary eggs) groups, respectively; *n* followed = 16 for the control (low-egg-intake) group without randomization.

[9] *n* followed = 142 and 149, and *n* randomly assigned = 176 and 174 for the intervention and control groups, respectively.

Figure 5 Characteristics of the trials (SZAJEWSKA ET AL. 2006)

There was no significant difference in birth weight and birth length, but n-3-supplementation was significantly associated with a greater head circumference of the infants in the supplemented group. There was no indication of any safety concerns with respect to the use of n-3 LC-PUFA supplementation in pregnancy.

In a Cochrane review (MAKRIDES ET AL. 2006) which aimed to estimate the effects of marine oil and other prostaglandin precursor supplementation during pregnancy on the risk of pre-eclampsia, preterm birth, low birth weight, small for gestational age (SGA) birth, and on other substantial measures of maternal morbidity and mortality for the child, some additional studies (D'ALMEIDA ET AL. 1992; ONWUDE ET AL. 1995) were considered. Data from this systematic review also indicates a small but consistent increase in the mean length of gestation associated with marine oil and other prostaglandin precursor supplementation during pregnancy (WMD: 2.5 d, 95 % CI: 1.03 - 4.07 d, 1621 subjects). Supplementation was also associated with a small

increase in birth weight that appears commensurate with the 2 – 3 day increase in the mean duration of gestation (WMD 47 g, 95 % CI: 1 - 93 g, 2440 subjects). Again, there was no indication of any safety concerns and the supplementation, although high doses were used, appeared to be safe.

DECSI & KOLETZKO (DECSI AND KOLETZKO 2005) summarized the results of 6 RCTs on the potential effects of different levels of n-3 FA supply in pregnancy on biochemical and developmental outcomes of mothers and their children. In addition to the studies of HELLAND ET AL. (2001), SANJURJO ET AL. (2004) AND SMUTS ET AL. (2003), they discussed two further RCTs published by DUNSTAN ET AL. (2004) and MONTGOMERY ET AL. (2003) which only reported biochemical outcomes (MONTGOMERY ET AL. 2003; DUNSTAN ET AL. 2004). No unequivocal effect of n-3 LC-PUFA supplementation on the length of pregnancy or on the basic anthropometric data of the newborn was demonstrated. In contrast, supplementation of DHA in relatively high doses (> 1 g/d) led to a significant increase in infant DHA values.

In another five RCTs, women were supplemented with various amounts of n-3 LC-PUFAs during pregnancy. They are of minor importance in relation to the present study because of a very small number of subjects studied, a very short intervention period during pregnancy or because they do not provide biochemical data and were originally designed to assess, if n-3 LC-PUFA supplementation could improve infant visual and cognitive development (CONNOR ET AL. 1996; OTTO ET AL. 2000; VELZING-AARTS ET AL. 2001; TOFAIL ET AL. 2006; JUDGE ET AL. 2007). As stated, there was no significant difference between the infant birth weights from mothers in the respective supplemented or control groups. In contrast, as also stated, the respective n-3 LC-PUFA supplement effectively increased maternal and infant n-3 LC-PUFA status.

A very recent study from Canada (INNIS AND FRIESEN 2008), a multicenter study from Europe (KRAUSS-ETSCHMANN ET AL. 2007) and a study from Germany (BERGMANN ET AL. 2007) support the former findings. In the canadian study, women were supplemented with 400 mg DHA/d from the 16th wk of gestation until delivery. There were no significant differences in infant birth weight or other pregnancy outcomes between the supplemented and the control group but supplementation effectively increased maternal RBC DHA status (INNIS AND FRIESEN 2008).

The European trial showed that supplementation of pregnant women from gestation wk 22 with 0.5 g DHA and 0.15 g EPA was associated with a significant increase in

maternal and cord blood plasma DHA and EPA content. No obvious differences among the intervention groups regarding infant birth outcomes and anthropometry were found (KRAUSS-ETSCHMANN ET AL. 2007).

Women in the German study were supplemented – together with vitamins, minerals and fructooligosaccharide - with 200 mg DHA/d from the 21 wk of gestation until the third month of lactation. Infant birth weight and length as well as infant anthropometry 1, 3 and 21 month after birth were measured. Infants in the supplemented group were on average 1 cm shorter than infants in the control group. There was no significant difference in birth weight between the two groups but at the age of 21 month, the infant body weight of the DHA supplemented group was lower by – 601 g (95 % CI: – 171; -1030 g) and BMI was lower by – 0.76 kg/m^2 (95 % CI: – 0.07; -1.46) compared to the controls (BERGMANN ET AL. 2007).

In some observational studies n-3 LC-PUFA intake during pregnancy has also been associated with a trend toward greater growth measures at birth (OLSEN AND JOENSEN 1985; OLSEN ET AL. 1995; ELIAS AND INNIS 2001). Furthermore, some cohort studies reported positive associations of higher fish intake by pregnant women with higher infant cognitive capacity, verbal intelligence quotient, fine motor, communication and social development scores (OKEN ET AL. 2005; HIBBELN ET AL. 2007). However, the effect of n-3 LC-PUFAs on fetal growth and development is discussed controversial as some studies reported an unfavorable effect, lower birth weight in particular (GRANDJEAN ET AL. 2001; RUMP ET AL. 2001). Concerns about potential health risks from the environmental contaminants found in fish were raised in a recent study in Denmark (HALLDORSSON ET AL. 2007) and should be taken into account (DOMINGO 2007).

3 Aim of the thesis

The purpose of the INFAT-Study is to examine the association of fatty acids in the mothers' diet during pregnancy and lactation with infant adipose tissue development during the first year of life in a prospective, randomized, controlled intervention trial with two parallel groups in pregnant and lactating women and their infants.

It is hypothesized that a reduced ratio of n-6 to n-3 LC-PUFAs in the maternal nutrition during pregnancy and lactation may lead to a less expansive development of infant adipose tissue. The women consumed either a diet with a ratio of n-6 to n-3 LC-PUFAs according to the average intake of pregnant women in Germany or a diet with a reduced ratio of n-6 to n-3 LC-PUFAs. The latter was achieved by increasing the n-3 LC-PUFA intake via supplementation with 1.2 g/d n-3 LC-PUFAs and reducing the n-6 LC-PUFA intake by normalizing the AA intake.

The primary outcome variable is the mass of adipose tissue in the newborn and infant assessed by SFT measurement. Secondary outcome variables include the relative contents of n-6 and n-3 LC-PUFAs in maternal, infant, and breast milk lipids, plus the pregnancy outcome parameters of the women and their infants.

The present work is an interim analysis of the INFAT-Study and the main objectives of the thesis are:

- To analyze whether a reduction of the n-6 to n-3 LC-PUFA ratio in the maternal diet is reflected in the fatty acid pattern of maternal, cord blood and breast milk lipids.
- To determine whether a reduction of the n-6 to n-3 LC-PUFA ratio in the maternal diet influences maternal anthropometry and biochemical parameters.
- To investigate whether a reduction of the n-6 to n-3 LC-PUFA ratio in the maternal diet has an impact on maternal and infant birth outcomes such as length of gestation and birth weight.

The present study is, to the best of our knowledge, the first study which was originally designed to investigate the effect of maternal n-3 LC-PUFA supplementation in combination with a reduced dietary AA intake on birth outcomes and infant adipose tissue development.

4 Materials and Methods

The study “The ***I***mpact of ***N***utritional ***F***atty acids during pregnancy and lactation for early human ***A***dipose ***T***issue development“ (INFAT-Study) was approved by the Ethics Committee (Faculty of Medicine) of the Technische Universtität München (Nr. 1479/06 / 2006/2/21).

4.1 Study design

The INFAT-Study was a prospective, randomized, controlled dietary intervention trial with two parallel groups in pregnant and lactating women and their infants to assess the impact of fatty acids in maternal nutrition on the infants’ development of adipose tissue. A detailed description of the study design was recently published (HAUNER ET AL. 2009).

The women consumed either a diet with a ratio of n-6 to n-3 LC-PUFAs according to the average intake of pregnant women in Germany or a diet with a reduced ratio of n-6 to n-3 LC-PUFAs. The latter was achieved by increasing the n-3 LC-PUFA intake via supplementation and reducing the n-6 LC-PUFA intake by nutrition.

The primary outcome variable was the mass of adipose tissue in the newborn and infant assessed by SFT measurement. Secondary outcome variables were the relative contents of n-6 and n-3 LC-PUFAs in maternal and infant plasma PL, red blood cells and breast milk plus pregnancy outcome parameters like duration of gestation and infant birth weight. The design and time schedule of the study is illustrated in Fig. 6. (see below) and all procedures during the course of the study including all visits and information on the samples that were taken under standardized conditions from both the mother and her infant are illustrated in the flow chart B.1 (see appendix).

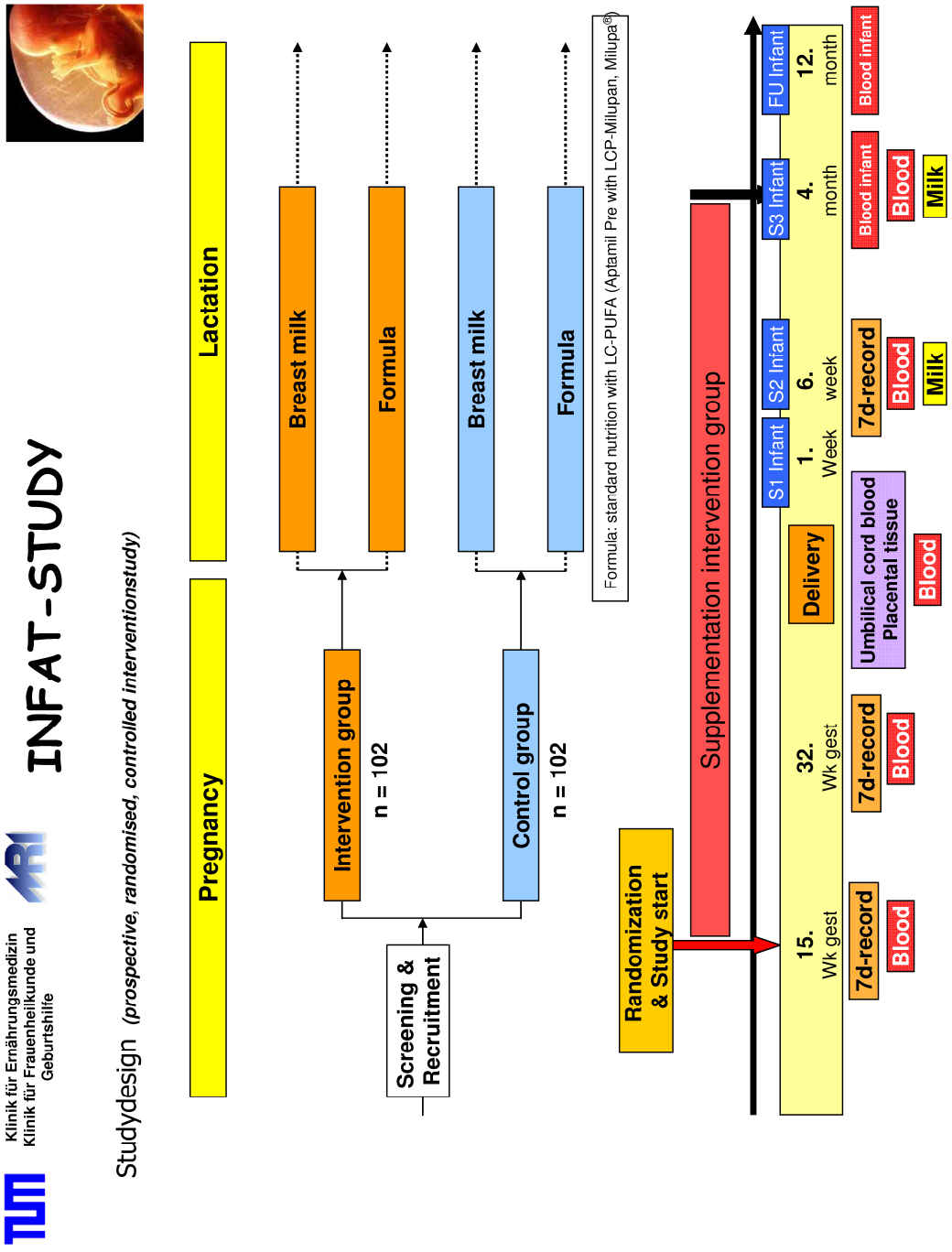

Figure 6 Study design

Eligible for the study were women who fulfilled the following inclusion criteria and who did not meet one of the following exclusion criteria:

Inclusion criteria:

- Gestation ≤ 15th wk at enrollment
- Singleton pregnancy
- Age between 18 and 45 years
- BMI before pregnancy between 18 and 30 kg/m^2
- Motivation and willingness to implement the nutritional intervention reliably

Exclusion criteria:

- High-risk pregnancy
- Hypertension
- Chronic diseases (e.g. diabetes mellitus) or diseases accompanied by maldigestion, malabsorption or elevated energy and nutritional requirement (e.g. gluten enteropathy), known metabolic defects (e.g. phenylketonuria)
- Psychiatric or psychosomatic diseases
- Use of fish-oil supplements since the beginning of pregnancy
- Hyperemesis gravidarum
- Alcohol or drug abuse

All participants received 50 Euros for their participation after successful completion of the study.

Women were first contacted for a telephone-screening by a member of the study team (see 4.2) and asked to come to the study center at Klinikum rechts der Isar (Visit 1) before the 15th wk of gestation for screening and randomization if they were considered eligible for the study.

Visit 1 represents the screening examination. The study details were carefully explained and the women received written information (appendices B.2 – B.4). Written informed consent of all participants was obtained. The screening examination included a detailed anamnesis and a detailed questionnaire on maternal lifestyle, education and any siblings. Maternal SFT was measured with a caliper (see 4.6).

At **Visit 2** (14th – 16th wk of gestation) the women were randomly assigned to the control or the intervention group. Randomization was performed using a random

envelope prepared by the Institute of Medical Statistics and Epidemiology of the Technical University of Munich and was conducted by a block length of 10 to ensure balanced group sample sizes during the whole recruitment period using SAS software Version 9.1. For the assessment of plasma PL and red blood cell fatty acids and a blood count, maternal venous blood was collected after an overnight fast (see 4.8). A first 7-day dietary record was filled in after careful instruction of the women by a member of the study team (appendices B.5 & B.6; see 4.5). Women in both groups then received dietary counseling on a healthy diet according to the guidelines of the German society of nutrition, which represents a healthy balanced diet. Treatment was started for both treatment groups:

- Intervention group:

  In order to reduce the ratio of n-6 to n-3 LC-PUFA intake during pregnancy and lactation, the intervention protocol combined two components: one is to increase n-3 LC-PUFA intake by asking the women to take a supplement (see 4.3) providing 1.2 g n-3 LC-PUFAs. The other is to reduce arachidonic acid intake to the recommended range of 50 to 90 mg per day. For this purpose, the women were advised - based on the guidelines of the German Society of Nutrition on a healthy balanced diet - to reduce their intake of AA-rich foods, particularly meat, meat products, and eggs (see 4.4 and appendix B.7).

- Control group:

  The women in the control group were advised to retain their habitual diet within the recommendations of the German society of nutrition. They did not receive any supplements and are not allowed to take any fish oil supplements.

If they wished to, the participants of both groups received individual nutrition counseling based on the 7-day dietary record.

At **Visit 3** (32nd wk of gestation) maternal venous blood was collected and the women of the intervention group were advised once again to reduce their AA-intake to the recommended range. Furthermore, all women were asked to fill in a second 7-day dietary protocol. Compliance was verified by counting leftover capsules and by the plausibility of the DHA increase in plasma PL and red blood cells. Maternal blood was collected at 32 weeks of gestation to avoid possible variables associated with labour and delivery.

At **birth**, pregnancy and birth outcomes of mother and infant, e.g. gestational age, birth weight and height, placental weight, mode of delivery, APGAR score and other data were documented in a standardized manner. Blood from the mother, umbilical cord blood, a piece of the umbilical cord and defined placental tissue samples were collected, aliquotted and immediately frozen at – 86°C (see 4.9 and 4.10).

Eligible for the study were healthy newborns who fulfilled the following inclusion criteria and who did not meet one of the following exclusion criteria:

Inclusion criteria for the newborns:

- Gestational age at birth between 37th and 42nd wk
- Appropriate size for gestational age (AGA)
- APGAR score > 7 at 5 min pp

Exclusion criteria for the newborns:

- Severe malformations or diseases
- Chromosomal anomaly
- Metabolic diseases

At **Visit S1** (2 – 8 days after delivery) SFT measurements of the newborn were performed and the upper arm circumference of the infant was measured (see 4.7.2). In case of maternal inability to breastfeed her infant, infant formula was provided for free in both groups (Aptamil Pre/1/2 with LCP-Milupan® and Aptamil HA Pre/1/2 with LCP-Milupan® of Milupa) until the 6th month pp. Every formula contains LA and ALA in a ratio of 5.4 / 1; 0.35 g AA / 100 g fatty acids and 0.2 g DHA / 100 g fatty acids.

At **Visit S2** (6 wks after birth) venous blood and breast milk samples were collected from the mother if she was breast feeding her infant and gave written, informed consent (appendix B.8). A third 7-day dietary record was requested. In the newborn, SFT measurements were performed as well as an assessment of subcutaneous fat by ultrasound (see 4.7.3). Furthermore, body height, weight, head and upper arm circumferences were measured. In a sub-sample of the newborns and, with maternal consent, whole body composition was assessed by magnetic resonance imaging (see 4.7.4).

At **Visit S3** (4 months after birth) the same anthropometric measurements were performed as described for Visit S2. In addition, a 1 – 4 ml blood sample of the infant was collected with maternal consent.

At **Visit S4** (12 months after birth), the final examination, SFT of the infant was measured and the subcutaneous fat was assessed by ultrasound. Furthermore, body height, weight, head and upper arm circumferences were measured. A 1 – 4 ml blood sample of the infant was collected again, with maternal consent.

Beyond the first year of life, follow-up examinations are planned at months 18, 24, 36, 48 and 60 after birth for the assessment of defined anthropometric parameters. Participation again requires the consent of both parents.

4.2 Recruitment

Recruitment started in July 2006 and was finished in July 2009. Gynecologists in private practices as well as out-patient clinics in the Munich area were contacted and invited to refer healthy pregnant women before the 14th wk of gestation to the study center at the University Hospital Klinikum rechts der Isar. In addition, the study was advertised in local newspapers (Süddeutsche Zeitung, Münchner Merkur) and pregnancy specific internet pages as well as in a monthly journal called “Baby & Familie”, which was produced for pregnant women and young families to give specific advice on topics related to pregnancy and baby care. The first screening was usually done by telephone or directly using a structured questionnaire.

4.3 Supplementation

The supplement was provided in capsule form (1 g), providing ~ 350 mg/g DHA and ~ 60 mg/g EPA from fish oil in glyceride form (Marinol-D40, Lipid Nutrition, Loders Crocklaan, The Netherlands, see appendix B.9). The women were asked to take 3 capsules / d in the evening. Three capsules provided ~ 30 kcal/d, 9 mg Vitamin E as an antioxidant, ~ 1.2 g n-3 LC-PUFAs and negligible amounts of LA and ALA compared with that in the usual diet. Apart from the n-3 LC-PUFAs, the capsules contained mainly C16:0, C18:0 and C18:1n-9. The decision for 1.2 g n-3 LC-PUFA was based on the calculations that daily consumption of the capsules would result in a ratio of n-6 to n-3 fatty acids of 3.5:1 in the diet of the intervention group compared to the ratio in the control group of ~ 7:1 (DGE 2004; MAX-RUBNER-INSTITUT 2008). The

choice of this ratio was based on guidelines for PUFA intake in pregnancy issued by various international authorities, which state that for optimum benefit, the ratio of n-6 to n-3 fatty acids should not be lower than 2:1 (KOLETZKO ET AL. 2007; KOLETZKO ET AL. 2008; KRIS-ETHERTON ET AL. 2009).

At Visit 2, the women were provided with the supplement for one month. They were carefully instructed about the storage and intake of the supplement. They were given a "capsule diary" in which they had to document the number of capsules they took and to document the reason why they did not take the supplement, if forgotten, as well as side effects or signs of illness. Forgotten capsules had to be preserved and were counted at the next visit at the study center. The women were asked to come to the study center one month later to pick up the supplement for the next 5 months. After delivery, they were provided for the third time with the supplement until the end of lactation. To support the intake of fish oil capsules and to maintain compliance, the women were called every 4 to 8 weeks by a member of the study team.

The supplement was stored at 2°C in a refrigerator at the study center. The storage temperature was controlled and documented regularly in the storage temperature log.

4.4 Dietary intervention

Women of both groups were advised to keep a healthy balanced diet according to the guidelines of the German Society for Nutrition. Both got a short dietary counseling upon beginning the study.

Dietary intervention was one of the two measures undertaken to reduce the n-6 to n-3 LC-PUFA ratio in maternal nutrition of the intervention group. Women in the intervention group were therefore advised to reduce their consumption of AA-rich foods, particularly meat, meat products and eggs in order to limit their AA-intake to 90 mg per day. The latter was primarily achieved by advising the women to limit their meat intake to 500 g (2 – 3 portions) per week. Written information about the AA content of common food and advice about how to replace AA-rich food adequately with food low in AA was provided (see appendix B.7). Great emphasis was placed on the identification of hidden sources of AA in foods or AA-rich foods, e.g. liver wurst or lard.

In both groups, the women were allowed to eat fish in usual quantities (2 – 3 times a week), paying attention to the AA content for the women in the intervention group.

4.5 Dietary intake

The dietary intake of the women was recorded at 15 and 32 wk of gestation and, in the case of breastfeeding, additionally at 6 and 16 wk pp by using a 7-day dietary record. All participants were instructed in detail about the use of the dietary record and how to record their dietary intake in difficult situations like dinner at a restaurant or during vacation (see appendices B.5 & B.6). The women were asked to estimate the consumed foods in amounts usually used in the household e.g. one tablespoon or 1 cup. Specific information on all sources of fat, including product brand names and types of fish, seafood, meat and meat products was collected. Dietary record data were analyzed with Prodi® 5.2. Expert (2006; Nutri Science GmbH, Wissenschaftliche Verlagsgesellschaft Stuttgart mbH). The intake of total kcal/d and the fat, carbohydrate and protein intake in % of the total energy intake as well as the AA intake per day and per 1000 kcal was calculated. This assessment also included information on all dietary supplements and drugs taken by the participants.

4.6 Maternal anthropometric measurements

4.6.1 Growth parameters and skinfold thickness

Body height and weight before pregnancy were taken from the “Mutterpass” at Visit 1, together with information about blood pressure, parity and gravidity. BMI [kg/m^2] before pregnancy was calculated.

Triceps, biceps, suprailiac and subscapular SFT was measured to the nearest 0.5 mm in triplicate on the left side of the body under standard conditions by using a skinfold caliper (Holtain Ltd. Croswell, Crymych, United Kingdom) that was operated with a constant pressure of 10 g/mm^2. For a given site, the mean of the three measurements was used for the SFT value. While the woman was standing in a relaxed position in front of the investigator, the biceps SFT was measured at the mid length (midway between the acromion and olecranon) of the ventral side of the upper arm. The triceps SFT was measured at the mid length of the dorsal side of the upper arm. Subscapular SFT was measured below the inferior angle of the left scapular at a diagonal in the natural cleavage of the skin. Suprailiac SFT was measured along the midaxillary line above the iliac crest. All measurements were made by lifting the skin with the thumb and index finger, with care taken not to include any underlying tissue. The caliper was left in place until a constant reading was obtained. The mid upper

arm circumference was measured on the left arm midway between the acromion and olecranon by using a standard measuring tape.

4.6.2 Body fat and lean body mass

Maternal body fat (BF) in % of total body weight, lean body mass (LBM) in % of total body weight, maternal BF in kg and LBM in kg were calculated with two equations (DURNIN AND WOMERSLEY 1974; VAN RAAIJ ET AL. 1988).

Maternal body density was estimated with a linear regression equation of body density x 10^3 [kg/m^3] from the logarithm of the SFT according to DURNIN AND WOMERSLY:

Density = c – m x log skinfold (mm)

Skinfold: sum of skinfolds (e.g. biceps SFT, triceps SFT)
c and m: variables depending on age, gender and skinfolds (given in tables, DURNIN & WOMERSLY (1974)).

Maternal fat mass in kg was calculated according to VAN RAAIJ ET AL:

$$W_{FM} = \frac{W_B}{100} \times \frac{\left(\frac{100}{D_B} - \frac{100}{D_{FFM}}\right)}{\left(\frac{1}{D_{FM}} - \frac{1}{D_{FFM}}\right)}$$

W_{FM} = weight of fat mass
W_B = body weigt
D_B = body density (calculation see above)
D_{FFM} = density of fat free mass
D_{FM} = density of fat mass, constant 0.900 x 10^3 kg/m^3

Because the density of fat free mass changes over the course of pregnancy, special equations are given for week 6, 12, 20, 24, 30, 34, 36 and 40 in the papers of VAN RAAIJ ET AL. (1988 AND 1989), based on the chart shown in figure 6: density and water content of maternal fat free body mass during pregnancy for a reference woman with no edema or leg edema only (continous line) and with generalized edema (broken line) according to VAN RAAIJ ET AL. (1988):

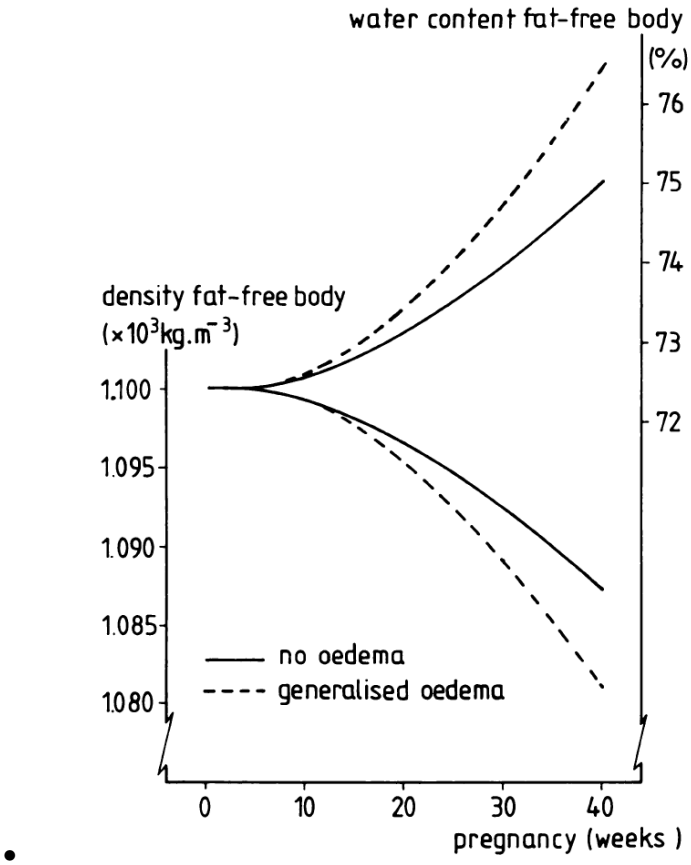

Figure 7 Density of maternal lean body mass (RAAIJ ET AL 1988)

Derivation of the equations for the 15th and 32nd wk of pregnancy using a cubic fit for the curves in Fig. 7 as proposed by LÖSCHL in her "Magister Thesis" (LÖSCHL 1996). The generation of a cubic fit seems to be a valuable approach for the precise assessment of the density of the fat free mass at any given week of pregnancy and was defined as follows:

$$y = 0.00015x^3 - 0.015x^2 + 0.065x + 1100$$

x = week of pregnancy
y = density of fat free mass

The following values result:

15th week: 1.0981×10^3 kg/m³

32nd week: 1.0916×10^3 kg/m³

By inserting the values for the 15th and 32nd week of pregnancy in the general equation for determining the fat mass meets to the following simplified results:

15th week of pregnancy:

$$W_{FM\,15} = \frac{W_B}{100} \times \left(\frac{498{,}9}{D_B} - 454{,}3 \right)$$

32nd week of pregnancy:

$$W_{FM\,32} = \frac{W_B}{100} \times \left(\frac{512{,}8}{D_B} - 469{,}7 \right)$$

4.7 Anthropometric measurements of infants

4.7.1 Growth parameters

Birth weight, birth length, head circumference (HC) and sex of the newborn were extracted from the maternal obstetric record. Appropriate weight, length and head circumference of the newborns for gestational age was defined on the basis of current age- and sex-related percentile charts as shown in appendix B.11 (VOIGT ET AL. 1996).

At visit S2, S3 and S4 the weight of the naked infant was measured to the nearest 10 g by using a standard balance (Babywaage Ultra MBSC-55, myweight®). Height was measured with a measuring stick (Säuglingsmessstab seca 207, seca, Pfaffenweiler) to the nearest 0.5 cm while the infant was supine with stretched legs. Furthermore, the Ponderal Index [kg/m^3] was calculated form the basic sizes and was used as a measure of relative weight-for-length and growth pattern.

4.7.2 Skinfold and circumference measurements

At visit S1, S2, S3 and S4 triceps, biceps, suprailiac and subscapular SFT was measured to the nearest 0.5 mm in triplicate on the left side of the body under standard conditions by using a standard skinfold caliper (Holtain Ltd. Croswell, Crymych, United Kingdom) that was operated with a constant pressure of 10 g/mm^2.

For a given site, the mean of the three measurements was used for the SFT value. While the infant was supine and the arm was slightly abducted and extended, biceps SFT was measured 2 cm proximal to the skin crease of the elbow. Triceps SFT was measured over the triceps, midway between the acromion and olecranon, with the arm slightly flexed and the infant lying on the right side. Subscapular SFT was measured below the inferior angle of the left scapular at a diagonal in the natural cleavage of the skin. Suprailiac SFT was measured along the midaxillary line above the iliac crest. All measurements were made by lifting the skin with the thumb and index finger, with care taken not to include any underlying tissue. The caliper was left in place until a constant reading was obtained. The mid upper arm circumference was measured on the left arm by using a standard measuring tape. All measurements for one infant were made by the same member of the study team.

Infant fat mass in g, body fat in % and fat mass/height^2 in kg/m^2 was estimated with 3 different equations (SLAUGHTER ET AL. 1988; WESTSTRATE AND DEURENBERG 1989; DEURENBERG ET AL. 1990), summarized in Fig. 8:

Reference	Equation	No. of skinfolds	Characteristics
Slaughter, M. H. et al (1988): Human Biology; Vol. 60, No.5:709-723	Male: % BF = 1,21 x ΣSFT-0,008 x ΣSFT^2-1,7 Female: % BF = 1,33 x ΣSFT-0,013 x ΣSFT^2-2,5	2 Skinfolds Sum of either triceps + subscapular or sum of triceps+ calf	prepubescent, pubescent and adults with ΣSFT < 35mm
Weststrate, J. A. and Deurenberg, P. (1989): Am J Clin Nutr; 50:1104-1115	Male & Female: % F = ({585-4.7[age(mo)^{0,5}}/D) -{550-45.1[age(mo)]^{0,5}} D = {1,1235+0,0016[age(mo)]^{0,5}} - S_1*log (skinfold thickness)	4 Skinfolds Sum of bicipital, tricipital, subscapular, and suprailical skinfold thickness in relation to total body density	For boys and girls aged 0 - 2 y Additional: gender-specific equations for boys and girls aged 2 - 18 y age and sex-specific reference values for skinfold thickness at given body fat percentage
Deurenberg P. et al (1990): Br. J. Nutr; 63:293-303	Male: d = 1,1133-0,0561 x log ΣSFT + 1.7 (age x 10^{-3}) Female: d = 1,1187-0,063 x log ΣSFT + 1.9 (age x 10^{-3})	4 Skinfolds Sum of bizeps, trizeps, subscapular, suprailiacal	Children with a mean age of 11 y

Figure 8 Equations for the assessment of infant fat mass

4.7.3 Assessment of subcutaneous and preperitoneal fat by ultrasound

At visit S2, S3 and S4 the infants' subcutaneous and preperitoneal fat thickness was measured by ultrasound using a high-resolution ultrasonographic system (Siemens Acuson Premium, Siemens Medical Solutions, Erlangen, Germany) with a 10 MHz linear probe (VFX 13-5, Siemens Medical Solutions, Erlangen, Germany) according to the method recently described by DURMUS and coworkers from Rotterdam, The Netherlands (DURMUS ET AL. 2009) . Briefly, the infant was examined in the supine

position. The thickness of the preperitoneal fat was measured on sagittal B-mode images ranging from the xiphoid process caudal along the linea alba. The preperitoneal fat thickness was defined as the thickness of the fat tissue between the liver surface and the linea alba. Transverse scanning was performed to measure the subcutaneous fat thickness, defined as the thickness of the fat tissue 1 cm lateral of the linea alba, between the skin-fat interface and the rectus abdominis muscle surface. The transducer was put on about 1cm ultrasound gel without pressure to avoid compression of the tissue. Both sagittal and transversal images were transferred to an offline workstation (Siemens Fujitsu, Munich, Germany) and measurements were performed with custom-built software using IDL 6.3 (Creaso, Gilching, Germany).

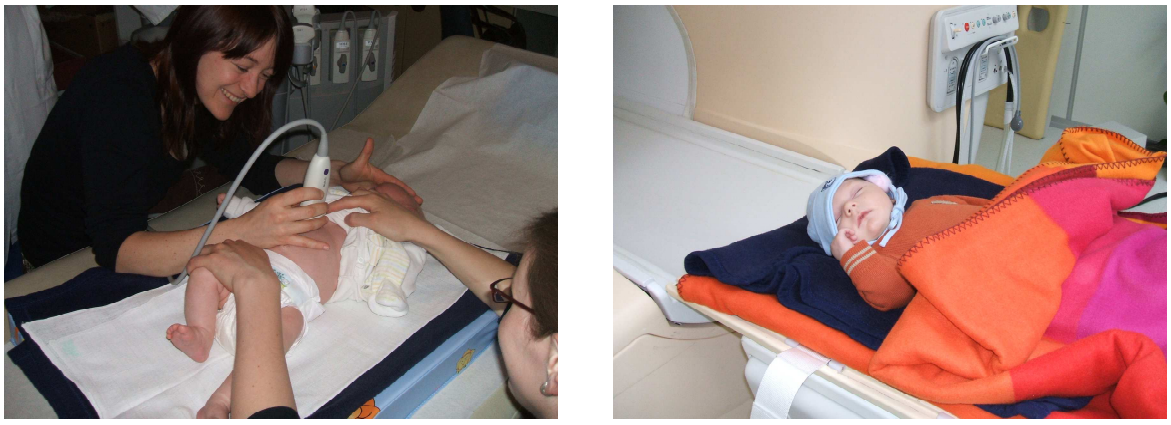

Figure 9 Assessment of infant fat mass by ultrasound (left) and MRI (right)

4.7.4 Assessment of adipose tissue volume by MRI

At visit S2 and S3 the infants' adipose tissue volume was measured by magnetic resonance imaging (MRI), using a 1.5 Tesla Siemens Avanto Scanner (Siemens Medical Solutions, Erlangen, Germany) with a four element surface coil in combination with an eight element spine coil (both TIM coils, Siemens Medical Solutions, Erlangen, Germany). The scanning procedure started when the infant was asleep, no sedative drugs were used. The infant was positioned supine on the platform in a small plastic bed and was swaddled to keep it as stationary as possible during the scan. Starting from the head, 100 axial intermediate-weighted images were acquired using a fat excitation turbo spin echo sequence with a total scan time of 4:56 minutes. For noise reduction, the scan was performed in "silent mode", using slow gradients. For reproducibility measurements, four scans were repeated three

times. The images were transferred to an offline workstation (Siemens Fujitsu, Munich, Germany) and measurements were performed with custom-built software using IDL 6.3 (Creaso, Gilching, Germany). Volumes of whole body fat, as well as visceral fat and subcutaneous fat of the body and of the extremities separately were determined using a semi-automatic segmentation technique. In a first step, whole body fat was automatically segmented by a threshold-based technique. Afterwards one trained user could correct this segmentation in case of local inhomogeneities of the magnetic field and consequent errors of the water saturation and fat excitation. The separation of the different fat depots was done manually as well. A detailed description and publication of the method is currently in progress.

4.8 Maternal blood count

At randomization (Visit 2), at 32nd wk of gestation (Visit 3) and additionally at 6 and 16 wks pp (if the mother was breastfeeding her infant) fresh venous blood samples were collected from each subject after fasting overnight. A laboratory investigation including a small blood count (hemoglobin, haematocrit, number of thrombocytes, leukocytes, red blood cells, MCH, MCV, MCHC, RDW), blood glucose concentration, triglyceride, total-, HDL- and LDL-cholesterol concentration and coagulation parameters (Quick, INR and PTT) was performed. All analyses were carried out by a commercial laboratory (Labor Dr. Tiller und Kollegen, München), using standard analytical methods and commercial kits.

4.9 Blood and milk fatty acid pattern analysis

4.9.1 Plasma Phospholipids

4.9.1.1 Lipid extraction

Lipids were extratcted according to the method of BLIGH AND DYER. Fresh venous blood samples were collected from each subject after fasting overnight and put in EDTA containing tubes (Sarstedt, Germany) and immediately centrifuged at 2000 x g for 10 minutes to separate erythrocytes and plasma. The plasma was then aliquotted and stored until analysis at – 86℃. Frozen plasma was thawed at room temperature and total lipids are extracted with chloroform/methanol/water (10:10:9, by vol) (see appendix A.1) (BLIGH AND DYER 1959).

4.9.1.2 Separation of lipid classes

The neutral and polar lipid classes were separated by high pressure liquid chromatography (HPLC) and fractionated via automatic fractionation sampling (BEERMANN 2003). An aliquot of the lipid extract was dissolved in chloroform/methanol (1:1, by vol) at a concentration of 0.5 - 2.5 mg/mL. The lipid class separation was completed with an HPLC Alliance 2695 Separation module from Waters (Waters GmbH, Eschborn, Germany) coupled with an PL-ELS 1000 evaporative light scattering detection system (Polymer Laboratories, Darmstadt, Germany). The detection was established at 30°C for the nebulizer and 50°C for the evaporator. For the lipid class separation a polyvinyl alcohol chemically bound stationary phase PVA Sil column (5 µm, 250 mm x 8 mm) (YMC Europe, Schermbeck, Germany) was used. The eluent system corresponded to that of CHRISTIE (CHRISTIE 1995): A: n-hexane; B: isopropanol / acetonitrile / chloroform / acetic acid (84:8:8:0.025; by vol); C: isopropanol / bidest. water / triethylamine (50:50:0.2, by vol). All applied solvents were of HPLC grade or supra-solvent quality (Merck Eurolab, Darmstadt, Germany). The solvent-gradient system was as follows: 0 - 1 min A/B/C (%) 80:20:0, 1 - 7 min A/B/C (%) 30:54:16, 7 - 13 min A/B/C (%) 30:54:16, 13 - 16 min A/B/C (%) 30:70:0, 16 - 19 min A/B/C (%) 80:20:0, 19 - 24 min A/B/C (%) 80:20:0. The flow rate was 2.5 mL/min (see appendix A.2). The distinct lipid classes were characterized by retention time. The distinct lipid species were collected with an automatic fraction-sampler from Waters by peak signal recognition. The fractions were collected in 5 mL lockable glass tubes.

4.9.1.3 Phospholipid fatty acid derivatization

The separated PL fraction of the HPLC fractionation was evaporated with nitrogen to dryness. For derivatization, the samples were dissolved in 2 mL methanol/hexane (4:1, vol/vol) plus 0.5% pyrogallol and were methylated (according to Lepage and Roy 1984) with 200 µL acetylchloride at 100°C, 1h; 5 mL 6 % K_2CO_3 were added and centrifuged for 10 min at 3200 x g. The upper hexane phase containing the fatty acid methyl ester (FAME) was separated (see appendix A.3).

4.9.1.4 Fatty acid methyl ester analysis

The FAME were analyzed by capillary gas chromatography (CGC) performed on the 6890N gas chromatograph (Agilent Technologies, Waldbronn, Germany) fitted with a cold-on-column injector to prevent fatty acid discrimination (BEERMANN ET AL. 2005). A

chemically bound 50 % cyanopropyl-methylpolysiloxane capillary column DB23, 60 m, I.D. 0.25 mm, film 0.25 µm (JW Scientific, Agilent Technologies, USA) was used for the separation of fatty acids. The chromatographic conditions were as follows: Injector (COC): 65 °C to 270 °C; carrier gas: hydro gen at a 40 cm/s flow. The signals were produced by a flame ionization detector at 250 °C. Fatty acids were identified according to their retention times relative to standards (GLC 85 standard mix, NuChekPrep, Inc. Elysian, Minnesota, USA). The temperature program was as follows: initial temperature 60 °C for 0.5 min; fro m 60 °C to 180 °C at 40 °C/min; 180 °C for 2 min; from 180 °C to 210 °C at 2 °C/min; 21 0 °C for 3 min; from 210 °C to 240 °C at 3 °C/min; 240 °C for 10 min (see appendices A .4 & A.5).

4.9.2 Red blood cells

4.9.2.1 Lipid derivatization

Fresh venous blood samples were collected from each subject after fasting overnight and put in EDTA containing tubes (Sarstedt, Germany) and immediately centrifuged at 2000 x g for 10 minutes to separate erythrocytes and plasma. The plasma was removed and the remaining erythrocytes are washed three times with 0.9% w/v NaCl solution, aliquotted and stored until analysis at – 86°C. Frozen erythrocytes were thawed at room temperature. For derivatization, 200 µL of each sample was dissolved in 2 ml methanol/hexane (4:1, vol/vol) plus 0.5% pyrogallol and was methylated according to LEPAGE AND ROY (1984) with 200 µL acetylchloride at 100°C, 1h; 5 mL 6 % K_2CO_3 were added and centrifuged for 10 min at 3200 x g. The upper hexane phase containing the FAME was separated.

4.9.2.2 Fatty acid methyl ester analysis

The FAME were analyzed by CGC according to 4.9.1.4.

4.9.3 Breast milk

4.9.3.1 Lipid derivatization

Fresh breast milk samples were collected after fasting overnight, aliquotted and immediately stored until analysis at – 86°C. Frozen milk was thawed at room temperature. For derivatization, 100 µL of each sample was dissolved in 2 ml methanol/hexane (4:1, vol/vol) plus 0.5% pyrogallol and was methylated according to LEPAGE AND ROY (1984) with 200 µL acetylchloride at 100°C, 1h; 5 m L 6 % K_2CO_3

were added and centrifuged for 10 min at 3200 x g. The upper hexane phase containing the FAME was separated.

4.9.3.2 Fatty acid methyl ester (FAME) analysis by capillary gas chromatography (CGC)

The FAME were analyzed by CGC according to 4.9.1.4.

4.10 Placental tissue preparation

Placental tissue was obtained shortly after delivery, thus minimizing analytic changes in the tissue. The tissue was placed on a cool work place (plate with freezer packs). Before starting the preparation the appearance of the placental tissue was assessed in a standardized manner (infarcted and/or calcified areas, any pathological abnormalities). A picture of the basal and the chorionic plate was taken. Placental preparation was carried out according to a standardized protocol (appendix A.6). Briefly, four stripes / arms of placental tissue of about 2 cm in the center were chosen although the umbilical cord was not located in the center (encasing at least four stripes, avoiding the outer areas / border) and the outer layers of the stripes (chorionic plate and basal plate) were removed. These four stripes were each divided in six cubes of 1 cm each. A small piece of each of these cubes was put into a cryo vial and immediately shock frozen in liquid nitrogen for later analysis of RNA, adipokine, fatty acid, DNA, and protein analysis. Another sample, as well as a piece of the umbilical cord and the amnion were fixed in formalin and then paraffin-embedded for later immunohistochemistry. A seven cm piece of the umbilical cord was cut out of the cord five cm distal of the onset of the cord and shock frozen in liquid nitrogen for later fatty acid analysis. After shock freezing, all samples were stored at – 86 ℃ until analysis .

4.11 Sample size calculation and statistical analysis

The calculation of sample size was based on the primary outcome (SFT measurements). According to recent publications, a mean sum of the 4 skinfolds of 30 ± 5 mm was expected (SCHMELZLE AND FUSCH 2002, RODRIGUEZ ET AL. 2005). It was assumed that there will be a difference of at least 5 mm in the sum of the four defined skin folds (subscapular, suprailiac, triceps, biceps) between intervention and control group at 4 months pp. To be able to detect a clinically significant difference of

5 mm between the groups at a two-sided α = 0.05 and a power of 80 % and assuming a drop-out rate of 30 % over the study period, a total of 102 women in each group was required. Thus, a total of 204 women had to be recruited for the study. The calculation was based on the nQuery Version 5 software programme.

Due to the exploratory nature of the statistical analyses performed within this work all tests were conducted in a non-parametric way which was more conservative and more economic with regard to the multiple test issue, since there was no need for particular proof of assumptions regarding the underlying data distribution (e.g. normality). A two-sided p value < 0.05 was considered statistically significant for the analysis of clinical outcome variables and fatty acid analysis. Bonferroni correction of p-values was applied to reduce multiple test issue within multiple sample analysis as time course and many group comparisons. Subject characteristics and clinical parameters between the groups were compared using the Mann-Whitney-U-Test for quantitative values and the Chi-Square-Test for categorical data.

Changes in maternal fatty acid status due to stage of gestation (15 or 32 wk) or stage of lactation (6^{th} and 16^{th} wk pp) were determined by using the Friedman-Test for more than 2 time points (e.g. fatty acid profile of maternal plasma PL) and the Wilcoxon-Test for the comparison of 2 time points (e.g. breast milk). Occurrence of missing values about the course of follow up was expected to be at random (independent of measurement level of any covariate to be investigated).

To determine associations between individual fatty acid proportions in maternal and cord blood plasma PL and RBCs and breast milk as well as fatty acid proportions and birth outcomes the Sperman-Rho correlation coefficient was calculated on the base of the entire study population.

All randomized participants were included in the Intention-to-treat-analysis whereas only those who do not violate the protocol are included in the Per-protocol-analysis. Protocol violaters are defined to be:

- Women who were randomized despite not fulfilling criteria
- Infants who were randomized despite not fulfilling criteria
- Infants for which no data were available at birth and / or for S2 (6^{th} wk pp) or S3 (4^{th} month pp)
- Women and infants who were excluded in the opinion of the investigator

Data presented in this interim analysis of the INFAT Study are based on an Intention-to-treat-analysis of all women randomized until august 2008.
All analyses are done with SPSS for WINDOWS (version 16; SPSS Inc., Chicago, IL).

4.12 Data management and legal requirements

Data from the participants were kept in coded form, randomly assigned to each participant. The investigator created a list in which the data are associated with the participants' full names. Data management was performed under consideration of current data protection laws. Data were collected using personal data questionnaires (Case Report Forms (CRFs) see appendix B.10). Data were saved in a data base. All adverse events reported by the participating women, and regularly assessed during the visits were carefully documented as requested in the CRF.

The trial was performed in accordance with the protocol, International Conference on Harmonization Good Clinical Practice guidelines (ICH-GCP, valid from 1997/1/17), the last revision of the declaration of Helsinki (october 2008, Seoul, South Korea) and applicable local regulatory requirements and laws.

The study protocol was registered at ClinicalTrials.gov Protocol Registration System (NCT00362089).

5 Results

5.1 Trial profile and sample size

A total of 173 women (n = 87 in the control and n = 86 in the intervention group) were involved in the study from August 2006 to August 2008 and included in this interim analysis. A total of 113 women (56 controls) gave birth to healthy infants during this period of time. 78 infants were able to attain the third visit pp (16. wk pp) and 26 infants were able to complete the study (12 month pp).

From the 173 women involved, 18 women (9 controls; 10.4 % of the women) quit and were excluded from the study before giving birth to their infants (Fig. 10). 2 women were excluded because they chose to take fish oil supplements despite being in the control group. One woman in the intervention group dropped out because of the strong taste of the capsules. 6 women in the intervention group and 4 women in the control group quit the study for personal reasons, 2 women the intervention group and 3 women in the control group quit the study because of lack of time. Another three women quit the study after delivery (2 controls) also as a result of lack of time. This corresponds to a drop-out rate of 12 %.

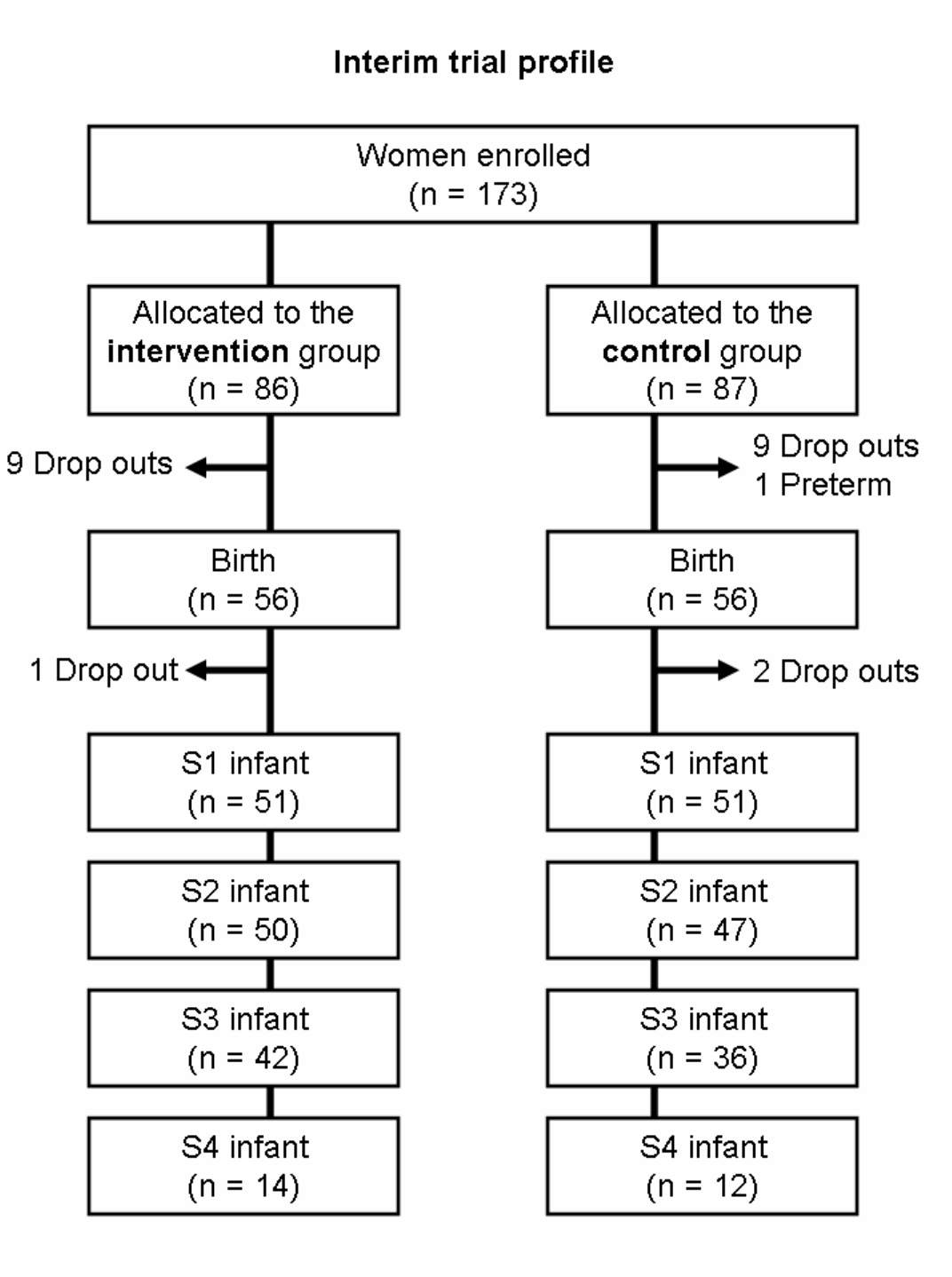

Figure 10 Trial profile

5.2 Baseline characteristics of the participants

The characteristics of the women are presented in table 1 and table 2. There were no significant differences in age, weight and BMI before pregnancy, blood pressure, parity, frequency of work-out, smoking, ethnicity, education or any other

sociodemographic variables between the women randomly assigned to one of the two groups.

Table 1 Maternal baseline characteristics 1

	group	n	mean ± SD	(range)	p*
Age of mother [y]	Intervention	86	31.7 ± 5.0	(21-45)	0.804
	Control	87	31.6 ± 4.4	(20-40)	
Weight before pregnancy [kg]	Intervention	86	63.2 ± 8.8	(45-91)	0.744
	Control	86	63.6 ± 8.1	(47-86)	
BMI before pregnancy [kg/m²]	Intervention	86	22 ± 2.8	(16.9-30.7)	0.244
	Control	86	22.4 ± 2.8	(17.5-30.2)	
Blood pressure systolic	Intervention	86	112 ± 11.8	(90-141)	0.985
	Control	86	112 ± 10.2	(90-136)	
Blood pressure diastolic	Intervention	86	68 ± 8	(50-85)	0.965
	Control	86	68 ± 8.7	(45-85)	
Cigarettes before pregnancy [nr/d] smokers only	Intervention	86	10 ± 7.8	(1-25)	0.501
	Control	86	12 ± 7.4	(1-25)	
Paternal BMI [kg/m²]	Intervention	84	24.9 ± 3.4	(16.9-40.1)	0.568
	Control	83	25.2 ± 3.2	(17.9-38.7)	
Maternal own PI [kg/m³]	Intervention	68	24.6 ± 2.7	(16.4-34.7)	0.839
	Control	64	25.4 ± 4.7	(19.9-51.8)	
Paternal own PI [kg/m³]	Intervention	52	24.2 ± 2.8	(17.7-32.5)	0.651
	Control	51	24.4 ± 3.2	(17.4-33.1)	

* Significantly different distribution between groups (Mann-Whitney-U-Test) $p < 0.05$

The study population was of Caucasian origin except for one woman (Asian origin), well-educated (67 % of the subjects had graduated their “Abitur”) and over 90 % of the women in both groups followed a “normal” typical western diet. Two women in each group followed a vegetarian diet, 2 women in each group followed a vegetarian diet with fish and two women in the intervention group (IG) had to follow a lactose free diet because of lactose intolerance. One woman in the control group (CG) followed a diet very low in fructose and histamines (“other diet” in table 2).

Table 2 Maternal baseline characteristics 2

			Intervention	Control	
	n		**%**	**%**	**p***
Graduation	172	Hauptschule	4.7	8.1	
		Realschule	29.1	23.3	0.495
		Gymnasium	66.3	68.6	
Parity	172	nulliparus	59.3	61.6	0.755
		multiparus	40.7	38.4	
Para	172	0	59.3	60.5	0.88
		1	34.9	31.4	
		2	5.8	7	
		3	0	1.2	
Mother is working	172		82.6	89.5	0.187
Work-out before pregnancy	172		54.7	58.1	0.645
Frequency of work-out	98	1h/wk	35.4	30	0.315
		2h/wk	27.1	36	
		3h/wk	25	20	
		4h/wk	8.3	2	
		5h/wk	4.2	12	
Work-out during pregnancy	172		44.2	33.7	0.159
Frequency of work-out during pregnancy	67	1h/wk	63.2	51.7	0.804
		2h/wk	21.1	27.6	
		3h/wk	7.9	7.5	
		4h/wk	7.9	13.8	
Smoking before pregnancy	172		17.4	23.3	0.344
Smoking during pregnancy	172		3.5	3.5	> 0.99
Specific diet	148	no	91.8	93.3	0.65
		vegetarian	2.7	2.7	
		vegie + fish	2.7	2.7	
		lactose free	2.7	0	
		other diets	0	1.3	
OGTT during pregnancy	120		38.8	38.8	> 0.99

* Significantly different distribution between groups (Chi-Square-Test) $p < 0.05$

Neither in the intervention group, nor in the control group did a severe adverse event (SAE) occur during the study. We observed 3 adverse events (AE) in the intervention group (pyelonephritis, early contractions, HELLP-Syndrome) and one in the control group (bleeding before the 28. wk gestation), which were harmless in all cases for the women and their infants (table 3).

Table 3 Adverse events

		Intervention	Control	
	n	**%**	**%**	**p***
Any adverse event during pregnancy or lactation	156	6.4	7.7	0.754
Any perinatal complications	103	3.9	9.6	0.437
Gestational diabetes mellitus	155	2.6	5.1	0.681

* Significantly different distribution between groups (Chi-Square-Test) $p < 0.05$

Two women in the intervention group and 4 women in the control group developed gestational diabetes mellitus (Table 3). They showed abnormal values in the oral glucose tolerance test (oGTT) during the third trimester of gestation. One woman in the intervention group and one in the control group had to use insulin to control her blood glucose concentration; the others were able to manage their blood glucose concentration following an adapted diet. Data of all six women were included in the interim analysis.

None of the infants had anomalies or metabolic diseases. 6 infants had perinatal infections or icterus (4 in the control and 2 in the intervention group) and were treated with antibiotics in case of an infection (Table 3).

5.3 Compliance, maternal dietary intake and the effect of dietary intervention

The women's adherence to the study protocol was quite good in general. 90 % of the women took the capsules regularly and missed less than 1 intake of the supplement per month. The remaining 10 % missed 1 or 2 intakes per month, i.e. 1 or 2 of 30 intakes. In very few cases the women missed more than 2 intakes because of gastrointestinal disorder, vacation or postpartum hospital stay.

In table 4 and appendix C.1, the mean ± SD and ranges of total dietary intake, protein, carbohydrate, fat and AA intakes at the 15^{th} and 32^{nd} wk of gestation are reported. Results are illustrated as boxplots in appendix D.1.

Table 4 Dietary intake

	group	n	15. wk gest		n	32. wk gest	
			mean ± SD	(range)		mean ± SD	(range)
Energy intake [kcal/d]	Intervention	68	1991 ± 338	(1125-2709)	26	2076 ± 324	(1670-2848)
	Control	70	1954 ± 325	(1235-2756)	22	2022 ± 462	(1237-2897)
Protein intake [%]	Intervention	68	14.9 ± 2.9	(9.2-27.6)	26	15 ± 2.4	(11-20)
	Control	70	14.9 ± 2	(11.5-20.2)	22	15.1 ± 2.5	(10.8-21.4)
Fat intake [%]	Intervention	68	31.9 ± 4.3	(19.4-42.4)	26	31.6 ± 4.4	(24.8-42.9)
	Control	70	33 ± 4.8	(19.8-42.6)	22	31.9 ± 5.1	(22.9-42.4)
Carbohydrate intake [%]	Intervention	68	51 ± 5.5	(28.9-63.6)	26	51 ± 4.5	(40.7-58.2)
	Control	70	49 ± 4.8	(37.7-61.8)	22	51.1 ± 6.1	(40.7-63.3)
AA intake [g/d]	Intervention	68	0.132 ± 0.09	(0.023-0.410)	26	0.109 ± 0.05 #	(0.046-0.289)
	Control	70	0.150 ± 0.09	(0.019-0.514)	22	0.169 ± 0.08 #	(0.034-0.352)
AA intake [mg/1000 kcal]	Intervention	68	67.1 ± 48	(11-231)	26	51 ± 22 #	(0.024-0.106)
	Control	70	77.2 ± 48	(13-231)	22	84 ± 42 #	(0.022-0.202)

Significantly different distribution between groups at 32nd wk (Mann-Whitney-U-Test) $p < 0.05$

The average daily energy intake for the 15th wk gestation was 1991 ± 338 kcal/d in the intervention group and 1954 ± 325 kcal/d in the control group within very similar ranges from ~ 1150 to ~ 2750 kcal/d for both groups. The average daily intake of protein was 15 ± 2.9 % of total energy intake in both groups; the average daily intake of carbohydrates was 51 ± 5.5 % in the IG and 49 ± 4.8 % in the CG and the average daily fat intake in % of total energy intake was 32 ± 4.3 % and 33 ± 4.8 % in the IG and the CG, respectively. The mean intake of AA was 132 ± 0.09 mg/d in the IG and 150 ± 0.09 mg/d in the CG. The mean intake of mg AA per 1000 kcal was 67 ± 48 in the IG and 77 ± 48 for the CG, respectively. There was no statistically significant difference in any of the described intakes between the IG and CG.

The average daily energy intake in the 32nd wk gestation was 2076 ± 324 kcal/d in the supplemented group and 2022 ± 462 kcal/d in the control group within ranges from ~ 1650 to ~ 2850 kcal/d in the IG and from ~ 1250 to ~ 2900 kcal/d in the CG. The average daily intake of protein was 15 ± 2.5 % of total energy intake in both groups; the average daily intake of carbohydrates was 51 ± 4.5 % in the IG and 51 ± 6.1 % in the CG. The average daily fat intake in % of total energy intake was 32 ± 4.5 % in both groups. The mean intake of AA was ~ 110 ± 0.05 mg/d in the IG and ~ 170 ± 0.08 mg/d in the CG. The mean intake of mg AA per 1000 kcal was 51 ± 22 in the IG and 84 ± 42 in the CG, respectively. There was no statistically significant difference in any of the described intakes between the IG and CG at the 32nd wk of gestation, except for the intake of AA in g/d and AA in mg/1000 kcal which were both significantly lower in the IG than in the CG ($p < 0.05$).

The proportion of macronutrients and the absolute intakes of energy and AA did not change significantly in both groups from the 15th to the 32nd wk of gestation (appendix C.1).

5.4 Maternal anthropometry

On the basis of reported prepregnancy weight, women in the intervention group gained on average 2.5 kg of weight during the first 15 wk of pregnancy, compared to 2.3 kg in the control group (table 1 & 5, appendix D.2). From wk 15 to wk 32 they gained 7.9 kg and 9.3 kg, respectively. The difference in body weight in the 15th (IG: 65.7 ± 9.4 kg; CG: 65.9 ± 7.9 kg) and the 32nd wk of pregnancy (IG: 73.6 ± 9 kg; CG: 75.2 ± 7.9 kg) between the 2 groups was not significant, as shown in appendix C.2.

Table 5 Maternal anthropometry

	group	n	15. wk gest		n	32. wk gest	
			mean ± SD	(range)		mean ± SD	(range)
Triceps SFT [mm]	Intervention	60	16.8 ± 4.5	(5.8 - 27.1)	39	17.5 ± 4.8	(8.6-27.3)
	Control	63	17.1 ± 4.7	(5.9-28.6)	45	18.1 ± 5.4	(7.6-33.7)
Biceps SFT [mm]	Intervention	60	9 ± 3.9	(2.4-24.3)	39	8.8 ± 3.8	(3.4-20)
	Control	63	9.1 ± 3.7	(2.5-28.6)	45	9.6 ± 4.2	(3.9-26.5)
Subscapular SFT [mm]	Intervention	60	13.4 ± 5.2	(5.4-29.3)	39	13.8 ± 4.6	(6.6-26.6)
	Control	63	13.1 ± 3.8	(7.1-23.1)	45	14.4 ± 3.9	(7.4-24)
Suprailiac SFT [mm]	Intervention	50	11.1 ± 5.2	(3-27.1)	34	12.8 ± 4.7	(5.5-22.7)
	Control	50	10.7 ± 4.8	(4.4-29.3)	38	13.3 ± 4.2	(5.1-22.2)
Arm circumference [cm]	Intervention	59	28.3 ± 2.8	(22-36.8)	39	28.4 ± 2.5 #	(24-35)
	Control	61	28.4 ± 2.8	(23.6-35.8)	45	29.9 ± 2.8 #	(24-36)
Body fat [% body weight]	Intervention	60	27.3 ± 4	(14.4-36.6)	36	25.5 ± 3.8	(16.1-33.2)
	Control	63	27.5 ± 3.7	(17.4-35.9)	42	26.4 ± 3.8	(18.6-35.8)
LBM [% body weight]	Intervention	60	72.1 ± 4.1	(63-85)	36	74 ± 4	(66-83)
	Control	63	72.1 ± 7.1	(47-94)	42	73 ± 3.8	(64-81)
Body fat [kg]	Intervention	60	17.4 ± 4.5	(7-32)	36	18.6 ± 4.6	(9-30)
	Control	63	16.9 ± 3.9	(8-29)	42	19.7 ± 4.7	(12-33)
LBM [kg]	Intervention	60	46 ± 5	(34-58)	36	54.3 ± 5	(45-65)
	Control	63	45.4 ± 6.2	(22-68)	42	55 ± 4	(47-62)
Body weight [kg]	Intervention	86	65.7 ± 9.4	(46-103)	55	73.6 ± 9	(58-96)
	Control	86	65.9 ± 7.9	(48-87)	55	75.2 ± 7.9	(58-94)

Significantly different distribution between groups at 32nd wk (Mann-Whitney-U-Test) $p < 0.05$

All measured SFT and the arm circumference of the women increased significantly during pregnancy ($p < 0.05$, table 5, appendix C.2), except for the biceps SFT in the CG (which increased only slightly) and the biceps SFT in the IG, which instead decreased slightly between the 15th and 32nd wk of pregnancy. There was neither a

statistically significant difference in any SFT between the two groups at the 15th wk gestation, nor at 32nd wk of gestation, in contrast to the arm circumference which was significantly lower in the IG compared to the CG at the 32nd wk of gestation ($p < 0.05$).

At wk 15, mean body fat, estimated in % of total body weight, was ~ 27.5 ± 4 %, for both groups. Until the 32nd wk, BF decreased slightly but significantly to 25.5 ± 3.8 % in the IG and to 26.4 ± 3.8 % in the CG (appendix C.2, $p < 0.05$). Lean body mass increased (significantly only for the IG) during the same time interval from 72 % in both groups to 74 ± 4 % in the IG and 73 ± 3.8 % in the CG. The differences between the two groups were not significant at any point. BF in kg increased significantly from 17.4 ± 4.5 kg to 18.6 ± 4.6 kg (+ 1.2 kg) at wk 32 in the IG and from 16.9 ± 3.9 kg to 19.7 ± 4.7 kg (+ 2.8 kg) in the CG ($p < 0.05$). LBM in kg also increased significantly in both groups from the 15th wk of gestation until the 32nd wk from ~ 45.5 kg to ~ 54.5 kg (= 8.3 kg (IG) and 9.6 kg (CG) of LBM gain, $p < 0.05$). Differences between the groups were neither significant for the 15th wk of gestation nor for the 32nd wk. Results are illustrated as boxplots in appendix D.2.

5.5 Maternal biochemical parameters

All observed changes in hematological parameters were within the normal ranges for pregnant and lactating women (according to the reference values: Labor Dr. Tiller und Kollegen). Overall results are presented in table 6 and illustrated in appendix D.3.

Table 6 Maternal biochemical parameters

	group	n	15. wk gest		n	32. wk gest		n	6. wk pp		n	16. wk pp		p*	p#	p§	p†	p‡
			mean ± SD	(range)		mean ± SD	(range)		mean ± SD	(range)		mean ± SD	(range)					
Erythrocytes [pL]	Intervention	83	4.2 ± 0.3	(3.5-5.2)	59	3.9 ± 0.2	(3.1-4.5)	42	4.4 ± 0.3	(3.9-5.2)	27	4.7 ± 0.3	(4.2-5.4)	> 0.99	> 0.99	0.363	> 0.99	< 0.001
	Control	84	4.2 ± 0.3	(3-5.3)	61	3.9 ± 0.3	(3.2-4.8)	35	4.6 ± 0.4	(3.5-5.6)	22	4.7 ± 0.3	(3.9-5.5)					< 0.001
Hb [g/dL]	Intervention	83	12.7 ± 0.9	(10.3-15.2)	59	11.8 ± 0.9	(8.9-14.3)	42	13 ± 0.9	(11.3-15.2)	27	13.5 ± 0.8	(11.7-15.1)	> 0.99	> 0.99	> 0.99	> 0.99	< 0.001
	Control	84	12.7 ± 0.9	(10.1-15)	61	11.8 ± 0.9	(10.1-14.7)	35	13.2 ± 0.9	(11.6-15.1)	22	13.5 ± 0.9	(11.9-15)					< 0.001
Hct [%]	Intervention	83	36.7 ± 2.7	(30.2-43.9)	59	34.8 ± 2.4	(26.6-40.6)	42	39.1 ± 2.6	(33.8-44.9)	27	39.9 ± 2.2	(36.4-44.5)	> 0.99	> 0.99	> 0.99	> 0.99	< 0.001
	Control	84	36.8 ± 2.9	(29.7-43.6)	61	34.9 ± 2.6	(30.1-43.6)	35	39.6 ± 2.3	(35.5-43.8)	22	39.8 ± 2.5	(34.9-43.2)					< 0.001
Thrombocytes [nL]	Intervention	83	242 ± 49	(139-458)	59	215 ± 39	(128-287)	42	239 ± 49	(133-379)	27	233 ± 41	(156-336)	> 0.99	> 0.99	0.525	0.725	0.003
	Control	84	236 ± 44	(153-351)	61	223 ± 52	(133-387)	35	259 ± 54	(163-398)	22	254 ± 48	(190-359)					0.002
Leucocytes [nL]	Intervention	83	8.1 ± 1.9	(4.1-13.7)	59	9.1 ± 2.2	(5.3-16.7)	42	5.5 ± 1.3	(3.3-10.4)	27	5.1 ± 0.9	(3.6-7.2)	> 0.99	0.451	0.479	0.545	< 0.001
	Control	84	8.1 ± 1.6	(4.3-12)	61	9.6 ± 2.1	(6.1-15)	35	6 ± 1.4	(3.9-10.3)	22	5.6 ± 1.3	(3.6-8.7)					< 0.001
Cholesterol [mg/dL]	Intervention	81	195 ± 30	(130-286)	59	270 ± 45	(194-393)	43	220 ± 39	(173-358)	27	195 ± 31	(123-247)	> 0.99	> 0.99	> 0.99	> 0.99	< 0.001
	Control	84	198 ± 34	(102-282)	60	276 ± 51	(182-416)	36	119 ± 39	(157-337)	22	206 ± 42	(147-274)					< 0.001
Triglycerides [mg/dL]	Intervention	81	103 ± 33	(43-232)	59	174 ± 62 #	(90-395)	42	64.4 ± 32	(35-198)	27	51 ± 13	(35-75)	0.429	< 0.001	0.054	0.190	< 0.001
	Control	84	114 ± 45	(42-335)	60	219 ± 63 #	(112-354)	36	81 ± 36	(35-194)	22	76 ± 48	(35-222)					< 0.001
HDL [mg/dL]	Intervention	81	77 ± 13	(45-118)	59	82.9 ± 15	(57-127)	43	77 ± 20	(36-127)	26	74 ± 16	(54-108)	> 0.99	> 0.99	0.396	> 0.99	< 0.001
	Control	84	77 ± 14	(40-118)	60	81 ± 16	(53-123)	36	71 ± 15	(49-115)	22	70 ± 15	(47-109)					< 0.001
LDL [mg/dL]	Intervention	81	108 ± 25	(66-183)	59	169 ± 43	(98-292)	43	131 ± 37	(82-230)	26	111 ± 22	(63-146)	> 0.99	> 0.99	> 0.99	> 0.99	< 0.001
	Control	84	107 ± 28	(29-181)	60	170 ± 43	(93-282)	36	133 ± 39	(80-229)	22	121 ± 38	(66-188)					< 0.001
LDL/HDL	Intervention	81	1.44 ± 0.4	(0.73-2.79)	59	2.18 ± 0.6	(0.96-4.14)	43	1.85 ± 0.8	(0.65-4.26)	26	1.55 ± 0.41	(0.76-2.37)	> 0.99	> 0.99	> 0.99	0.936	< 0.001
	Control	84	1.42 ± 0.4	(0.41-2.8)	60	2.18 ± 0.6	(0.84-3.72)	36	1.97 ± 0.7	(0.84-3.82)	22	1.8 ± 0.69	(0.74-3.28)					< 0.001
Quick/INR	Intervention	63	1.01 ± 0.04	(0.91-1.12)	55	0.99 ± 0.04	(0.91-1.09)	41	1.03 ± 0.05	(0.92-1.15)	28	1.04 ± 0.06	(0.92-1.14)	0.639	0.787	0.695	> 0.99	0.046
	Control	66	1.01 ± 0.07	(0.8-1.52)	58	0.99 ± 0.07	(0.89-1.44)	36	1.04 ± 0.06	(0.96-1.29)	22	1.04 ± 0.05	(0.98-1.17)					0.001
Blood glucose [mg/dL]	Intervention	66	81 ± 10	(64-106)	54	83 ± 10	(65-115)	42	85 ± 12	(67-112)	27	83 ± 9	(66-100)	> 0.99	> 0.99	> 0.99	> 0.99	0.001
	Control	80	82 ± 13	(51-121)	56	83 ± 12	(63-115)	34	84 ± 11	(68-112)	19	84 ± 13	(68-116)					0.192

* Significantly different distribution between groups at 15th wk (Mann-Whitney-U-Test) $p < 0.05$
# Significantly different distribution between groups at 32nd wk (Mann-Whitney-U-Test) $p < 0.05$
§ Significantly different distribution between groups 6 wks pp (Mann-Whitney-U-Test) $p < 0.05$
† Significantly different distribution between groups 16 wks pp (Mann-Whitney-U-Test) $p < 0.05$
‡ Significant change over time (Friedman-Test)

5.5.1 Maternal blood count

Maternal erythrocyte concentration decreased from 4.2 ± 0.3 per pL at the 15th wk of gestation to 3.9 ± 0.3 per pL at the 32nd wk of gestation in both groups. After delivery, the number of erythrocytes increased significantly to 4.4 ± 0.3 per pL and to 4.6 ± 0.4 per pL in the IG and CG, respectively, to reach 4.7 ± 0.3 per pL in both groups 16 wks pp ($p < 0.05$). The concentrations were not significantly different between the two groups at study entry, the 32nd wk of gestation, nor at 6 and 16 wks pp.

Maternal hemoglobin concentration followed a very similar pattern in both groups. It decreased from the baseline value of 12.7 ± 0.9 g/dL at week 15 of pregnancy to 11.8 ± 0.9 g/dL at week 32 and rose significantly to 13 ± 0.9 and 13.2 ± 0.9 g/dL after delivery in the IG and CG, respectively, and further to 13.5 ± 0.9 g/dL in both groups at 16 wks pp ($p < 0.05$). The hemoglobin concentration did not differ significantly between the groups at any of the sampling points.

Maternal hematocrit values decreased from ~ 36.7 % at the 15th wk of gestation to ~ 34.8 % at the 32nd wk of gestation in both groups. After delivery, the hematocrite increased to 39.1 % and to 39.6 % in the IG and CG, respectively, to reach ~ 39.9 % in both groups 16 wks pp. There were no significant differences between the groups at any of the sampling points.

The number of thrombocytes per nL fell from the 15th wk of gestation to the 32nd wk of gestation from 242 to 215 in the IG and from 236 to 223 in the CG. After delivery, it increased significantly to 239 ± 49 at 6 wks pp in the IG and to 259 ± 54 in the CG ($p < 0.05$). The number remained similar during the course of lactation and was 233 ± 41 in the IG and 254 ± 48 in the CG 16 wks after delivery. The observed differences between the groups were not statistically significant.

Maternal leucocytes increased from 8.1 per nL at week 15 of gestation in both groups to 9.1 ± 2.2 in the IG and 9.6 ± 2.1 in the CG at week 32. After delivery, the number decreased significantly in both groups and remained lower than the baseline value (5.5 and 5.1 in the IG and 6 and 5.6 in the CG at 6 and 16 wks pp, $p < 0.05$). There were no statistically significant differences between the groups observed during the study.

5.5.2 Blood lipids

Measured lipid parameters were not different between the two groups at study entry but changed significantly during the course of pregnancy and lactation in both groups compared to the baseline values ($p < 0.05$).

As shown in table 6 and illustrated in figure 11, plasma TAGs increased enormously during the course of pregnancy in both groups, to decline markedly postpartum. In the IG, plasma TAG concentrations increased approximately 70 % from 103 ± 33 to 174 ± 62 mg/L during pregnancy and then decreased almost 100 % to 64 ± 32 and 51 ± 13 mg/dL after delivery. In the CG, TAG concentration increased almost 100% from 114 ± 45 to 219 ± 63 mg/dL during pregnancy and decreased more than 100 % to 81 ± 36 and 76 ± 48 mg/dL after delivery. I.e. observed TAG concentrations followed the same pattern in both groups but were significantly lower at 32nd wk of gestation in the IG ($p < 0.05$). The difference observed at 6 and 16 wks pp was no longer statistically different between the two groups. Figure 11 shows a comparison of maternal TAG concentrations over the course of pregnancy and lactation in the IG and the CG. Blood sample 1 corresponds to the 15th wk of gestation, number 2 to the 32nd wk, number 3 to the 6th wk pp and number 4 to the 16th wk pp:

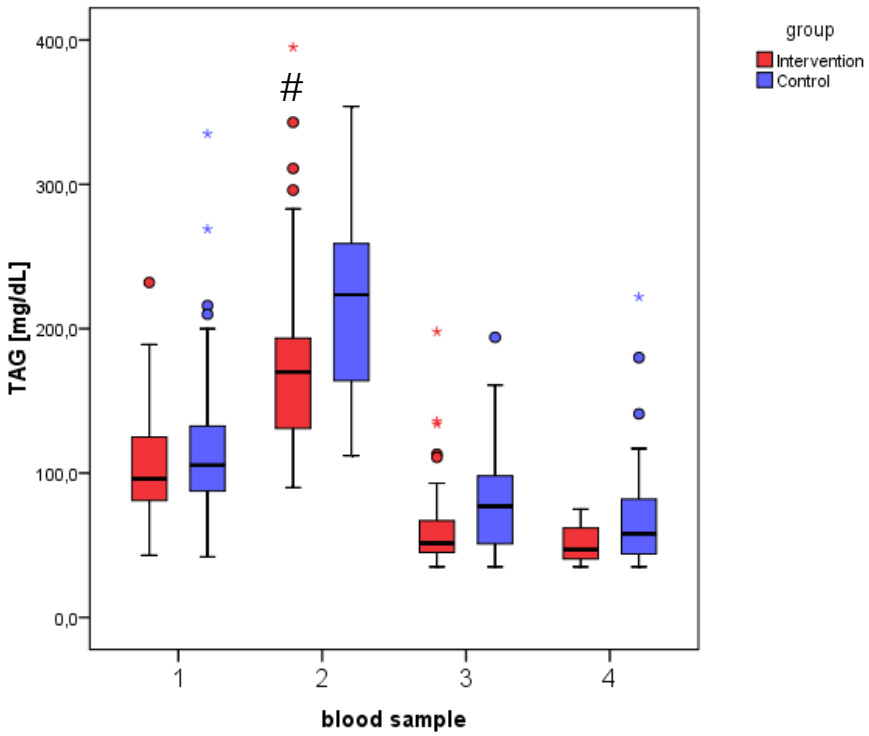

Figure 11 Maternal triacylglyceride concentration

= significantly lower in the IG, $p < 0.05$

1: 15th wk; 2: 32nd wk; 3: 6th wk pp; 4: 16th wk pp

n (IG/CG) = 83/84 (1); 59/61 (2); 42/35 (3); 27/22 (4)

Plasma cholesterol concentration also increased significantly from 195 – 200 ± 30 mg/dL to 270 – 275 ± 47 mg/dL during the course of pregnancy, with a slower decline in the TAGs after delivery, reaching baseline values at 16 wks pp ($p < 0.05$). Plasma cholesterol concentrations did not differ significantly between the two groups at any studied time point.

The HDL concentration remained very similar to the initial value of ~ 75 – 80 ± 14 mg/dL at 15th wk gestation during the whole course of pregnancy and lactation. There was no significant difference between the two groups at any time point, but the minor changes over time were statistically significant ($p < 0.05$).

The LDL concentration in both groups increased significantly from ~ 110 ± 27 mg/dL to ~ 170 ± 43 mg/dL until delivery and declined thereafter to ~ 110 ± 22 mg/dL in the IG and to 120 ± 38 mg/dL in the CG, reaching concentrations similar to study entry ($p < 0.05$). Plasma LDL concentrations did not differ significantly between the groups at study entry, at the 32nd wk of pregnancy, 6 and 16 wks pp.

The ratio of LDL/HDL concentration increased significantly from 1.43 ± 0.4 at the 15th wk of gestation to 2.18 ± 0.6 at 32nd wk of gestation in both groups and declined after delivery to 1.5 ± 0.4 in the IG and to 1.8 ± 0.7 in the CG ($p < 0.05$). There was no significant difference between the two groups at any of the four time points.

5.5.3 Blood glucose and coagulation

The Quick/INR ratio in maternal blood almost remained constant throughout pregnancy and lactation. The slight but significant change from 1.01 to 0.99 from the 15th to the 32nd wk of pregnancy and then to 1.03 and 1.04 at 6 and 16 wks pp showed an almost identical trend in both groups with no significant differences between the two groups at any of the four studied time points ($p < 0.05$).

The average fasting blood glucose concentration slightly rose from the 15th wk of pregnancy to the 32nd wk from 81 ± 10 to 83 ± 10 mg/dL in the IG group and from 82 ± 13 to 83 ± 12 in the CG (statistically significant only for the IG, $p < 0.05$). Up to 6 weeks pp the blood glucose level still rose slightly to 85 ± 12 mg/dL in the IG and to 84 ± 11 mg/dL in the CG. The values tended to decrease slightly till the 16th wk pp in both groups. The differences between the IG and the CG were not significant at any time point studied.

5.6 Fatty acid pattern in maternal plasma PL and red blood cells

In each blood sample 60 FA were detected (C 4:0 – C 24:1n-9). Results are shown in table 7 & 8. Boxplots in appendices D.4 & D.5 show the changes in maternal plasma PL and red blood cell fatty acids, as well as the sum of n-3 FA, n-6 FA, SFA, MUFA and PUFA and the ratio of LA/ALA, LA/AA, ALA/EPA and AA/DHA during the course of pregnancy and lactation. All values are presented in % by weight.

Maternal blood plasma samples in the IG and the CG were available for 64 and 62 women at study entry, 42 and 39 at the 32nd wk of gestation, 33 and 24 at the 6th wk pp and 16 and 18 at the 16th wk pp. Maternal red blood cell samples in the IG and the CG were available for 63 and 62 women at study entry, 42 and 40 at the 32nd wk of gestation, 34 and 25 at the 6th wk pp and 19 and 17 at the 16th wk pp.

5.6.1 Plasma PLs

There were no statistically significant differences in the fatty acid composition of plasma PLs between the IG and the CG at study entry as illustrated in table 7.

Table 7 Maternal plasma PL fatty acid profile

	group	n	15. wk gest		n	32. wk gest		n	6. wk pp		n	16. wk pp						
			mean ± SD	(range)		mean ± SD	(range)		mean ± SD	(range)		mean ± SD	(range)	p*	p#	p§	p†	p‡
18:2w6	Intervention	64	18.22 ± 2.40	(12.87-23.84)	42	17.38 ± 1.94#	(13.03-21.12)	33	18.73 ± 1.91	(14.90-23.07)	16	19.97 ± 2.35	(15.76-24.11)	> 0.99	0.006	0.195	> 0.99	0.043
	Control	62	18.02 ± 2.27	(12.41-22.71)	39	18.98 ± 2.15	(14.36-22.94)	24	19.96 ± 2.01	(16.99-23.79)	18	20.04 ± 2.06	(17.22-24.82)					0.003
18:3w3	Intervention	64	0.24 ± 0.08	(0.10-0.41)	42	0.27 ± 0.07	(0.16-0.43)	33	0.19 ± 0.08	(0.08-0.48)	16	0.19 ± 0.06	(0.08-0.33)	0.256	> 0.99	> 0.99	> 0.99	0.009
	Control	63	0.22 ± 0.08	(0.07-0.52)	40	0.25 ± 0.08	(0.11-0.48)	24	0.18 ± 0.06	(0.10-0.35)	18	0.18 ± 0.06	(0.12-0.34)					0.009
20:3w6	Intervention	64	3.40 ± 0.64	(1.83-4.62)	42	2.78 ± 0.60#	(1.45-4.02)	33	2.25 ± 0.49§	(1.25-3.40)	16	2.31 ± 0.45†	(1.44-3.23)	> 0.99	0.000	< 0.001	0.009	< 0.001
	Control	63	3.49 ± 0.70	(1.89-5.18)	40	3.53 ± 0.67	(2.00-4.47)	24	3.21 ± 0.57	(2.32-4.50)	18	3.03 ± 0.72	(1.51-4.13)					0.028
20:4w6	Intervention	64	10.34 ± 1.45	(7.80-13.79)	42	7.75 ± 0.99#	(5.87-10.00)	33	8.84 ± 1.19§	(6.37-10.77)	16	8.53 ± 1.13†	(6.52-10.86)	> 0.99	0.000	0.001	0.047	< 0.001
	Control	63	10.37 ± 1.37	(6.58-13.34)	40	9.05 ± 1.27	(6.33-11.79)	24	10.47 ± 1.65	(6.62-13.40)	18	9.93 ± 1.57	(7.27-11.92)					0.001
20:5w3	Intervention	64	0.73 ± 0.47	(0.28-2.49)	42	1.46 ± 0.63#	(0.64-3.43)	33	1.92 ± 1.02§	(0.92-6.49)	16	1.75 ± 0.75	(0.56-3.47)	> 0.99	0.000	< 0.001	0.083	0.001
	Control	63	0.68 ± 0.37	(0.28-2.19)	40	0.55 ± 0.25	(0.22-1.32)	24	0.92 ± 0.36	(0.45-1.76)	18	1.15 ± 0.53	(0.55-2.17)					0.002
22:4w6	Intervention	64	0.45 ± 0.09	(0.27-0.66)	42	0.24 ± 0.05#	(0.15-0.37)	33	0.23 ± 0.05§	(0.14-0.36)	16	0.23 ± 0.06†	(0.14-0.39)	0.362	0.000	< 0.001	< 0.001	< 0.001
	Control	63	0.48 ± 0.11	(0.26-0.77)	40	0.41 ± 0.10	(0.26-0.65)	24	0.41 ± 0.09	(0.22-0.58)	18	0.40 ± 0.10	(0.22-0.57)					0.011
22:5w3	Intervention	64	0.82 ± 0.22	(0.00-1.37)	42	0.70 ± 0.17	(0.45-1.17)	33	0.72 ± 0.13§	(0.47-1.01)	16	0.77 ± 0.13†	(0.59-1.08)	0.166	> 0.99	0.001	0.005	0.159
	Control	63	0.91 ± 0.22	(0.49-1.55)	40	0.69 ± 0.12	(0.47-0.91)	24	0.86 ± 0.12	(0.60-1.06)	18	0.96 ± 0.15	(0.68-1.21)					< 0.001
22:6w3	Intervention	64	5.06 ± 1.13	(2.35-8.35)	42	8.37 ± 1.14#	(5.64-10.59)	33	7.19 ± 1.13§	(4.62-10.75)	16	6.47 ± 1.28†	(4.25-9.01)	> 0.99	0.000	< 0.001	< 0.001	< 0.001
	Control	63	4.80 ± 1.03	(2.63-8.22)	40	4.63 ± 0.91	(2.81-6.70)	24	3.02 ± 0.79	(1.45-4.14)	18	3.49 ± 1.87	(1.44-8.41)					< 0.001
transFA	Intervention	64	0.23 ± 0.08	(0.06-0.41)	42	0.21 ± 0.07	(0.06-0.46)	33	0.26 ± 0.10	(0.15-0.66)	16	0.27 ± 0.09	(0.13-0.48)	> 0.99	0.503	> 0.99	> 0.99	0.015
	Control	63	0.23 ± 0.09	(0.05-0.59)	40	0.23 ± 0.07	(0.13-0.41)	24	0.26 ± 0.08	(0.13-0.50)	18	0.25 ± 0.10	(0.10-0.53)					0.018
w3LCP	Intervention	64	6.78 ± 1.50	(3.86-10.63)	42	10.72 ± 1.53#	(7.76-14.76)	33	9.99 ± 1.32§	(6.63-13.37)	16	9.15 ± 1.99†	(5.98-12.28)	> 0.99	0.000	< 0.001	0.001	< 0.001
	Control	63	6.56 ± 1.37	(3.84-10.89)	40	6.03 ± 1.16	(3.68-8.80)	24	4.95 ± 1.08	(2.89-6.65)	18	5.76 ± 2.19	(3.13-10.63)					< 0.001
w6LCP	Intervention	64	15.21 ± 1.67	(11.50-18.94)	42	11.55 ± 1.32#	(8.70-13.74)	33	11.99 ± 1.45§	(9.12-14.32)	16	11.73 ± 1.50†	(8.86-14.02)	> 0.99	0.000	< 0.001	0.004	< 0.001
	Control	63	15.40 ± 1.66	(9.38-18.29)	40	14.14 ± 1.67	(11.33-17.93)	24	14.97 ± 1.77	(10.53-18.14)	18	14.12 ± 2.02	(9.88-16.78)					0.023
SAFA	Intervention	64	45.06 ± 0.67	(43.58-46.76)	42	46.05 ± 1.01	(44.19-50.96)	33	45.56 ± 0.83	(43.55-47.20)	16	45.52 ± 0.74	(43.89-46.55)	> 0.99	0.623	> 0.99	> 0.99	0.003
	Control	63	45.45 ± 2.33	(43.75-63.02)	40	45.82 ± 0.95	(44.26-50.25)	24	45.41 ± 0.70	(44.02-46.56)	18	45.37 ± 0.77	(43.67-46.65)					0.419
MUFA	Intervention	64	13.98 ± 1.13	(10.37-17.87)	42	13.60 ± 1.71#	(4.62-15.77)	33	13.03 ± 0.90§	(11.05-14.94)	16	12.93 ± 0.83†	(11.24-14.24)	> 0.99	0.009	0.003	0.015	< 0.001
	Control	63	13.85 ± 1.29	(8.97-17.02)	40	14.30 ± 1.89	(4.53-17.40)	24	13.98 ± 0.91	(12.46-15.52)	18	13.96 ± 1.07	(11.80-15.84)					0.543
PUFA	Intervention	64	40.53 ± 1.32	(37.61-44.73)	42	39.99 ± 1.31	(37.24-44.24)	33	40.96 ± 0.96§	(39.08-43.24)	16	41.10 ± 1.01	(39.78-43.18)	> 0.99	0.106	0.025	0.154	0.016
	Control	63	39.99 ± 2.71	(25.70-43.77)	40	39.02 ± 3.04	(22.22-44.76)	24	40.17 ± 1.03	(37.74-41.99)	18	40.22 ± 1.14	(37.42-42.24)					0.1
18:2w6/18:3w3	Intervention	64	85.02 ± 34.72	(39.28-200.81)	42	69.33 ± 20.86	(35.43-108.28)	33	111.5 ± 38.23	(36.33-234.60)	16	119.2 ± 53.55	(55.98-272.35)	0.565	0.105	> 0.99	> 0.99	< 0.001
	Control	63	91.97 ± 41.86	(0.00-295.28)	39	83.48 ± 29.19	(35.66-185.64)	24	120.0 ± 35.09	(54.83-196.14)	18	120.3 ± 38.45	(58.40-180.94)					< 0.001
18:2w6/20:4w6	Intervention	64	1.81 ± 0.41	(1.08-2.86)	42	2.29 ± 0.45	(1.49-3.44)	33	2.17 ± 0.45	(1.44-3.21)	16	2.40 ± 0.54	(1.70-3.58)	> 0.99	0.659	0.121	0.213	< 0.001
	Control	63	1.75± 0.44	(0.00-2.91)	39	2.17 ± 0.53	(1.42-3.49)	24	1.98 ± 0.54	(1.39-3.48)	18	2.09 ± 0.53	(1.53-3.41)					0.123
18:3w3/20:5w3	Intervention	64	0.4 ± 0.18	(0.08-0.94)	42	0.20 ± 0.07#	(0.07-0.37)	33	0.11 ± 0.06§	(0.03-0.32)	16	0.13 ± 0.08†	(0.05-0.34)	> 0.99	0.000	< 0.001	0.023	< 0.001
	Control	63	0.38 ± 0.18	(0.08-1.11)	40	0.54 ± 0.30	(0.16-1.86)	24	0.22 ± 0.08	(0.08-0.36)	18	0.17 ± 0.04	(0.10-0.24)					< 0.001
20:4w6/22:6w3	Intervention	64	2.15 ± 0.59	(1.08-4.28)	42	0.95 ± 0.22#	(0.62-1.55)	33	1.27 ± 0.31§	(0.77-2.14)	16	1.39 ± 0.41†	(0.90-2.18)	0.854	0.000	< 0.001	< 0.001	< 0.001
	Control	63	2.25 ± 0.52	(1.07-3.90)	40	2.01 ± 0.42	(1.29-3.32)	24	3.68 ± 1.03	(1.66-6.53)	18	3.50 ± 1.46	(0.89-5.84)					< 0.001

Values for fatty acids are expressed as % of total fatty acids (wt %)

transFA: tC14:1n5; tC16:1n7; tC17:1n7; tC18:1n9/7; ttC18:2n6
SFA: C4:0 - C27:0
MUFA: sum of all cis-FA with one double bond
PUFA: sum of all cis-FA with two or more double bonds
n6LCP: C20:2n6; DHyLA; AA; C22:2n6; C22:4n6; C22:5n6
n3LCP: C20:3n3; C20:4n3; EPA; C21:5n3; C22:3n3; n3-DPA; DHA

* Significantly different distribution between groups at 15th wk (Mann-Whitney-U-Test) $p < 0.05$
# Significantly different distribution between groups at 32nd wk (Mann-Whitney-U-Test) $p < 0.05$
§ Significantly different distribution between groups 6 wks pp (Mann-Whitney-U-Test) $p < 0.05$
† Significantly different distribution between groups 16 wks pp (Mann-Whitney-U-Test) $p< 0.05$
‡ Significant change over time (Friedman-Test) $p < 0.05$

LA content increased slightly but statistically significantly from study entry to the 16th wk pp in both groups ($p < 0.05$). The LA content at the 32nd wk of gestation was significantly lower in the IG compared to the CG ($p < 0.05$).

ALA content decreased significantly from study entry to the 16th wk pp in both groups ($p < 0.05$). There were no differences in the ALA content between the groups at any of the four time points during pregnancy and lactation.

DHyLA levels also decreased significantly from study entry to the 16th wk pp in both groups ($p < 0.05$). DHA supplementation resulted in a more pronounced decrease compared to the CG. The DHyLA content in the PLs of the women in the IG was therefore significantly lower during pregnancy and lactation, as compared to the women in the CG ($p < 0.05$).

n-3 LC-PUFA supplementation and a reduced intake of AA resulted in a significant lower content of AA at the 32nd wk of gestation and at 6 and 16 wks pp compared to the CG, although the AA content decreased significantly in both groups during the course of pregnancy (figure 12, $p < 0.05$). After delivery, the level of AA remained almost constant until the 16th wk pp.

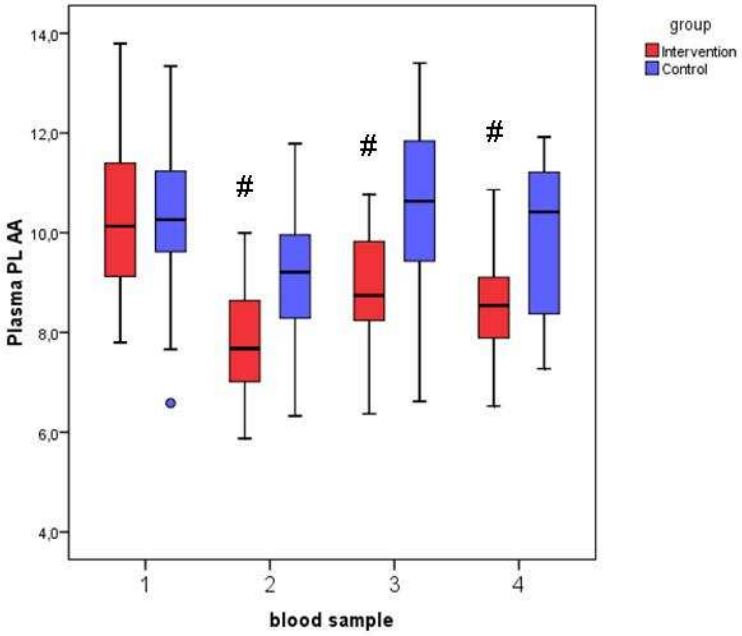

Figure 12 Maternal plasma AA content

= significantly lower in the IG, $p < 0.05$

1: 15th wk; 2: 32nd wk; 3: 6th wk pp; 4: 16th wk pp

n (IG/CG) = 63/64 (1); 42/40 (2); 33/24 (3); 16/18 (4)

Compared to the CG, there was a significant increase of the EPA content in maternal plasma PL throughout pregnancy and lactation in the IG ($p < 0.05$). In the CG, the content of EPA slightly decreased from study entry to the 32nd wk of gestation, increased after delivery, and reached higher values as the original baseline value.

A 50 % decrease of adrenic acid was observed after supplementation compared to the control group, resulting in significant lower adrenic acid levels throughout pregnancy and lactation, although a slight but significant decrease in the adrenic acid level of the CG during the course of pregnancy and lactation was also observed ($p < 0.05$).

The n-3 DPA content decreased slightly (significant only for the CG) during pregnancy and increased thereafter to reach their original baseline values. At the 6th and the 16th wk pp, the n-3 DPA content was significantly lower in the IG compared to the CG ($p < 0.05$).

DHA content in plasma PL increased significantly after supplementation until delivery and declined slightly thereafter ($p < 0.05$). In the CG, the DHA content did not change clearly during the course of pregnancy but also declined after delivery. Therefore, the DHA level in the IG was significantly higher (twice as high as in the CG) in the 32nd wk of pregnancy and during lactation compared to the control group ($p < 0.05$).

No statistically significant differences in the sum of trans-FA, SFA and PUFA (except of the PUFA content 6 wks pp, which was significantly higher in the IG compared to the CG, $p < 0.05$) between the two groups were observed at any of the 4 time points. Moreover, there were no major changes in the fatty acid levels during the course of pregnancy and lactation. The MUFA level was slightly but significantly lower after n-3 LC-PUFA supplementation compared to the CG ($p < 0.05$).

n-3 LCP (long-chain polyunsaturated fatty acid) content in plasma PL increased significantly after fish oil supplementation until delivery and declined slightly thereafter ($p < 0.05$). In the CG, the n-3 LCP content did not clearly change during the course of pregnancy but also declined after delivery. Therefore, the n-3 LCP level in maternal plasma PL in the IG was significantly higher (twice as high as in the CG) in the 32nd wk of pregnancy and during lactation compared to the control group ($p < 0.05$).

In contrast, the n-6 LCP content was significantly lower in the IG at 32nd wk gestation and in the 6th and 16th wk pp, compared to the CG because supplementation resulted in a more pronounced decline from baseline values onwards ($p < 0.05$).

The ratio of LA/ALA significantly decreased from study entry to delivery and increased thereafter to values almost twice as high as the baseline values in both groups ($p < 0.05$). There was no statistical difference between the two groups at any studied time point.

The ratio of LA/AA significantly increased from study entry to delivery and during lactation in both groups ($p < 0.05$). There was no statistical difference between the two groups at any studied time point.

The ratio of ALA/EPA decreased significantly after supplementation with n-3 LC-PUFAs from study entry to delivery and further until 16 wks pp ($p < 0.05$). In the CG a slight increase from study entry to delivery was observed, followed by a decline until 16 wks pp, resulting in a significantly lower ratio in the 32nd wk of pregnancy, 6 and 16 wks pp compared to the control group ($p < 0.05$).

Supplementation with n-3 LC-PUFAs resulted in a decrease of the AA/DHA ratio from 2.15 ± 0.6 to 0.95 ± 0.2 from study entry to the 32nd wk of gestation. During this time period, the ratio in the CG did not change significantly. From delivery to the 16th wk pp, the ratio in the IG slightly increased to 1.27 ± 0.3 and almost doubled in the CG to 3.68 ± 1 6 weeks after delivery and to 1.39 ± 0.4 and 3.5 ± 1.5 at 16 weeks pp, respectively. Women in the IG had significantly lower ratios of AA/DHA compared to the CG (2 = 32nd wk gestation; 3 = 6th wk pp; 4 = 16th wk pp), except for the baseline value at study entry (1) (figure 13, $p < 0.05$):

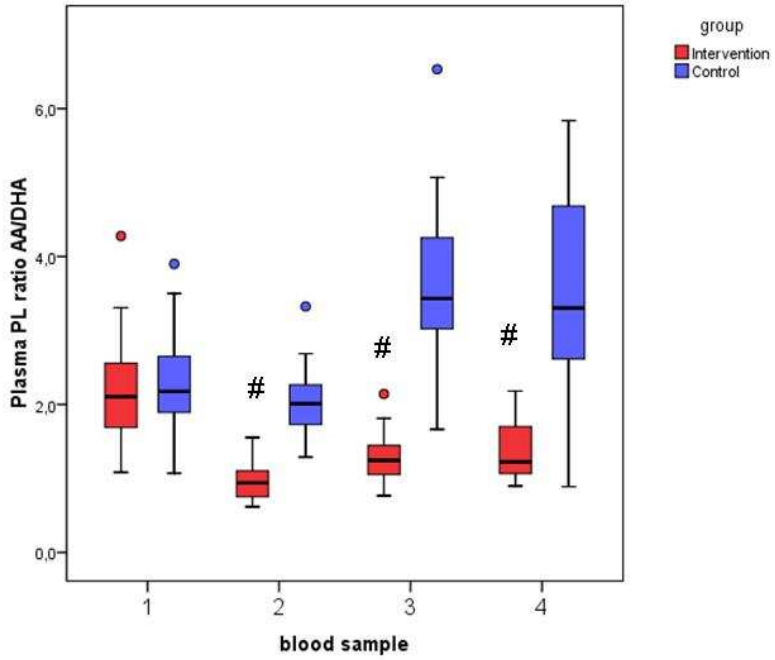

Figure 13 Maternal plasma AA/DHA ratio

= significantly lower in the IG, $p < 0.05$

1: 15th wk; 2: 32nd wk; 3: 6th wk pp; 4: 16th wk pp

n (IG/CG) = 63/64 (1); 42/40 (2); 33/24 (3); 16/18 (4)

Taken together, the effect of n-3 LC-PUFA supplementation was to increase the maternal plasma PL contents of DHA and EPA and to reinforce the physiologic decrease of AA and adrenic acid during pregnancy and lactation, resulting in a remarkable, significant decrease of the AA/DHA ratio compared to the CG ($p < 0.05$).

In the control group the EPA and DHA levels decreased during the course of pregnancy instead, as well as the AA and adrenic acid levels, but the latter two to a less pronounced extent as in the IG.

After DHA supplementation, the highest relative fatty acid changes were observed for EPA (+ 100 %), DHA (+ 65 %), adrenic acid (- 47 %) and the ratio of AA/DHA (- 56 %).

5.6.2 Red blood cells

There were no statistically significant differences in the fatty acid composition of RBCs between the IG and the CG at study entry as illustrated in table 8.

Table 8 Maternal RBC fatty acid profile

	group	n	15. wk gest		n	32. wk gest		n	6. wk pp		n	16. wk pp						
			mean ± SD	(range)		mean ± SD	(range)		mean ± SD	(range)		mean ± SD	(range)	p*	p#	p§	p†	p‡
18:2w6	Intervention	63	7.13 ± 1.41	(3.04-9.92)	42	6.32 ± 1.27	(3.42-8.11)	34	7.37 ± 1.49	(3.95-10.24)	19	8.04 ± 1.87	(3.35-10.36)	0.984	0.176	0.769	> 0.99	< 0.001
	Control	62	7.53 ± 1.03	(4.30-9.55)	40	6.65 ± 1.78	(2.98-9.41)	25	7.85 ± 1.47	(3.91-10.33)	17	7.90 ± 1.86	(3.66-10.54)					0.001
18:3w3	Intervention	63	0.12 ± 0.02	(0.08-0.17)	42	0.12 ± 0.02	(0.08-0.17)	34	0.10 ± 0.02	(0.06-0.19)	19	0.11 ± 0.03	(0.08-0.17)	0.637	> 0.99	0.926	> 0.99	0.001
	Control	62	0.12 ± 0.03	(0.05-0.32)	40	0.12 ± 0.02	(0.08-0.17)	25	0.11 ± 0.02	(0.07-0.15)	17	0.10 ± 0.02	(0.07-0.14)					0.005
20:3w6	Intervention	62	1.49 ± 0.47	(0.28-2.82)	42	1.21 ± 0.43#	(0.33-1.89)	34	1.11 ± 0.42	(0.25-1.90)	19	1.11 ± 0.40	(0.34-1.75)	> 0.99	0.042	0.061	0.315	0.004
	Control	62	1.56 ± 0.38	(0.45-2.34)	40	1.45 ± 0.61	(0.34-2.43)	25	1.40 ± 0.51	(0.32-2.61)	17	1.35 ± 0.53	(0.30-2.17)					0.896
20:4w6	Intervention	63	11.17 ± 3.54	(1.58-14.90)	42	8.05 ± 3.30#	(1.47-11.95)	34	9.11 ± 3.56§	(1.45-13.90)	19	9.10 ± 3.60	(1.61-12.96)	0.575	0.001	0.042	0.073	< 0.001
	Control	62	11.90 ± 2.69	(3.64-15.15)	40	9.64 ± 4.22	(1.40-13.34)	25	10.64 ± 3.51	(1.77-14.16)	17	10.91 ± 4.37	(1.23-14.62)					0.014
20:5w3	Intervention	63	0.38 ± 0.21	(0.00-0.88)	42	0.65 ± 0.38#	(0.04-1.51)	34	0.79 ± 0.43§	(0.00-1.75)	19	0.91 ± 0.49	(0.11-1.67)	> 0.99	< 0.001	0.003	0.061	0.002
	Control	62	0.39 ± 0.17	(0.06-0.87)	40	0.30 ± 0.19	(0.00-0.72)	25	0.41 ± 0.22	(0.00-0.84)	17	0.53 ± 0.33	(0.01-1.15)					0.013
22:4w6	Intervention	63	2.61 ± 0.94	(0.28-4.31)	42	1.42 ± 0.62#	(0.23-2.37)	34	1.49 ± 0.72§	(0.16-3.67)	19	1.34 ± 0.65†	(0.26-2.45)	> 0.99	< 0.001	< 0.001	0.008	0.003
	Control	62	2.81 ± 0.74	(0.84-4.48)	40	2.29 ± 1.09	(0.38-3.60)	25	2.34 ± 0.85	(0.38-3.58)	17	2.31 ± 1.04	(0.32-3.55)					0.116
22:5w3	Intervention	63	1.66 ± 0.64	(0.13-2.23)	42	1.23 ± 0.63	(0.09-2.06)	34	1.29 ± 0.62	(0.10-2.04)	19	1.28 ± 0.63†	(0.12-1.84)	> 0.99	0.288	0.902	0.030	0.014
	Control	62	1.77 ± 0.53	(0.32-2.46)	40	1.43 ± 0.74	(0.10-2.33)	25	1.46 ± 0.58	(0.13-2.24)	17	1.75 ± 0.80	(0.09-2.61)					0.089
22:6w3	Intervention	63	4.32 ± 1.82	(0.23-7.51)	42	6.59 ± 3.35#	(0.26-10.61)	34	6.67 ± 3.05§	(0.50-9.66)	19	6.10 ± 2.84†	(0.61-9.24)	> 0.99	< 0.001	< 0.001	0.006	0.003
	Control	62	4.24 ± 1.41	(0.55-6.86)	40	3.97 ± 2.16	(0.16-7.21)	25	3.66 ± 1.55	(0.29-5.50)	17	3.04 ± 1.67	(0.13-5.79)					0.258
transFA	Intervention	63	0.24 ± 0.06	(0.15-0.39)	42	0.24 ± 0.06	(0.14-0.36)	34	0.23 ± 0.06	(0.15-0.36)	19	0.24 ± 0.06	(0.15-0.34)	> 0.99	> 0.99	> 0.99	> 0.99	0.015
	Control	62	0.23 ± 0.04	(0.14-0.33)	40	0.25 ± 0.05	(0.16-0.34)	25	0.24 ± 0.06	(0.12-0.36)	17	0.25 ± 0.05	(0.17-0.35)					0.733
w3LCP	Intervention	63	6.43 ± 2.60	(0.37-10.15)	42	8.53 ± 4.30#	(0.44-13.25)	34	8.81 ± 4.02§	(0.61-12.94)	19	8.35 ± 3.90†	(0.85-12.16)	> 0.99	< 0.001	0.001	0.022	0.01
	Control	62	6.48 ± 2.00	(0.97-10.09)	40	5.76 ± 3.06	(0.26-10.05)	25	5.59 ± 2.28	(0.42-7.95)	17	5.39 ± 2.71	(0.26-9.09)					0.526
w6LCP	Intervention	63	16.13 ± 5.01	(2.30-21.09)	42	11.33 ± 4.48#	(2.22-16.57)	34	12.36 ± 4.79§	(1.99-20.87)	19	12.15 ± 4.70†	(2.32-16.87)	0.559	< 0.001	0.003	0.036	0.001
	Control	62	17.23 ± 3.78	(5.25-22.08)	40	14.33 ± 6.21	(2.37-20.40)	25	15.27 ± 4.92	(2.63-20.14)	17	15.33 ± 6.04	(1.99-20.12)					0.071
SAFA	Intervention	63	49.68 ± 7.46	(44.26-72.25)	42	52.25 ± 8.42	(45.50-70.47)	34	50.92 ± 8.24	(44.14-70.34)	19	51.29 ± 8.44	(45.28-70.89)	> 0.99	> 0.99	> 0.99	> 0.99	0.372
	Control	62	48.43 ± 4.81	(44.35-66.81)	40	51.61 ± 9.01	(44.61-70.46)	25	50.12 ± 7.04	(44.72-67.92)	17	50.71 ± 8.88	(44.72-72.42)					0.216
MUFA	Intervention	63	19.98 ± 1.40	(16.73-23.86)	42	20.92 ± 1.61	(18.27-24.74)	34	19.91 ± 1.70	(16.94-23.80)	19	19.54 ± 1.75	(17.07-23.28)	0.538	> 0.99	0.254	0.990	0.002
	Control	62	19.70 ± 1.50	(16.58-23.92)	40	20.99 ± 1.97	(18.11-25.62)	25	20.51 ± 1.36	(18.67-24.39)	17	20.02 ± 1.48	(17.36-22.86)					0.004
PUFA	Intervention	63	29.87 ± 8.55	(5.99-36.51)	42	26.35 ± 9.76	(6.42-34.77)	34	28.70 ± 9.75	(7.32-36.80)	19	28.70 ± 10.05	(6.70-35.81)	0.609	> 0.99	0.926	> 0.99	0.042
	Control	62	31.41 ± 6.03	(10.64-35.68)	40	26.93 ± 10.73	(6.14-35.28)	25	28.89 ± 8.20	(7.18-35.55)	17	28.79 ± 9.87	(6.01-35.21)					0.085
18:2w6/18:3w3	Intervention	63	62.61 ± 17.98	(25.43-112.99)	42	51.56 ± 12.39	(25.48-79.71)	34	76.53 ± 23.16	(37.51-122.38)	19	77.99 ± 23.74	(26.49-106.01)	0.256	0.602	> 0.99	> 0.99	< 0.001
	Control	62	68.63 ± 17.51	(25.04-146.82)	40	55.38 ± 16.93	(24.20-84.04)	25	75.69 ± 17.86	(29.75-112.61)	17	78.35 ± 22.03	(33.27-126.80)					0.001
18:2w6/20:4w6	Intervention	63	0.73 ± 0.33	(0.45-1.93)	42	0.97 ± 0.50	(0.59-2.41)	34	1.02 ± 0.60	(0.43-2.73)	19	1.06 ± 0.48	(0.69-2.26)	> 0.99	0.104	0.689	0.205	0.002
	Control	62	0.67 ± 0.18	(0.46-1.29)	40	0.89 ± 0.49	(0.52-2.38)	25	0.87 ± 0.44	(0.53-2.21)	17	0.97 ± 0.70	(0.48-2.97)					0.052
18:3w3/20:5w3	Intervention	61	0.49 ± 0.63	(0.14-4.20)	42	0.41 ± 0.57#	(0.08-3.05)	33	0.21 ± 0.24§	(0.06-1.17)	19	0.24 ± 0.33	(0.05-1.14)	> 0.99	0.001	0.001	0.061	0.001
	Control	62	0.37 ± 0.26	(0.13-1.68)	37	0.70 ± 0.81	(0.17-3.48)	24	0.35 ± 0.31	(0.14-1.34)	17	0.72 ± 1.78	(0.10-7.51)					0.02
20:4w6/22:6w3	Intervention	63	3.06 ± 1.27	(1.44-7.79)	42	1.52 ± 0.80#	(0.93-5.62)	34	1.56 ± 0.53§	(0.97-3.37)	19	1.68 ± 0.47†	(1.19-2.63)	> 0.99	< 0.001	< 0.001	< 0.001	< 0.001
	Control	62	3.05 ± 0.85	(1.74-6.56)	40	3.14 ± 1.49	(1.62-8.76)	25	3.33 ± 1.12	(2.11-6.61)	17	4.44 ± 1.84	(2.06-9.73)					0.036

Values for fatty acids are expressed as % of total fatty acids (wt %)

transFA: tC14:1n5; tC16:1n7; tC17:1n7; tC18:1n9/7; ttC18:2n6
SFA: C4:0 - C27:0
MUFA: sum of all cis-FA with one double bond
PUFA: sum of all cis-FA with two or more double bonds
n6LCP: C20:2n6; DHyLA; AA; C22:2n6; C22:4n6; C22:5n6
n3LCP: C20:3n3; C20:4n3; EPA; C21:5n3; C22:3n3; n3-DPA; DHA

* Significantly different distribution between groups at 15th wk (Mann-Whitney-U-Test) $p < 0.05$
\# Significantly different distribution between groups at 32nd wk (Mann-Whitney-U-Test) $p < 0.05$
§ Significantly different distribution between groups 6 wks pp (Mann-Whitney-U-Test) $p < 0.05$
† Significantly different distribution between groups 16 wks pp (Mann-Whitney-U-Test) $p< 0.05$
‡ Significant change over time (Friedman-Test) $p < 0.05$

LA content decreased slightly but statistically significantly from study entry to delivery and increased thereafter to the 16th wk pp in both groups ($p < 0.05$). No statistically significant differences between the groups were observed.

ALA content decreased slightly but significantly from study entry to the 16th wk pp in both groups ($p < 0.05$). There were no statistically significant differences in the ALA content between the groups at any studied time point during pregnancy and lactation.

DHyLA levels also decreased (significant only for the IG, $p < 0.05$) from study entry to the 16th wk pp in both groups. The DHyLA content at the 32nd wk of gestation was significantly lower in the IG compared to the CG ($p < 0.05$).

There was a significantly lower content of AA in the IG in the 32nd wk of gestation and at 6 wks pp, compared to the CG, although the AA content decreased significantly in both groups during the course of pregnancy ($p < 0.05$). After delivery, the level of AA increased slightly and remained almost stable until the 16th wk pp.

Compared to the CG, supplementation with n-3 LC-PUFA resulted in a significant increase of the EPA content throughout pregnancy and lactation, whereas the content in the CG slightly decreased from study entry to the 32nd wk of gestation, increased after delivery, and then reached higher values than at baseline ($p < 0.05$). The content of EPA was significantly higher after supplementation at the 32nd wk of gestation and 6 wks pp compared to the control group ($p < 0.05$).

A 50 % decrease of adrenic acid was observed after supplementation compared to the control group, resulting in significant lower adrenic acid levels throughout pregnancy and lactation ($p < 0.05$), although a slight (but not significant) decrease in the adrenic acid level in the CG was observed, too.

The n-3 DPA content decreased slightly (significant only for the IG, $p < 0.05$) during pregnancy and lactation, and increased thereafter slightly in the CG only from the 6th to the 16th wk pp. No statistically significant differences between the groups were observed.

DHA content increased significantly after supplementation during the course of pregnancy and lactation ($p < 0.05$). In contrast to the plasma PLs, the relative increase was only ~ 50 % (figure 14). In the CG, the DHA content declined during the course of pregnancy and lactation. Therefore, the DHA level in maternal RBCs in the

IG was significantly higher in the 32nd wk of pregnancy and during lactation compared to the control group ($p < 0.05$).

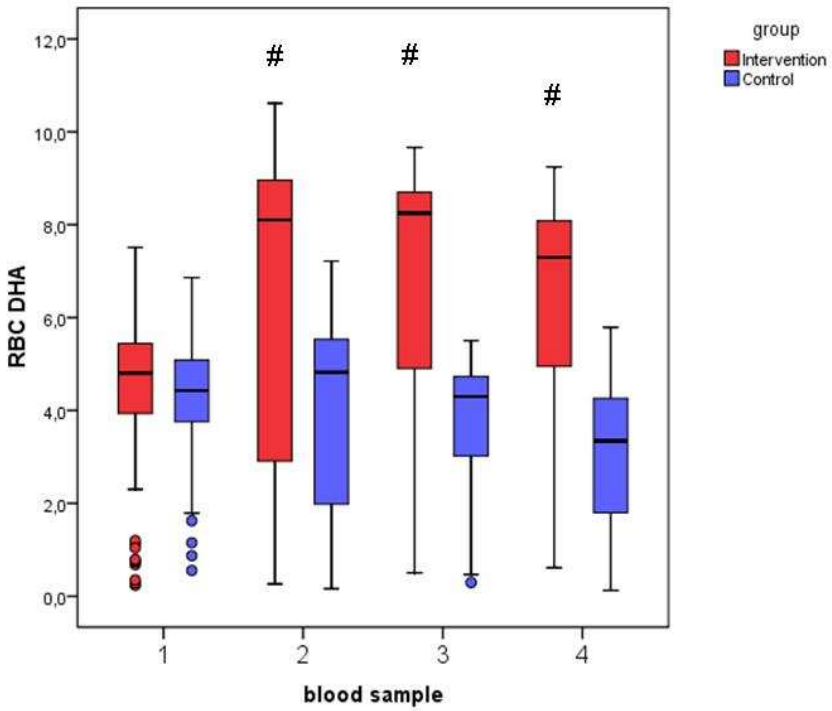

Figure 14 Maternal RBC DHA content

= significantly higher in the IG, $p < 0.05$

1: 15th wk; 2: 32nd wk; 3: 6th wk pp; 4: 16th wk pp

n (IG/CG) = 63/62 (1); 42/40 (2); 34/25 (3); 10/17 (4)

No statistically significant differences in the sum of trans-FA, SFA, MUFA, PUFA, the ratio of LA/ALA and LA/AA between the two groups were observed at any of the 4 time points. Moreover, there were no major changes in the fatty acid levels during the course of pregnancy and lactation, except for an increase of the LA/ALA ratio after delivery ($p < 0.05$).

n-3 LCP content in RBCs increased significantly after supplementation until the 6th wk of gestation and slightly declined thereafter ($p < 0.05$). In the CG, the n-3 LCP content decreased during the course of pregnancy and lactation. Therefore, the n-3 LCP level was significantly higher in the IG at the 32nd wk of pregnancy and during lactation compared to the control group ($p < 0.05$).

In contrast, the n-6 LCP content was significantly lower in the IG at 32nd wk gestation and in the 6th and 16th wk pp, compared to the CG because supplementation resulted in a more pronounced decline from baseline onwards ($p < 0.05$).

The ratio of ALA/EPA decreased significantly after delivery in the IG, reaching half of the content compared to baseline values. In the CG an increase from study entry to delivery was observed, followed by a decline until 6 wks pp, resulting in a significantly lower ratio in the 32nd wk of pregnancy and the 6th wk pp in the IG, compared to the CG ($p < 0.05$).

Supplementation with n-3 LC-PUFAs resulted in a decrease of 50 % of the AA/DHA ratio (figure 15) from 3.06 ± 1.3 to 1.52 ± 0.8 from study entry (1) to the 32nd wk of gestation (2). During this period of time, the ratio in the CG did not change significantly. From delivery to the 16th wk pp (4), the ratio in the IG slightly increased to 1.68 ± 0.5 and almost doubled in the CG to 4.44 ± 1.8 at 16 wks pp (4). Women in the IG had significantly lower ratios of AA/DHA except for the baseline value at study entry compared to the CG ($p < 0.05$).

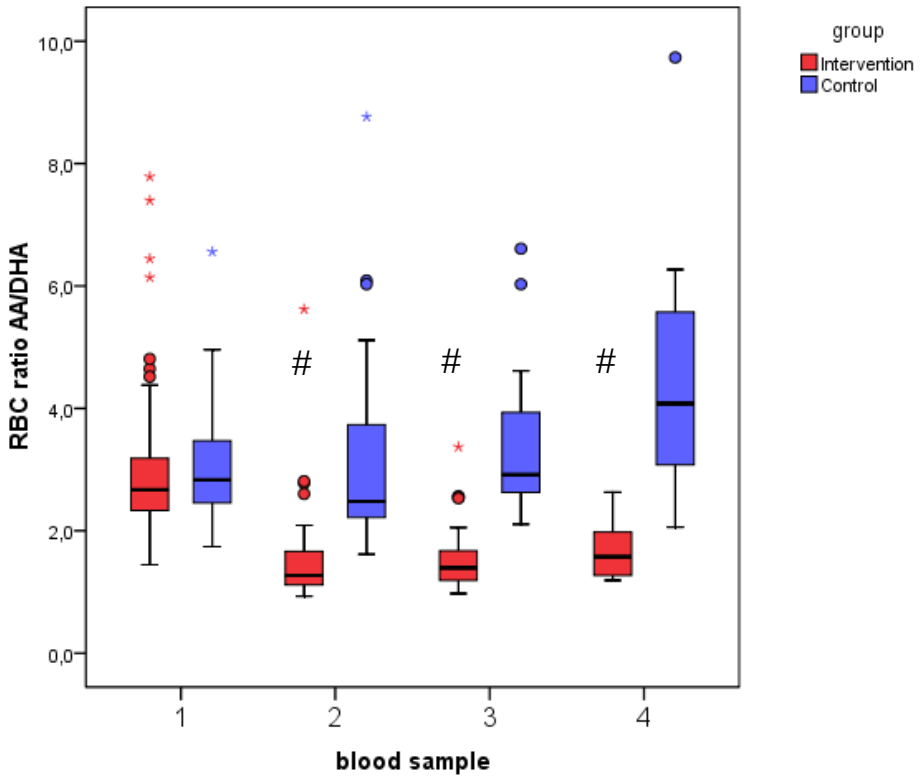

Figure 15 Maternal RBC ratio of AA/DHA

= significantly lower in the IG, $p < 0.05$

1: 15th wk; 2: 32nd wk; 3: 6th wk pp; 4: 16th wk pp

n (IG/CG) = 63/62 (1); 42/40 (2); 34/25 (3); 10/17 (4)

Comparable to the changes in plasma PL, the effect of n-3 LC-PUFA supplementation was to increase the maternal RBC contents of DHA and EPA and to reinforce the physiologic decrease of AA and Adrenic acid during pregnancy and lactation, resulting in a remarkable, significant decrease of the AA/DHA ratio compared to the CG ($p < 0.05$). In the control group the EPA and DHA levels decreased during the course of pregnancy instead, as well as the AA and adrenic acid levels, but the latter two to a less pronounced extent as in the IG.

After n-3 LC-PUFA supplementation, very similar to the changes in PL, albeit to a lesser extent, the highest relative fatty acid changes were observed for EPA (+ 70 %), DHA (+50 %), adrenic acid (- 46 %) and the ratio of AA/DHA (- 50 %).

5.6.3 Relation of PL and RBC fatty acids in maternal blood samples

Both at the 15th and the 32nd wk of gestation, there were significant positive correlations between PL EPA and DHA contents and significant negative correlations between PL AA and EPA, as well as AA and DHA (see table C.4 appendix, $p < 0.05$).

Both at the 15th and the 32nd wk of gestation, there were also strong significantly positive correlations between RBC EPA and DHA contents and – in contrast to the FA pattern in PL - also weak significantly positive correlations between RBC AA and EPA, as well as AA and DHA ($p < 0.05$).

Both at the 15th and the 32nd wk of gestation, there were significantly positive correlations between PL EPA and DHA as well as RBC EPA and DHA contents and significantly negative correlations between PL EPA and DHA as well as RBC AA ($p < 0.05$). The PL AA content correlated significantly positively with RBC AA content and significantly negatively with RBC EPA and DHA content ($p < 0.05$).

5.6.4 Relation of PL and RBC fatty acids and blood lipids

15 wk gestation: There are no statistically significant correlations between blood lipids and fatty acid composition of PL or RBCs (see table appendix C.5).

32 wk gestation: There are weak significantly positive correlations between the TAG concentration and PL AA content and the AA/DHA ratio, and weak significantly negative correlations between the TAG concentration and PL EPA and DHA content ($p < 0.05$).

5.7 Fatty acid pattern in cord blood plasma PL and red blood cells

5.7.1 Plasma PL

Umbilical cord plasma samples were available for 19 newborns in the IG and for 18 in the CG as illustrated in table 9.

Table 9 Cord blood plasma PL fatty acid profile

	group	n	cord blood		
			mean ± SD	(range)	p*
18:2w6	Intervention	19	6.73 ± 0.81	(5.19-8.18)	0,346
	Control	18	6.44 ± 1.07	(4.96-8.68)	
18:3w3	Intervention	19	0.02 ± 0.02	(0.00-0.06)	0,078
	Control	18	0.01 ± 0.02	(0.00-0.05)	
20:3w6	Intervention	19	4.75 ± 0.47	(3.79-5.49)	0,784
	Control	18	4.83 ± 0.71	(3.45-6.06)	
20:4w6	Intervention	19	14.85 ± 1.74*	(12.44-17.69)	< 0.001
	Control	18	17.77 ± 1.57	(14.50-20.52)	
20:5w3	Intervention	19	0.98 ± 0.43*	(0.13-1.68)	< 0.001
	Control	18	0.27 ± 0.12	(0.08-0.53)	
22:4w6	Intervention	19	0.64 ± 0.16*	(0.43-0.92)	< 0.001
	Control	18	0.84 ± 0.19	(0.60-1.36)	
22:5w3	Intervention	19	0.50 ± 0.19	(0.20-0.89)	0,213
	Control	18	0.41 ± 0.18	(0.15-0.78)	
22:6w3	Intervention	19	7.95 ± 1.34*	(5.73-10.38)	0,002
	Control	18	6.04 ± 1.78	(3.05-9.60)	
transFA	Intervention	19	0.16 ± 0.14	(0.03-0.48)	0,201
	Control	18	0.09 ± 0.09	(0.00-0.27)	
w3LCP	Intervention	19	9.56 ± 1.54*	(7.21-11.86)	< 0.001
	Control	18	6.77 ± 1.97	(3.28-10.52)	
w6LCP	Intervention	19	21.38 ± 1.63*	(18.99-24.37)	< 0.001
	Control	18	25.07 ± 1.52	(22.25-27.77)	
SAFA	Intervention	19	48.04 ± 0.61	(47.09-49.23)	0,564
	Control	18	47.96 ± 0.79	(46.91-50.08)	
MUFA	Intervention	19	13.84 ± 1.28	(11.57-16.27)	0,412
	Control	18	13.41 ± 1.57	(10.52-15.86)	
PUFA	Intervention	19	37.76 ± 1.44	(35.83-40.74)	0,171
	Control	18	38.37 ± 1.45	(36.23-40.75)	
18:2w6/18:3w3	Intervention	10	199.6 ± 65.95	(126.40-300.51)	0,54
	Control	5	241.0 ± 91.24	(124.62-375.44)	
18:2w6/20:4w6	Intervention	19	0.46 ± 0.09*	(0.30-0.60)	0,002
	Control	18	0.37 ± 0.08	(0.24-0.54)	
20:4w6/22:6w3	Intervention	19	1.93 ± 0.44*	(1.41-3.03)	< 0.001
	Control	18	3.19 ± 0.98	(1.90-5.56)	

Values for fatty acids are expressed as % of total fatty acids (wt %)

transFA: tC14:1n5; tC16:1n7; tC17:1n7; tC18:1n9/7; ttC18:2n6
SFA: C4:0 - C27:0
MUFA: sum of all cis-FA with one double bond
PUFA: sum of all cis-FA with two or more double bonds
n6LCP: C20:2n6; DHyLA; AA; C22:2n6; C22:4n6; C22:5n6
n3LCP: C20:3n3; C20:4n3; EPA; C21:5n3; C22:3n3; n3-DPA; DHA

* Significantly different distribution betw. groups (Mann-Whitney-U-Test) p < 0.05

As demonstrated in table C.10 (appendix), the neonatal plasma PL FA levels (% of total FAs) of DHyLA, AA, Adrenic acid, DHA, the sum of n-6- and n-3-LC-PUFAs and the sum of SFAs are higher than the levels in maternal plasma. No significant differences in plasma PL contents of LA, ALA, DHyLA, n-3 DPA, trans-FA, SFA, MUFA and PUFA were found between the two treatment groups (table 9). In contrast, the EPA, DHA and n-3 LCP levels as well as the ratio of LA/AA were significantly higher in the IG than in the CG ($p < 0.05$). Moreover, the AA, Adrenic acid and n-6 LCP levels, as well as the AA/DHA ratio, were significantly lower in the IG than in the CG (figure 16, $p < 0.05$). Taken together, n-3 LC-PUFA supplementation resulted in a 200 % (three-fold) higher level of EPA, a 30 % higher level of DHA and a 15 % lower level of AA in cord blood plasma PL compared to the CG. Results are also illustrated as boxplots in appendix D.6.

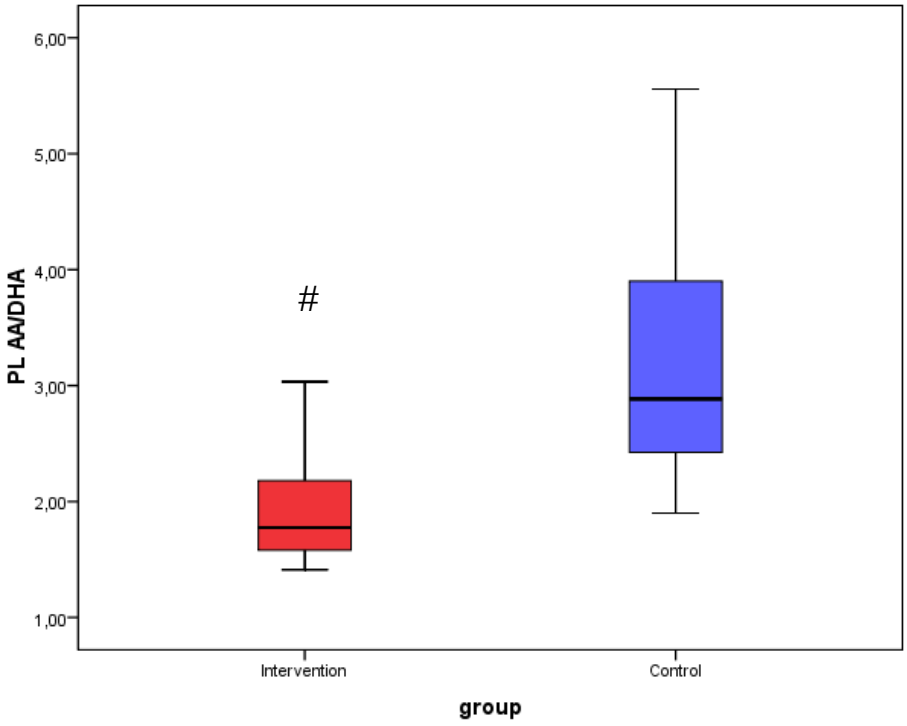

Figure 16 Cord blood plasma PL ratio of AA/DHA

= significantly lower in the IG, $p < 0.05$

n (IG/CG) = 19/18

5.7.2 Red blood cells

Umbilical cord RBC samples were available for 30 newborns in the IG and for 28 in the CG. Results are shown in appendix C.3. In most of the cases, the neonatal RBC FA proportions (% of total FAs) are lower than the maternal FA proportions

throughout pregnancy (table C.10, appendix), except the relative concentration of the SFAs, which were higher than the maternal values. No significant differences in RBC contents of AA, Adrenic acid, trans-FA, n-6 LCPs and the ratio of LA/AA were found between the two treatment groups. The LA, DHyLA, EPA, n-3 DPA, DHA and n-3 LCP and PUFA contents as well as the ratio of LA/ALA were significantly higher in the IG than in the CG ($p < 0.05$). Moreover, the ALA, SFA, and MUFA contents, as well as the ALA/EPA and the AA/DHA ratio, were significantly lower in the IG compared to the CG ($p < 0.05$). The n-3 LC-PUFA supplementation resulted in an EPA level five times higher than in the control group, a 100 % higher (two-fold increase) DHA level and a reduction of the AA/DHA ratio from 4 to 2.2. Surprisingly, the average AA content of cord RBCs of the IG was higher than in the CG albeit not statistically significant. Results are also illustrated as boxplots in appendix D.7.

5.7.3 Relation of maternal and cord blood fatty acid pattern

There are significant, strong, positive correlations between maternal plasma PL AA, EPA and DHA content and their respective fatty acid levels in cord blood PL (see table appendix C.6). Figure 17 illustrates the correlation between maternal and cord blood DHA content ($r = 0.599$; $p < 0.01$). The correlations between maternal and cord blood FAs in RBCs are less pronounced but still significant (except for the AA content, $p < 0.05$). Furthermore, a high maternal PL DHA content correlates negatively with cord blood PL AA content, but there was no considerable correlation between maternal PL AA content and cord blood PL DHA content. This was also true for RBCs but also to a lesser extent.

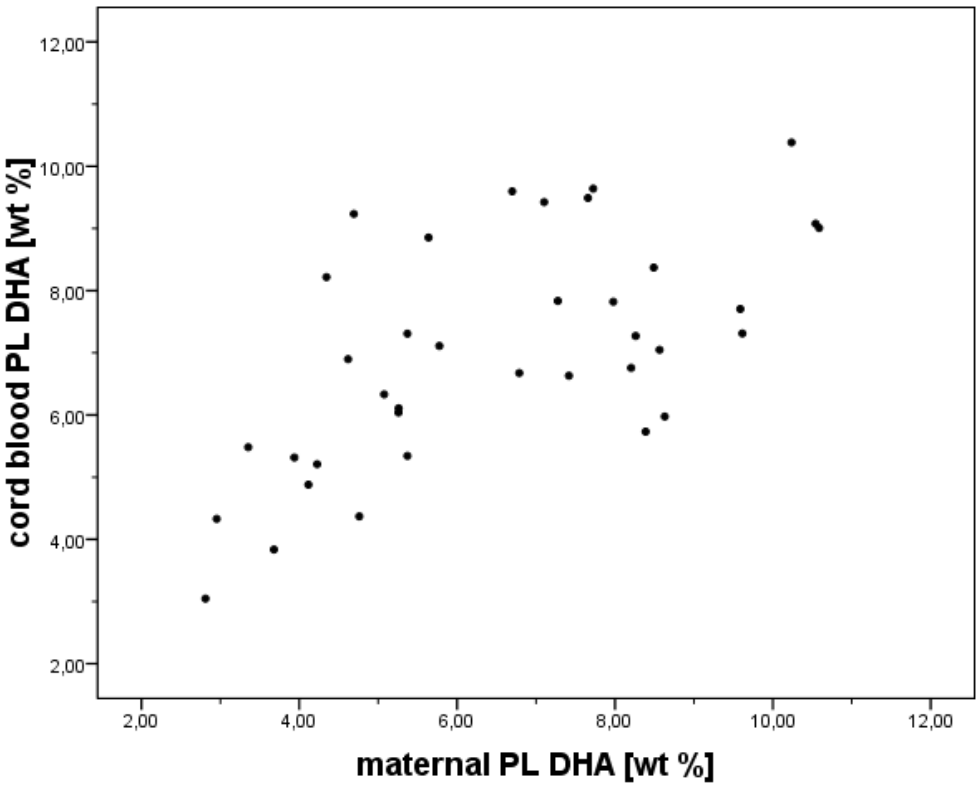

Figure 17 Association of maternal PL and cord blood PL DHA content (n = 37)

Strong significantly positive correlations are also observed between maternal RBC AA, EPA and DHA content and their respective fatty acid levels in cord blood PL. This was also true for maternal PL fatty acids and cord blood RBCs, except for AA (data not shown).

5.8 Fatty acid pattern in infant red blood cells 16 weeks pp

Infant RBC samples from the 16th wk pp were available for 16 infants in the IG and for 14 infants in the CG (see appendix table C.3 and boxplots D.7). No significant differences in RBC contents of LA, ALA, DHyLA, AA, EPA, DHA, trans-FA, n-3 LCP, SFA, MUFA and PUFA and in the ratio of LA/ALA and ALA/EPA were found between the two groups. In contrast, Adrenic acid, n-3 DPA, and n-6 LCP contents as well as the ratio of AA/DHA were significantly lower in the IG than in the CG, in contrast to the LA/AA ratio which was higher in the IG than in the CG ($p < 0.05$).

5.9 Fatty acid pattern in breast milk

The content of selected FAs in breast milk at 6 wks (n = 76) and 16 wks (n = 50) pp is illustrated in table 10 and in appendix D.8.

Table 10 Breast milk fatty acid profile

	group	n	6. wk pp		n	16. wk pp				
			mean ± SD	(range)		mean ± SD	(range)	p#	p†	p‡
18:2w6	Intervention	40	10.99 ± 2.85	(5.03-22.81)	28	11.59 ± 2.53	(6.30-16.73)	> 0.99	0,466	0,904
	Control	36	11.15 ± 2.36	(5.95-17.15)	22	10.96 ± 2.36	(7.78-17.50)			0,958
18:3w3	Intervention	40	1.06 ± 0.52	(0.49-2.69)	28	1.07 ± 0.47	(0.44-2.29)	0,302	0,226	0,719
	Control	36	0.87 ± 0.28	(0.41-1.62)	22	0.89 ± 0.41	(0.46-2.23)			0,192
20:3w6	Intervention	40	0.33 ± 0.07	(0.21-0.50)	28	0.28 ± 0.08	(0.19-0.57)	0,117	0,274	0,002
	Control	36	0.37 ± 0.09	(0.22-0.64)	22	0.31 ± 0.08	(0.21-0.54)			< 0.001
20:4w6	Intervention	40	0.44 ± 0.08	(0.23-0.61)	28	0.40 ± 0.06	(0.29-0.56)	> 0.99	> 0.99	0,013
	Control	36	0.44 ± 0.08	(0.30-0.60)	22	0.39 ± 0.07	(0.24-0.53)			< 0.001
20:5w3	Intervention	40	0.21 ± 0.20#	(0.08-1.15)	28	0.16 ± 0.06†	(0.09-0.38)	< 0.001	< 0.001	0,442
	Control	36	0.08 ± 0.04	(0.03-0.23)	22	0.07 ± 0.04	(0.04-0.20)			0,903
22:4w6	Intervention	40	0.08 ± 0.02#	(0.04-0.12)	28	0.08 ± 0.02	(0.04-0.15)	0,012	0,422	0,029
	Control	36	0.10 ± 0.02	(0.05-0.14)	22	0.08 ± 0.02	(0.05-0.13)			< 0.001
22:5w3	Intervention	40	0.25 ± 0.13#	(0.14-0.89)	28	0.21 ± 0.06†	(0.10-0.37)	< 0.001	0,003	0,361
	Control	36	0.18 ± 0.06	(0.11-0.43)	22	0.17 ± 0.05	(0.11-0.34)			0,092
22:6w3	Intervention	40	1.50 ± 0.83#	(0.48-5.75)	28	1.20 ± 0.39†	(0.30-1.93)	< 0.001	< 0.001	0,031
	Control	36	0.28 ± 0.15	(0.13-0.78)	22	0.22 ± 0.09	(0.13-0.45)			0,114
transFA	Intervention	40	0.64 ± 0.29	(0.28-2.04)	28	0.59 ± 0.23	(0.19-1.39)	> 0.99	> 0.99	0,701
	Control	36	0.62 ± 0.14	(0.36-1.00)	22	0.61 ± 0.20	(0.39-1.22)			0,217
w3LCP	Intervention	40	2.17 ± 1.34#	(0.79-9.20)	28	1.71 ± 0.49†	(0.68-2.66)	< 0.001	< 0.001	0,049
	Control	36	0.66 ± 0.26	(0.38-1.67)	22	0.58 ± 0.20	(0.41-1.20)			0,039
w6LCP	Intervention	40	1.24 ± 0.18	(0.70-1.70)	28	1.08 ± 0.17	(0.76-1.47)	> 0.99	> 0.99	< 0.001
	Control	36	1.24 ± 0.22	(0.86-1.75)	22	1.06 ± 0.20	(0.70-1.62)			< 0.001
SAFA	Intervention	40	44.30 ± 4.47	(36.56-57.94)	28	45.35 ± 4.55	(37.42-58.95)	> 0.99	0,548	0,032
	Control	36	44.33 ± 3.69	(38.04-54.00)	22	43.93 ± 4.34	(36.14-54.41)			0,375
MUFA	Intervention	40	38.97 ± 3.57	(29.97-45.25)	28	37.98 ± 3.55†	(30.48-45.99)	0,144	0,008	0,178
	Control	36	40.50 ± 2.95	(30.50-46.31)	22	41.36 ± 3.90	(33.42-50.17)			0,903
PUFA	Intervention	40	15.62 ± 3.42#	(7.75-29.08)	28	15.61 ± 2.87†	(9.62-21.12)	0,040	0,030	0,29
	Control	36	14.07 ± 2.57	(7.99-19.94)	22	13.63 ± 2.58	(10.15-20.08)			0,59
18:2w6/18:3w3	Intervention	40	11.70 ± 4.16#	(3.91-22.33)	28	12.54 ± 5.31	(4.64-26.10)	0,050	0,452	0,4
	Control	36	13.92 ± 4.42	(6.73-23.26)	22	14.00 ± 4.84	(5.24-24.97)			0,244
18:2w6/20:4w6	Intervention	40	25.41 ± 7.51	(11.66-54.85)	28	29.45 ± 7.74	(16.13-52.46)	0,720	> 0.99	0,124
	Control	36	26.02 ± 5.57	(15.52-37.89)	22	28.69 ± 6.66	(15.42-41.54)			0,01
18:3w3/20:5w3	Intervention	40	6.31 ± 2.82#	(0.76-13.45)	28	7.22 ± 4.02†	(2.47-20.31)	< 0.001	< 0.001	0,737
	Control	36	13.71 ± 6.34	(4.12-31.71)	22	12.84 ± 4.54	(7.46-21.63)			0,339
20:4w6/22:6w3	Intervention	40	0.34 ± 0.14#	(0.11-0.78)	28	0.38 ± 0.19†	(0.20-1.00)	< 0.001	< 0.001	0,428
	Control	36	1.81 ± 0.62	(0.58-2.85)	22	1.94 ± 0.64	(0.96-3.21)			0,614

Values for fatty acids are expressed as % of total fatty acids (wt %)

transFA: tC14:1n5; tC16:1n7; tC17:1n7; tC18:1n9/7; ttC18:2n6
SFA: C4:0 - C27:0
MUFA: sum of all cis-FA with one double bond
PUFA: sum of all cis-FA with two or more double bonds
n6LCP: C20:2n6; DHyLA; AA; C22:2n6; C22:4n6; C22:5n6
n3LCP: C20:3n3; C20:4n3; EPA; C21:5n3; C22:3n3; n3-DPA; DHA

Significantly different distribution between groups 6 wks pp (Mann-Whitney-U-Test) p < 0.05
† Significantly different distribution between groups 16 wks pp (Mann-Whitney-U-Test) p < 0.05
‡ Significant change over time (Wilcoxon-Test) p < 0.05

Over the course of lactation, between the 6th and the 16th wk pp, a significant decrease of DHyLA, AA, n-3 LCPs and n-6 LCPs in both groups as well as a significant decrease of DHA and SFA in the IG were observed ($p < 0.05$).

n-3 LC-PUFA supplementation resulted in a significantly higher level of EPA (0.21 ± 0.2 % in the IG compared to 0.08 ± 0.04 % in the CG), n-3 DPA, DHA (1.5 ± 0.8 % in the IG compared to 0.28 ± 0.15 % in the CG), n-3 LCP and PUFAs compared to the

CG in the 6th wk pp ($p < 0.05$). Also, the ratio of LA/ALA, ALA/EPA and AA/DHA and the level of adrenic acid were significantly lower in the IG compared to the CG ($p < 0.05$).

The fatty acid composition of the milk in the 16th wk pp showed a very similar pattern compared to the 6th wk pp, except the differences between the groups in the adrenic acid content and the ratio of LA/ALA, which were no longer statistically significant. In contrast, the MUFA content was significantly lower in the IG compared to the CG ($p < 0.05$). The levels of DHA (1.2 ± 0.4 % in the IG and 0.22 ± 0.1 % in the CG) and EPA (0.16 ± 0.06 % in the IG and 0.07 ± 0.04 % in the CG) in the intervention group were still significantly higher than in the control group ($p < 0.05$).

Taken together, n-3 LC-PUFA supplementation resulted in a remarkable, significant increase in EPA and DHA in breast milk both in the 6th and the 16th wk pp ($p < 0.05$). Supplementation had no effect on the AA content of breast milk at any of the two time points.

5.9.1 Relation of maternal PL and RBC fatty acid and BM fatty acid pattern

Both in the 6th and in the 16th wk pp, there were significant, strong, positive correlations between maternal PL AA, EPA and DHA levels and their respective levels in breast milk ($p < 0.05$). This held also true for RBC fatty acids albeit to a lesser extent (appendix C.7). Figure 18 illustrates the correlation between maternal PL DHA content and BM DHA content 16 wks pp ($r = 0.939$, $p < 0.01$). There was no considerable association between maternal RBC AA and BM AA levels.

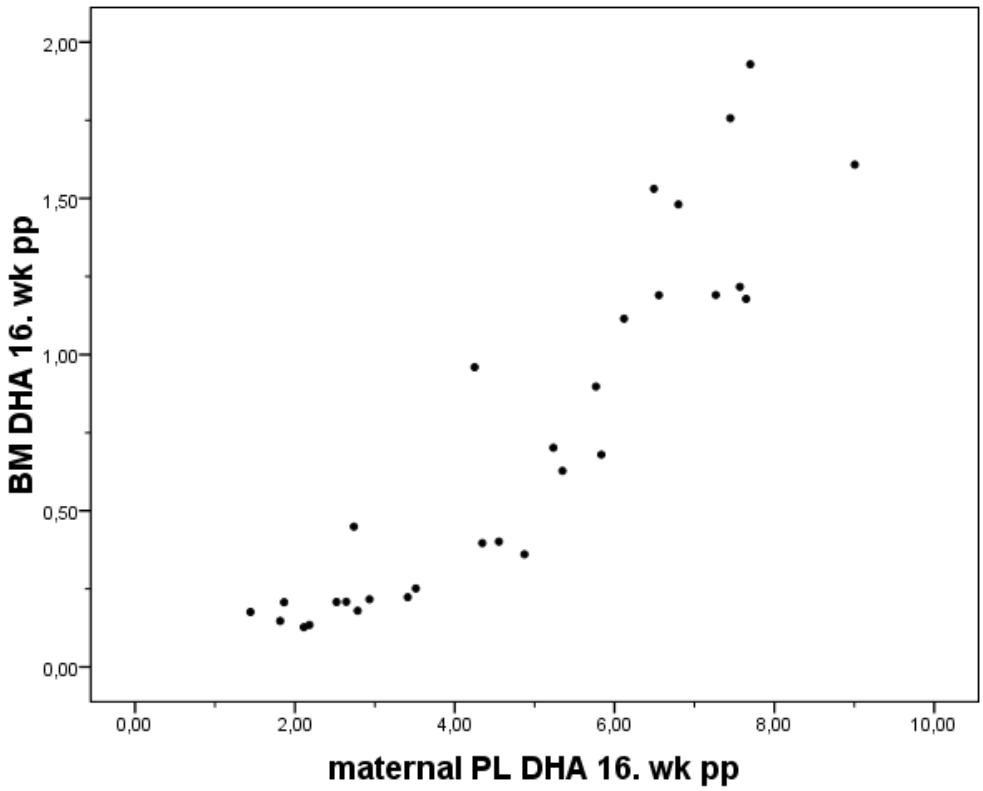

Figure 18 Association of maternal PL and breast milk DHA content (n = 30)

PL AA and, to a lesser extent RBC AA, were negatively correlated to breast milk EPA and DHA levels.

In contrast, there was no considerable correlation between PL or RBC DHA content and the AA content in breast milk.

5.10 Birth outcomes

A total of 113 women gave birth to healthy infants during the time period of August 2006 to August 2008. Infants were classified as “intervention group” and “control group”, according to which group their mothers were part of.

5.10.1 Mother

Mean gestational weight gain in women of the IG (13.7 ± 3.8 kg) was significantly lower than in the CG (16.1 ± 4.7 kg) as illustrated in table 11 ($p < 0.05$). There were no significant correlations between GWG and maternal weight or BMI before pregnancy, frequency of work-out during pregnancy, energy intake, birth weight of the infant, parity, maternal age or PL and RBC levels of AA and DHA (data not

shown). After adjustment for prepregnancy BMI, parity, gestational age in days and infant birth weight (see appendix C.9) the effect of the supplementation could be confirmed as independent and even became a little bit stronger (2.5 kg difference between the two groups). The second variable which had a significant effect on GWG in the linear regression model was the maternal prepregnancy BMI. The higher the maternal BMI, the lower the GWG (decrease per BMI unit: 368 g).

Table 11 Maternal birth outcomes

	group	n	mean ± SD	(range)	p*
Blood loss [ml]	Intervention	55	377 ± 167	(200-1000)	0.985
	Control	55	364 ± 123	(200-700)	
Gestational weight gain [kg]	Intervention	50	13.7 ± 3.8	(4-21)	0.02*
	Control	53	16.1 ± 4.7	(7-30)	
Gestational age [days]	Intervention	56	280 ± 7.8	(258-296)	0.14
	Control	56	277 ± 8.6	(255-291)	

* Significantly different distribution between groups (Mann-Whitney-U-Test) $p < 0.05$

No significant difference was observed for any other maternal birth outcome such as blood loss during delivery (table 11), fetal lie, birth mode, inition of labour, primary and secondary cesarean section and vacuum extraction after spontaneous onset of delivery and after inition of delivery (table 12) between the two groups.

Table 12 Birth outcomes

			Intervention	Control	
	n		**%**	**%**	**p***
Fetal lie	112	cephalic	89.3	91.1	
		breech	7.1	7.1	> 0.99
		transverse	3.6	1.8	
Birth mode	112	spontanous	51.8	53.6	
		cesarean section	28.6	33.9	0.559
		vaccum extraction	19.6	12.5	
Gestational age in weeks	112	37	1.8	5.4	0.096
		38	3.6	10.7	
		39	12.5	10.7	
		40	26.8	41.1	
		41	39.3	17.9	
		42	14.3	14.3	
		43	1.8	0	
Inition of labour	112		26.8	14.3	0.102
Primary CS	112		14.3	16.1	0.792
Secondary CS	112		14.3	17.9	0.607
Vaccum extraction after spontanous onset of delivery	112		14.3	8.9	0.376
Vaccum extraction after inition of delivery	112		5.4	3.6	> 0.99
Infant sex	112	female	48.2	50	0.85
Mother started breastfeeding	111		94.5	94.5	> 0.99

* Significantly different distribution between groups (Chi-Square-Test) $p < 0.05$

Although there seems to be no significant difference in the frequency of inition of labour between the two groups, the difference (IG: n = 15; CG: n = 8) may be important or relevant for clinical issues.

A slight increase of the duration of gestation was observed under n-3 LC-PUFA supplementation (IG: 280 ± 8 d; CG: 277 ± 9 d, see table 11) but this difference was not statistically significant compared to the CG and was of no major clinical relevance. There was no correlation of the length of gestation and the PL or RBC AA and DHA levels in maternal and neonatal blood lipids (data not shown).

The occurrence of minor complications during labour was not statistically different between the IG and the CG (table 13).

Table 13 Birth complications

		Intervention	Control	
	n	**%**	**%**	**p***
Any complication during labour	111	30.9	28.6	0.788
Cessation of labour	112	14.3	8.9	0.376
Pathological CTG	110	16.4	20	0.621
Placental retention	111	5.5	0	0.118

* Significantly different distribution between groups (Chi-Square-Test) $p < 0.05$

There were no statistically significant differences observed in cessation of labour, pathological cardiotokographs (CTG) and placental retention between the two groups. Although the difference in the occurrence of placental retention (IG: n = 3; CG: n = 0) was not statistically different, it may be of clinical importance in light of the severity of the incident. Results are also illustrated as boxplots in appendix D.9.

5.10.2 Infant

All infants were born between the 37th and 42nd wk of gestation except one infant in the control group who had to be excluded because of preterm birth (32. wk gestation). Randomisation took place around wk 15 of gestation, without knowledge of infant sex. By chance, 50 % of the infants in the control group and 48.2 % of the infants in the intervention group were girls.

Birth outcomes are presented in tables 14 & 15 and are illustrated in detail as boxplots in appendix D.9.

Table 14 Infant birth outcomes

	group	n	mean ± SD	(range)	p*
Birth weight [g]	Intervention	56	3490 ± 502	(2450-4850)	0.912
	Control	56	3460 ± 498	(2200-4965)	
Birth lenght [cm]	Intervention	56	51.7 ± 2	(48-57)	0.292
	Control	56	52 ± 2.5	(46-57)	
Head circumference [cm]	Intervention	56	34.9 ± 1.4	(32-39)	0.696
	Control	56	35.1 ± 1.2	(32-38)	
Ponderal Index [kg/m³]	Intervention	56	25.1 ± 2.5	(19.5-30.3)	0.122
	Control	56	24.4 ± 2.3	(20.1-30.8)	
pH-value cord blood	Intervention	55	7.25 ± 0.08	(7.04-7.41)	0.045*
	Control	52	7.28 ± 0.08	(7.1-7.48)	
APGAR score at 5 min	Intervention	56	9.7 ± 0.5	(8-10)	0.866
	Control	56	9.6 ± 0.6	(8-10)	
Placental weight [g]	Intervention	45	521 ± 109	(230-773)	0.335
	Control	42	552 ± 111	(400-800)	

* Significantly different distribution between groups (Mann-Whitney-U-Test) $p < 0.05$

The mean birth weight was 3490 ± 502 g in the IG and 3460 ± 498 g in the CG. The mean birth length was 51.7 ± 2 cm and 52 ± 2.5 cm for the IG and CG, respectively. Head circumference was 34.9 ± 1.4 cm in the IG and 35.1 ± 1.2 cm in the CG. Ponderal index was 25.1 ± 2.5 kg/m^3 and 24.4 ± 2.3 kg/m^3 in the CG. Mean placental weight was 521 ± 109 g for the IG and 552 ± 111 g for the CG and correlated positively with birth weight ($r = 0.538$; $p = 0.01$). There were no statistically significant differences in infant birth weight (figure 19), birth length, head circumference, Ponderal index (figure 20), placental weight and APGAR score at 5 min between the two groups. The pH-value in cord blood at delivery was significantly lower in the IG (7.25) than in the CG (7.28), which was of no clinical importance ($p < 0.05$).

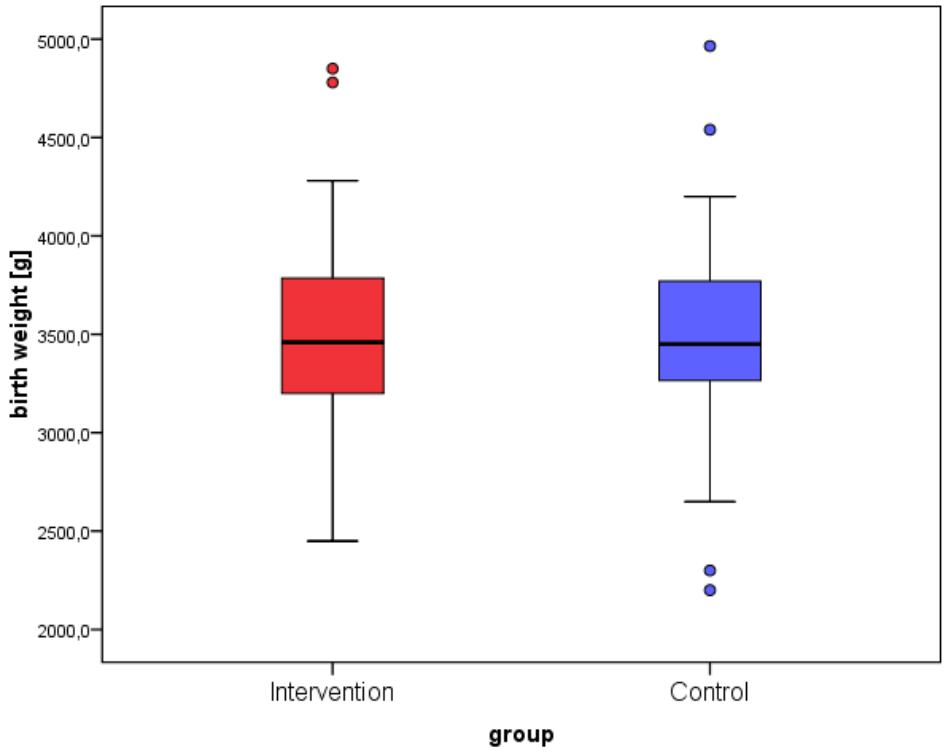

Figure 19 Birth weight (n = 46)

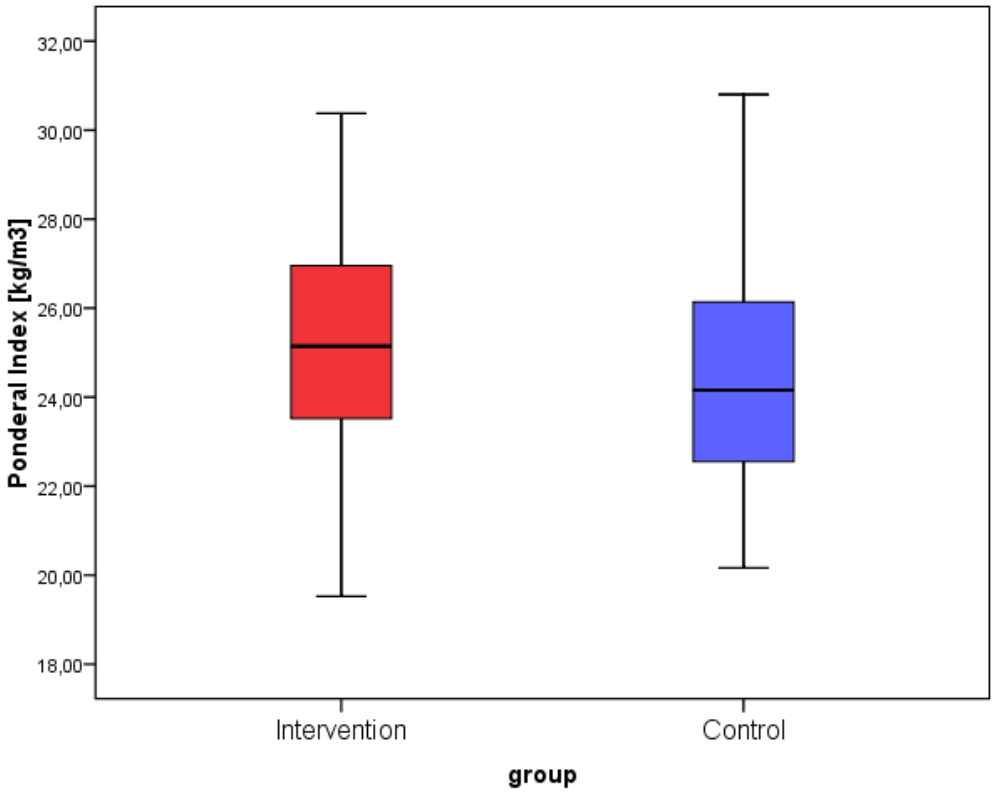

Figure 20 Ponderal Index (n = 46)

Also, birth weight was not significantly correlated to maternal plasma PL and RBC AA and DHA, anthropometry of the mother or dietary intake (data not shown), but correlated positively with parity (r = 0.327; p = 0.01) and duration of gestation (r = 0.314; p = 0.01). There were no considerable associations between cord blood AA, EPA and DHA (PL and RBC) and infant birth weight or Ponderal Index except for a significant negative correlation between cord blood plasma DHA content and PI (r = -0.427, p = 0.008). The associations could be confirmed in an adjusted linear regression analysis (adjusted for prepregnancy BMI, parity, gestational age in days and GWG). Supplementation therefore did not have a significant effect on infant birth weight or PI (appendix C.9).
In contrast, the number of previous births (parity) and the gestational age [d] were confirmed to be independent variables which were associated with birth weight (~ 180 g per birth and 25 g per day).

As shown in table 15, 7.1 % of the infants in the IG and 10.7 % of the infants in the CG were born with a birth weight < 10th Percentile of growth standards for Germany and classified as small for gestational age (SGA) (defined on the basis of current age- and sex-related percentile charts (VOIGT ET AL. 1996). 3.6 % of the infants in the IG and 5.4 % in the CG were born with a birth weight < 5th Percentile. 10.7 % of the infants in the IG and 14.3 % of the infants in the CG were classified as large for gestational age (LGA) (birth weight > 90th Percentile) and 8.9 % in the IG and 5.4 % in the CG were born with a birth weight > 95th Percentile. Neither the ratio of SGA and LGA infants nor the percentage of infants with a birth weight < 5th Percentile or > 95th Percentile was significantly different between the IG and the CG.

Table 15 Birth weight percentiles

		Intervention	Control	
	n	**%**	**%**	**p***
Birth weight <10. Percentile	112	7.1	10.7	0.508
Birth weight <5. Percentile	112	3.6	5.4	> 0.99
Birth weight >90. Percentile	112	10.7	14.3	0.568
Birth weight >95. Percentile	112	8.9	5.4	0.716

* Significantly different distribution between groups (Chi-Square-Test) p < 0.05

94.5 % of mothers in both groups decided to start breast feeding their infants after delivery. Infants in both groups were exclusively breast-fed on average for 143 ± 76

days or 20.5 ± 11 wks (table 16). Most of the women continued breast feeding after the introduction of complementary feeding. In the IG, mothers were breast feeding their infants on average for 221 ± 136 days or 32 ± 19 wks, mothers in the CG for 268 ± 100 days or 38 ± 14 wks.

Table 16 Breast feeding duration

	group	n	mean ± SD	(range)	p*
Exclusive breastfeeding [d]	Intervention	15	143 ± 75	(24-241)	0.937
	Control	16	143 ± 76	(20-321)	
Exclusive breastfeeding [wk]	Intervention	15	20.4 ± 10.8	(3-34)	0.937
	Control	16	20.5 ± 11	(3-46)	
Breastfeeding [d]	Intervention	13	221 ± 136	(24-476)	0.369
	Control	13	268 ± 100	(101-405)	
Breastfeeding [wk]	Intervention	13	32 ± 19	(3-68)	0.369
	Control	13	38 ± 14	(14-58)	

* Significantly different distribution between groups (Mann-Whitney-U-Test) p = < 0.05

5.11 Infant anthropometry and body fat during the first week pp

SFT measurement was carried out during the first 6 days pp (mean: 4.4 days for both groups; table 17) in 102 of the 112 infants, with 11 exceptions where the SFT could only be measured within the first 9 days pp. 10 measurements are missing because we were not able to measure the SFT within the first 10 days after delivery. The distribution of the day of SFT measurement was comparable for the two groups (4.4 ± 2.2 days).

Results of the SFT measurements are presented in table 17. Mean triceps SFT was 4.7 ± 0.8 mm in the IG and 4.6 ± 0.8 mm in the CG. Mean biceps SFT was 3.4 ± 0.5 mm in the IG and 3.3 ± 0.6 mm in the CG. Mean subscapular SFT was 4.4 ± 1 mm in the IG and 4.4 ± 0.9 mm in the CG. Mean suprailiacal SFT was 3.2 ± 0.6 mm in the IG and 3 ± 0.7 mm in the CG.There were no statistically significant differences in the triceps, biceps, subscapular and suprailiac SFT and the arm circumference between the two groups. Results are also shown as boxplots in appendix D.10.

Table 17 Skinfold Thickness at S1

	group	n	mean ± SD	(range)	p*
Age infant at S1	Intervention	51	4.4 ± 2.2	(1-10)	0.949
	Control	51	4.4 ± 2.2	(1-9)	
Triceps SFT [mm]	Intervention	51	4.7 ± 0.8	(3.1-6.9)	0.828
	Control	51	4.6 ± 0.8	(2.9-7.3)	
Biceps SFT [mm]	Intervention	51	3.4 ± 0.5	(2.4-4.5)	0.331
	Control	51	3.3 ± 0.6	(2.3-4.7)	
Subscapular SFT [mm]	Intervention	51	4.4 ± 1	(2.8-7.9)	0.851
	Control	51	4.4 ± 0.9	(2.9-7.6)	
Suprailiac SFT [mm]	Intervention	51	3.2 ± 0.6	(2.3-5.3)	0.143
	Control	51	3 ± 0.7	(2-6.7)	
Arm circumference [cm]	Intervention	51	10.40 ± 1.02	(8.5-12.5)	0.994
	Control	49	10.38 ± 1.07	(8-13)	

* Significantly different distribution between groups (Mann-Whitney-U-Test) $p < 0.05$

Infant fat mass in g, body fat in % and fat mass/height^2 in kg/m^2 was estimated with 3 equations (table 18). There were no statistically significant differences in the estimates between the IG and the CG in any of the investigated items. But there were obviously great differences in the absolute amount of fat mass in g, body fat in % and fat mass/height^2 when comparing the result from the three different equations. Using the equation of WESTSTRATE (1989) resulted in the highest values for all three items, followed by the results of DEURENBERGS (1990) equation and SLAUGHTERS (1988) equation which resulted in the lowest values.

Table 18 Estimation of infant fat mass

	group	n	mean ± SD	(range)	p*
Fat mass [g] Weststrate	Intervention	51	761 ± 111	(539-1067)	0.733
	Control	51	761 ± 112	(484-1092)	
Fat mass / height² [kg/m²] Weststrate	Intervention	51	2.85 ± 0.3	(2.16-3.61)	0.451
	Control	51	2.8 ± 0.26	(2.26-3.39)	
Body fat [% BW] Weststrate	Intervention	51	13.7 ± 2.8	(8-20.5)	0.491
	Control	51	13.3 ± 2.8	(7.3-19.7)	
Fat mass [g] Deurenberg	Intervention	51	402 ± 129	(191-774)	0.891
	Control	51	396 ± 125	(158-757)	
Fat mass / height² [kg/m²] Deurenberg	Intervention	51	1.49 ± 0.4	(0.8-2.7)	0.676
	Control	51	1.45 ± 0.4	(0.6-2.3)	
Body fat [% BW] Deurenberg	Intervention	51	11.4 ± 2.4	(7.1-17.7)	0.743
	Control	51	11.3 ± 2.4	(5.9-16.3)	
Fat mass [g] Slaughter	Intervention	51	343 ± 113	(159-701)	0.792
	Control	51	345 ± 107	(135-662)	
Fat mass / height² [kg/m²] Slaughter	Intervention	51	1.27 ± 0.4	(0.7-2.4)	0.987
	Control	51	1.26 ± 0.3	(0.5-2.1)	
Body fat [% BW] Slaughter	Intervention	51	9.8 ± 2.2	(5.6-15.5)	0.776
	Control	51	9.8 ± 2.2	(5-14.4)	

* Significantly different distribution between groups (Mann-Whitney-U-Test) $p < 0.05$

As expected there are significant positive associations between all four skinfolds and body fat of the infants in % of total body weight. Correlations were strongest for the equation of WESTSTRATE (1989), followed by the equations of DEURENBERG (1990) and the equation of SLAUGHTER (1988) (see appendix C.8). There were also significant positive correlations between all four skinfolds and the infants' fat mass/height^2, only the order of the equations changed ($p < 0.05$). Correlations were highest for the DEURENBERG (1990) equation, followed by SLAUGHTERS (1988) equation and the equation of WESTSTRATE (1989).

5.11.1 Association of cord blood fatty acids and infant anthropometry

Weak but significantly negative correlations were observed between cord blood plasma PL DHA and Ponderal Index ($r = - 0.427$, $p = 0.008$) and infant triceps SFT ($r = - 0.426$, $p = 0.01$). Moreover, there were weak but significant negative associations between cord blood PL DHA levels and infant body fat in % of total body weight as well as fat mass/height^2 (for all three equations, data not shown). In contrast, there

was no significant correlation between cord blood RBC AA, EPA or DHA level and infant anthropometry except for subscapular SFT, which showed a weak negative correlation to cord blood RBC EPA level (r = - 0.316, p = 0.017) and DHA level (r = - 0.265, p = 0.046).

5.11.2 Association of maternal PL and RBC fatty acids and infant anthropometry

We did not observe any significant correlation between infant SFT or body fat in the first few days after delivery and AA, EPA or DHA level in maternal PL and RBCs in the 32nd wk of gestation (data not shown).

5.11.3 Association of maternal and infant anthropometry

No significant correlation between newborn SFT or anthropometry and maternal SFT and anthropometry at the 32nd wk of gestation could be observed. There was neither a correlation between maternal BF or LBM (in % of total body weight and in kg) and the newborns BF (in % of total body weight and in g or the fat mass/height^2), nor a correlation of maternal SFT and infant SFT or anthropometry (data not shown).

6 Discussion

6.1 Baseline characteristics and compliance

The study population was composed of Caucasian women who had normal pregnancies and delivered term, healthy babies. The compliance of the women with regard to the supplementation and the dietary intervention was very good in general and the supplement did not have any side-effects. The study achieved a high short-term follow-up rate of 88 % at visit S2 (6 wks pp), which was in the range of what would be considered “acceptable” and as a RCT of “high-quality”, for the purpose of “Evidence-Based Medicine” (FEWTRELL ET AL. 2008).

There were no significant differences in any of the maternal baseline characteristics or socio-demographic variables between the two groups, confirming successful randomization, and assuring the comparability of the two groups.

6.2 Dietary intake

The average daily intake of total energy and the proportion of macronutrients did not differ between the two groups and were within the recommended ranges of a healthy, balanced diet during pregnancy, as recommended by the German society of nutrition, both in the 15th and the 32nd wk of gestation. Depending on the age, the personal activity level, weight and height of the women, the German society of nutrition recommends an average energy intake of 1900 – 2200 kcal/d, consisting of ~ 15 % protein, ~ 55 % carbohydrates and ~ 30 % fat (DGE 2004). The fat intake of the women was slightly higher as recommended (~ 32 ± 4 % in both groups) and the carbohydrate intake was slightly lower than the recommended 55 % (~ 51 ± 5 % in both groups). There was neither a significant increase in total energy intake nor a shift in the proportion of the macronutrients from the 15th to the 32nd wk of pregnancy. Table 19 shows a comparison of dietary macronutrient and AA intakes from previous studies in early and late pregnancy of women in Europe, North America and Australia (GODFREY ET AL. 1996; MATHEWS ET AL. 1999; WIJENDRAN ET AL. 1999; LOOSEMORE ET AL. 2004; THOMAS ET AL. 2006; ANDREASYAN ET AL. 2007; KNUDSEN ET AL. 2008; RADESKY ET AL. 2008).

Table 19 Comparison of dietary intake during pregnancy

Reference	early pregnancy				
	Energy [kcal/d]	Protein [%]	Fat [%]	Carbohydrates [%]	AA [mg/d]
Godfrey et al. (1996) ‡	2329 (1882, 2789)	14.7 (13.3, 16.5)	35.7 (32.5, 38.7)	49.4 (46.2, 53.4)	—
Mathews et al. (1999) ‡	2044 (1755, 2305)	14.5 (13.1, 16.0)	37.8 (34.7, 40.9)	47.3 (44.1, 50.4)	—
Radesky et al. (2008) *	2049 ± 674	—	28.1 ± 5.4	55.6 ± 7.1	—

Reference	late pregnancy				
	Energy [kcal/d]	Protein [%]	Fat [%]	Carbohydrates [%]	AA [mg/d]
Godfrey et al. (1996) ‡	2314 (1970, 2729)	14.7 (13.3, 16)	36.4 (33.3, 39.1)	49.0 (46.1, 52.2)	—
Mathews et al. (1999) ‡	2197 (1824, 2660)	15.7 (14.0, 17.3)	33.7 (30.0, 38.0)	50.1 (46.1, 53.7)	—
Loosemore et al. (2004) *	2168 ± 585	15 ± 4.5	31.7 ± 6.8	54 ± 8.13	156 ± 87
Andreasyan et al. (2007) ‡	2912 (2282, 3654)	16.3 (14.7, 18.1)	36.2 (33, 39.3)	45.2 (41.1, 49.4)	—
Knudsen et al. (2008) *	2804 ± 479	13.8 ± 2.2	34.8 ± 5.6	50.9 ± 5.7	—
Wijendran et al. (1999) *	2007 ± 300	16.3 ± 2.7	32.9 ± 6.7	52.9 ± 6.6	106 ± 52
Thomas et al. (2006) *	2213 ± 521.9	14.5 ± 3.5	37.3 ± 5.3	47.48 ± 6.09	120 ± 70

*** = mean ± SD; ‡ = median (lower, upper quartile)**

The mean intake of energy and the intake of fat (in % of total energy intake) in the present study were slightly lower compared to previous studies. The intake of protein (in % of total energy intake) and the intake of carbohydrates (in % of total energy intake) were in accordance with the intakes reported for pregnant women in the previous trials. The mean intakes of AA in mg/d of the women in the control group in the present study were similar to the intake reported by LOOSEMORE ET AL (2004), in contrast to WIJENDRAN ET AL (1999) and THOMAS ET AL. (2006) who reported lower mean intakes of 106 ± 52 mg/d and 120 ± 70 mg/d, respectively.

Dietary intervention in the present study resulted in a significant difference in the intake of AA in mg/d and AA in mg/1000 kcal in the 32nd wk of gestation between the two groups, with a significantly lower intake of AA in the intervention group compared to the controls (109 ± 0.05 mg/d vs. 170 ± 0.08 mg/d and 51 ± 22 mg/1000 kcal vs. 84 ± 42 mg/1000 kcal, respectively, $p < 0.05$). Dietary intervention and dietary counseling were therefore effective in reducing the AA intake of pregnant women significantly, at least compared to the dietary intake records from the 32nd wk of gestation. However, the participation in a nutritional study and the open study design might have caused “underreporting” of consumed (AA-rich) foods in the intervention group. Moreover, the results – particularly the absolute amounts of AA in mg - have

to be interpreted cautiously due to the poor data quality of the AA content in the Prodi®-Database. Both the women in the IG and the women in the CG followed a typical western style diet with a n-6 to n-3 PUFA ratio of ~ 7:1 in the control group (DGE 2004; BAUCH ET AL. 2006; MAX-RUBNER-INSTITUT 2008). By successfully reducing the AA intake, and supplementing the women in the IG, an effective reduction of the n-6 to n-3 PUFA ratio from ~ 7:1 to ~ 3.5:1 could be achieved as originally intended.

6.3 Maternal anthropometry

6.3.1 Skinfold Thickness and body composition

This is the first study investigating the effect of n-3 LC-PUFA supplementation on maternal anthropometry during pregnancy and lactation. Thickness of triceps, subscapular and suprailiac skinfolds increased significantly ($p < 0.05$) during pregnancy in both groups without any significant difference between the groups. For example, triceps SFT in the IG increased from 16.8 ± 4.5 mm to 17.5 ± 4.8 mm and from 17.1 ± 4.7 to 18.1 ± 5.4 mm in the CG. This was in line with results of triceps SFT in previous trials (FORSUM ET AL. 1989; THAME ET AL. 2007) that ranged from 16.8 ± 5 mm (VAN RAAIJ ET AL. 1989) to 23.5 ± 8.6 mm (PAXTON ET AL. 1998). Values for the other SFT were also in line with previous trials. In contrast, biceps SFT slightly decreased during pregnancy in both groups (< 0.05). Arm circumference was significantly lower in the IG compared to the CG in the 32nd wk of gestation ($p < 0.05$).

The sum of the four SFT measured was used to estimate body density using the equation of DURNIN & WOMERSLEY (1974). Maternal fat mass was then derived using a specific equation to convert body density to fat mass. Pregnancy-specific equations have been developed by VAN RAAIJ ET AL. (1988) to convert maternal body density to fat mass. These equations account for the altering composition of the maternal fat free mass components through pregnancy, as the expected increase in water in lean body mass that leads to a lower density of the lean body mass relative to that in non-pregnant women. Estimates of total body fat in the present study at wk 15 of pregnancy (17.4 ± 4.5 kg and 16.9 ± 3.9 kg in the intervention group and the control group, respectively) were not significantly different between the two groups and were similar to the results of previous trials: 17.8 ± 4.9 kg (VAN RAAIJ ET AL. 1989), 17.9 ±

5.4 kg (LEDERMAN ET AL. 1997) and 16.5 ± 3.6 kg (SOLTANI AND FRASER 2000). Estimates of total body fat in the present study at wk 32 of pregnancy (18.6 ± 4.6 kg and 19.7 ± 4.7 kg in the intervention group and the control group, respectively) were not significantly different between the two groups and were similar to the results of previous trials: 19.3 ± 5.5 kg (VAN RAAIJ ET AL. 1989) and 21.7 ± 5.8 kg (LEDERMAN ET AL. 1997). These results are also in line with a study of PAXTON ET AL. (1998) who compared four equations for the estimation of body composition from SFT to a four component-model of body composition in pregnant women. The obtained values for body fat of women in this trial ranged from 17.7 to 23.5 kg at week 14 of pregnancy and from 21.9 to 28.7 kg at week 37 of pregnancy. Slightly higher values were reported in another study of adolescent and mature women in the 15th and the 35th wk of pregnancy (values for mature women (mean age: 28 y): 24.5 ± 8 kg and 25.9 ± 7.8 kg, respectively) (THAME ET AL. 2007).

Therefore, supplementation with n-3 LC-PUFAs seems to have no pronounced effect on SFT in pregnancy and therfore on the physiological changes of body fat mass, at least until the 32nd wk of gestation. However, it can not be ruled out that minor differences might have occurred, which could not be detected by SFT measurements.

6.3.2 Gain in total body weight

When tallying up the weight gain from wk 15 to 32 (= estimated body fat in kg + estimated LBM in kg; 9.5 kg in the IG and 12.4 kg in the CG), the estimated weight gain was higher than the actual weight gain, assessed by weighing of the women at the 32nd wk of gestation (weight gain of 7.9 kg and 9.3 kg in the IG and CG, respectively). This indicates that the equations of DURNIN & WOMERSLY (1974) and VAN RAAIJ (1989) slightly overestimated the gain in weight. This was also reported in a study of PAXTON ET AL. (1998) in which the estimation of body fat with the equation of DURNIN AND WOMERSLY (1974) resulted in higher values compared to an estimation using a four-component model of body composition (23.5 ± 8.5 kg versus 21.4 ± 9.0 kg).

Thus, the estimation of body fat and lean body mass from anthropometric equations in pregnancy seems to be rather imprecise and may only serve as a tool for comparison of two groups, using approximated values. The equation was not precise

enough to allow individual predictions of FM and LBM from SFT in the present collective of women.

6.3.3 Gain in fat mass

Relatively few observational studies have focused on the maternal fat changes during pregnancy (DURNIN 1987; FORSUM ET AL. 1988; VAN RAAIJ ET AL. 1989; LEDERMAN ET AL. 1997; SOHLSTROM AND FORSUM 1997; PAXTON ET AL. 1998; SOLTANI AND FRASER 2000; THAME ET AL. 2007). They show a great variation probably due to methodological variations, and different times of measurements. Total fat gain from week 15 to week 32 of gestation increased significantly from 17.4 ± 4.5 kg to 18.6 ± 4.6 kg (+ 1.2 kg) in the IG and from 16.9 ± 3.9 kg to 19.7 ± 4.7 kg (+ 2.8 kg) in the CG ($p < 0.05$). Again, the difference between the groups did not reach statistical significance. These findings were lower than the results of FORSUM ET AL. (1988): 4.5 kg, of SOHLSTRÖM AND FORSUM (1997): 5.5 ± 3.2 kg, SOLTANI AND FRASER (2000): 4.6 ± 3.3 kg, THAME ET AL. (2007): 3.7 ± 2.7 kg, LEDERMANN ET AL. (1997): 3.8 ± 3.5 kg and PAXTON ET AL. (1998): 3.3 ± 4.3 kg. They were however in line with the findings of VAN RAAIJ et al. (1989): 2.0 ± 2.6 kg and DURNIN (1987): 2.1 kg.

6.3.4 General remarks

The use of anthropometry to estimate body fat in pregnant women presents certain problems and limitations even though the equations account for the altering composition of the maternal fat free mass like the lower density of the lean body mass relative to that in non-pregnant women. Skin compressibility may be altered during pregnancy by edema or hormone-based changes in connective tissues for example and may therefore lead to increased SFT, resulting in an overestimation of local fat. As pregnancy advances, it also becomes increasingly difficult to measure SFT at the trunk. Despite its limitations it has been used – and is used - in many studies to estimate body fat in pregnant women, last but not least because of undeniable advantages like the easy accessibility and its low costs.

6.4 Maternal biochemical parameters and blood lipids

The present study was, - to the best of our knowledge, - the first RCT in which the effect of n-3 LC-PUFA supplementation on maternal biochemical parameters and blood lipids during pregnancy and lactation was reported in detail. In none of the

studies cited in chapter 2.10, was the association of these parameters with n-3 LC-PUFA supplementation reported. Available information about the effect of n-3 LC-PUFA supplementation on blood lipids comes only from studies in non-pregnant subjects.

Several hematological and biochemical parameters were analyzed for safety and tolerance evaluation. Over time, we observed significant physiologic changes in all parameters ($p < 0.05$), which were all within the reference ranges for pregnancy and lactation. The plasma TAG level increased ~ 2-fold from ~ 100 mg/dL to 220 mg/dL in the CG and to 170 mg/dL in the IG (~ 200 %) and the cholesterol level increased by ~ 50 % in both groups from ~ 190 mg/dL to 280 mg/dL during the course of pregnancy. The HDL and LDL levels also increased during the course of pregnancy, reaching 20 % and 30 % higher levels than at the beginning of pregnancy, respectively. The values of women in the control group of the present study are consistent with many previous studies on lipids and lipoprotein profiles in uncomplicated pregnancies (KNOPP ET AL. 1982; DESOYE ET AL. 1987; VAN STIPHOUT ET AL. 1987; MONTELONGO ET AL. 1992; PIECHOTA AND STASZEWSKI 1992; BALLOCH AND CAUCHI 1993; MAZURKIEWICZ ET AL. 1994; ALVAREZ ET AL. 1996; SATTAR ET AL. 1997; DE ARCOS ET AL. 1998; BRIZZI ET AL. 1999; HERRERA ET AL. 2004; LAIN AND CATALANO 2007; LIPPI ET AL. 2007; BASARAN 2009).

With the exception of the TAG concentration, the analyzed parameters did not differ between the groups. Supplementation with n-3 LC-PUFAs coupled with dietary intervention resulted in a significantly lower TAG concentration in women of the intervention group in the 32nd wk of gestation compared to the control group ($p < 0.05$). This is in agreement with previous studies in non-pregnant subjects, reporting a TAG reduction after EPA and / or DHA supplementation in the range of 1.6 – 4 g/d in healthy subjects (NELSON ET AL. 1997; LAIDLAW AND HOLUB 2003; GEPPERT ET AL. 2006), whereas other studies with lower DHA intakes of 0.7 g/d and 0.75 – 1.5 g/d could not show a reduction of the TAG concentration (CONQUER AND HOLUB 1998; DI STASI ET AL. 2004; THEOBALD ET AL. 2004). However, a TAG lowering effect of DHA supplementation was also shown by 3 reviews and a meta-analysis (HARRIS 1997; BALK ET AL. 2006; LIEN 2009; RYAN ET AL. 2009). The most likely mechanism for the TAG-lowering effect is the inhibition of hepatic TAG synthesis mediated via activation of PPARα by n-3 LC-PUFAs, increased FA oxidation and altered metabolism of enzymes (e. g. lipoprotein lipase) involved in the uptake and esterification of FAs

(NESTEL 2000; EVANS ET AL. 2004). In the latter cited reviews and meta-analysis, it was also shown that n-3 LC-PUFA supplementation has no effect on serum cholesterol concentration and that the HDL- as well as the LDL-cholesterol concentrations slightly increased after supplementation. However, in the present study, no effect of n-3 LC-PUFA supplementation on HDL and LDL could be shown. First, this might be due to the smaller sample size of the present study compared to the large number of subjects included in the meta-analyses and secondly to the larger amount of n-3 LC-PUFAs applied in the reviewed studies.

It is also interesting to note that, supplementation did not show any effect on blood glucose concentration and blood coagulation (assessed as prothrombin time or INR). This is in line with several previous studies in non-pregnant subjects, recently summarized (LIEN 2009), showing no effect of n-3 LCPUFA supplementation on blood glucose concentration and blood coagulation. Again, this lack of data about n-3 LC-PUFA supplementation on blood glucose concentration and blood coagulation in pregnancy questions the comparison with the present data but may nevertheless be used as a clue.

In summary, supplementation did not have a pronounced effect on maternal biochemichal parameters and blood lipids except of decreasing the TAG concentration. The comparison of the present results with previous studies is difficult because there is a lack of data on biochemical parameters in n-3 LC-PUFA supplemented women.

6.5 Fatty acid pattern of maternal plasma PL and red blood cells

The relative FA concentration (% by wt) in PLs and RBCs changed in a specific manner for each individual fatty acid during the course of pregnancy and lactation in the non-supplemented control group. In particular, the decline of AA, EPA, adrenic acid and n-3 DPA over the course of pregnancy, as well as the increase of DHA in the first trimester, followed by a slight decrease of DHA until delivery, are in accordance with the longitudinal maternal FA changes observed in previous studies of normal pregnancy (AL ET AL. 1990; AL ET AL. 1995; BERGHAUS ET AL. 1998; WIJENDRAN ET AL. 1999; ELIAS AND INNIS 2001; RUMP ET AL. 2001; VLAARDINGERBROEK AND HORNSTRA 2004; PANKIEWICZ ET AL. 2007; STEWART ET AL. 2007; VAN EIJSDEN ET AL. 2008; DONAHUE ET AL. 2009).

Supplementation with 1050 mg DHA and 150 mg EPA per day combined with a decrease of the AA intake from the 15th wk of gestation until the 16th wk pp in healthy women resulted in a significant reduction of the n-6- to n-3 LC-PUFA ratio in maternal plasma PL and RBCs until supplementation was terminated 16 wks pp ($p < 0.05$). The ratio of AA/DHA decreased significantly from the 15th wk of gestation to the 32nd wk of gestation from 2.15 ± 0.6 to 0.95 ± 0.2 (- 56 %) in the PLs and from 3.1 ± 1.3 to 1.5 ± 0.8 in the RBCs (- 50 %) ($p < 0.05$). Also, supplementation significantly increased the DHA and EPA content of plasma PLs and RBCs and reinforced the physiologic decrease of the AA and adrenic acid content ($p < 0.05$). The highest relative fatty acid changes in the intervention group were observed for EPA (+ 100 %), followed by DHA (+ 65 %), adrenic acid (- 47 %) and the ratio of AA/DHA (- 56 %) in the plasma PLs, as illustrated in figure 21 (values presented as means ± SD versus wk 15 of gestation). AA decreased by 25 % in the IG and 12.5 % in the CG.

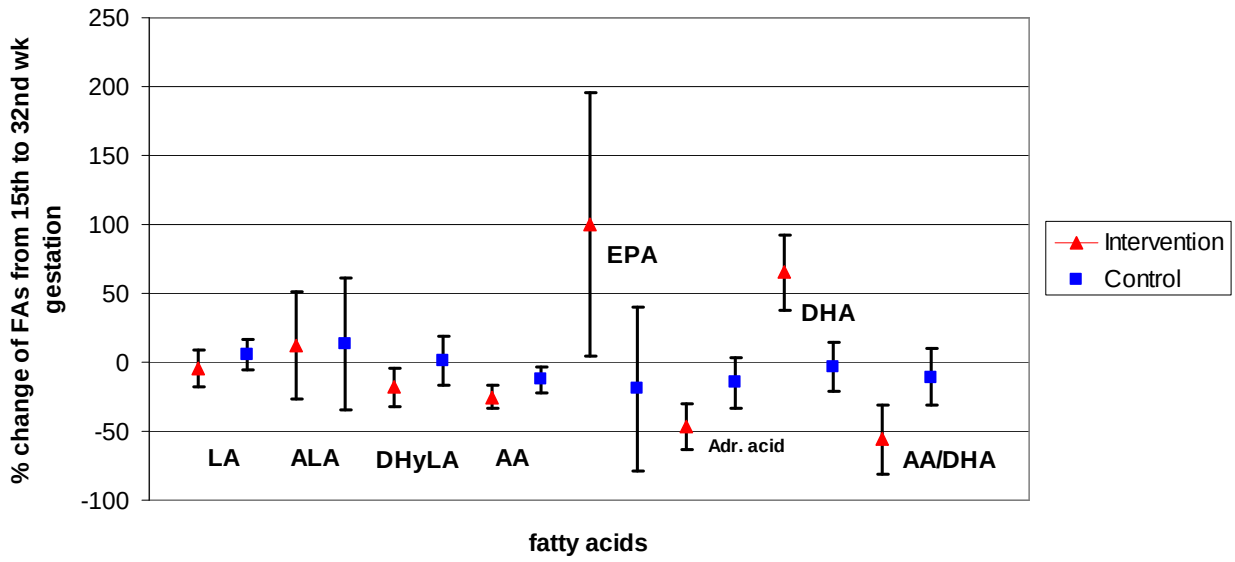

Figure 21 Change of plasma PL fatty acids in % from baseline to 32nd wk gestation

Very similarly to the changes in PLs, albeit to a lesser extent, the highest relative fatty acid changes in RBCs were also observed for EPA (+ 70 %), DHA (+50 %), adrenic acid (- 46 %) and the ratio of AA/DHA (- 50 %). AA decreased by 28 % in the IG and 19 % in the CG.

The small difference in the degree of change between PLs and RBCs probably results from the slower FA turnover in RBCs compared to plasma PLs, in

combination with physiological changes in plasma volume and total RBC mass during pregnancy. Nevertheless, the RBC fatty acid pattern was useful to provide valid estimates of differences in FA intake over the past weeks and months (KATAN ET AL. 1997). This change in the maternal RBC FA pattern and its strong correlation to the change in maternal PLs, emphasizes the substantial impact of the dietary n-6/n-3 FA ratio reduction on the maternal metabolism. Dietary DHA and EPA obviously replace the n-6 PUFAs LA, DHyLA, AA and adrenic acid in both maternal PLs and RBCs. The observed pronounced increase in EPA levels of PLs and RBCs after supplementation with 1.05 g DHA and 0.15 g EPA in the study, appears to reflect retroconversion through a ß-oxidation reaction of DHA, as also suggested in previous studies (BROSSARD ET AL. 1996; CONQUER AND HOLUB 1997; VIDGREN ET AL. 1997; GEPPERT ET AL. 2006).

The direct comparability of the present study with previous n-3 LC-PUFA supplementation RCTs during pregnancy and lactation often turns out to be difficult because of the different blood sampling time-points, the different blood lipid fractions analyzed, the variable onset and length of supplementation and particularly the varying doses and dosage forms of the LC-PUFA supplements. As summarized in chapter 2.10, the provided daily amount of n-3 LC-PUFAs in previous trials ranged from 200 mg to 1200 mg DHA and from 40 mg to 1800 mg EPA, combined in various amounts and ratios. According to the dose of DHA/d, the trials can be classified into two major groups: low DHA supplementation (< 500 mg/d) (D'ALMEIDA ET AL. 1992; MALCOLM ET AL. 2003; MONTGOMERY ET AL. 2003; SMUTS ET AL. 2003; SMUTS ET AL. 2003; SANJURJO ET AL. 2004; BERGMANN ET AL. 2007; JUDGE ET AL. 2007; KRAUSS-ETSCHMANN ET AL. 2007; INNIS AND FRIESEN 2008) and high DHA supplementation (> 900 mg/d) (OLSEN ET AL. 1992; ONWUDE ET AL. 1995; VAN HOUWELINGEN ET AL. 1995; CONNOR ET AL. 1996; HELLAND ET AL. 2001; DUNSTAN ET AL. 2004; TOFAIL ET AL. 2006). They all have in common, that DHA supplementation, regardless of the amount of DHA, effectively increased the proportion of DHA in plasma and RBCs. A cross-study meta-regression analysis of 12 studies on the effect of DHA supplementation on plasma PL FA concentration by ARTERBURN ET AL. (2006) showed that plasma PL concentrations increase in a dose-dependent, saturable manner up to ~ 2 g/d DHA, i. e. doses > 2 g/d would probably not show an effect anymore. Studies with fish oils containing both DHA and EPA showed increases in both DHA and EPA in plasma PLs. Also, AA concentrations, as expected, decreased dose-dependently in response

to the combined supplementation, but to a lesser extent (ARTERBURN ET AL. 2006). In some of the previous RCTs in pregnancy, as cited above, reduced levels of plasma and RBC n-6 LC-PUFAs were observed after n-3 LC-PUFA supplementation (VAN HOUWELINGEN ET AL. 1995; HELLAND ET AL. 2001; VELZING-AARTS ET AL. 2001; MALCOLM ET AL. 2003; DUNSTAN ET AL. 2004; INNIS AND FRIESEN 2008), although the reduction was not significant in two studies (VELZING-AARTS ET AL. 2001; MALCOLM ET AL. 2003). Studies of pregnant vegetarians, who only consume ¼ of the AA amount of omnivore women (~ 40 mg/d) due to the refusal of meat consumption show, that the proportion of AA in maternal and infant blood lipids as well as in breast milk does not differ from the proportion in women who follow a typical western style diet. This was probably due to a higher endogenous synthesis (FINLEY ET AL. 1985; SPECKER ET AL. 1987; REDDY ET AL. 1994; LAKIN ET AL. 1998). Neither the reduced levels of AA in some of the previous RCTs, nor the lower AA intake of vegetarians had any undesirable effects on infant health, the latter most likely explained by the “normal” concentrations of AA in maternal blood lipids. As recently summarized by KOLETZKO (2008), the endogenous synthesis of AA is far more effective than of DHA, resulting in a greater metabolic control and stability of AA as of DHA levels in human blood and tissues. Reassurance on the safety of provision of DHA without AA is also provided by recent meta-analyses, which found no harmful effects of the supplementation on infant development (KOLETZKO 2008).

Therefore, it can be assumed that the observed reduction of the AA levels in maternal plasma PLs and RBCs during pregnancy and lactation in the present study should be regarded as safe for the participants and their infants. As described below the reduction of AA in maternal blood lipids did not have any detrimental effects on infant health.

The present results meet our expectations and confirm the good compliance of the women. Supplementation with 1.2 g n-3 LC-PUFAs from week 15 of pregnancy until 16 wks pp, combined with a reduction of the dietary AA intake was therefore an effective means to enhance maternal n-3 LC-PUFA content in both maternal plasma PLs and RBCs and to decrease the ratio of n-6/n-3 LC-PUFAs and the ratio of AA/DHA, respectively.

6.6 Breast milk

6.6.1 Fatty acid pattern

n-3 LC-PUFA supplementation combined with a decrease in the AA intake doubled the concentration of EPA and resulted in a significant, 5-fold increase of the DHA concentration in breast milk both in the 6th and the 16th wk pp compared to the BM of mothers in the control group (1.5 ± 0.8 % DHA (IG) versus 0.28 ± 0.15 % DHA (CG), 6 wks pp; $p < 0.05$). Interestingly, supplementation had no effect on the AA content of breast milk at either of the two time points.

The distribution of SFA, MUFA, PUFA and specific LC-PUFAs like AA and DHA are, apart from the EPA, n-3 DPA and DHA levels in the BM of supplemented mothers, in agreement with numerous other studies on the fatty acid composition of breast milk and reflect a typical western style diet of Middle European women (CHARDIGNY ET AL. 1995; GENZEL-BOROVICZENY ET AL. 1997; JENSEN 1999; PRECHT AND MOLKENTIN 1999; FIDLER AND KOLETZKO 2000; LAURITZEN ET AL. 2001; BRENNA ET AL. 2007). The content of LC-PUFAs in BM was highest in colostrum and declines during the course of lactation (SALA-VILA ET AL. 2005; MINDA ET AL. 2004; Yu ET AL. 1998; GENZEL-BOROVICZENY ET AL. 1997; BOERSMA ET AL. 1991; HARZER ET AL. 1983). This could also be confirmed in the present study, with significant lower values of DHyLA, AA, adrenic acid and DHA (only significant for the IG), and consecutively the sum of n-3 and n-6 LC-PUFAs in the 6th wk pp compared to the levels in the 16th wk pp in both groups of women ($p < 0.05$). In a recent meta-analysis, BRENNA ET AL. (2007) reviewed the AA and DHA concentration in mature human breast milk worldwide. The mean levels of DHA and AA in BM were 0.32 ± 0.22 % and 0.47 ± 0.13 %, respectively. This corresponds to a ratio of AA/DHA of approximately 1.5, but as the correlation between DHA and AA seems to be low in BM, the ratio shows a high degree of variability (BRENNA ET AL. 2007). Milk DHA content appears to be closely linked to maternal dietary DHA intake, with dose-dependent linear increases in BM concentrations with increased maternal intake (MAKRIDES ET AL. 1996). The biological variation of milk AA content is among the lowest of all FAs, while those of EPA and DHA constitute the highest (SMIT ET AL. 2002; YUHAS ET AL. 2006). In two very recent studies of DHA and DHA plus AA supplementation in pregnancy and lactation, it was shown, that also the AA content of milk is sensitive to long-term dietary AA intake,

but milk DHA seems to be more responsive to dietary intake than milk AA (WESELER ET AL. 2008; VAN GOOR ET AL. 2009).

The increased secretion of EPA, n-3 DPA and DHA in BM of the supplemented women in the present study agreed with previous observations from n-3 LC-PUFA supplementation RCTs in lactation (HARRIS ET AL. 1984; HENDERSON ET AL. 1992; MAKRIDES ET AL. 1996; HELLAND ET AL. 1998; FIDLER ET AL. 2000; HELLAND ET AL. 2001; LAURITZEN ET AL. 2004; JENSEN ET AL. 2005; WESELER ET AL. 2008; VAN GOOR ET AL. 2009). Consistent with these earlier reports, n-3 LC-PUFA supplementation did not reduce the concentration of AA in BM in the present study which indicates that the AA content of BM seems to be tightly controlled (BRENNA ET AL. 2007). Nevertheless, supplementation resulted also in a pronounced decrease in the AA/DHA ratio of BM, both in the 6^{th} and in the 16^{th} wk pp (AA/DHA ratio in the 6^{th} wk pp: 0.35 ± 0.14 (IG) and 1.8 ± 0.6 (CG); 16^{th} wk pp: 0.38 ± 0.2 (IG) and 1.95 ± 0.6 (CG)). This is of major importance as both DHA and AA are indispensable FAs for normal growth and development of the newborn infant.

6.6.2 Implications for the breast-fed infant

Although the fetus and newborn infant is capable of converting of the precursor FAs LA and ALA into AA and DHA, the enzymatic systems involved seem unable to supply sufficient LC-PUFAs to meet the requirements until 4 - 6 month pp and therefore the infant is dependent on the supply through dietary intake via breast milk or infant formula (UAUY ET AL. 2000; CARNIELLI ET AL. 2007; HADDERS-ALGRA 2008). This was also supported by studies, in which infant blood lipid DHA content could be improved by supplementing the lactating mother, confirmed by a strong correlation between BM and infant plasma PLs or RBCs LC-PUFA concentrations (GIBSON ET AL. 1997; JENSEN ET AL. 2000; HELLAND ET AL. 2001; LAURITZEN ET AL. 2004). It was also well known that the inclusion of DHA in infant formulas increases DHA in a dose-dependent manner in the blood lipids of infants fed formula (MAKRIDES ET AL. 1995; CARLSON ET AL. 1996; INNIS ET AL. 1996; BIRCH ET AL. 1998).

One of the major concerns in the debate regarding safety of n-3 LC-PUFA supplementation in pregnancy and lactation has been the question as to whether or not increased levels of n-3 LC-PUFAs in BM or infant formulas had an influence on growth of the infant as observed by CARLSON ET AL. (1992) for the first time, reporting, that marine oil supplemented formula-fed preterm infants did not grow as well as controls. They showed that the AA content of infant plasma positively correlated with

infant anthropometry and the high EPA content of the used supplement was suspected of decreasing the AA level, which could be important for infant growth via its growth stimulating effect of PGE_2 *in vitro* (SELLMAYER AND KOLETZKO 1999). Furthermore, there has been two reports that fetal plasma AA was positively associated with birth weight (KOLETZKO AND BRAUN 1991; WOLTIL ET AL. 1998).

In two large, recent reviews by SIMMER ET AL. on supplementation of infant formula with LC-PUFAs in preterm infants and infants born at term (SIMMER ET AL. 2008; SIMMER ET AL. 2008) there was no evidence that supplementation of formula with n-3 and n-6 LC-PUFAs impaired the growth of the infants. The meta-analysis of relevant studies in term infants also did not show any effect of LC-PUFA supplementation on growth. In the meta-analysis of RCTs in preterm infants, four out of thirteen studies reported benefits of LC-PUFAs on growth at different postnatal ages, whereas two trials (CARLSON ET AL. 1992; CARLSON ET AL. 1996) suggested that LC-PUFA supplemented infants grew less well than controls, possibly due to a reduction in AA levels that occur when n-3 supplements are used without n-6 supplements. Recent trials with addition of AA to the supplement have reported no significant negative effect on growth (UAUY ET AL. 1990; CARLSON ET AL. 1996; VANDERHOOF ET AL. 2000; INNIS ET AL. 2002). Thus, the available data does not indicate that an adverse effect on weight gain should be a serious concern in relation to n-3 LC-PUFA supplementation, particularly if AA is supplied in sufficient amounts. Recent recommendations of the EU Commission Directive on infant formula for the LC-PUFA supply of infants support the supplementation of AA and DHA to infant formula on condition that DHA supplementation does not exceed 0.5 % of total FAs and that levels of AA are at least equal to those of added DHA (KOLETZKO ET AL. 2008).

As 95 % of the women in both groups in the present study decided to breast-feed their infants and that the duration of exclusive breast-feeding was on average 20 wks, the intervention was expected to exert strong influence on the amount of n-3 LC-PUFAs ingested by the infants. If the increased n-3 LC-PUFA intake of infants in the IG will also be reflected in the infant plasma PLs and RBCs 4 month after delivery, and moreover, if differences in the FA composition of infant blood lipids will be associated with differences in the adipose tissue development remains to be investigated in future analysis.

In summary, supplementation resulted in an increase of EPA and DHA content in BM, leading to a reduced ratio of AA/DHA without affecting the AA concentration.

Available data does not suggest any safety concerns of this change for the breast-fed infant.

6.7 Fatty acid pattern of cord blood

The present study shows, that n-3 LC-PUFA supplementation during pregnancy results in a significant increase of cord blood PL and RBC DHA and EPA concentration which was followed by a pronounced decrease of the AA/DHA-ratio. This finding is consistent with a number of previous studies that have demonstrated improved neonatal DHA status after maternal supplementation (VAN HOUWELINGEN ET AL. 1995; CONNOR ET AL. 1996; BOROD ET AL. 1999; HELLAND ET AL. 2001; VELZING-AARTS ET AL. 2001; KRAUSS-ETSCHMANN ET AL. 2007). In contrast, several other studies of low-dose n-3 LC-PUFA supplementation (≤ 200 mg DHA/d) did not find an improvement of the n-3 LC-PUFA status of the infants after maternal supplementation (MALCOLM ET AL. 2003; MONTGOMERY ET AL. 2003; SMUTS ET AL. 2003; SANJURJO ET AL. 2004). A daily intake of ≤ 200 mg DHA/d seems to enhance maternal DHA status but does not have any impact on neonatal DHA status. Only a dietary intake of more than 500 mg DHA/d was shown to enhance neonatal DHA status. The observed plasma PL and RBC FA pattern of the infants in the control group was in agreement with several previous studies from Europe and North America (SANDERS AND REDDY 1992; DECSI ET AL. 2001; ELIAS AND INNIS 2001; RUMP ET AL. 2001; MINDA ET AL. 2002; VLAARDINGERBROEK AND HORNSTRA 2004), including one systematic review, summarizing 19 data sets, describing FA composition of cord blood PLs (MINDA ET AL. 2002). Only DECSI ET AL. (2001) reported slightly lower levels of AA and DHA in newborn plasma PLs from Austria, compared to the present study. The comparison with several other studies reporting newborn FA patterns in cord blood is hardly possible because firstly, FA concentrations are sometimes not given in percentage of total FAs, but in mg/L and secondly, because the fatty acid pattern of other plasma lipid classes than PLs (Cholesterol ester, TAG) were analyzed.

There was a strong linear correlation between the maternal (32^{nd} wk) and neonatal PL and RBC PUFA pattern in both the IG and the CG (appendix C.6), with stronger correlations between maternal and infant PLs than between RBCs. This was also shown previously in non-supplemented mother-infant pairs (RUYLE ET AL. 1990; AL ET AL. 1995; BERGHAUS ET AL. 2000; GHEBREMESKEL ET AL. 2000; ELIAS AND INNIS 2001; RUMP AND HORNSTRA 2001; DE VRIESE ET AL. 2003; HERRERA ET AL. 2004;

VLAARDINGERBROEK AND HORNSTRA 2004; PANKIEWICZ ET AL. 2007; ALVINO ET AL. 2008), as well as in supplemented women (VAN HOUWELINGEN ET AL. 1995; CONNOR ET AL. 1996; HELLAND ET AL. 2001; VELZING-AARTS ET AL. 2001; KRAUSS-ETSCHMANN ET AL. 2007).

In agreement with these studies is the finding, that – on the one hand - the concentration of the LC-PUFAs DHyLA, AA, adrenic acid and DHA was higher in the infant, compared to the maternal blood. On the other hand, the concentration of their essential precursors LA and ALA was found to be lower in fetal PLs and RBCs, compared to maternal PLs and RBCs. This phenomenon is called “biomagnification” (CRAWFORD ET AL. 1976). It has been related to the capability of the placenta and the fetus to increase the percentage of LC-PUFAs in the fetal circulation, i.e. to provide the fetus and infant with FAs of particular importance for development and the rapid LC-PUFA accretion of the growing brain and other neural tissues. The fetus is able to synthesize SFAs and MUFAs from glucose and ketone bodies but depends on placental supply of EFAs (KIMURA 1989). While LA and ALA can be further desaturated in humans, no activity of both the delta-6- and delta-5-desaturases could be detected in human placental tissue (CHAMBAZ ET AL. 1985). Therefore, LC-PUFAs in the fetal circulation appear to be synthesized either by the mother or the fetus. Although LC-PUFA biosynthesis is active in the newborn, there are indications that this capacity is limited (DEMMELMAIR ET AL. 1995; UAUY ET AL. 2000; CARNIELLI ET AL. 2007; HADDERS-ALGRA 2008). This indicates a selective materno-fetal placental transport for certain LC-PUFAs: The preferential uptake of LC-PUFAs was confirmed by HAGGARTY ET AL. (1999) who reported a selective, preferential transport of DHA > AA > ALA > LA across the human placenta. Further evidence was provided by LARQUÉ ET AL. (2003) who showed in a study with radioactive labeled FAs a preferential uptake of DHA in placental tissue. As a result, the proportion of LCPs is higher, and the proportion of their parent EFAs is lower in cord blood samples compared to maternal plasma.

After a close examination of the DHA proportions in maternal and fetal plasma PLs and RBCs it was striking, that the proportion of DHA was higher in neonates compared to their mothers only in the control group. This was also shown by DUNSTAN ET AL. (2004) who suggested that this loss of concentration gradient between the mother and her infant in the supplemented group indicates that fetal levels of DHA could be approaching saturation, regardless of the DHA levels in

maternal blood. On the other hand, this implies that when DHA stores are low in less-well nourished women, supplementation with DHA may primarily affect the fetal DHA status, because DHA is then preferentially transported to the fetus (LARQUE ET AL. 2006).

n-3 LC-PUFAs are widely regarded as healthy and being beneficial to human health, and there is no doubt that they are essential for infant development. However, n-6 LC-PUFAs, such as AA are also indispensable for infant development. In humans, several studies have reported a positive association between cord blood plasma AA and infant birth weight. In preterm infants and several clinical trials of formula feeding, AA and the combination of AA and DHA was related to growth (KOLETZKO AND BRAUN 1991; CARLSON ET AL. 1993; HAMOSH AND SALEM 1998; ELIAS AND INNIS 2001; INNIS ET AL. 2002; HEIRD AND LAPILLONNE 2005; INNIS 2005). A study by RUMP ET AL., in contrast, did not support the concept that EFAs such as AA and DHA serve as fetal growth factors, they could in contrast show that the proportions of AA and DHA in umbilical cord plasma PLs were negatively related to neonatal size and weight at birth (RUMP ET AL. 2001). In the INFAT-Study, supplementation was associated with a decrease of maternal DHyLA, AA and Adrenic acid content in plasma PLs and RBCs. The impact on neonatal n-6 LC-PUFA status could also be shown clearly but was less consistent. In the plasma PLs, the AA and Adrenic acid level in cord blood of “supplemented” infants was significantly lower than the content in cord blood of “non-supplemented” infants. This was in contrast not true for RBCs, in which all n-6 LC-PUFA levels are higher in the infants of the IG, compared to the infants in the control group, although not significantly. This finding, although unexpected, was also observed in a study on the influence of a vegetarian diet on the EFA status in cord plasma PLs (SANDERS AND REDDY 1992). This suggests an association of maternal vegetarian diet – as was aimed to achieve in the intervention group in the INFAT-Study – with an increased AA proportion in cord blood. A possible explanation could be a higher conversion of LA into AA in vegetarians, who are known to consume more LA in their diet and have higher proportions of LA in all tissues and blood lipids compared to omnivores (SANDERS ET AL. 1978; SANDERS 2009). Interestingly, the proportion of LA was significantly higher in cord blood RBC of the IG (2.97 ± 0.7 %), compared to the CG (2.19 ± 0.7 %) in the INFAT-Study, too ($p < 0.05$, table C.3 appendix).

No relationship was found between the amount of AA and DHA in infant blood lipids and birth weight or growth in the present study so far. However, these results have to be interpreted with caution as the number of infants (IG = 19, CG = 18) was very limited. Furthermore, the collection of cord blood samples in a standardized manner was very difficult and presented some irregularities, regarding the classification of the cord blood into venous or arterial blood and the time elapsed until storage and refreezing may have occurred.

Nevertheless it could be demonstrated in the present study, that maternal supplementation effectively enhances fetal and infant n-3 LC-PUFA status, while lowering the AA concentration, which results in a lower ratio of AA/DHA, at least, in the infants PLs. Maternal supplementation resulted in an AA/DHA ratio of infant blood lipids of ~ 2 : 1 in the IG and ~ 3 : 1 in the CG. Whether the reduction in infant blood was reflected in infant tissue and has clinical relevant properties and was reflected in adipose tissue development remains to be elucidated.

6.8 Maternal and infant birth outcomes and infant body fat

6.8.1 Maternal GWG and anthropometry

The results of the present study suggest that the n-3 LC-PUFA supplementation from the 15th wk of pregnancy until delivery affects maternal gestational weight gain. Mean GWG of women in the IG (13.7 ± 3.8 kg) was significantly lower compared to the CG (16.1 ± 4.7 kg) ($p < 0.05$). Considering the period of the study, GWG in the present study is consistent with the GWG in other studies: 10.7 kg (SCHIESSL ET AL. 2009), 15.1 ± 5.9 kg (NOHR ET AL. 2008), 11.7 kg (DURNIN 1987) and 10.9 ± 4.7 kg (SOLTANI AND FRASER 2000) from ~ 10 – 40 wks of pregnancy or values from prepregnancy to wk 40 +: 13.6 ± 6.1 kg (LEDERMAN ET AL. 1997), 13.8 kg (SADURSKIS ET AL. 1988), 11.8 ± 3.7 kg (VAN RAAIJ ET AL. 1989), 15.8 ± 7.7 kg (SOHLSTROM AND FORSUM 1997). GWG of both groups in the present study also lay within the ranges of “Recommended gestational weight gain” of the IOM which are illustrated in table 20.

Table 20 Recommended GWG (IOM, 2009)

	BMI [kg/m²]	recommended GWG [kg]
Underweight	**< 18.5**	**12.5 - 18**
Normalweight	**18.5 - 24.9**	**11.5 - 16**
Overweight	**25 - 29.9**	**7 - 11.5**
Obese	**> 29.9**	**5.5 - 9**

Also, a significant, inverse relationship between maternal prepregnancy BMI and GWG in both groups was observed (decrease per BMI unity: 368 g, $p < 0.05$), which was also shown in numerous studies (BERGMANN ET AL. 2003; CEDERGREN 2007; NOHR ET AL. 2008; IOM 2009; SCHIESSL ET AL. 2009).

The wide range of values showed, that GWG varies greatly among women. GWG was composed of total body water accretion, protein and fat accretion, the increase of amniotic fluid and placental and fetal growth but with great individual differences among the study participants in the proportions of the individual GWG components. Total body water accretion averages about 7 – 8 l, plus up to 3 l with generalized edema, resulting in an increased hydration of fat free mass in pregnancy (HYTTEN AND LEITCH 1971). Approximately 40 % of GWG accounts for fat mass accretion, of which ~ 75 % is deposited subcutaneously (BUTTE ET AL. 2003). In two studies, using four-component body composition models, GWG was positively correlated with both, the increase in maternal fat and fat free mass. Birth weight of the infants in contrast was only shown to be related to the gain in maternal fat free mass, but not with the gain in FM (LEDERMAN ET AL. 1997; BUTTE ET AL. 2003). The difference in GWG between the two treatment groups in the present study was not observed until shortly before delivery. Up to the 32nd wk of gestation, there were no significant differences in maternal weight, body fat and lean body mass between the two groups of women, although women in the control group already had a slightly higher amount of body fat compared to the women in the intervention group. Unfortunately, the SFT of the women was not measured until shortly before delivery and it was therefore not possible to make a statement on the composition of the 2.4 kg of supplemental GWG in the control group. The present study was not designed to accurately measure maternal body composition throughout pregnancy and therefore one can only

speculate about the nature of the additional GWG and the underlying metabolic mechanisms.

Compared to previous n-3 LC-PUFA supplementation studies in pregnancy and lactation (see 2.10), the present analysis was the *only one* showing a significantly lower GWG of the supplemented women compared to the women in the control group. In most studies, there is not even any data on GWG given. Also, GWG is not mentioned in the two recent meta-analysis of n-3 LC-PUFA supplementation during pregnancy (MAKRIDES ET AL. 2006; SZAJEWSKA ET AL. 2006). Only in four of the studies, information on GWG was given, but in none of the trials, a significant difference in GWG was observed between the two treatment groups (SMUTS ET AL. 2003; SANJURJO ET AL. 2004; BERGMANN ET AL. 2007; JUDGE ET AL. 2007). Only JUDGE ET AL. (2007) observed – in contrast to the present study – a slightly higher mean GWG in the supplemented women but this finding did not reach statistical significance.

The total amount of GWG is determined by many factors. Aside from physiological factors; psychological, behavioral, family, social, cultural, and environmental factors can also have an impact on GWG. The two most obvious explanations of the difference in GWG would be a difference in the level of physical activity and a difference in energy intake between the women of the two groups. However, both factors have to be eliminated because there were no differences observed between the women of the two groups concerning these variables, although data rely on self reported information by the women and may therefore be imprecise and subject to bias.

An undeniable influence on the results was the fact that the women were not blinded to the treatment groups. On the one hand, this could have caused a veritable change in dietary habits and level of physical activity or may have led to an under- or over reporting of dietary intake and physical activity on the other hand.

Plausible reasons whereby n-3 LC-PUFAs might affect GWG include:

1) An altered production of eicosanoids due to the competition for cyclooxygenase and lipoxygenase and their role in vascular permeability and endothelial function.

2) Differences in utilization and deposition of n-3 LC-PUFAs in metabolism.

3) Effects on the expression of genes involved in FA metabolism.

1) Many of the components of GWG are directly related to physiologic alterations in maternal physiology and the metabolic and endocrine system. Adaptation in maternal renal physiology during gestation is one of the mechanisms accounting for the increase in plasma volume and hence total body water retention during gestation. There is a large increase of tubular sodium reabsorption during pregnancy, promoted by increased aldosterone and other hormones. Renin and angiotensin levels increase five- to tenfold above pregravid levels (IOM 2009). On the one hand, the adrenal gland remains responsive to the trophic action of angiotensin II, on the other hand human pregnancy is characterized by a less sensitive pressor responsiveness to vasopressor substances like angiotensin II. This may be a probable explanation for the expansion of plasma volume during pregnancy. It was suggested that although a variety of factors may mediate this blunted pressor responsiveness, the most likely mechanism appears to be the localized production of prostaglandins within endothelium and/or vascular smooth muscle (GANT ET AL. 1974; CUNNINGHAM ET AL. 1975; EVERETT ET AL. 1978; GANT ET AL. 1987). They suggested that a reduced refractoriness to angiotensin II may be one of the risk factors for developing pregnancy-induced hypertension, a leading symptom of preeclampsia. Hypertension in pregnancy is in turn often associated with increased occurrence of edema, which is also one of the main symptoms of preeclampsia. Modification of the eicosanoid balance with agents such as low-dose aspirin decrease the sensitivity to angiotensin II and thus may possibly restore the refractoriness to angiotensin II and avoid pregnancy-induced hypertension and edema by lowering the blood pressure through vasodilatation (BROWN ET AL. 1990; ADAIR ET AL. 1996). Both aspirin as well as supplementation with n-3 LC-PUFAs in pregnancy are able to alter eicosanoid metabolism and result in a shift of the eicosanoid synthesis from the series-2-eicosanoids (TXA_2 and PGI_2) to the series-3-eicosanoids (TXA_3 and PGI_3) in platelets and vascular endothelial cells, respectively. TXA_3 appears to be less biologically active than TXA_2 regarding its vasoconstrictive properties (BROWN ET AL. 1990; SECHER AND OLSEN 1990; SORENSEN ET AL. 1993; ADAIR ET AL. 1996). n-3 LC-PUFA supplementation in the present study might therefore have been involved in the prevention of capillary hemodynamics (i.e. elevated blood pressure) that favors the movement of fluid from the vascular space into the interstitium, one of the major events occurring in the development of edema. Proteinuria, edema and weight loss

after delivery were not documented in the present study. However, there was no significant difference in blood pressure observed between the two groups. Blood pressure was extracted from the “Mutterpass” and not measured in a standardized manner in the present study, which puts the validity of the information on blood pressure in question. A preventive effect of the incidence of edema, but not of hypertension and preeclampsia was observed in one single study with a combined supplementation of fish oil and gamma-linolenic acid, a precursor of EPA and DHA (D'ALMEIDA ET AL. 1992). The hypothesis that the difference in GWG consisted to a large extent of fluid would only be true assuming that fluid retention occurs additionally to the expansion of plasma volume in the control group and that the expansion of plasma volume is similar in both groups apart from that.

2) A second approach whereby the intervention may influence GWG, comes from studies of n-3 LC-PUFA supplementation in rodents and non pregnant volunteers, in which the consumption of diets rich in n-3 LC-PUFAs has been associated with differences in nutrient intake, absorption, utilization and deposition of n-3 LC-PUFAs in the metabolism, such as reduced food intake, increased energy expenditure and following lower body weight or fat mass compared to the control group (PARRISH ET AL. 1991; BELZUNG ET AL. 1993; HILL ET AL. 1993; COUET ET AL. 1997; BAILLIE ET AL. 1999; WANG ET AL. 2002; BEERMANN ET AL. 2003; RUZICKOVA ET AL. 2004; KRATZ ET AL. 2009).

There were no differences in energy intake, and there was little reason to expect a difference in fat absorption between the two treatment groups.

However, the metabolic pathway of n-3 LC-PUFAs differs from that of other FAs and it is hypothesized that n-3 LC-PUFAs modulate fat oxidation and storage by stimulating peroxisomal ß-oxidation, resulting in an increased basal fatty acid oxidation and/or by increasing resting energy expenditure (SCHUTZ ET AL. 1989; SCHUTZ ET AL. 1992; MADSEN ET AL. 1999; BEERMANN ET AL. 2003; KRATZ ET AL. 2009). In theory, an increase in peroxisomal ß-oxidation, which is less energy-efficient than mitochondrial oxidation, will result in an increase in energy expenditure (DELANY ET AL. 2000; LAPILLONNE ET AL. 2003). In a small study (COUET ET AL. 1997) healthy subjects were supplemented with 6 g/d fish oil over three weeks. While weight loss was similar, the subjects lost more fat mass and had an increased basal lipid oxidation, when consuming n-3 LC-PUFAs, compared to the control group. In another small short term weight loss intervention trial n-3 LC-PUFA supplementation

did not result in a different loss of weight or fat mass between the treatment groups, but stimulated the hepatocytic ß-oxidation (BEERMANN ET AL. 2003). Further evidence comes from a very recent weight loss study in young, overweight and obese adults from Iceland, where a dose-response relationship between cod consumption (rich in n-3 LC-PUFAs) and weight loss was found (RAMEL ET AL. 2009).

These results are in contrast, however, to a very recent study which could not show a significant effect of n-3 LC-PUFA supplementation on plasma leptin and ghrelin concentrations, appetite, resting energy expenditure or body weight and fat mass in overweight or moderately obese men and women after a n-3 LC-PUFA rich diet (1.4 % of total energy provided as n-3 LC-PUFAs) (KRATZ ET AL. 2009). Also, in an other study (KREBS ET AL. 2006), analyzing cardiovascular endpoints in overweight women randomized to a weight loss diet with or without n-3 LC-PUFAs, weight loss was independent of the n-3 LC-PUFA content of the diets. If a modulated fat oxidation and/or increased resting energy expenditure contributed to the difference in GWG in the two groups remains speculative and should be investigated with appropriate techniques.

3) Last but not least, n-3 LC-PUFAs play a key role in the control of gene expression. They enhance hepatic FA oxidation and inhibit FA synthesis and VLDL secretion, in part, by regulating gene transcription. Recently, it was established that key transcription factors, like PPARα, SREBP-1 (sterol regulatory element binding protein), ChREBP (carbohydrate regulatory element binding protein) and MLX (Max-like factor X), are regulated by n-3 PUFAs, which in turn control levels of proteins involved in lipid and carbohydrate metabolism (JUMP ET AL. 2008). Numerous studies in animals and cell systems have demonstrated that fats rich in n-3 PUFAs suppress hepatic lipogenesis, reduce hepatic TAG release and induce FA oxidation in both liver and skeletal muscle (CLARKE 2000; LAPILLONNE ET AL. 2003). DHA has recently been established as a key controller of hepatic lipid synthesis by suppression of nuclear SREBP-1 which, in turn suppresses lipogenesis (reviewed in JUMP ET AL. 2008). It was shown that when added to a saturated-fat diet, n-3 LC-PUFAs increased basal metabolic rate and total energy expenditure. Within minutes of ingestion, n-3 LC-PUFAs upregulate genes involved in lipid oxidation and downregulated genes involved in lipogenesis. Intake of a diet rich in n-3 LC-PUFAs may promote fat utilization rather than storage (JONES AND SCHOELLER 1988; PRICE ET AL. 2000). Taken together, these metabolic alterations may result in a lower rate of fat

deposition or weight gain and may have contributed to the difference GWG in the present study.

It is not yet possible to draw a valid conclusion or even to establish causality between the effects of n-3 LC-PUFA supplementation and GWG from the present study by now. Further mechanistic and clinical studies are needed to verify the presented associations and their underlying mechanisms.

Furthermore, both leptin and adiponectin are correlated with various components of maternal metabolism such as energy expenditure and adiposity, although there are no direct mechanisms described, relating to maternal GWG. Indirectly, these cytokines may however influence GWG through their effects on maternal insulin sensitivity and other metabolic pathways (HIGHMAN ET AL. 1998; OKEREKE ET AL. 2004; WINZER ET AL. 2004). A further investigation of these associations is planned, as part of the present study.

For the mother, ample evidence exists to suggest that higher GWG is not only linearly associated with post partum weight retention and later risk for overweight, but also with an increased risk of caesarean delivery and instrumental delivery (CEDERGREN 2006; DEVADER ET AL. 2007; NOHR ET AL. 2008) also described in a recent review and in the re-examined guidelines of weight gain during pregnancy from the IOM (VISWANATHAN ET AL. 2008; IOM 2009). These findings could not be confirmed in the present study probably because GWG in both groups was within the recommended ranges of the IOM (table 20) and did not exceed “normal” mean weight gains in Europe and North America.

6.8.2 Maternal GWG and implications for the infant

We also need to ask what the consequences of maternal GWG for the infants are. Why does a significantly higher GWG in the CG not result in a higher BW of the infants in the CG, compared to the IG, as suggested in some previous studies? As reviewed above (2.7), evidence to date suggests strongly, but still far from proving the causality, that GWG is associated with BW and BMI in the offspring. However, an association of GWG above the ranges recommended by the IOM (2009) and a higher BMI of the infants seems to be more evident. Both, the GWG of the IG as well as the GWG of the CG in the present study, despite a difference of 2.5 kg, are within the recommended GWG ranges of the IOM. Therefore one would not expect a dramatic

effect of the difference in GWG between the two treatment groups on infant BW and later BMI, taking further into account that BW is influenced by numerous different factors. Nevertheless, we need to consider whether excessive GWG is one of the risk factors for obesity in the offspring and limiting the rates of GWG may therefore be a means for an early prevention of childhood obesity. At the same time, very low rates of GWG may also increase the risk of preterm delivery and as such there has previously been an emphasis to promote sufficient GWG in an effort to reduce low-BW deliveries and adverse perinatal outcomes (IOM 1990). Since the increase in the obesity epidemic and the increase in the percentage of women gaining more than the IOM weight gain recommendations, the role of GWG in obesity in the offspring has received more attention and should be further investigated in RCTs that are designed to modify GWG, including follow-up of the children. Such studies may provide new strategies to stop excessive weight gain. In the present study, the number of women and their infants was probably too small to obtain valid results in this regard. It is desirable that the weight gain of the infants will be followed during childhood, as it is intended in a follow-up study.

6.8.3 Length of gestation

A slight increase of the duration of gestation was observed in the n-3 LC-PUFA supplemented women (IG: 280 d; CG: 277 d), but this difference was not statistically significant and was of no major clinical relevance in any way. Nevertheless, this result is consistant with previous observational and interventional studies, suggesting that a higher n-3 LC-PUFA intake during pregnancy may result in a small increase in the duration of gestation (OLSEN AND JOENSEN 1985; ELIAS AND INNIS 2001; RUMP ET AL. 2001; JENSEN 2006; JUDGE ET AL. 2007; CETIN AND KOLETZKO 2008). The rationale for this effect is that a high intake of n-3 LC-PUFAs may result in a modification of the production of prostaglandins involved in the initiation of spontaneous delivery, namely in a reduction of PGE_2 and $PGF_{2\alpha}$ derived from AA, and therefore a prolongation of gestation. The effect of n-3 LC-PUFA supplementation on duration of gestation has been evaluated in numerous RCTs, with inconsistent results (OLSEN ET AL. 1992; ONWUDE ET AL. 1995; HELLAND ET AL. 2001; MALCOLM ET AL. 2003; SMUTS ET AL. 2003). Two recent meta-analyses showed a slight but significant prolongation of gestation by 1.6 and 2.6 days, respectively (MAKRIDES ET AL. 2006; SZAJEWSKA ET AL. 2006), although differences in terms of baseline n-3 LC-PUFA levels, amount and

duration of intervention existed. In a more recent multicentre trial of fish oil supplementation during pregnancy it was confirmed that the delaying effect of fish oil supplementation on timing of spontaneous delivery depends on the baseline intake of fish, only having an effect in women with low or middle habitual fish intake, whereas no effect was observed in women reporting a high fish intake at baseline (OLSEN ET AL. 2007). Due to the fact that the habitual fish (and therefore the n-3 LC-PUFA) intake of German women seems to be rather low compared to the worldwide diversity of fish intake (DENOMME ET AL. 2005; BAUCH ET AL. 2006; HIBBELN ET AL. 2006; MUSKIET ET AL. 2006), the slight increase in the length of gestation in the present study seems to be perfectly plausible, supporting the hypothesis of a modifying effect of a high n-3 LC-PUFA intake regarding prostaglandin synthesis.

6.8.4 Placental retention

Noteworthy, although not statistically significant, was a difference in the incidence of placental retention, a severe birth complication. Three women in the intervention group had placental retention after normal birth, in contrast to no women in the control group. Previous uterine abrasion, abortion and cesarean section are some of the risk factors which are associated with an elevated risk of a placental retention in subsequent births. Unfortunately, data on these previous interventions have not been collected in the present study. Therefore, it is not possible to ascribe the placental retentions entirely to the n-3 LC-PUFA supplementation, which may be responsible for a modulation of the uterine contraction during and after delivery through the action of $PGF_{2\alpha}$ which is derived from AA and is known to stimulate the uterine contractility and plays an important role in spontaneous labor at term (LUNDSTROM AND GREEN 1978; BYGDEMAN ET AL. 1991). Rats fed diets containing high amounts of n-3 LC-PUFAs have reduced *in vivo* production of $PGF_{2\alpha}$ and this is also assumed to be true in humans (GALLI ET AL. 1980; OLSEN ET AL. 1986). In other words, the intervention could have reduced the formation of $PGF_{2\alpha}$ in the supplemented women, which would possibly have led to less strong contractions of the uterus after labor, which in turn may be one possible reason for placental retention. This remains very speculative, most importantly because this has not been described previously in any of the RCTs of n-3 LC-PUFA supplementation in pregnancy and could only be ascertained by measuring the magnitude of the uterine contractions during labor.

6.8.5 Birth weight

Few studies on the effect of dietary n-3 and n-6 LC-PUFAs on childhood obesity have been performed and none of them started as early in fetal development as the present study. In very few of the studies, infant anthropometry and body composition were defined as the primary outcome parameters and the study design was developed to investigate effects of maternal LC-PUFA supplementation on infant anthropometry. To the best of our knowledge, this is the first RCT supplementing pregnant women with n-3 LC-PUFAs in which infant anthropometry and body composition was analyzed in detail by the use of SFT measurements, ultrasonography and MRI. n-3 LC-PUFA supplementation in combination with a decrease of dietary AA intake, was shown to influence neonatal LC-PUFA status, but did not have any effect on infant birth outcomes, except a clinically irrelevant difference in the pH-value in cord blood between the two groups. While n-3 LC-PUFA supplementation and subsequent AA and DHA status have been correlated to birth weight, birth length and head circumference in previous studies, there were no significant associations found in the present study. This was further confirmed in a linear regression analysis for infant birth weight, adjusted for prepregnancy BMI of the mother, parity, gestational age and gestational weight gain. This is in line with some (HELLAND ET AL. 2001; MALCOLM ET AL. 2003; SANJURJO ET AL. 2004; BERGMANN ET AL. 2007; JUDGE ET AL. 2007; KRAUSS-ETSCHMANN ET AL. 2007; INNIS AND FRIESEN 2008) randomized, controlled supplementing trials, although others have found a minor, but significantly greater head circumference and a higher birth weight of the infants in the supplemented groups (OLSEN ET AL. 1992; SMUTS ET AL. 2003; MAKRIDES ET AL. 2006; SZAJEWSKA ET AL. 2006). However, the small increase in birth weight reported in a meta-analysis by MAKRIDES ET AL. 2006 rather appears to be explained by the observed 2 - 3 day longer gestational length and one has to keep in mind that there are lots of other factors influencing birth outcomes for which we are not able to control for in the present study like the genetic variation for example.

As shown in previous studies, infant birth weight was significantly associated with parity and the length of gestation. First-born infants tend to be lighter than second- and later-born infants, with demonstrated differences in mean birth weight between first- and later-born infants of as much as 200 g, which is very similar to the observed difference of 180 g in the present study. Parity has a complex effect, one in relation to the pre-pregnancy weight increase with increasing parity, the second, independent

of weight, associated to a better adaptation of the maternal vascular system to the transfer of energy to the uterus, placenta and fetus in multiparous women (MACLEOD AND KIELY 1988; COGSWELL AND YIP 1995; GUIHARD-COSTA ET AL. 2004).

6.8.6 Infant anthropometry

n-3 LC-PUFA supplementation in combination with a decrease of dietary AA intake did also not have an obvious impact on SFT of the newborns. There were no statistically significant differences in the triceps, biceps, subscapular and suprailiac SFT and the arm circumference between the infants of the two groups. This seems to be plausible because there was no difference shown between the two groups concerning the birth weight of the infants. Birth weight strongly correlates with SFT, as also shown in the present study. Apart from that, the observed SFT values (e.g. ticeps: 4.7 ± 0.8 mm (IG), 4.6 ± 0.8 mm (CG), table 17) are in accordance with previous published results of SFT in neonates (CATALANO ET AL. 1995; SCHMELZLE AND FUSCH 2002; CATALANO ET AL. 2003; KOO ET AL. 2004; RODRIGUEZ ET AL. 2004; OLHAGER AND FORSUM 2006; SEWELL ET AL. 2006).

Because there is a strong correlation of total body fat with subcutaneous AT, equations converting SFT into total body fat have been established for this purpose and have been compared and validated with other methods in infants and children (SLAUGHTER ET AL. 1988; WESTSTRATE AND DEURENBERG 1989; DEURENBERG ET AL. 1990; WELLS ET AL. 1999; SCHMELZLE AND FUSCH 2002; ELBERG ET AL. 2004). Nevertheless, the existing published equations are all associated with large random errors or significant systematic errors as revealed in a comparison and validation study of several equations used to estimate infant body composition (REILLY ET AL. 1995). Prediction of body fatness by measurement of SFT in infants was associated with large errors at the individual level and therefore it should be emphasized that none of the three equations used in the present study necessarily gives the true figure for AT mass. The values are rather means of comparing different groups. Infant fat mass in g, body fat in % and fat mass/height^2 in kg/m^2 was estimated with 3 different equations published by WESTSTRATE & DEURENBERG (1989), DEURENBERG ET AL. (1990) and SLAUGHTER ET AL. (1988). There were no statistically significant differences in estimations between the two groups when fat was estimated with the same equation, but there were great differences in the absolute amount of fat mass in g, body fat in % and fat mass/height^2 when the 3 equations were compared.

The reason why three different equations were used in the present study is due to the fact that no appropriate equations for the estimation of fat mass from skinfolds exist for newborns. The present equations were all developed for the use in older children and are less appropriate for the assessment in newborns (Fig. 8). The equation of SLAUGHTER ET AL. (1988) was developed for prepubescent children up to adults with a sum of the four skinfolds < 35 mm, and the equation of DEURENBERG ET AL. (1990) for children with a mean age of 11 y. The equation of WESTSTRATE AND DEURENBERG (1989) in contrast was developed for infants and children between 0 and 2 years of age at least and should predict infant fat mass in the most accurate way. Nevertheless all three equations were used in previous trials to estimate infant fat mass and are therefore used in the present study because of comparability. In the present study the equation of WESTSTRATE & DEURENBERG (1989) resulted in the highest values for all three items (e. g. 761 ± 112 g fat mass in both groups), followed by the results of DEURENBERGS (1990) equation (402 ± 129 g (IC) and 396 ± 125 g (CG) fat mass). SLAUGHTERS' (1988) equation resulted in the lowest values (343 ± 113 g (IC) and 345 ± 107 g (CG) fat mass) (table 18).

The ranges of body fat, reported in previous studies (CATALANO ET AL. 1995; BUTTE ET AL. 2000; FOMON AND NELSON 2002; SCHMELZLE AND FUSCH 2002; CATALANO ET AL. 2003; HARRINGTON ET AL. 2004; KOO ET AL. 2004; OLHAGER AND FORSUM 2006; SEWELL ET AL. 2006) ranged between 360 ± 198 g (CATALANO ET AL. 2003) and 440 ± 220 g (SCHMELZLE AND FUSCH 2002) except one study which reported an infant fat mass of 757 ± 219 g (HARRINGTON ET AL. 2004).

On the basis of the present results, it is not possible to draw a conclusion which equation estimates infant fat mass "the best" because a reliable "control" or validated "gold standard" method is lacking. The results have to be compared with the results from the US and MRI measurements of infant adipose tissue which are currently be performed. Based on these data, it even may be possible to generate a new equation for the precise estimation of infant fat mass from SFT in the present collective of newborn infants.

Furthermore, infant body composition was not associated with maternal body composition before or during pregnancy which is in agreement with a study showing that infant body composition at 2 weeks of age (FFM, FM or % FM) was not correlated with maternal body composition before or after pregnancy or with maternal gain in total body water, FFM and FM during pregnancy (BUTTE ET AL. 2003).

Moreover, there are contradictory results coming from similar studies. In another study supplementation of preterm infants with both, AA and DHA, it was observed that the supplemented infants had less fat mass and greater lean body mass than infants in the control group (GROH-WARGO ET AL. 2005). RCTs, in which lactating mothers have been supplemented with n-3 LC-PUFAs after delivery, supplementation did not show any effect on infant anthropometry or body composition (JENSEN ET AL. 1999; HELLAND ET AL. 2001). However, in a study from Denmark supplementation with 4.5 g fish oil /d during lactation was associated with an increase of the BMI and the waist circumference at 2.5 y of age in term infants although there was no association of supplementation and anthropometry at birth and the first year of life (LAURITZEN ET AL. 2005). This finding of a „late effect“ was also observed by a more recent study but in contrast to LAURITZEN ET AL. it was shown, that DHA supplementation (200 mg/d) of pregnant women from the 21st wk of gestation until three month pp resulted in a significant lower BMI and weight of their infants, compared to the control group (BERGMANN ET AL. 2007).

The potential of SFT measurement for quick and inexpensive comparison of body composition between two groups of children without claiming for “true values”, may be further affected in the present study by the open study design. The measurements were made by 3 observers who were not blinded to the treatment groups. This may have had an effect on the results and should be taken into account when interpreting the results. However, the present results do not suggest that maternal n-3 LC-PUFA supplementation and the subsequent decrease in the plasma PL and RBC ratio of AA/DHA does have any impact on fetal adipose tissue development and the mass of AT at birth so far, although conclusions have to be drawn very carefully at this early stage of the study.

So far, any anti-adipogenic effects of n-6 and n-3 LC-PUFAs or their ratio have not been established and the overall effect of the treatment in the present study should be further investigated in a long term follow-up of the infants.

7 Conclusion and perspectives

The supplementation with n-3 LC-PUFAs in the present study was well tolerated and appeared safe for both mothers and their infants. A significant decrease of the AA intake could be achieved by dietary counseling in the intervention group, which led – in combination with the n-3 LC-PUFA supplementation, to a pronounced decrease of the n-6 to n-3 LC-PUFA ratio of maternal dietary intake. The Intervention resulted in a remarkably decrease of the n-6 to n-3 LC-PUFA ratio in both maternal plasma PLs and RBCs. n-6 and n-3 LC-PUFAs were transferred across the placenta into the fetal circulation so that at the time of birth, newborn blood levels of n-3 LC-PUFAs and the ratio of n-6 to n-3 LC-PUFAs strongly correlated to maternal concentrations, resulting in a decreased ratio of n-6 to n-3 LC-PUFAs and an enhanced EPA and DHA content in cord blood PLs and RBCs. Breast milk FA pattern was also responsive to the changes in maternal fatty acid patterns, resulting in a dramatic increase of DHA, without affecting AA, as well as to a decrease of the n-6 to n-3 LC-PUFA ratio. No significant associations of the newborns DHA and AA status with birth weight were found in the present study and intervention did not so far show an impact on infant anthropometry. The present preliminary results do not suggest that maternal n-3 LC-PUFA supplementation does have any impact on fetal adipose tissue development and the mass of AT at birth so far, although conclusions have to be drawn very carefully at this early stage of the study.

Now that obesity is the most burdensome and costly nutritional condition worldwide, questions naturally arise about infancy as a key periode for development of obesity and its consequences. The associations between maternal fatty acid intake and the development of adipose tissue in the infants are certainly of a very complex nature and there are numerous variables (such as the genetic variation) influencing birth outcomes which we were not able to control for in the present study. Nevertheless, the lack of an apparent impact on the newborns birth weight and anthropometry at the present point of time should not be regarded as an absence hereof. In light of the epidemiological evidence from the Dutch Famine study (RAVELLI ET AL. 1998) and similar findings which suggest an association between certain nutritional circumstances in fetal development and later health outcomes, the importance of the perinatal period for long-term health implications is clearly demonstrated. The impact of an altered ratio of n-6 to n-3 LC-PUFAs in maternal nutrition during pregnancy and

lactation may therefore not appear until childhood or even adolescence. Although not detectable at the moment, the impact of the maternal nutrition may appear during the follow-up of the collective of children in the INFAT-Study.

A reduction of the n-6 to n-3 fatty acid ratio in the maternal nutrition during fetal development may therefore lead to later long-term consequences for body composition and for the prevention of developing overweight and obesity. We are only at the beginning to understand how nutrition derived factors like fatty acids may drive these processes for example by altering placental function or by the control of the epigenetic regulation of gene expression. The concept of nutritional programming fits well into the concept of primordial prevention, i. e. prevention of overweight and obesity in the first place, from the very beginning of the food chain.

Whether the reduction of the n-6 to n-3 LC-PUFA ratio will in fact be associated with the infants' body composition and adipose tissue development in the future remains to be investigated in a follow up of the participating infants.

In addition, it is perhaps not only the birth weight or adipose tissue mass in and of itself that has the most important impact on later development of overweight and obesity. Rapid weight gain in infancy (ONG AND LOOS 2006) and maternal gestational weight gain (OKEN ET AL. 2008) have shown to be directly associated with BMI and risk of obesity in adolescence. Intervention in the present study resulted in a significantly lower GWG compared to the control group. It is not yet possible to draw a valid conclusion or even to establish causality between the effects of n-3 LC-PUFA supplementation and GWG from the present study by now. The number of women and their infants included in the present interim analysis was probably too small to obtain valid results in this regard and the final analysis of the study results should be awaited. But nevertheless this finding serves as a first clue and a new possibility to help women to gain the optimal amount of weight during pregnancy in order to facilitate an optimal development of their infants.

The present data therefore represent a very well defined and characterized collective of infants as basis to track and observe infant adipose tissue and body composition development according to alterations in maternal dietary fatty acid intake in future.

8 Summary

Background: Childhood obesity is a serious public health problem predisposing the affected infants to adult obesity and its associated diseases. The first influences on adipose tissue development are known to take place even during the fetal period and early infancy. Eicosanoids derived from long-chain polyunsaturated fatty acids appear to play a key role in the differentiation process of adipocytes and the hyperplastic development of adipose tissue. While eicosanoids derived from the n-6 LC-PUFA arachidonic acid seem to stimulate the hyperplastic development of adipose tissue, those derived from the n-3 LC-PUFAs seem instead to inhibit this process.

Objective: The present work (as part of the INFAT-Study) investigated whether the composition of fatty acids, and in particular the reduction of the n-6 to n-3 fatty acid ratio in the maternal diet during pregnancy and lactation, will lead to a reduction of the same ratio in maternal and infant blood lipids and in breast milk and directly thereafter to a less expansive development of adipose tissue in infants. Tolerance and safety, as well as maternal dietary intake and anthropometry were evaluated as well.

Design: A randomized, controlled intervention trial with two parallel groups was performed. 173 pregnant women aged 20 – 45 years consumed either a diet with a ratio of n-6 to n-3 LC-PUFAs according to the average intake of pregnant women in Germany or a diet with a reduced ratio of n-6 to n-3 LC-PUFAs from the 15th week of pregnancy until 4 months post partum. The latter was achieved by increasing the n-3 LC-PUFA intake via supplementation with 1.2 g/d n-3 LC-PUFAs (DHA + EPA) and a reduction of the n-6 LC-PUFA intake by normalizing the AA intake. The fatty acid profile of maternal plasma phospholipids and red blood cells was determined in the 15th and 32nd week of pregnancy and in the 6th and 16th wk post partum. The fatty acid profile of cord blood was determined at delivery and the fatty acid profile of breast milk was determined in the 6th and the 16th wk post partum. Maternal biochemical parameters (e.g. Hb and cholesterol), dietary intake, anthropometry and birth outcomes as well as newborn birth outcomes and anthropometry were also measured.

Results: Supplementation together with dietary intervention significantly decreased the AA intake and the ratio of n-6 to n-3 LC-PUFAs in the maternal diet (from 7:1 to ~ 3.5:1). The intervention significantly increased the DHA and significantly decreased the AA content in maternal plasma PL and RBC compared to the control group, resulting in a significantly lower ratio of AA/DHA (32^{nd} wk of gestation: 0.95 (IG) vs 2.0 (CG) in maternal PLs, $p < 0.05$).

At the time of birth, the cord blood fatty acid profile strongly correlated with the maternal fatty acid profile, and likely explains the significantly lower ratio of AA/DHA in the intervention group compared to the control group (1.93 (IG) vs 3.2 (CG), $p < 0.05$). Breast milk FA pattern was also responsive to the changes in maternal fatty acid patterns, resulting in a significantly increase of DHA, without affecting the AA content, and therefore resulting in a decrease of the AA/DHA ratio (0.3 (IG) vs 1.8 (CG), $p < 0.05$).

No significant associations of the newborns' DHA and AA status with birth weight and anthropometry were found in the present study. Supplementation also had no significant effect on maternal and infant birth outcomes (e.g. length of gestation) except a significant lower gestational weight gain of the women in the intervention group (13.7 kg (IG) vs 16.1 kg (CG)).

Conclusion: Supplementation with 1.2 g n-3 LC-PUFAs from week 15 of pregnancy until 16 wks post partum, combined with a reduction of the dietary AA intake was an effective means of decreasing the ratio of n-6 to n-3 LC-PUFAs (AA/DHA) in maternal and cord blood PLs and RBCs, as well as in breast milk. The changes in the balance of n-6 to n-3 LC-PUFAs did not have any impact on newborn anthropometry and body fat. These data provide a basis for the future investigation of infant anthropometry and adipose tissue development as affected by maternal diet in pregnancy and lactation. It also suggests a new approach for controlling gestational weight gain in order to improve infant development.

Danksagung

Diese Arbeit wäre nicht möglich gewesen, ohne die Beteiligung vieler Kollegen und Freunde, denen ich an dieser Stelle danken möchte.

An erster Stelle möchte ich mich ganz herzlich bei meinem Doktorvater Prof. Hans Hauner bedanken. Er hat mir die Möglichkeit gegeben und das Vertrauen entgegengebracht dieses interessante Thema selbständig zu bearbeiten. Ich danke ihm für die angenehme Betreuung und große Unterstützung der Arbeit.

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Ganz besonders danke ich allen Eltern mit ihren Kindern, die an der Studie teilgenommen haben. Sie ermöglichten, dass diese Arbeit überhaupt zustande kommen konnte und haben mir jeden Tag ein Lächeln ins Gesicht gezaubert.

Appendix A: Analytical methods:

Chemikalien und Materialien:

Acetlylchlorid (Merck®)

Acetoniril (Merck®)

Chloroform $CHCl_3$ (99 %, Merck®)

Essigsäure CH_3COOH (zur Analyse, Merck®)

Isopropanol (Merck®)

Kaliumcarbonat-Lösung, K_2CO_3; 6%ig (Merck®)

Methanol CH_3OH (wasserfrei, Merck®)

Natriumsulfat Na_2SO_4 (wasserfrei, Merck®)

n-Hexan C_6H_{14} (Merck®)

Pyrogallol (Merck®)

Standard (GLC 85 standard mix, NuChekPrep, Inc. Elysian, Minnesota, USA)

Stickstoff N_2 (technisch, Linde®)

Triethylamin (Merck®)

Wasser, bidest. H_2O

A.1 Lipid extraction modified according to Bligh & Dyer

- 1 ml Frauenmilch (entspricht ca. 35 mg Fett) oder 1ml Plasma (entspricht ca. 6 mg Fett) in ein Lösungsmittel-resistentes Extraktionsröhrchen geben
- 2,5 ml Methanol und 1,25 ml Chloroform zugeben, kräftig schütteln
- mind. 15 min. bei 37℃ im Wasserbad inkubieren
- auf Raumtemperatur abkühlen lassen
- 1,25 ml H_2O bidest. und 1,25 ml Chloroform zugeben, kräftig schütteln

- mind. 15 min. im Eisbad kühlen
- 40 min. bei 4000 Upm zentrifugieren

untere Chloroformphase möglichst quantitativ mit einer Hamilton-Spritze abziehen (dabei etwas Luft mit der Spritze ansaugen, welche beim Durchdringen der Methanol/Wasser-Phase und der Eiweißschicht wieder ausgeblasen wird, um das Mitschleppen störender Substanzen zu verhindern)

Der Lipidextrakt kann nun direkt weiterverarbeitet werden oder zur Lagerung eingefroren werden

Anmerkung:

Treten Phasentrennungsprobleme auf, sollte mehrere Male mit Chloroform ausgeschüttelt, zentrifugiert und abgezogen werden, um eine größtmögliche Lipidausbeute zu erzielen.

In einigen Fällen reicht auch schon aus, die Zentrifugationstemperatur zu erhöhen bzw. zu erniedrigen.

A.2 Separation of lipid classes with HPLC

- An aliquot of the lipid extract is dissolved in chloroform / methanol (1:1, by vol) at a concentration of 0.5 - 2.5 mg/mL. The lipid class separation is completed with an HPLC Alliance 2695 Separation module from Waters (Waters GmbH, Eschborn, Germany) coupled with an PL-ELS 1000 evaporative light scattering detection system (Polymer Laboratories, Darmstadt, Germany).
- The detection is established at 30℃ for the nebulizer and 50℃ for the evaporator.
- A polyvinyl alcohol chemically bound stationary phase PVA Sil column (5 µm, 250 mm x 8 mm) (YMC Europe, Schermbeck, Germany) is used.
- The eluent system is as follows: A: n-hexane; B: isopropanol / acetonitrile / chloroform / acetic acid (84:8:8:0.025; by vol); C: isopropanol / bidest. water / triethylamine (50:50:0.2, by vol). The solvent-gradient system is as follows: 0 - 1 min A/B/C (%) 80:20:0, 1 - 7 min A/B/C (%) 30:54:16, 7 - 13 min A/B/C (%) 30:54:16, 13 - 16 min A/B/C (%) 30:70:0, 16 - 19 min A/B/C (%) 80:20:0, 19 - 24 min A/B/C (%) 80:20:0. The flow rate is 2.5 mL/min.
- The distinct lipid classes are characterized by retention time and are collected with an automatic fraction-sampler from Waters by peak signal recognition.
- The fractions are collected in 5 mL lockable glass tubes.

A.3 Derivatisation according to Lepage & Roy

- eine 3 - 6 mg Fett entsprechende Menge Lipidextrakt in ein Veresterungs-röhrchen (Schraubverschluss Teflondichtung) geben
- Lösungsmittel unter Stickstoffstrom abblasen
- 2 ml Methanol / Hexan 4 : 1 dazu pipettieren
- Spatelspitze Pyrogallol als Antioxidant zugeben
- unter Schütteln auf einem Vortexmischer langsam 200 µl Acetylchlorid zugeben
- Röhrchen gut verschließen und für 1h bei 100℃ im Heizblock reagieren lassen
- Proben im Wasser- oder Eisbad auf Raumtemperatur abkühlen lassen
- 5 ml 6%ige K_2CO_3-Lösung zugeben, kräftig schütteln
- 10 min. bei 3200 Upm zentrifugieren
- Die obere Phase (Hexan) abziehen und evtl. mit Hexan verdünnen.
- Probe kann nun direkt am GC analysiert werden

Anmerkung:

1. Vorsicht, Probe wird bei der Zugabe von Acetylchlorid heiß. Bei zu schneller Zugabe kann die Probe aus dem Gefäß spritzen. Schutzbrille und Handschuhe tragen!
2. Bei der Zugabe der Kaliumcarbonat-Lösung kann die Probe aufschäumen. Am besten bei der Zugabe leicht schütteln.

A.4 Capillary gas chromatography of methylated fatty acids

- The FAME are analyzed by CGC performed on the 6890N gas chromatograph (Agilent Technologies, Waldbronn, Germany) fitted with a cold-on-column injector to prevent fatty acid discrimination. A chemically bound 50 % cyanopropyl-methylpolysiloxane capillary column DB23, 60 m, I.D. 0.25 mm, film 0.25 µm (JW Scientific, Agilent Technologies, USA) is used.
- The chromatographic conditions are as follows:
- Injector (COC): 65 ℃ to 270 ℃
- Carrier gas: hydrogen at a 40 cm/s flow.
- The signals are produced by a flame ionization detector at 250 ℃.

- Fatty acids are identified according to their retention times relative to standards (GLC 85 standard mix, NuChekPrep, Inc. Elysian, Minnesota, USA).
- The temperature program is as follows: initial temperature 60 ℃ for 0.5 min; from 60 ℃ to 180 ℃ at 40 ℃ / min; 180 ℃ for 2 m in; from 180 ℃ to 210 ℃ at 2 ℃ / min; 210 ℃ for 3 min; from 210 ℃ to 240 ℃ at 3 ℃ / min; 240 ℃ for 10 min.

A.5 Capillary gas chromatography conditions

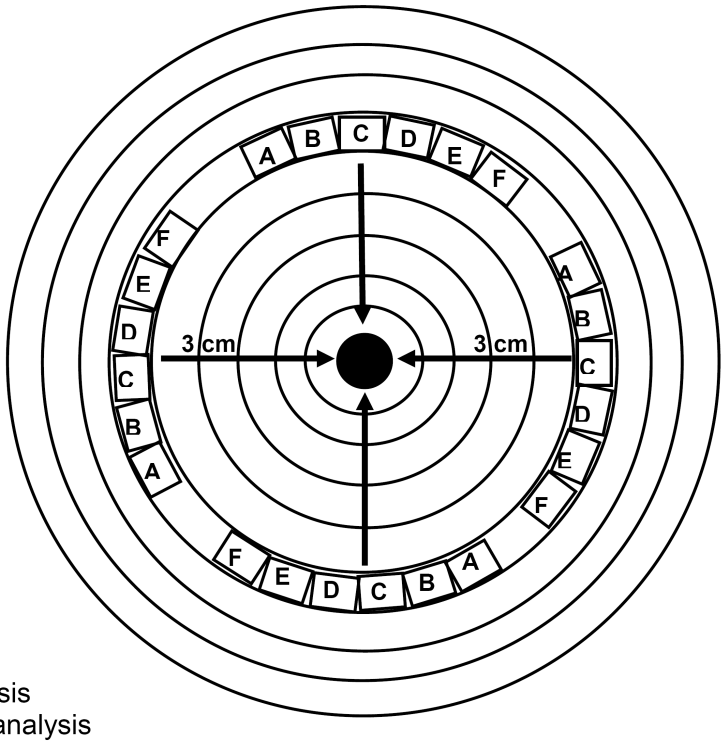
	Agilent, 6890N GC		
Länge der Säule [m]	60		
Autosampler	Agilent		
Säule	DB 23 (Agilent) I. D. 0,25 mm film: 0,25 µm		
Trägergas	H_2		
V_{lin} [cm/sec.]	40		
Injektionsmodus	Split 1:20		
Brennergase	Luft und H_2		
Injektortemperatur [℃]	270		
Detektortemperatur [℃]	250		
Detektor	FID		
Injektionsvolumen [µl]	1		
Temperaturprogramm	Heizrate [℃/min]	Temp. [℃]	Isotherm [min]
		60	0.5
	40	180	2
	2	210	3
	3	240	10

A.6 Placenta preparation

__ __ __ Zentrums Nr.	__ __ __ __ Teilnehmer Nr.

Instruction on the preparation of placental tissue:

Choose 4 stripes/ arms in the center although the umbilical cord is not located in the center (encasing at least 4 stripes, avoiding the outer areas / border) and remove the outer layers of the stripes (chorionic plate and basal plate). Divide the stripes in six cubes à 1 cm. Divide each cube one time and put it into cryo vials (1 cm x 0.5 cm x 2.5 cm). Immediately store the vials in liquid nitrogen.



A: RNA analysis
B: adipokine analysis
C: fatty acid analysis
D: DNA, Protein analysis
E: reserve
F: histology

Appendix B: Documents used in the study

B.1 Flow chart

Anhang A1: Flow Chart Version B

Schwangerschaft					
Studienwoche	-1	0	2 bis 16	18	23-28
SSW	12.-13.	14.	16. bis 30.	32.	Geburt
Parameter \ Visite	V1 (Screening)	V2 (Randomisierung)	Telefonischer Kontakt 2-wöchentlich	V3	
Ernährungsintervention[1]		x	x	x	x
Aufklärungsgespräch	x				
Einverständniserklärung	x				
Anamnese (Gewicht, BMI, Alter, Blutdruck, Gravida, Para)	x				
Blutentnahme[2]		x		x	x
Randomisierung		x			
7-Tage-Ernährungsprotokoll[3]		x		x	
Ernährungsberatung[4]		x		x	
routinemäßig vorgesehene gynäkologische Untersuchung	x				
Postnatal					
Studienwoche	23-28	1 pp	6 pp	16 pp	52 pp
Alter des Säuglings	Geburt	3.-5.Tag	6. Woche	4. Monat	12. Monat
Parameter \ Visite	S0 (Geburt)	S1	S2	S3	FU
Ernährungsintervention MM-Gruppe[5]	x	x	x	x	x
Blutentnahme aus der Nabelschnur[6]	x				
Nabelschnur und Plazentagewebe[7]	x				
Geburtsparameter[8]	x				
Hautfaltendickenmessung Säugling[9]		x	x	x	x
Ultraschallmessungen[10]			x	x	x
MRT[11]			x	x	
Größe, Gewicht, Kopf- u. Oberarmumfang Säugling	x	x	x	x	x
Blutentnahme Säugling (falls möglich)[12]				x	
Muttermilchprobe der Stillenden[13]			x	x	
7-Tage-Ernährungsprotokoll der Stillenden[14]			x		
Ernährungsberatung				x	
Blutentnahme der Stillenden[15]			x	x	

[1] Gruppenspezifische Ernährungsberatung bzw. Supplementierung und Überprüfung der Compliance während der Schwangerschaft

[2] 4x 9ml EDTA-Vollblut zur Bestimmung von Triglyzeriden, HDL-, LDL-, Gesamtcholesterin, kleines Blutbild, Eisen, B-Vitamine, Fettsäuren, Oxidationsprodukte der Fettsäuren (nur bei einer Subgruppe von max. 40 Frauen), Immunparameter (IFNg, IL-1b, IL-4, IL-6, IL-18, IL-22, TGF-b, IL-10, Chemokine wie IL-8, MIP-1, IP-10, Gro-a)

[3] 7-Tage-Schätzprotokoll in der 13. SSW

[4] Ernährungsberatung basierend auf dem 7-Tage-Schätzprotokoll

[5] Gruppenspezifische Ernährungsberatung bzw. Supplementierung und Überprüfung der Compliance während der Stillzeit

[6] Arterielles Nabelschnurblut zur Bestimmung der Fettsäuren und Immunparameter (IFNg, IL-1b, IL-4, IL-6, IL-18, IL-22, TGF-b, IL-10 und evtl. Chemokine wie IL-8, MIP-1, IP-10, Gro-a)

[7] Proteomanalytik, Immunparameter falls ausreichend Blut und Gewebe vorhanden (IFNg, IL-1b, IL-4, IL-6, IL-18, IL-22, TGF-b, IL-10, Chemokine wie IL-8, MIP-1, IP-10, Gro-a)

[8] Geburtsmodus, Kindslage, pH-Wert, Plazentagewicht, Gestationsalter, APGAR-Score nach 5 Minuten

[9] Biceps-, Triceps-, Subscapular-, Suprailiacal-Hautfalten mit Holtain-Caliper

[10] Ultraschallmessungen zur Bestimmung des subcutanen und visceralen Fettanteils; für die Überprüfung der Reproduzierbarkeit der Methodik wird bei einer Subgruppe von jeweils 20 Säuglingen pro Muttermilch-Gruppe im 4. Monat pp eine dreimalige Messwiederholung durchgeführt.

[11] MR-Messungen zur Bestimmung der Körperfettmasse bei einer Subgruppe von jeweils 20 Säuglingen pro Muttermilch-Gruppe

[12] falls möglich ca. 4 ml EDTA-Blut zur Bestimmung von Fettsäuren

[13] ca. 20 ml Muttermilch zur Bestimmung von Fettsäuren, Oxidationsprodukten

[14] 7-Tage-Schätzprotokoll

[15] 4x 9ml EDTA-Vollblut nur bei Frauen die Stillen (nicht formulaenähren) zur Bestimmung von Triglyzeriden, HDL-, LDL-, Gesamtcholesterin, kleinem Blutbild, Eisen, B-Vitaminen, Fettsäuren, Oxidationsprodukten (nur bei einer Subgruppe von max. 40 Frauen), Immunparameter (IFNg, IL-1b, IL-4, IL-6, IL-18, IL-22, TGF-b, IL-10, Chemokine wie IL-8, MIP-1, IP-10, Gro-a)

B.2 Flyer für Schwangere

Else Kröner-Fresenius-Zentrum für Ernährungsmedizin
des Klinikums rechts der Isar
der Technischen Universität München
Direktor: Univ.-Prof. Dr. Hans Hauner

Kontakt:

Wenn Sie Interesse haben an der Studie teilzunehmen würden wir uns sehr freuen!

Bitte sprechen Sie uns unter folgender Adresse an:
Für weiterführende Auskünfte stehen wir Ihnen jederzeit gerne zur Verfügung!

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des Klinikums rechts der Isar
der Technischen Universität München
Direktor: Univ.-Prof. Dr. Hans Hauner

Information

Die Bedeutung des Fettsäurenmusters in der mütterlichen Nahrung während Schwangerschaft und Stillzeit für die frühe Fettgewebsentwicklung beim Menschen

The **i**mpact of the **n**utritional **f**atty acids during pregnancy and lactation for early human **a**dipose **t**issue development

INFAT-Studie

Else Kröner-Fresenius-Zentrum für Ernährungsmedizin
des Klinikums rechts der Isar
der Technischen Universität München
Direktor: Univ.-Prof. Dr. Hans Hauner

Liebe werdende Mütter!

Wir möchten Sie hiermit über eine klinische Studie informieren, bei der der Einfluss der mütterlichen Ernährung während der Schwangerschaft und Stillzeit auf die Entwicklung des Fettgewebes des Säuglings untersucht wird.

Während der Schwangerschaft erhält das im Mutterleib heranwachsende Kind alle nötigen Nährstoffe für Wachstum und Entwicklung von der Mutter. Die Nährstoffe werden aus dem mütterlichen Blutkreislauf über die Plazenta und die Nabelschnur zum Kind transportiert. Während der ersten Lebensmonate erhält der Säugling dann alle Nährstoffe aus der Muttermilch. Das Angebot an Nährstoffen für das Kind ist im Wesentlichen von der Ernährung der Mutter während Schwangerschaft und Stillzeit abhängig.

Es werden verschiedene Einflüsse der mütterlichen Ernährung auf die Entwicklung des Kindes diskutiert. So gibt es derzeit auch Grund zur Vermutung, dass sich die Qualität der Fette in der mütterlichen Ernährung auf die kindliche Fettgewebsentwicklung auswirkt. Diese Auswirkungen kann man z. B. über Hautfaltenmessungen beim Kind erfassen.
Einige Fettsäuren sind für den Körper essentiell, das heißt, sie müssen mit der Nahrung aufgenommen werden, da sie nicht selbst vom Körper hergestellt werden können. Die essentiellen Fettsäuren lassen sich in 2 Gruppen unterteilen, die Omega-6- und die Omega-3-Fettsäuren. Omega-3-Fettsäuren werden auch oftmals als „Fischöle" bezeichnet, da sie besonders reichlich in fetten Meeresfischen, wie z. B. Hering, Makrele und Lachs enthalten sind. Omega-3-Fettsäuren sind unter anderem für die neurologische Entwicklung (Gehirn und Nerven) und die Entwicklung der Sehschärfe Ihres Kindes besonders wichtig und können eine optimale Entwicklung fördern.

Es gibt aber auch erste Hinweise darauf, dass Omega-3-Fettsäuren einen schützenden Einfluss haben könnten auf ein übermäßig starkes Wachstum von frühkindlichen Fettzellen und dass eine damit verbundene spätere Entwicklung von Übergewicht und Fettsucht (Adipositas) im Kindesalter eventuell sogar zu vermeiden wäre.

Eine hohe Zufuhr von Arachidonsäure (Omega-6-Fettsäure) hingegen, die durch eine Ernährung mit einem hohen Anteil von Fleisch und Wurstwaren gekennzeichnet ist, scheint das Wachstum von Fettzellen eher zu fördern.

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Mit dieser Studie wollen wir nun versuchen zu klären, wie sich der Einfluss einer normalen Ernährung der Mutter während Schwangerschaft und Stillzeit im Vergleich zu einer Ernährung mit einem hohen Anteil an Fischölen (Omega-3-Fettsäuren) und wenig Fleisch und Wurstwaren (Arachidonsäure) auf die Entwicklung des kindlichen Fettgewebes auswirkt.

Wie:	Alle interessierten Frauen werden zufällig in eine Interventions- oder eine Kontrollgruppe eingeteilt. Die Frauen in der Interventionsgruppe erhalten Weichkapseln mit Fischöl und sollten sich möglichst arachidonsäurearm ernähren. Die Frauen in der Kontrollgruppe behalten ihre gewohnte Ernährung bei. Alle Frauen erhalten eine kostenlose Ernährungsberatung und werden während der Schwangerschaft begleitend beraten. Nach der Geburt entscheiden Sie sich, ob Sie Ihr Kind stillen oder mit Formulanahrung ernähren möchten. Die Formulanahrung wird Ihnen kostenlos zur Verfügung gestellt.
Wann:	Beginn ist in der 14. Schwangerschaftswoche. Die Studie erstreckt sich über die gesamte Schwangerschaft bis zum 4. Monat nach der Geburt. 12 Monate nach der Geburt findet eine Abschlussuntersuchung statt.
Was:	Zur Kontrolle der Blutfettwerte und Bestimmung der Fettsäuren in Ihrem Blut sind 3 Blutabnahmen vorgesehen. Die Körperfettmasse des Neugeborenen und Säuglings wird indirekt über die Bestimmung der Hautfaltendicke (Bizeps, Trizeps, suprailiacal, subscapular) mit Hilfe eines Kalipers sowie Ultraschall zu 3 definierten Zeitpunkten nach der Geburt gemessen. Bei einer kleinen Gruppe von Kindern wird der Körperfettgehalt mit Hilfe von Magnetresonanztomographie bestimmt. Alle Untersuchungen sind schnell und einfach durchführbar und mit keinerlei Schmerzen für Ihr Kind verbunden.
Wo:	Die Ernährungsberatung, die Blutentnahmen sowie die Messungen des Körperfettes Ihres Kindes finden im Klinikum rechts der Isar statt.
Wer:	Studienleitung: Prof. Dr. med. H. Hauner, Klinikum rechts der Isar

Wir würden uns sehr freuen, wenn Sie Interesse an dem Studienthema haben und an der Studie teilnehmen möchten!

Für weitere Informationen und Auskünfte melden Sie sich bitte bei uns! (siehe Rückseite)

B.3 Teilnehmerinformation

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Teilnehmerinformation

STUDIENTITEL:
Die Bedeutung des Fettsäurenmusters in der mütterlichen Nahrung während Schwangerschaft und Stillzeit für die frühe Fettgewebsentwicklung beim Menschen

Sehr geehrte Studienteilnehmerin,

wir möchten Sie hiermit über eine klinische Studie informieren, bei der der Einfluss der mütterlichen Nahrung während der Schwangerschaft und Stillzeit auf die Entwicklung des Fettgewebes des Säuglings untersucht wird.

Während der Schwangerschaft erhält das im Mutterleib heranwachsende Kind alle nötigen Nährstoffe für Wachstum und Entwicklung von der Mutter. Die Nährstoffe werden aus dem mütterlichen Blutkreislauf über die Plazenta und die Nabelschnur zum Kind transportiert. Während der ersten Lebensmonate erhält dann der Säugling alle Nährstoffe aus der Muttermilch. Diese wird von den Milchdrüsen gebildet und mit Nährstoffen aus dem mütterlichen Blut versorgt. Die optimale Entwicklung des Säuglings ist jedoch nur dann gesichert, wenn die erforderlichen Nährstoffe im mütterlichen Blut vorhanden sind. Deren Angebot wiederum ist im Wesentlichen von der Ernährung der Mutter während der Schwangerschaft und der Stillzeit abhängig.

Es werden verschiedene Einflüsse der Ernährung der Mutter auf die Zusammensetzung der Muttermilch und damit auf die Entwicklung des Kindes diskutiert. So gibt es derzeit auch Grund zur Vermutung, dass sich die Qualität der Fettzufuhr der Mutter auf die kindliche Fettgewebsentwicklung auswirkt. Diese Auswirkungen kann man zum Beispiel über Hautfaltenmessungen erfassen und an den roten Blutzellen untersuchen.

Einige Fettsäuren sind für den Körper essentiell, das heißt, sie müssen mit der Nahrung aufgenommen werden, da sie nicht selbst vom Körper hergestellt werden können. Die essentiellen Fettsäuren lassen sich in 2 Gruppen unterteilen, die Omega-6- und die Omega-3-Fettsäuren. Omega-3-Fettsäuren werden auch oftmals als „Fischöle" bezeichnet, da sie besonders reichlich in fetten Meeresfischen, wie zum Beispiel Hering, Makrele und Lachs enthalten sind. Omega-3-Fettsäuren sind unter anderem für die neurologische Entwicklung (Gehirn und Nerven) und die Entwicklung der Sehschärfe Ihres Kindes besonders wichtig und können eine optimale Entwicklung fördern.

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Es gibt aber auch erste Hinweise dafür, dass Omega-3-Fettsäuren einen schützenden Einfluss haben könnten auf ein übermäßig starkes Wachstum von frühkindlichen Fettzellen und dass eine damit verbundene spätere Entwicklung von Übergewicht und Fettsucht (Adipositas) im Kindesalter eventuell sogar zu vermeiden wäre.

Eine hohe Zufuhr von Arachidonsäure (Omega-6-Fettsäure) hingegen, die durch eine Ernährung mit einem hohen Anteil von Fleisch und Wurstwaren gekennzeichnet ist, fördert eher das Wachstum von Fettzellen. Beim Menschen liegen dazu allerdings noch keine genauen Untersuchungen vor.

Mit dieser Studie wollen wir nun versuchen zu klären, wie sich der Einfluss einer normalen Ernährung der Mutter (Kontrollgruppe) während Schwangerschaft und Stillzeit im Vergleich zu einer Ernährung mit einem hohen Anteil an Fischölen (Omega-3-Fettsäuren) und wenig Fleisch und Wurstwaren (Arachidonsäure) (Interventionsgruppe) auf die Entwicklung des kindlichen Fettgewebes auswirkt.

Gemessen wird dies über die Hautfaltendicken, das Körpergewicht und die Körpergröße Ihres Kindes sowie mittels Ultraschall und bei einem Teil der Kinder mittels Magnetresonanztomographie (MRT). Alle diese Untersuchungen sind völlig ungefährlich und schmerzfrei für Ihr Kind. Es kommt zu keiner Strahlenbelastung.

Weiterhin versuchen wir zu klären, welchen Einfluss diese unterschiedlichen Ernährungsweisen auf den Aufbau der roten Blutzellen und deren Gehalt an Fettsäuren sowie auch auf den Gehalt an Fettsäuren in der Muttermilch haben.

2. Ablauf der Studie

Wenn Sie sich zur Teilnahme entschließen, ist es erforderlich, dass Sie die beiliegenden Einverständniserklärungen unterschreiben und datieren. Eine Einverständniserklärung dürfen Sie behalten, die andere bekommt der für Sie zuständige Studienarzt dieser Studie ausgehändigt.

Die Untersuchungen und Blutabnahmen sowie die Ernährungsberatungen finden am Klinikum rechts der Isar statt.

Die Dauer der Studie beträgt etwa 54 Wochen (= 1 Jahr + 2 Wochen).
Die Studie wird nur in München an insgesamt ca. 200 freiwilligen, gesunden, schwangeren und stillenden Frauen und deren Säuglingen durchgeführt.

<u>Voruntersuchung (Screening) Besuch V1:</u>

Während der 12. bis 13. Schwangerschaftswoche (SSW) werden Ihr Frauenarzt oder wir Sie über diese Studie informieren.
Hierbei wird auch geprüft, ob Sie die Eignungskriterien für die Aufnahme in die Studie erfüllen. Dazu erhalten Sie eine ausführliche Information über den Hintergrund und das Vorgehen der Studie. Erst nachdem Sie sich zur Teilnahme an der Studie bereit

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erklärt haben und die Einverständniserklärungen unterschrieben haben, wird Ihre medizinische Vorgeschichte erhoben und Sie werden zu den Medikamenten befragt, die Sie aktuell einnehmen oder über die letzten 6 Monate eingenommen haben.

Es erfolgt eine körperliche Untersuchung, bei der Ihr Körpergewicht, Ihre Körpergröße, Ihre Hautfaltendicke an definierten Körperstellen (Bizeps, Trizeps, subscapular) und Ihr Blutdruck kontrolliert werden.

Um Ihre Ernährungsgewohnheiten zu erfassen, bitten wir Sie, eine Woche lang über Ihre Ernährung Buch zu führen. Sie bekommen dazu nach der Untersuchung ein 7-Tage-Ernährungsprotokoll sowie eine Anleitung zum Ausfüllen von unseren Mitarbeitern ausgehändigt. Dort schreiben Sie eine Woche lang alles auf, was Sie essen und trinken. Nach Abgabe des Protokolls werden Sie zu einer persönlichen, individuell auf Sie abgestimmten Ernährungsberatung in das Klinikum rechts der Isar eingeladen.

<u>Start der Studie (Randomisierung) Besuch V2:</u>

Wenn nach Auswertung der Voruntersuchung festgestellt wurde, dass Sie die Eignungskriterien für die Studie erfüllen, werden Sie für die Studie randomisiert. Das heißt, Sie werden per Zufallsgenerator (so, als ob Sie eine Münze werfen) in eine der beiden Ernährungsgruppen (Kontrollgruppe oder Interventionsgruppe) eingeteilt.
Die Randomisierung findet in der Klinik für Ernährungsmedizin am Klinikum rechts der Isar statt. Weder Sie noch der für Sie zuständige Arzt haben Einfluss auf diese Einteilung.

Gleichzeitig wird Ihnen eine Blutprobe abgenommen (für Routineuntersuchungen wie „kleines Blutbild", Blutfettwerte sowie für die Bestimmung der Fettsäuren, molekularbiologische und genetischen Analysen (RNA, DNA)). Für die Blutprobe wird Ihnen Blut (insgesamt ca. 40 ml, das sind ca. 4 Esslöffel) aus der Armvene entnommen.
Nach der Probenentnahme findet die persönliche, individuell auf Sie abgestimmte Ernährungsberatung statt.

Wenn Sie in die Kontrollgruppe eingeteilt wurden, würden wir Sie bitten, sich entsprechend Ihren persönlichen Ernährungsgewohnheiten möglichst ausgewogen und gesund nach den Regeln der Deutschen Gesellschaft für Ernährung zu ernähren.
Bei Einteilung in die Interventionsgruppe sollten Sie sich ebenfalls möglichst gesund und ausgewogen nach den Regeln der Deutschen Gesellschaft für Ernährung ernähren, zusätzlich aber möglichst wenig Fleisch und Wurstwaren konsumieren, Raps- und/oder Leinöl als bevorzugte Ölquellen verwenden und zusätzlich ein Nahrungsergänzungsmittel in Form von drei Fischölkapseln pro Tag (Marinol™ D-40 von Loders Croklaan) einnehmen. Dieses Präparat wird Ihnen kostenlos von uns zur Verfügung gestellt, so dass Ihnen für die Dauer der Studie keine zusätzlichen Kosten entstehen.
Sie sollten möglichst versuchen, die jeweilige Ernährungsweise während der gesamten Schwangerschaft und bis zum 4. Lebensmonat Ihres Kindes einzuhalten, das heißt über einen Zeitraum von 52 Wochen. Zur Erleichterung der täglichen Praxis erhalten Sie von uns praktische Tipps für eine gesunde Ernährung während der Schwangerschaft und Vorschläge für die Lebensmittelauswahl sowie Rezepte.

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Die Frauen, die der Interventionsgruppe zugeteilt werden, beginnen nach der Ernährungsberatung im Klinikum mit der Einnahme der Fischölkapseln. Mit Beginn der Kapseleinnahme sollte auch die arachidonsäurearme Ernährung eingehalten werden.

Während der folgenden Wochen erkundigen wir uns telefonisch in regelmäßigen Abständen bei Ihnen, wie Sie mit der für Sie geplanten Ernährungsweise zurechtkommen und bieten Ihnen eine Wiederholung der persönlichen Ernährungsberatung an.

<u>Verlauf der Studie, Besuch V3:</u>

In der <u>32. SSW</u> werden Sie zusätzlich gebeten, ein zweites Ernährungsprotokoll zu führen und über 7 Tage genau zu notieren, was Sie essen und trinken. Außerdem ist wieder eine Blutentnahme vorgesehen. Es wird Ihnen nochmals Blut (insgesamt ca. 40 ml, das sind ca. 4 Esslöffel) abgenommen, um den Einfluss Ihrer Ernährung auf die Zellwand der roten Blutkörperchen zu untersuchen sowie molekularbiologische und genetische Analysen (RNA, DNA) vorzunehmen.
Wenn Sie möchten, bieten wir Ihnen eine erneute Ernährungsberatung an.

<u>Geburt, S0:</u>

Noch vor der Geburt bzw. der Einleitung der Geburt wird Ihnen Blut abgenommen (2 Röhrchen à 9 ml), um zu untersuchen, welchen Einfluss die vorgeburtlichen Veränderungen auf Ihre Fettsäuren, Fettgewebshormone und Abwehrbotenstoffe haben.

Die Hebammen und Ärzte werden nach der Geburt die so genannten Geburtsparameter (Geburtsweg, Plazentagewicht, Schwangerschaftsdauer, Anzahl der vorangegangenen Schwangerschaften, Fehlgeburten, usw.) von Ihnen sowie den APGAR-Score (Aussehen, Puls, Gesichtsausdruck, Atmung, Reflexe) Ihres Kindes und die Körpergröße, das Körpergewicht und den Kopfumfang Ihres Kindes messen.

Unmittelbar nach der Entbindung wird von den Hebammen aus der Nabelschnur kindliches Blut gewonnen, ohne Ihr Kind dabei stechen zu müssen. Auch in dieser Blutprobe wollen wir die Fettsäuren in den roten Blutkörperchen untersuchen sowie molekularbiologische und genetische Analysen vornehmen (RNA, DNA). Zusätzlich sollen diese molekularbiologischen und geneitschen Analysen auch an einem kleinen Stück der Nabelschnur (ca. 5 cm) erfolgen. Hierzu wird ein Nabelschnurstück entnommen und tiefgefroren.
Gleichzeitig werden aus der nicht mehr benötigten Plazenta mehrere kleine Stückchen Gewebe entnommen (ca. 2 x 2 cm, etwa so groß wie ein Würfel). Das Gewebe wird entsprechend der Analysenmethode weiterverarbeitet, tiefgefroren und später am Lehrstuhl für Ernährungsmedizin der Technischen Universität München in Weihenstephan analysiert. Dabei sollen verschiedene molekularbiologische und genetische Analysen (RNA, DNA) durchgeführt werden.

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Falls Sie Ihr Kind stillen, sollten Sie auch über den Verlauf der Stillzeit bis zum 4. Lebensmonat Ihres Kindes versuchen, diejenige Ernährungsweise beizubehalten, die Sie während der Schwangerschaft eingehalten haben (gesunde, ausgewogene Ernährung (Kontrollgruppe) oder gesunde, ausgewogene Ernährung mit wenig Arachidonsäure und einem Nahrungsergänzungsmittel in Form von Fischölkapseln (Interventionsgruppe).

Falls es Ihnen nicht möglich sein sollte, Ihr Kind zu stillen, erhalten Sie bis zum 6. Lebensmonat Ihres Kindes von uns kostenlos eine Säuglingsmilchnahrung.
Sowohl für den Fall, dass Sie der Ernährungsgruppe zugeteilt wurden, die sich ausgewogen und gesund, nach den Regeln der Deutschen Gesellschaft für Ernährung ernähren soll (Kontrollgruppe), als auch als Teilnehmerin in der Gruppe, die wenig Fleisch und Wurstwaren verzehrt und Fischölkapseln einnimmt (Interventionsgruppe), erhalten Sie kostenlos die Nahrung Aptamil Pre bzw. Aptamil HA Pre mit LCP-Milupan von der Firma Milupa. LCP ist eine internationale Abkürzung und steht für „langkettige, mehrfach ungesättigte Fettsäuren".

3. bis 5. Tag nach der Geburt, Besuch S1:

Um eine genaue Untersuchung der körperlichen Entwicklung Ihres Kindes vornehmen zu können, werden wir noch auf der Neugeborenenstation sogenannte Hautfaltendickenmessungen durchführen. Dazu wird eine Hautfaltenzange (Kaliper) verwendet, mit der wir am Oberarm (Bizeps und Trizeps), am Schulterblatt und seitlich des Nabels jeweils dreimal hintereinander eine Hautfaltenmessung vornehmen. Außerdem wird der Oberarmumfang mit einem Maßband bestimmt.
Alle Messungen werden von geschulten Untersuchern durchgeführt und sind mit keinerlei Schmerzen oder Verletzungen Ihres Kindes verbunden.

6. Woche nach der Geburt, Besuch S2:

Bei Ihrem Kind werden ein zweites Mal die Hautfaltendicken gemessen sowie Körpergröße, Körpergewicht, Kopf- und Oberarmumfang bestimmt. Es wird ebenfalls eine Ultraschallmessung zur Bestimmung des Fettgewebes durchgeführt.

Bei einem zufällig ausgewählten kleinen Teil der Kinder (40 von 200 Kindern) wird zusätzlich eine Magnetresonanztomographie (MRT) durchgeführt. Auch diese Untersuchung ist nicht schädlich oder schmerzhaft für Ihr Kind und dient nur der genauen Bestimmung des Fettgewebes. Ihr Kind wird nach dem Stillen oder Füttern schlafend in den Tomographen gelegt. Normalerweise kommt es bei einer solchen Untersuchung zu lauten Geräuschen. Für Ihr Baby wird deshalb eine geräuscharme Einstellung gewählt und es bekommt zusätzlich einen Gehörschutz angelegt.

Wenn Sie Ihr Kind stillen, würden wir Sie bitten, ein letztes Mal über 7 Tage genau aufzuschreiben, was Sie essen und trinken. Sie bekommen die Ergebnisse dieses Ernährungsprotokolls in Form einer persönlichen Ernährungsberatung in der 16. Woche nach der Geburt mitgeteilt.

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Um den Einfluss Ihrer Ernährung auf die roten Blutzellen und die Muttermilch während der Stillzeit zu untersuchen, würden wir bei Ihnen eine dritte Blutentnahme (40 ml) vornehmen und Sie bitten, uns eine Probe Muttermilch (20 ml = 3 Esslöffel) zur Verfügung zu stellen.

16. Woche nach der Geburt (4. Lebensmonat), Besuch S3:

Bei Ihrem Kind werden wir das dritte Mal die Hautfaltendickenmessungen vornehmen und Körpergröße, Körpergewicht und Kopf- sowie Oberarmumfang bestimmen. Gleichzeitig findet die Ultraschallmessung statt.

Wie in der 6. Woche nach der Geburt findet bei dem zufällig ausgewählten Teil der Kinder eine MRT-Messung statt.
Bei den Kindern, die für die MRT-Untersuchung ausgewählt wurden, sind zusätzlich zu der normalen Ultraschalluntersuchung drei Untersuchungstermine innerhalb einer Woche vorgesehen, um die Ultraschallmessungen mit denen der MRT-Messungen vergleichen zu können.

Zu diesem Zeitpunkt würden wir uns wünschen, von Ihnen die Genehmigung zu erhalten, Ihrem Kind aus der Vene eine Blutprobe (max. 4 ml = 1 Teelöffel) zu entnehmen, um die Auswirkung der Ernährung Ihres Kindes auf die Entwicklung der roten Blutzellen untersuchen zu können. Der Einstich ist zwar mit einem kleinen Schmerz verbunden, jedoch erleidet Ihr Kind durch die Blutentnahme keinerlei Schaden. Die Blutproben werden im Labor des Lehrstuhls für Ernährungsmedizin an der Technischen Universität München-Weihenstephan untersucht.

Wenn Sie Ihr Kind stillen, erhalten Sie von uns eine auf Sie persönlich abgestimmte Ernährungsberatung zu dem 7-Tage-Ernährungsprotokoll, das Sie in der 6. Woche nach der Geburt geführt haben.

Um den Einfluss Ihrer Ernährung auf die roten Blutzellen und die Muttermilch während der Stillzeit zu untersuchen, würden wir bei Ihnen eine vierte Blutentnahme (40 ml) vornehmen und Sie bitten, uns eine zweite Probe Muttermilch (20 ml = 3 Esslöffel) zur Verfügung zu stellen. Die Blutprobe wird außerdem für molekularbiologische und genetische Analysen (RNA, DNA) verwendet.

Diesen Termin sollten Sie auch dann versuchen wahrzunehmen, wenn Sie vorzeitig aus der Studie ausgeschieden sind.

Abschlussbesuch Woche 50 – 52 (12. Lebensmonat), Besuch FU:

Bei diesem letzten Besuch im Rahmen der Studie werden bei Ihrem Kind die Hautfaltendickenmessungen vorgenommen, Körpergröße, Körpergewicht und Kopf- sowie Oberarmumfang bestimmt und eine Ultraschalluntersuchung durchgeführt.

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Zu diesem Zeitpunkt würden wir nochmals versuchen, Ihrem Kind Blut abzunehmen, um die Auswirkung der Ernährung Ihres Kindes auf die Fettsäuren und Fettgewebshormone untersuchen zu können.

Diesen Besuch sollten Sie auch dann versuchen wahrzunehmen, wenn Sie die Studie vorzeitig beendet haben.

3. Nutzen der Studie

Durch die Teilnahme an dieser Studie ermöglichen Sie es uns, dass Informationen gewonnen werden, wie sich eine bestimmte Ernährung während der Schwangerschaft und Stillzeit auf die Fettgewebsentwicklung von Säuglingen auswirkt.
Dadurch kann eventuell auf die Entstehung von Übergewicht und Fettsucht (Adipositas) im frühen Kindesalter Einfluss genommen werden und dem zunehmenden Übergewicht bei Kindern Einhalt geboten werden.
Diese Informationen können in Zukunft auch anderen schwangeren und stillenden Frauen zugute kommen, die für eine optimale Entwicklung ihrer Säuglinge sorgen möchten.

4. Aufwandsentschädigung und Kosten

Wenn Sie sich bereit erklären, an der Studie teilzunehmen und die Studie bis zum 12. Lebensmonat Ihres Kindes erfolgreich beendet haben, erhalten Sie eine Aufwandsentschädigung in Höhe von 50 Euro. Sollten Sie zu der Gruppe der zufällig ausgewählten Frauen gehören, deren Säugling an der Magnetresonanztomographie-Messung und den Ultraschallmessungen teilnimmt, erhalten Sie dafür eine zusätzliche Aufwandsentschädigung in Höhe von 100 Euro.

Alle Besuche und Untersuchungen, die im Rahmen dieser Studie stattfinden, sind für Sie und Ihre Krankenkasse völlig kostenfrei.

5. Risiken und Nebenwirkungen

Zur Entnahme der Blutproben muss bei Ihnen und - Ihre besondere Zustimmung vorausgesetzt - bei Ihrem Kind eine kleine Nadel in eine Armvene eingeführt werden. An der Blutentnahmestelle können leichte Schmerzen auftreten und es können sich ein Bluterguss und eine Schwellung bilden. Es ist auch möglich, dass eine Infektion an der Entnahmestelle auftritt, oder dass Sie ohnmächtig werden, wobei diese Vorfälle absolut selten sind.

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Aufgrund der Einnahme der Fischölkapseln kann es in seltenen Fällen zu Störungen im Magen-Darm-Trakt kommen, wie Aufstoßen (verbunden mit einem Fischgeruch oder Geschmack), Übelkeit, Erbrechen, Blähungen, Durchfall oder Verstopfung. In sehr seltenen Fällen wurde bei der Einnahme von Fischölkapseln ähnlicher Zusammensetzung eine etwas verlängerte Blutungszeit beobachtet.

6. Ihr Recht auf Information und/oder Rücktritt von der Studie

Sie haben jederzeit das Recht, Fragen über die Studie zu stellen. Während der Studie werden Ihnen der Prüfarzt bzw. seine Mitarbeiter die Fragen zu der Studie beantworten. Wenn Sie Fragen zu Ihren Rechten als Teilnehmer an einem wissenschaftlichen Forschungsprojekt haben, wenden Sie sich bitte an Herrn Professor Dr. Hans Hauner, Klinik für Ernährungsmedizin, Klinikum rechts der Isar, Tel: 089 / 4140-6771.

Ihre Teilnahme an dieser Studie ist freiwillig. Sie können die Teilnahme verweigern oder jederzeit, ohne dafür einen Grund angeben zu müssen, von der Studie zurücktreten. Selbstverständlich werden Sie weiterhin von einem Frauenarzt betreut, unabhängig von Ihrer Entscheidung zur weiteren Teilnahme an dieser Studie.

Wenn Sie von der Studie zurücktreten möchten nachdem Sie bereits mit einer der beiden Ernährungsweisen begonnen haben, oder von Ihrem Arzt aus medizinischen Gründen aus der Studie ausgeschlossen werden, sollten Sie nach Möglichkeit an einer Abschlussuntersuchung durch Ihren Studienarzt teilnehmen. Der Arzt wird hierfür Aufzeichnungen zu Ihrem Gesundheitszustand machen.

7. Verwendung der Studienergebnisse und Datenschutz

Alle im Rahmen dieser klinischen Studie erhobenen Teilnehmer- und Krankheitsdaten werden entsprechend den Bestimmungen des Bundesdatenschutzgesetzes und der ärztlichen Schweigepflicht streng vertraulich behandelt. Die Weitergabe und Auswertung der Daten erfolgt ohne Angabe Ihres Namens (d.h. pseudonymisiert); es wird nur Ihre Studiennummer verwendet. Diese Daten können in einem Computer gespeichert und verarbeitet werden.

Die Einwilligung zur Teilnahme an dieser Studie schließt die Aufzeichnung Ihrer im Rahmen dieser Studie erhobenen Krankheitsdaten durch beteiligte Ärzte ein sowie die Weitergabe dieser Daten an den Leiter dieser klinischen Prüfung, Herrn Prof. Dr. med. Hans Hauner, einschließlich dessen Vertreter und Auftragnehmer sowie die zuständigen Überwachungsbehörden und Ethikkommissionen.

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Sie haben das Recht, jegliche Informationen, die sich auf die Teilnahme an dieser Studie beziehen, einzusehen. Dies betrifft Informationen über Ihre allgemeine Gesundheit, jegliche aufgetretenen Nebenwirkungen und alle Testergebnisse, die während dieser Studie gesammelt worden sind.

8. Vorzeitiges Ausscheiden aus der Studie

Der Studienarzt kann Sie auch ohne Ihre Zustimmung auffordern, Ihre Teilnahme an der Studie abzubrechen. Sie werden aus der Studie ausgeschlossen, wenn es Ihnen nicht möglich sein sollte, eine der beiden Ernährungsweisen einzuhalten oder wenn es den Anschein hat, als würde Ihnen durch die Studie ein medizinischer Schaden drohen. Wenn Sie gebeten werden, von der Studie zurückzutreten, werden die Gründe mit Ihnen besprochen und Sie sollten zu einer Abschlussuntersuchung in die Klinik kommen.

Kontaktadresse für weitere Informationen sowie im Notfall:

Für weitere Fragen im Zusammenhang mit dieser klinischen Studie stehen Ihnen der Studienarzt und seine Mitarbeiter gerne zur Verfügung. Auch Fragen, die Ihre Rechte als Teilnehmerin dieser klinischen Studie betreffen, werden Ihnen gerne beantwortet.

Ihr Studienarzt ist:

Name der/des Ärztin/Arztes:_____

Adresse der Praxis / Klinik: Klinik für Ernährungsmedizin, Ismaninger Str. 22

81675 München

Telefonnummer: 089-4140-6782

NOTFALL-Nr: **0163-1738420**

B.4 Einverständniserklärung

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Einverständniserklärung

„Die Bedeutung des Fettsäurenmusters in der mütterlichen Nahrung während Schwangerschaft und Stillzeit für die frühe Fettgewebsentwicklung beim Menschen“

Ich erkläre mich hiermit einverstanden, an der oben genannten Studie teilzunehmen. Ich wurde über Wesen, Bedeutung und Tragweite der Studie aufgeklärt. Ich habe die Teilnehmerinformation gelesen und fühle mich ausreichend informiert.
Ich hatte genügend Gelegenheit, Fragen zur Untersuchung zu stellen und meine Fragen wurden ausreichend beantwortet. Ich wurde ferner darauf hingewiesen, dass es mir jederzeit freisteht, mein Einverständnis ohne Angabe von Gründen zurückzuziehen, ohne dass mir dadurch irgendwelche Nachteile entstehen.

Ich bin damit einverstanden, dass insgesamt 3 Blutentnahmen erfolgen für den Fall, dass ich mein Kind nicht stillen kann und 5 Blutentnahmen durchgeführt werden, wenn ich mein Kind stille. Die maximale Blutmenge beträgt pro Abnahme ca. 40 ml. Über mögliche Risiken, wie z. B. Bluterguss oder Infektionen an der Einstichstelle, wurde ich aufgeklärt. Ich bin damit einverstanden, dass Größe, Gewicht, Kopf- und Oberarmumfang und Hautfaltendicken meines Kindes sowie Ultraschallmessungen zur Bestimmung des Körperfettgehalts insgesamt viermal gemessen bzw. durchgeführt und dokumentiert werden. Falls es nach Rücksprache mit dem zu betreuenden Kinderarzt möglich sein sollte, bin ich damit einverstanden, dass meinem Kind im vierten Lebensmonat (max. 4 ml) und im 12. Lebensmonat (max. 9 ml) Blut abgenommen wird. Sämtliche Blut- und Gewebeproben (Nabelschnur, Plazenta) werden für die Analyse von Fettsäuren, Fettgewebshormonen, molekularbiologische und genetische Analysen (RNA, DNA) verwendet. Ferner erkläre ich mich einverstanden, zweimal ein 7-Tage-Ernährungsprotokoll während der Schwangerschaft zu führen. Falls ich mein Kind stillen werde, erkläre ich mich dazu bereit, ein drittes 7-Tage-Ernährungsprotokoll während der Stillzeit zu führen. Während der Stillzeit werde ich versuchen, zu zwei verschiedenen Zeitpunkten (6. Woche nach der Geburt, 4. Monat nach der Geburt) jeweils eine Probe Muttermilch (ca. 20 ml) zur Verfügung zu stellen.

Ich erkläre mich damit einverstanden, dass die im Rahmen der klinischen Prüfung erhobenen Daten/Angaben über meine Gesundheit auf Fragebögen und elektronischen Datenträgern aufgezeichnet und ohne Namensnennung (pseudonymisiert) weitergegeben werden an:

a) den Auftraggeber (Sponsor) oder beauftragte Unternehmen der Studie zur wissenschaftlichen Auswertung, Bewertung von unerwünschten Ereignissen oder Beantragung der Zulassung.
b) die zuständige(n) Überwachungsbehörde(n) (Landesamt / -ämter) oder Bezirksregierung (en) und Ethikkommission(en).

Außerdem erkläre ich mich damit einverstanden, dass ein autorisierter und zur Verschwiegenheit verpflichteter Beauftragter des Auftraggebers, der zuständigen inländischen und ausländischen Überwachungs- und Zulassungsbehörden in meine beim Prüfarzt vorhandenen personenbezogenen Daten Einsicht nimmt, soweit dies für die

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Überprüfung der Studie notwendig ist. Für diese Maßnahme entbinde ich den Prüfarzt von der ärztlichen Schweigepflicht.
Die Einwilligung zur Erhebung und Verarbeitung der Angaben über meine Gesundheit ist unwiderruflich. Ich bin bereits darüber aufgeklärt worden, dass ich jederzeit die Teilnahme an der klinischen Prüfung beenden kann. Im Falle dieses Widerrufs erkläre ich mich damit einverstanden, dass die bis zu diesem Zeitpunkt gespeicherten Daten ohne Namensnennung weiterhin verwendet werden dürfen, soweit dies erforderlich ist.

Eine Kopie der Teilnehmerinformation und der Einverständniserklärung habe ich erhalten.

München, den _____

_____ _____
Name der Studienteilnehmerin Unterschrift

_____ _____
Name der aufklärender Person / des Arztes Unterschrift

B.5 Anleitung 7-Tage Schätzprotokoll

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Liebe Studienteilnehmerin!

Das Ernährungsprotokoll soll Ihre individuellen Ernährungsgewohnheiten erfassen und widerspiegeln. Deshalb ist es wichtig, dass Sie das Ernährungsprotokoll möglichst genau und ehrlich ausfüllen. Bitte ändern Sie während der Protokollführung Ihre Ernährungsgewohnheiten nicht und essen Sie so normal wie möglich.

Bitte notieren Sie ***alle*** **Lebensmittel** und **Getränke**, die Sie in den kommenden sieben Tagen verzehren. Wenn möglich, sollten Sie Ihr Ernährungsprotokoll immer mit sich führen und alle Speisen und Getränke sofort eintragen, auch wenn das Protokoll dabei schmutzig wird. Tragen Sie auch Lebensmittel ein, die Sie im Restaurant oder zwischen den Mahlzeiten zu sich nehmen. Auch kleine Snacks, wie Bonbons oder ein Apfel, sollten ebenso wenig fehlen wie Vitamin- und Mineralstoffpräparate, die Sie im Rahmen Ihrer Schwangerschaft einnehmen oder Medikamente, die Sie verordnet bekommen haben.

Notieren Sie bitte Ihren **Namen**, das **Datum** und den **Wochentag** auf jeder Seite und dem Deckblatt.

- ➔ In die ***erste Spalte*** tragen Sie bitte ein, **wann und wo** Sie die Mahlzeit zu sich nehmen (z. B. zu Hause, in der Kantine, im Restaurant, unterwegs…).
- ➔ In die ***zweite Spalte*** tragen Sie bitte genau ein, **wie viel** Sie von einem Lebensmittel verzehren. Dies geschieht in „haushaltsüblichen Maßen", zum Beispiel geben Sie die Portionen in „Scheiben", „Esslöffeln" oder „Tassen" bzw. „Bechern" an, wie im Gespräch mit Ihnen vereinbart.
  Es ist nicht nötig, die Lebensmittel zu wiegen bzw. abzumessen. Wenn Wiegen für Sie in bestimmten Situationen keinen Mehraufwand bedeutet, können Sie dies gerne tun.
  Wenn Angaben auf der Verpackung zu finden sind (z. B. bei Jogurt oder Fertiggerichten), ist es sinnvoll, diese anzugeben.
  Gerichte – wenn möglich – nach einzelnen Zutaten aufschlüsseln. Bei Mahlzeiten mit der Familie kann es schwierig sein, die Zutaten einzelner Speisen mengenmäßig genau zu ermitteln. Hier genügt es, wenn Sie die Zutaten komplett angeben und dann angeben, wie viel Sie davon verzehrt haben (z. B. ein Viertel).
- ➔ Die ***dritte Spalte*** soll die **genaue Bezeichnung des Lebensmittels** enthalten, z. B.:
  „Edamer, 40 % F.i.Tr." anstatt „Käse", „Roggenmischbrot" statt „Brot" oder „Apfel, Boskop, geschält" statt „Apfel". Wenn möglich, geben Sie bitte auch immer den Marken- oder Sortennamen an.
- ➔ In der ***vierten*** Spalte tragen Sie bitte die **Zubereitungsart** des Essens ein. Die Zubereitungsart sollte näher und möglichst genau beschrieben werden, z. B. bei Fleisch und Fisch: „paniert" oder „gebraten in Rapsöl" oder bei Gemüse "in Olivenöl angebraten" oder „gedünstet".

Angabe des Fettgehalts:

- Bei Milch- und Milchprodukten (Jogurt, Käse, Milch, usw.) in % Fett (z. B. Milch, 1,5 % Fett) oder % Fettgehalt i. Tr. (z. B. Camembert 60 % i. Tr.)
- Bei Fleisch- und Wurstwaren Einteilung in fett, mager, fettreduziert (z. B. „Schweineschinken mager, gekocht" oder „Speck, durchwachsen")

Angabe des Brat-, Salat- und Aufstrichfettes bezüglich Sorte und Menge:

- z. B. Butter, Sonnenblumenmargarine, Diätmargarine (Handelsnamen angeben)
- oder: Rapsöl, Olivenöl, Leinöl (Handelsnamen angeben)

Zubereitungsart:

- gekocht, gebraten, frittiert, gegrillt, gedünstet, gedämpft, überbacken, paniert, gebunden…

Nähere Beschreibung des Lebensmittels (Art, Sorte, Markenname usw.):

- Fleisch: von welchem Tier (Rind, Schwein, Huhn) und Stück (Brust, Filet, usw.)
- Wurst und Käse: z. B. Salami, Fleischwurst, Schinken (gekocht oder geräuchert) oder Camembert, Gouda, Mozzarella, Feta, Appenzeller, usw.
- Soße / Suppe: Bratensoße gebunden, Holländische Soße, Essig-Öl-Salatsoße, Jogurtdressing, Suppe mit Fleischeinlage, Cremesuppe, usw.
- Kuchen: Hefekuchen, Streuselkuchen, Sahnetorte, Obstkuchen (Mürbteig, Biskuit)
- Getränke: Unterscheidung von Saft und Nektar, Markennamen angeben, z. B. bei Bier

Angabe über die verwendeten Lebensmittel: frisch, Tiefkühlkost oder Konserven

- Zum Beispiel bei Kompott oder Gemüse

Im Anhang finden Sie ein **Beispiel** für ein ausgefülltes Protokoll.
Wir wünschen Ihnen gutes Gelingen und bedanken uns ganz herzlich für Ihre tatkräftige Unterstützung!

Sollten beim Ausfüllen des Protokolls Fragen oder Unsicherheiten auftreten, stehen wir Ihnen jederzeit gerne zur Verfügung!

Prof. Dr. med. H. Hauner und Studienteam
Ansprechpartnerinnen:
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B.6 Beispiel für einen Tagesplan

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Beispiel für einen Tagesplan – arachidonsäurearm mit Fisch

Frühstück / Zwischenmahlzeit:

Zeit	Menge	Lebensmittel/Getränk	Zubereitung
	4 Esslöffel (50 g)	Früchtemüsli ohne Zucker	
	1	Orange	
	1 Becher (200 ml)	Milch, 1,5 % Fett	
	1 Becher	Kaffee mit Zucker	
	2 Gläser	Orangensaftschorle	
	1	Mehrkornbrötchen	
	1 Teelöffel	Pflanzenmargarine	
	2 Teelöffel	Aprikosenmarmelade	
	1	Möhre	roh, geraspelt

Mittagessen / Zwischenmahlzeit:

Zeit	Menge	Lebensmittel/Getränk	Zubereitung
	1 Portion (200 g)	Seelachs	in Butter gebraten
	1 Portion (200 g)	Reis	gekocht
	500 g	Brokkoli, Möhre, Blumenkohl	gedünstet, mit etwas Butter
	1	Banane	
	150 g	Paprika	roh
	1 Becher	Kaffee mit Milch (1,5 % Fett)	

Abendessen / Spätmahlzeit:

Zeit	Menge	Lebensmittel/Getränk	Zubereitung
	1 Scheibe	Roggenvollkornbrot	
	2 Scheiben	Weizenmischbrot	
	2 Teelöffel	Butter	
	1	Tomate (als Brotbelag)	
	2 Scheiben	Edamer 30 % Fett	
	1 Portion	Feldsalat	
	1 Esslöffel	Olivenöl	
	1 Esslöffel	Obstessig	
	1	Apfel, Boskop	ungeschält

Und nicht vergessen: reichlich Mineralwasser und Tee trinken!

Finale Version 06. Februar 2006

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Ernährungsempfehlungen für arachidonsäurearme Ernährung

Liebe Studienteilnehmerin!

Sie nehmen an einer Studie teil, die den Einfluss der mütterlichen Ernährung während Schwangerschaft und Stillzeit auf die Fettgewebsentwicklung Ihres Kindes untersucht. Ein besonderes Augenmerk liegt dabei auf der Zusammensetzung der Nahrungsfette, die Sie zu sich nehmen.

Wir bitten Sie, während der Studienzeit einige Grundregeln zu beachten und möchten Ihnen gerne ein paar Tipps zu gesunder Ernährung mit auf den Weg geben.
Das Merkblatt soll auch eine kleine Hilfestellung für den Alltag sein und Ihnen erleichtern, die Empfehlungen ohne großen Aufwand umzusetzen.

Während der Studie sollten Sie maximal **90 mg Arachidonsäure pro Tag (= 630 mg Arachidonsäure pro Woche) zu sich nehmen**.

Aus der essentiellen Fettsäure Linolsäure kann der menschliche Körper Arachidonsäure (AA) bilden. In der Nahrung kommt sie fast ausschließlich in tierischen Lebensmitteln wie z. B. in Fleisch oder Eiern vor. Pflanzliche Lebensmittel enthalten keine Arachidonsäure.

Das heißt, Obst und Gemüse, Hülsenfrüchte, Kartoffeln, Brot und Getreide, Nudeln, Müsli, Nüsse und pflanzliche Öle enthalten keine Arachidonsäure und können uneingeschränkt verzehrt werden.

In der Schwangerschaft besteht ein etwas erhöhter Energiebedarf, der allerdings häufig überschätzt wird. So empfiehlt die Deutsche Gesellschaft für Ernährung während der Schwangerschaft eine Mehrzufuhr von lediglich 250 kcal pro Tag. Aufgrund der zahlreichen Stoffwechselvorgänge im mütterlichen und kindlichen Organismus (Wachstum des Feten und der Plazenta, Neubildung von mütterlichem Gewebe) besteht während der Schwangerschaft und Stillzeit jedoch ein deutlich erhöhter Bedarf an Vitaminen und Mineralstoffen. Da der Bedarf an den einzelnen essentiellen Nährstoffen höher ist als der Energiebedarf an sich, ist eine Ernährung mit hoher Nährstoffdichte erforderlich. Das heißt, es sollten vorwiegend Lebensmittel verzehrt werden, die besonders reich an bestimmten Inhaltsstoffen aber nicht sehr energiereich sind. Beispiele wären frisches Gemüse, das viel Vitamine und Ballaststoffe aber wenig Energie enthält oder fettreduzierter Käse, der gegenüber normalem Käse weniger Kalorien, dafür aber mehr Eiweiß und Kalzium enthält.

Milch- und Milchprodukte enthalten sehr wenig Arachidonsäure aber viel **Kalzium**, das für den Knochen- und Zahnaufbau Ihres Kindes sehr wichtig ist. Der Verzehr von Milch und Milchprodukten muss nicht eingeschränkt werden. Besonders zum Verzehr geeignet sind fettreduzierte Produkte, wie z. B. Jogurt oder Milch mit 1,5 % Fett, Magerquark, Buttermilch und Hartkäse mit niedrigem Fettgehalt, wie z. B. Emmentaler, Parmesan oder Appenzeller. Wer keine Milch mag, eine Kuhmilchallergie oder eine Laktoseunverträglichkeit hat, kann auf kalziumangereicherte Sojaprodukte ausweichen oder laktosefreie Milch trinken.

Der **Eiweißbedarf** einer Schwangeren erhöht sich ab dem 4. Monat um ca. 10 g pro Tag. Hierzulande besteht jedoch keine Eiweißunterversorgung, im Gegenteil, junge Frauen nehmen eher zu viel Eiweiß zu sich. Um Ihren Bedarf an **Eiweiß** zu decken, kombinieren Sie am besten Kartoffeln und Getreide mit Milchprodukten oder Hülsenfrüchten (z. B. Pellkartoffeln mit Quark, Kartoffelgratin mit Käse, Vollkornbrot mit Käse, Milchreis oder Grießbrei, Linsensuppe mit Brot, Müsli mit Milch oder Jogurt...). Essen Sie ca. 1-2 x pro Woche arachidonsäurearmen Fisch (z. B. Forelle, Hering, Seelachs, Scholle oder Kabeljau).

Der **Fettbedarf** einer Schwangeren ist nur unwesentlich höher als der einer nicht schwangeren Frau. Hierzulande wird der Bedarf problemlos erreicht, oft sogar überschritten. Achten Sie besonders auf die **Qualität der Fette**. Verwenden Sie hochwertige Pflanzenöle wie Rapsöl, Leinöl und Olivenöl und verzichten Sie eher auf Sonnenblumen-, Maiskeim- und Distelöl. Vermeiden Sie fettige Snacks, versteckte Fette in Knabbereien, tierische Fette in Wurstprodukten und Süßwaren.

Kohlenhydrate und Ballaststoffe sollten mindestens 50 % Ihrer Energieaufnahme ausmachen. Dazu gehört ein reichlicher Verzehr von (Vollkorn-)Brot, Nudeln, Kartoffeln und Reis sowie frischem Obst und Gemüse. Ballaststoffe sind wichtig für eine gute Verdauung und kommen vor allem in Vollkornprodukten (z. B. Vollkornbrot, Vollkornnudeln, Vollkornreis), faserreichem Gemüse und Obst vor, wie z. B. in Beeren, Gurken, Paprika, Karotten, Äpfeln, Orangen, Birnen, Pilzen sowie in Trockenfrüchten und Hülsenfrüchten (z. B. Erbsen, Linsen). Gleichzeitig liefern Vollkornprodukte, Obst und Gemüse wichtige Vitamine und Mineralstoffe.

Um Heißhungergefühlen vorzubeugen, sollten Sie regelmäßig kleine **Zwischenmahlzeiten** im Verlauf des Tages einplanen. Geeignet wären z. B.:

- Frisches Obst oder Trockenfrüchte (1 Apfel, 1 Orange, getrocknete Feigen, Aprikosen…)
- 1 Naturjogurt (mit frischen Früchten)
- 1 kleines Müsli
- Früchteriegel
- 1 Scheibe Vollkornbrot oder Brötchen, dünn mit Marmelade oder Käse belegt
- 1 großes Glas Fruchtsaft oder 1 Milchshake

In der Schwangerschaft wird für den Fetus, die Plazenta und das größere mütterliche Blutvolumen zusätzliches **Eisen** benötigt. Mit einer ausgewogenen Ernährung kann der erhöhte Bedarf jedoch sichergestellt werden. Fleisch ist die beste Eisenquelle. Es genügt, 2 – 3 mal pro Woche eine kleine Portion Fleisch (ca. 150 g) und 2 – 3 mal eine Portion fettarme Wurst (ca. 30 g) zu essen. Dies entspricht gleichzeitig den Ernährungsempfehlungen für eine arachidonsäurearme Kost. Eisen aus pflanzlichen Lebensmitteln wird weniger gut vom Körper aufgenommen. Die Aufnahme lässt sich jedoch steigern, wenn man während einer Mahlzeit eisenreiche Lebensmittel wie z. B. Linsen, Hirse oder Vollkornbrot mit Vitamin C-reichen

Lebensmitteln wie z. B. Orangensaft, Erdbeeren, Kiwi oder Paprika, kombiniert. Trinken Sie z. B. einfach ein Glas Orangensaft zum Essen. Vermeiden Sie unmittelbar zu den Mahlzeiten schwarzen Tee oder Kaffee zu trinken, weil diese die Eisenaufnahme behindern können.

Eine ausreichende Versorgung mit **Folat** / **Folsäure** ist wichtig für die Vorbeugung von Neuralrohrdefekten sowie Lippen-, Kiefer- und Gaumenspalten. Gute Folatlieferanten sind grüne Gemüse wie Brokkoli, Spinat, Fenchel, grüne Blattsalate, Tomaten, aber auch Hülsenfrüchte, Weizen- oder Sojasprossen, Vollkornbrot sowie Erdbeeren und Orangen. Folat ist sehr empfindlich gegenüber Licht und Hitze, deswegen sollten folatreiche Lebensmittel, wenn möglich, roh verzehrt werden, z. B. als Salat, Rohkost und frisches Obst. Zusätzlich ist es empfehlenswert, jodiertes Speisesalz mit Fluorid und Folsäure zum Kochen und Würzen zu verwenden.

Jod ist wichtig für eine normale körperliche und geistige Entwicklung Ihres Kindes. Schon im Mutterleib ist das Ungeborene auf die Jodversorgung durch die Mutter zur eigenen Hormonproduktion angewiesen. Leider ist Deutschland ein Jodmangelgebiet und die meisten Lebensmittel leisten keinen nennenswerten Beitrag zur Jodversorgung. Ausnahmen sind Milch und Milchprodukte sowie Meeresfische wie Seelachs, Kabeljau und Scholle, die Sie wenigstens 1 – 2 mal pro Woche verzehren sollten. Zur Verbesserung der Jodversorgung sollten Sie jodiertes Speisesalz zum Kochen und Backen verwenden sowie nach Rücksprache mit Ihrem Frauenarzt Jodtabletten einnehmen.

Bitte sprechen Sie auch mit Ihrem Frauenarzt über eine eventuell notwendige Einnahme von Vitamin- und Mineralstoffpräparaten.

Im Folgenden finden Sie eine Liste über den Arachidonsäuregehalt von ausgewählten Lebensmitteln. Sie soll Ihnen erleichtern, Ihre tägliche Arachidonsäureaufnahme abzuschätzen und dabei helfen, Ihre Arachidonsäureaufnahme zu reduzieren.

Arachidonsäuregehalt ausgewählter Lebensmittel:

Lebensmittel	**Arachidonsäuregehalt** (mg/100 g)	**90 mg AA (Tageshöchstmenge) sind in diesen Lebensmitteln in folgender Menge enthalten**
Fleisch		
Kalbfleisch (Filet)	55	150 g
Rindfleisch (Filet)	30	250 - 300 g
Hühnchenbrust (mit Haut)	160	50 g
Hühnchenschenkel (mit Haut)	330	30 g
Putenbrust (ohne Haut)	50	170 g
Schweinefleisch (Filet)	50	170 g
Schweinefleisch	160	50 g
Schweinespeck (durchwachsen)	250	30 g
Schweineleber	490	20 g
Schweineniere	350	25 g
Lammfleisch (Muskelfleisch)	10	900 g

Wurst & Schinken		
Gekochter Schinken (Schwein)	50	170 g
Fleischwurst und Würstchen	120	70 g
Geräucherter Schinken	130	60 g
Leberwurst	200	45 g
Milch & Milchprodukte		
Buttermilch	1	Relativ arachidonsäurearm, kalziumreich, täglicher Verzehr empfehlenswert, z. B. 2 Gläser Milch (1,5 %), 1 Scheibe Käse (30 %), 1 Portion Brie (30 %) pro Tag
Fettreduzierte Milch 1,5 % Fett	2	
Jogurt 1,5 % Fett	2	
Jogurt 3,5 % Fett	4	
Vollmilch 3,5 % Fett	4	
Speisequark 20 % Fett i. Tr.	5	
Saure Sahne 10 % Fett	11	
Camembert 30 % Fett i. Tr.	13	
Schnittkäse 45 % Fett i. Tr.	28	
Emmentaler 45 % Fett i. Tr.	30	
Schlagsahne 30 % Fett	32	
Camembert 60 % Fett i. Tr.	34	
Fette und Öle		
Butter	115	70 g
Schweineschmalz	1700	5 g
Pflanzenmargarine	-	Frei von Arachidonsäure
Pflanzenöl, versch. Sorten	-	
Fisch und Meeresfrüchte		
Seelachs (Köhler)	11	Relativ arachidonsäurearm, 1 – 2 Portionen pro Woche empfehlenswert
Kabeljau (Dorsch)	17	
Zander	20	
Forelle	25	
Hering (Atlantik)	37	
Scholle	60	150 g
Garnele, Krabbe, Miesmuschel	70	120 g
Ölsardine	90	100 g
Karpfen	120	70 g
Makrele	170	50 g
Lachs	190	45 g
Rotbarsch	240	35 g
Thunfisch (Dose)	244	35 g
Schillerlocken	600	15 g
Ei		
Hühnerei	70	120 g
1 Hühnerei ca. 50 g	35	2 Eier
Hühnereigelb	210	40 g

Wir bitten Sie, höchstens 2 – 3 Portionen Fleisch (150 g) und Wurst (30 g) pro Woche zu essen und dabei darauf zu achten, möglichst arachidonsäurearmes Fleisch zu bevorzugen (Lammfilet bzw. Filet vom Rind, Kalb und Schwein, sowie Huhn oder Pute ohne Haut und magerer Schinken bzw. Geflügelwurst).

Achten Sie bitte auf **„versteckte" tierische Fette** und **Ei** in Fertiggerichten und Backwaren, z. B. in Kuchen, Plätzchen, Pralinen und Schokoriegeln.

Auch **Soßen** und **Tiefkühlkost** (z. B. Lasagne, Rahmgeschnetzeltes, Chicken wings usw.) enthalten oft versteckte Fette.

Bitte achten Sie auch auf die Verwendung von Fleisch und Ei in **Fast Food** und **Snacks** (Currywurst, Döner Kebab, Hamburger, Big Mac, Spaghetti Bolognese…).

Im Anhang finden Sie Vorschläge und Beispiele zur Lebensmittelauswahl für eine arachidonsäurearme Ernährung.

Bitte bewahren Sie die Ihnen zur Verfügung gestellten Weichkapseln immer im **Kühlschrank** auf und entnehmen Sie immer nur die zum Verbrauch bestimmte Menge.

Sollten während der Studie Unsicherheiten oder Fragen auftreten, stehen wir Ihnen jederzeit gerne zur Verfügung!

Prof. Dr. med. H. Hauner und Studienteam

Ansprechpartnerinnen:

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B.8 Einverständniserklärung Muttermilchentnahme

Else Kröner-Fresenius-Zentrum für
Ernährungsmedizin
des Klinikums rechts der Isar
der Technischen Universität München
Direktor: Univ.-Prof. Dr. Hans Hauner

Gewinnung einer Probe Muttermilch

Sehr geehrte Studienteilnehmerin,

um den Einfluss der Ernährung der Mutter während der Stillzeit auf die Zusammensetzung der Muttermilch und die Versorgung des Säuglings mit Energie und wichtigen Nährstoffen zu untersuchen, ist es notwendig, von einer Muttermilchmahlzeit eine kleine Probe (ca. 20 ml) zu entnehmen.

Die Probe Muttermilch muss aus einer gesamten Muttermilchmahlzeit entnommen werden, da sich die Zusammensetzung der Muttermilch im Verlauf des Stillens etwas verändert und nicht alle Nährstoffe immer in derselben Konzentration vorhanden sind.

Die Milch wird - bevor Sie mit dem Stillen beginnen - mit Hilfe einer speziellen, sterilisierten Milchpumpe in eine Trinkflasche abgepumpt. Die Brustwarzen müssen vor dem Abpumpen sorgfältig gereinigt werden. Es dürfen keinesfalls Fettrückstände auf der Haut (z. B. von Pflegemitteln) in die Muttermilch gelangen. Beide Seiten der Brust müssen wie beim Stillen völlig entleert werden.

Die gesammelte Milch in der Trinkflasche wird danach durch Schütteln vermischt und anschließend werden ca. 20 ml in das beschriftete Muttermilchprobengefäß abgegossen. Dieses wird sofort bis zur Analyse tiefgefroren, um mögliche Veränderungen der Muttermilch auszuschließen.

Nach der Probenentnahme kann die abgepumpte Milch mit dem Fläschchen selbstverständlich an den Säugling verfüttert werden.

Rückstände von Spülmitteln, Desinfektionsmitteln oder Präparaten zur Kaltsterilisation können empfindliche Nährstoffe in der Muttermilch verändern. Daher dürfen zur Reinigung der Muttermilchpumpe und der dazugehörigen Trinkflasche nur heißes Wasser und eine nur für diese Milchpumpe bestimmte Flaschenbürste verwendet werden. Nach jedem Gebrauch wird die Pumpe durch Auskochen oder eine vergleichbare Methode sterilisiert.

Else Kröner-Fresenius-Zentrum für
Ernährungsmedizin
des Klinikums rechts der Isar
der Technischen Universität München
Direktor: Univ.-Prof. Dr. Hans Hauner

Ich habe die Teilnehmerinformation zur Gewinnung einer Probe Muttermilch gelesen und fühle mich ausreichend informiert. Ich hatte genügend Gelegenheit Fragen zu stellen und meine Fragen wurden ausreichend beantwortet. Ich erkläre mich mit dem Ablauf der oben beschriebenen Gewinnung einer Probe Muttermilch einverstanden. Ich wurde ferner darauf hingewiesen, dass es mir jederzeit frei steht, mein Einverständnis ohne Angabe von Gründen zurückzuziehen, ohne dass mir dadurch irgendwelche Nachteile entstehen.
Ich erkläre mich bereit zwei Mal während der Stillzeit (6. Woche und 4. Monat nach der Geburt) ca. 20 ml Muttermilch zur Verfügung zu stellen.

Eine Kopie der Teilnehmerinformation und der Einverständniserklärung habe ich erhalten.

München, den _____

_____ _____
Name der Studienteilnehmerin Unterschrift

_____ _____
Name der aufklärender Person / des Arztes Unterschrift

Sollten Probleme auftreten oder Sie noch Fragen haben, stehen wir Ihnen jederzeit gerne zur Verfügung!

Prof. Dr. med. H. Hauner und Studienteam

Ansprechpartnerinnen:

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B.9 Supplement Marinol D-40

LIPID NUTRITION

PRODUCT SPECIFICATIONS

MARINOL™ D-40

Concentrated Natural Fish Oil rich in DHA

PRODUCT DESCRIPTION:

Marinol™ D-40 is concentrated natural fish oil with a high content of docosahexaenoic acid (DHA) in glyceride form.

TECHNICAL CHARACTERISTICS:

Analysis	AOCS test method	Specifications		
Appearance	Lovibond color (cell 1 ″)	< 60	yellow	
	Ce 13e-92	< 6	red	
Free fatty acid	Ca 5a-40	Max. 0.5	%	
Peroxide Value	Cd 8b-90	Max. 2	meq/Kg	

FATTY ACID COMPOSITION:

DHA C22:6	Ce 1b-89	Min. 39.5	%*	Min. 310	mg/g**
EPA C20:5	Ce 1b-89	Max. 8	%*	Max. 65	mg/g**
DHA: EPA % Ratio		Min. 5			

TOCOPHEROLS:

Mixed Natural Tocopherols	3.0	mg/g

* Values given in %: relative peak area in chromatogram.
** Values given in mg/g: absolute amount of free fatty acid per g of glyceride oil.

Lipid Nutrition Offices – Europe, The Netherlands: Phone: +31 75 629 29 11 Fax: +31 75 629 25 64 - U.S.A.: Phone: +1 815 730 5200 Fax: +1 815 730 5202 - Malaysia/Asia Pacific. Phone: + 603 89 47 8888 Fax: +603 89 47 8889
www.lipidnutrition.com E-mail: lipid.nutrition@croklaan.com

B.10 Ausschnitt CRF

INFAT - Studie

CASE REPORT FORM

Die Bedeutung des Fettsäurenmusters in der mütterlichen Nahrung während Schwangerschaft und Stillzeit für die frühe Fettgewebsentwicklung beim Menschen

The impact of nutritional fatty acids during pregnancy and lactation for early human adipose tissue development

Zentrums Nr. |__|__|__| Teilnehmer Nr. |__|__|__|__|

Vertraulich ■ Finale Version G ■ 31. Jan 2008

Zentrums Nr. Teilnehmer Nr.

V1 / Woche –1 Besuchsdatum: __ __/__ __ __/__ __ __ __
Screening Tag Monat Jahr

Demographische Daten Demog_1

Datum der Einverständniserklärung: |__|__| |__|__| |__|__|__|__| (Tag / Monat / Jahr)

Alter: |__|__| Jahre

Ethnische Herkunft:
- Kaukasisch ❑
- Schwarz ❑
- Asiatisch, Orientalisch ❑
- Andere ❑

Wenn Andere, spezifizieren _____

Betreuende/r Gynäkologe/in: _____

Seite 1

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Zentrums Nr. Teilnehmer Nr.

V1 / Woche –1
Screening

Anamnese Anam_1

Entbindungstermin: |__|__| |__|__| |__|__|__|__| (Tag / Monat / Jahr)

A01: Schwangerschaftswoche: |__|__|

A02: Gewicht vor SS-Beginn: |__|__|,|__| kg

A03: Größe: |__|__|__| cm

A04: BMI vor SS: |__|__|,|__| kg/m²

A05: Aktuelles Gewicht: |__|__|,|__| kg

A06: Gravida: |__|

A07: Para: |__|

A08: Blutdruck: Systolisch |__|__|__| mmHg / Diastolisch |__|__|__| mmHg

Hautfalten 1 HF_1

HF01: Trizeps: |__|__|,|__| mm

HF02: Bizeps: |__|__|,|__| mm

HF03: Subscapular: |__|__|,|__| mm

HF04: Suprailiacal: |__|__|,|__| mm

HF05: Armumfang: |__|__|,|__| cm

Seite 2

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Zentrums Nr. Teilnehmer Nr.

V1 / Woche –1
Screening

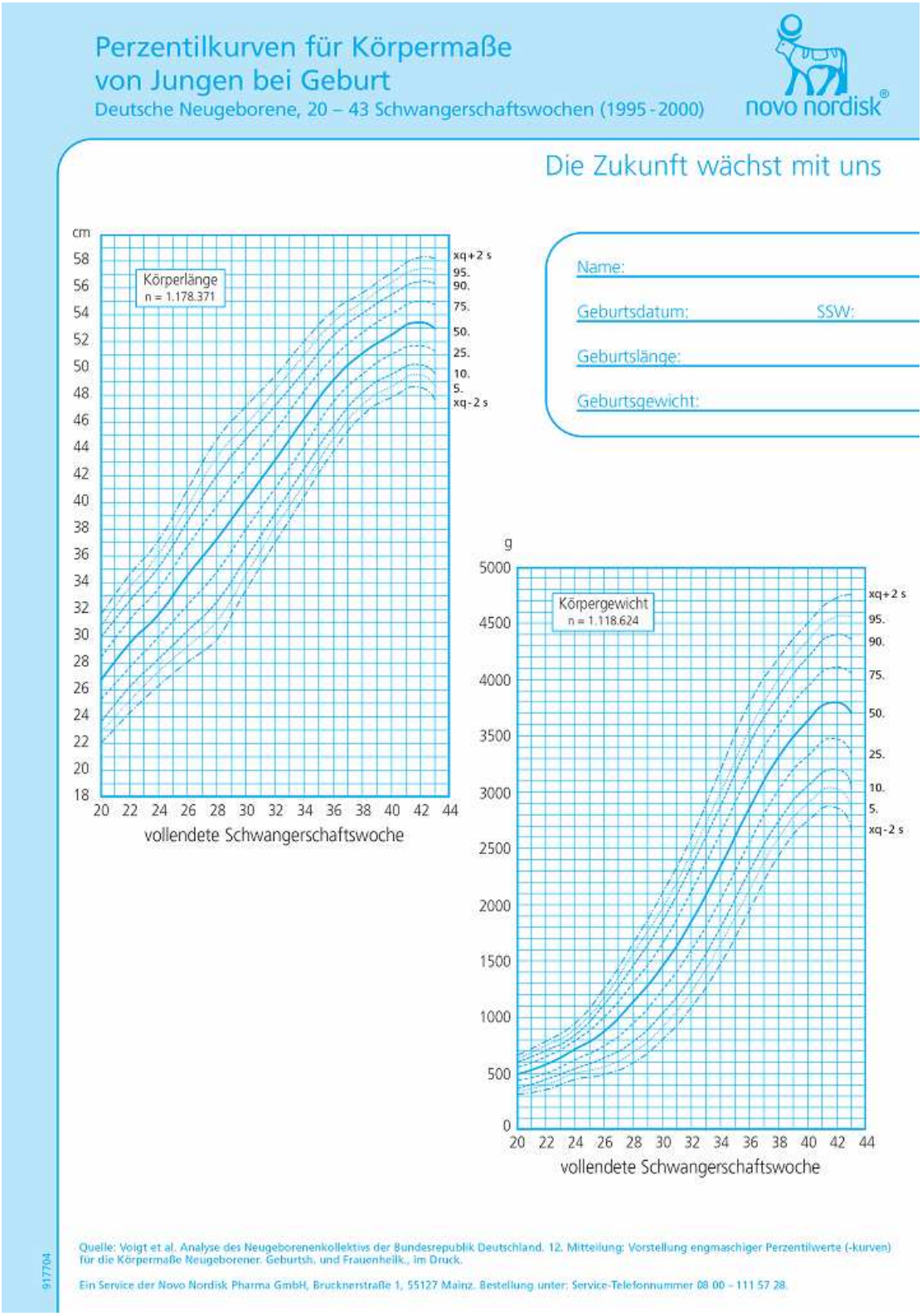
7-Tage-Ernährungsprotokoll Ep_1

EP01: Einweisung in das 7-Tage-Ernährungsprotokoll: Ja ❑ Nein ❑

Seite 3

Vertraulich ■ Finale Version G ■ 31. Jan. 2008

B.11 Perzentilkurve Geburt Größe & Gewicht



Appendix C: Tables

C.1 Dietary intake

	group	n	15. wk gest		n	32. wk gest		p*	p#	P‡
			mean ± SD	(range)		mean ± SD	(range)			
nutrient intake [kcal/d]	Intervention	68	1991 ± 338	(1125-2709)	26	2076 ± 324	(1670-2848)	0.842	> 0.99	0.904
	Control	70	1954 ± 325	(1235-2756)	22	2022 ± 462	(1237-2897)			0.485
protein intake [%]	Intervention	68	14.9 ± 2.9	(9.2-27.6)	26	15 ± 2.4	(11-20)	> 0.99	> 0.99	0.819
	Control	70	14.9 ± 2	(11.5-20.2)	22	15.1 ± 2.5	(10.8-21.4)			0.987
fat intake [%]	Intervention	68	31.9 ± 4.3	(19.4-42.4)	26	31.6 ± 4.4	(24.8-42.9)	0.286	> 0.99	0.968
	Control	70	33 ± 4.8	(19.8-42.6)	22	31.9 ± 5.1	(22.9-42.4)			0.615
carbohydrate intake [%]	Intervention	68	51 ± 5.5	(28.9-63.6)	26	51 ± 4.5	(40.7-58.2)	0.099	> 0.99	0.968
	Control	70	49 ± 4.8	(37.7-61.8)	22	51.1 ± 6.1	(40.7-63.3)			0.408
AA intake [g/d]	Intervention	68	0.132 ± 0.09	(0.023-0.410)	26	0.109 ± 0.05 #	(0.046-0.289)	0.193	0.013	0.716
	Control	70	0.150 ± 0.09	(0.019-0.514)	22	0.169 ± 0.08 #	(0.034-0.352)			0.795
AA intake [mg/1000 kcal]	Intervention	68	67.1 ± 48	(11-231)	26	51 ± 22 #	(0.024-0.106)	0.152	0.004	0.798
	Control	70	77.2 ± 48	(13-231)	22	84 ± 42 #	(0.022-0.202)			0.808

* Significantly different distribution between groups at 15th wk (Mann-Whitney-U-Test) $p < 0.05$
# Significantly different distribution between groups at 32nd wk (Mann-Whitney-U-Test) $p < 0.05$
‡ Significant change over time (Wilcoxon-Test) $p < 0.05$

C.2 Maternal anthropometry

	group	n	15. wk gest		n	32. wk gest		p*	p#	p‡
			mean ± SD	(range)		mean ± SD	(range)			
triceps SFT [mm]	Intervention	60	16.8 ± 4.5	(5.8 - 27.1)	39	17.5 ± 4.8	(8.6-27.3)	> 0.99	> 0.99	0.036
	Control	63	17.1 ± 4.7	(5.9-28.6)	45	18.1 ± 5.4	(7.6-33.7)			0.024
biceps SFT [mm]	Intervention	60	9 ± 3.9	(2.4-24.3)	39	8.8 ± 3.8	(3.4-20)	> 0.99	0.584	0.289
	Control	63	9.1 ± 3.7	(2.5-28.6)	45	9.6 ± 4.2	(3.9-26.5)			0.722
subscapular SFT [mm]	Intervention	60	13.4 ± 5.2	(5.4-29.3)	39	13.8 ± 4.6	(6.6-26.6)	0.836	0.661	< 0.001
	Control	63	13.1 ± 3.8	(7.1-23.1)	45	14.4 ± 3.9	(7.4-24)			0.002
suprailiac SFT [mm]	Intervention	50	11.1 ± 5.2	(3-27.1)	34	12.8 ± 4.7	(5.5-22.7)	> 0.99	> 0.99	0.001
	Control	50	10.7 ± 4.8	(4.4-29.3)	38	13.3 ± 4.2	(5.1-22.2)			< 0.001
arm circumference [cm]	Intervention	59	28.3 ± 2.8	(22-36.8)	39	28.4 ± 2.5 #	(24-35)	> 0.99	0.029	0.001
	Control	61	28.4 ± 2.8	(23.6-35.8)	45	29.9 ± 2.8 #	(24-36)			< 0.001
body fat [% body weight]	Intervention	60	27.3 ± 4	(14.4-36.6)	36	25.5 ± 3.8	(16.1-33.2)	> 0.99	0.662	0.020
	Control	63	27.5 ± 3.7	(17.4-35.9)	42	26.4 ± 3.8	(18.6-35.8)			0.011
LBM [% body weight]	Intervention	60	72.1 ± 4.1	(63-85)	36	74 ± 4	(66-83)	> 0.99	0.667	0.030
	Control	63	72.1 ± 7.1	(47-94)	42	73 ± 3.8	(64-81)			0.054
body fat [kg]	Intervention	60	17.4 ± 4.5	(7-32)	36	18.6 ± 4.6	(9-30)	> 0.99	0.611	< 0.001
	Control	63	16.9 ± 3.9	(8-29)	42	19.7 ± 4.7	(12-33)			< 0.001
LBM [kg]	Intervention	60	46 ± 5	(34-58)	36	54.3 ± 5	(45-65)	> 0.99	0.503	< 0.001
	Control	63	45.4 ± 6.2	(22-68)	42	55 ± 4	(47-62)			< 0.001
body weight [kg]	Intervention	86	65.7 ± 9.4	(46-103)	55	73.6 ± 9	(58-96)	> 0.99	0.494	< 0.001
	Control	86	65.9 ± 7.9	(48-87)	55	75.2 ± 7.9	(58-94)			< 0.001

* Significantly different distribution between groups at 15th wk (Mann-Whitney-U-Test) $p < 0.05$
# Significantly different distribution between groups at 32nd wk (Mann-Whitney-U-Test) $p < 0.05$
‡ Significant change over time (Wilcoxon-Test) $p < 0.05$

C.3 Infant red blood cell fatty acid profile

	group	n	cord blood		n	16. wk pp			
			mean ± SD	(range)		mean ± SD	(range)	p*	p#
18:2w6	Intervention	30	2.97 ± 0.76*	(1.33-4.24)	16	3.99 ± 1.43	(2.17-7.99)	< 0.001	0,314
	Control	28	2.19 ± 0.77	(0.02-3.47)	14	4.64 ± 1.44	(2.07-7.10)		
18:3w3	Intervention	30	0.04 ± 0.03*	(0.00-0.10)	16	0.11 ± 0.02	(0.07-0.15)	0,008	> 0.99
	Control	28	0.07 ± 0.04	(0.01-0.13)	14	0.11 ± 0.03	(0.05-0.16)		
20:3w6	Intervention	30	2.04 ± 0.89*	(0.44-3.52)	16	0.49 ± 0.29	(0.15-1.45)	0,002	0,102
	Control	28	1.26 ± 0.66	(0.53-2.57)	14	0.71 ± 0.36	(0.38-1.43)		
20:4w6	Intervention	30	8.82 ± 3.93	(1.82-13.82)	16	2.77 ± 1.89	(0.93-8.93)	0,108	0,062
	Control	28	6.56 ± 5.11	(1.81-16.32)	14	5.41 ± 4.24	(1.75-14.35)		
20:5w3	Intervention	30	0.27 ± 0.18*	(0.00-0.60)	16	0.11 ± 0.14	(0.00-0.61)	< 0.001	0,906
	Control	28	0.05 ± 0.07	(0.00-0.22)	14	0.07 ± 0.08	(0.00-0.28)		
22:4w6	Intervention	30	1.97 ± 0.92	(0.41-3.39)	16	0.48 ± 0.35#	(0.16-1.59)	0,158	0,004
	Control	28	1.58 ± 1.29	(0.40-4.10)	14	1.31 ± 1.24	(0.35-4.03)		
22:5w3	Intervention	30	0.55 ± 0.33*	(0.04-1.24)	16	0.18 ± 0.22#	(0.00-0.92)	< 0.001	0,028
	Control	28	0.38 ± 1.04	(0.01-5.60)	14	0.45 ± 0.45	(0.09-1.38)		
22:6w3	Intervention	30	4.66 ± 2.61*	(0.39-8.57)	16	1.16 ± 1.43	(0.23-6.25)	< 0.001	> 0.99
	Control	28	2.19 ± 2.34	(0.32-7.08)	14	1.48 ± 1.57	(0.23-5.59)		
transFA	Intervention	30	0.23 ± 0.13	(0.10-0.51)	16	0.27 ± 0.07	(0.14-0.43)	0,064	> 0.99
	Control	28	0.29 ± 0.15	(0.13-0.59)	14	0.27 ± 0.10	(0.08-0.38)		
w3LCP	Intervention	30	5.52 ± 3.09*	(0.42-10.36)	16	1.47 ± 1.79	(0.29-7.82)	< 0.001	> 0.99
	Control	28	2.64 ± 3.12	(0.36-12.92)	14	2.04 ± 2.09	(0.33-7.19)		
w6LCP	Intervention	30	14.13 ± 6.09	(2.94-21.22)	16	3.99 ± 2.63#	(1.31-12.64)	0,092	0,05
	Control	28	10.42 ± 7.64	(3.27-24.96)	14	7.91 ± 6.13	(2.58-19.68)		
SAFA	Intervention	30	58.85 ± 8.49*	(47.77-75.87)	16	67.74 ± 5.37	(52.95-76.67)	0,04	0,248
	Control	28	65.03 ± 9.82	(42.86-75.81)	14	63.01 ± 8.22	(47.96-72.63)		
MUFA	Intervention	30	17.99 ± 1.58*	(14.73-20.98)	16	22.16 ± 2.27	(18.06-27.41)	0,044	> 0.99
	Control	28	19.01 ± 1.64	(15.79-21.76)	14	21.78 ± 1.71	(18.99-24.67)		
PUFA	Intervention	30	22.70 ± 9.76*	(4.92-33.94)	16	9.61 ± 5.68	(3.98-28.56)	0,032	0,21
	Control	28	15.37 ± 11.14	(3.97-39.22)	14	14.74 ± 9.34	(5.12-32.66)		
18:2w6/18:3w3	Intervention	28	94.73 ± 64.86*	(16.57-228.94)	16	38.02 ± 23.14	(15.77-116.75)	0,008	0,798
	Control	26	52.59 ± 54.48	(11.13-200.96)	14	51.26 ± 35.45	(15.08-128.15)		
18:2w6/20:4w6	Intervention	30	0.40 ± 0.15	(0.25-0.73)	16	1.65 ± 0.42#	(0.89-2.34)	0,554	0,01
	Control	27	0.48 ± 0.22	(0.19-0.86)	14	1.13 ± 0.41	(0.40-1.64)		
18:3w3/20:5w3	Intervention	27	0.51 ± 0.81*	(0.04-2.75)	14	1.83 ± 1.42	(0.11-4.93)	0,002	> 0.99
	Control	18	1.88 ± 1.86	(0.08-6.07)	11	1.86 ± 1.26	(0.16-3.84)		
20:4w6/22:6w3	Intervention	30	2.26 ± 0.92*	(1.51-6.14)	16	3.13 ± 1.16#	(1.43-6.13)	< 0.001	0,004
	Control	28	4.09 ± 1.24	(1.90-5.95)	14	4.68 ± 1.42	(2.57-7.51)		

Values for fatty acids are expressed as % of total fatty acids (wt %)

transFA: tC14:1n5; tC16:1n7; tC17:1n7; tC18:1n9/7; ttC18:2n6
SFA: C4:0 - C27:0
MUFA: sum of all cis-FA with one double bond
PUFA: sum of all cis-FA with two or more double bonds
n6LCP: C20:2n6; DHyLA; AA; C22:2n6; C22:4n6; C22:5n6
n3LCP: C20:3n3; C20:4n3; EPA; C21:5n3; C22:3n3; n3-DPA; DHA

* Significantly different distribution between groups in cord blood (Mann-Whitney-U-Test) $p < 0.05$
# Significantly different distribution between groups 16 wks pp (Mann-Whitney-U-Test) $p < 0.05$

C.4 Spearman-Rho correlations between maternal PL and RBC fatty acids

	wk 15	wk 32
	(n = 125)	(n = 82)
PL AA - RBC AA	,372**	,323**
PL EPA - RBC EPA	,610**	,499**
PL DHA - RBC DHA	,548**	,543**
PL AA - PL EPA	ns	-,392**
PL AA - PL DHA	ns	-,471**
PL EPA - PL DHA	,534**	,824**
RBC AA - RBC EPA	,252**	,332**
RBC AA -RBC DHA	,329**	,379**
RBC EPA - RBC DHA	,820**	,944**
PL AA - RBC EPA	-,216*	-,417**
PL AA - RBC DHA	ns	-,390**
PL EPA - RBC AA	ns	-,432**
PL EPA - RBC DHA	,368**	,389**
PL DHA - RBC AA	ns	-,375**
PL DHA - RBC EPA	,473**	,542**

* Significant correlations; p < 0.05
** Significant correlations; p < 0.01
ns = not significant

C.5 Spearman-Rho correlations between maternal PL FAs and blood lipids

	wk 15	wk 32
	(n = 122)	(n = 82)
PL AA - TAG	ns	,342**
PL EPA - TAG	ns	-,417**
PL DHA - TAG	ns	-,315**

** Significant correlations; p < 0.01
ns = not significant

C.6 Spearman-Rho correlations between maternal (32nd wk) and cord blood PL and RBC fatty acids

	PL n = 37; RBC n = 58
mat. PL AA - cb PL AA	,725**
mat. PL EPA - cb PL EPA	,784**
mat. PL DHA - cb PL DHA	,599**
mat. RBC AA - cb RBC AA	ns
mat. RBC EPA - cb RBC EPA	,554**
mat. RBC DHA - cb RBC DHA	,452**
mat. PL AA - cb PL EPA	-,403*
mat. PL AA - cb PL DHA	ns
mat. PL EPA - cb PL AA	-,661**
mat. PL EPA - cb PL DHA	,543**
mat. PL DHA - cb PL AA	-,583**
mat. PL DHA - cb PL EPA	,788**
mat. RBC AA - cb RBC EPA	ns
mat. RBC AA - cb RBC DHA	ns
mat. RBC EPA - cb RBC AA	,279*
mat. RBC EPA - cb RBC DHA	,395**
mat. RBC DHA - cb RBC AA	,343**
mat. RBC DHA - cb RBC EPA	,560**

* Significant correlations; $p < 0.05$
** Significant correlations; $p < 0.01$
ns = not significant; mat. = maternal; cb = cord blood

C.7 Spearman-Rho correlations between maternal PL + RBC FAs and BM FAs

	wk 6 pp (n = 54)	wk 16 pp (n = 30)
PL AA - BM AA	,554**	,556**
PL EPA - BM EPA	,924**	,916**
PL DHA - BM DHA	,811**	,939**
RBC AA - BM AA	ns	,395*
RBC EPA - BM EPA	,557**	,584**
RBC DHA - BM DHA	,511**	,611**
RBC AA - BM EPA	ns	ns
RBC AA - BM DHA	ns	ns
RBC EPA - BM AA	ns	ns
RBC EPA - BM DHA	,524**	,536**
RBC DHA - BM AA	ns	ns
RBC DHA - BM EPA	,457**	,530**
PL AA - BM EPA	-,420**	-,455*
PL AA - BM DHA	-,498**	-,518**
PL EPA - BM AA	ns	ns
PL EPA - BM DHA	,852**	,766**
PL DHA - BM AA	ns	ns
PL DHA - BM EPA	,681**	,835**

* Significant correlations; $p < 0.05$
** Significant correlations; $p < 0.01$
ns = not significant

C.8 Spearman-Rho correlations between infant SFT and body fat at S1

	(n = 102)
% BF (Weststrate) - Ticeps SFT	,833**
% BF (Weststrate) - Biceps SFT	,855**
% BF (Weststrate) - subscapular SFT	,880**
% BF (Weststrate) - suprailiac SFT	,810**
% BF (Deurenberg) - Ticeps SFT	,768**
% BF (Deurenberg) - Biceps SFT	,790**
% BF (Deurenberg) - subscapular SFT	,841**
% BF (Deurenberg) - suprailiac SFT	,818**
% BF (Slaugther) - Ticeps SFT	,684**
% BF (Slaugther) - Biceps SFT	,593**
% BF (Slaugther) - subscapular SFT	,775**
% BF (Slaugther) - suprailiac SFT	,643**
FM/height2 (Weststrate) - Ticeps SFT	,404**
FM/height2 (Weststrate) - Biceps SFT	,371**
FM/height2 (Weststrate) - subscapular SFT	,411**
FM/height2 (Weststrate) - suprailiac SFT	,341**
FM/height2 (Deurenberg) - Ticeps SFT	,778**
FM/height2 (Deurenberg) - Biceps SFT	,770**
FM/height2 (Deurenberg) - subscapular SFT	,835**
FM/height2 (Deurenberg) - suprailiac SFT	,769**
FM/height2 (Slaugther) - Ticeps SFT	,725**
FM/height2 (Slaugther) - Biceps SFT	,614**
FM/height2 (Slaugther) - subscapular SFT	,788**
FM/height2 (Slaugther) - suprailiac SFT	,637**

* Significant correlations; p < 0.05
** Significant correlations; p < 0.01
ns = not significant

C.9 Linear Regression analysis tables

gestational weight gain [kg]

Modell		Nicht standardisierte Koeffizienten		Standardisierte Koeffizienten	
		B	Standardfehler	Beta	Signifikanz
unadjusted	(Konstante)	11.378	1.331		.000
	group	2.373	.849	.268	.006
adjusted	(Konstante)	15.342	15.373		.321
	Body Mass Index before pregnancy in kg/m2	-.368	.155	-.227	.020
	para	.378	.745	.050	.613
	gestational age in days	.003	.058	.006	.957
	birth_weight	.001	.001	.089	.415
	group	2.555	.848	.289	.003

a. Abhängige Variable: weight gain in pregnancy; korr. R-Quadrat = 0.098

birth weight [g]

Modell		Nicht standardisierte Koeffizienten		Koeffizienten	
		B	Standardfehler	Beta	Signifikanz
unadjusted	(Konstante)	3520.893	149.528		.000
	group	-30.446	94.570	-.031	.748
adjusted	(Konstante)	-3224.233	1542.666		.039
	Body Mass Index before pregnancy in kg/m2	-18.556	16.174	-.104	.254
	group	1.687	90.489	.002	.985
	para	178.488	73.906	.214	.018
	gestational age in days	24.652	5.336	.410	.000
	weight gain in pregnancy	8.456	10.327	.077	.415

a. Abhängige Variable: birth_weight; korr. R-Quadrat = 0.224

Ponderal Index [kg/m3]

Modell		Nicht standardisierte Koeffizienten		Koeffizienten	
		B	Standardfehler	Beta	Signifikanz
unadjusted	(Konstante)	25.788	.728		.000
	group	-.674	.460	-.138	.146
adjusted	(Konstante)	11.626	8.386		.169
	Body Mass Index before pregnancy in kg/m2	-.065	.088	-.073	.461
	group	-.869	.492	-.180	.080
	para	.695	.402	.168	.087
	gestational age in days	.053	.029	.178	.070
	weight gain in pregnancy	.045	.056	.082	.427

a. Abhängige Variable: Ponderal Index in kg/m3; korr. R-Quadrat = 0.07

C.10 Comparison of maternal and cord blood fatty acids

		mother 15. wk gest		mother 32. wk gest		cord blood	
		PL	RBC	PL	RBC	PL	RBC
	group	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD
18:2w6 (LA)	Intervention	18.22 ± 2.40	7.13 ± 1.41	17.38 ± 1.94	6.32 ± 1.27	6.73 ± 0.81	2.97 ± 0.76
	Control	18.02 ± 2.27	7.53 ± 1.03	18.98 ± 2.15	6.65 ± 1. 78	6.44 ± 1.07	2.19 ± 0.77
18:3w3 (ALA)	Intervention	0.24 ± 0.08	0.12 ± 0.02	0.27 ± 0.07	0.12 ± 0.02	0.02 ± 0.02	0.04 ± 0.03
	Control	0.22 ± 0.08	0.12 ± 0.03	0.25 ± 0.08	0.12 ± 0.02	0.01 ± 0.02	0.07 ± 0.04
20:3w6 (DHyLA)	Intervention	3.40 ± 0.64	1.49 ± 0.47	2.78 ± 0.60	1.21 ± 0.43	4.75 ± 0.47	2.04 ± 0.89
	Control	3.49 ± 0.70	1.56 ± 0.38	3.53 ± 0.67	1.45 ± 0.61	4.83 ± 0.71	1.26 ± 0.66
20:4w6 (AA)	Intervention	10.34 ± 1.45	11.17 ± 3.54	7.75 ± 0.99	8.05 ± 3.30	14.85 ± 1.74	8.82 ± 3.93
	Control	10.37 ± 1.37	11.90 ± 2.69	9.05 ± 1.27	9.64 ± 4. 22	17.77 ± 1.57	6.56 ± 5.11
20:5w3 (EPA)	Intervention	0.73 ± 0.47	0.38 ± 0.21	1.46 ± 0.63	0.65 ± 0.38	0.98 ± 0.43	0.27 ± 0.18
	Control	0.68 ± 0.37	0.39 ± 0.17	0.55 ± 0.25	0.30 ± 0.19	0.27 ± 0.12	0.05 ± 0.07
22:4w6 (Adrenic a.)	Intervention	0.45 ± 0.09	2.61 ± 0.94	0.24 ± 0.05	1.42 ± 0.62	0.64 ± 0.16	1.97 ± 0.92
	Control	0.48 ± 0.11	2.81 ± 0.74	0.41 ± 0.10	2.29 ± 1.09	0.84 ± 0.19	1.58 ± 1.29
22:5w3 (n-3 DPA)	Intervention	0.82 ± 0.22	1.66 ± 0.64	0.70 ± 0.17	1.23 ± 0.63	0.50 ± 0.19	0.55 ± 0.33
	Control	0.91 ± 0.22	1.77 ± 0.53	0.69 ± 0.12	1.43 ± 0.74	0.41 ± 0.18	0.38 ± 1.04
22:6w3 (DHA)	Intervention	5.06 ± 1.13	4.32 ± 1.82	8.37 ± 1.14	6.59 ± 3.35	7.95 ± 1.34	4.66 ± 2.61
	Control	4.80 ± 1.03	4.24 ± 1.41	4.63 ± 0.91	3.97 ± 2.16	6.04 ± 1.78	2.19 ± 2.34
transFA	Intervention	0.23 ± 0.08	0.24 ± 0.06	0.21 ± 0.07	0.24 ± 0.06	0.16 ± 0.14	0.23 ± 0.13
	Control	0.23 ± 0.09	0.23 ± 0.04	0.23 ± 0.07	0.25 ± 0.05	0.09 ± 0.09	0.29 ± 0.15
w3LCP	Intervention	6.78 ± 1.50	6.43 ± 2.60	10.72 ± 1.53	8.53 ± 4.30	9.56 ± 1.54	5.52 ± 3.09
	Control	6.56 ± 1.37	6.48 ± 2.00	6.03 ± 1.16	5.76 ± 3.06	6.77 ± 1.97	2.64 ± 3.12
w6LCP	Intervention	15.21 ± 1.67	16.13 ± 5.01	11.55 ± 1.32	11.33 ± 4.48	21.38 ± 1.63	14.13 ± 6.09
	Control	15.40 ± 1.66	17.23 ± 3.78	14.14 ± 1.67	14.33 ± 6.21	25.07 ± 1.52	10.42 ± 7.64
SAFA	Intervention	45.06 ± 0.67	49.68 ± 7.46	46.05 ± 1.01	52.25 ± 8.42	48.04 ± 0.61	58.85 ± 8.49
	Control	45.45 ± 2.33	48.43 ± 4.81	45.82 ± 0.95	51.61 ± 9.01	47.96 ± 0.79	65.03 ± 9.82
MUFA	Intervention	13.98 ± 1.13	19.98 ± 1.40	13.60 ± 1.71	20.92 ± 1.61	13.84 ± 1.28	17.99 ± 1.58
	Control	13.85 ± 1.29	19.70 ± 1.50	14.30 ± 1.89	20.99 ± 1.97	13.41 ± 1.57	19.01 ± 1.64
PUFA	Intervention	40.53 ± 1.32	29.87 ± 8.55	39.99 ± 1.31	26.35 ± 9.76	37.76 ± 1.44	22.70 ± 9.76
	Control	39.99 ± 2.71	31.41 ± 6.03	39.02 ± 3.04	26.93 ± 10.73	38.37 ± 1.45	15.37 ± 11.14
18:2w6/18:3w3	Intervention	85.02 ± 34.72	62.61 ± 17.98	69.33 ± 20.86	51.56 ± 12.39	199.6 ± 65.95	94.73 ± 64.86
	Control	91.97 ± 41.86	68.63 ± 17.51	83.48 ± 29.19	55.38 ± 16.93	241.0 ± 91.24	52.59 ± 54.48
18:2w6/20:4w6	Intervention	1.81 ± 0.41	0.73 ± 0.33	2.29 ± 0.45	0.97 ± 0.50	0.46 ± 0.09	0.40 ± 0.15
	Control	1.75± 0.44	0.67 ± 0.18	2.17 ± 0.53	0.89 ± 0.49	0 .37 ± 0.08	0.48 ± 0.22
18:3w3/20:5w3	Intervention	0.4 ± 0.18	0.49 ± 0.63	0.20 ± 0.07	0.41 ± 0.57	not detected	0.51 ± 0.81
	Control	0.38 ± 0.18	0.37 ± 0.26	0.54 ± 0.30	0.70 ± 0.81	not detected	1.88 ± 1.86
20:4w6/22:6w3	Intervention	2.15 ± 0.59	3.06 ± 1.27	0.95 ± 0.22	1.52 ± 0.80	1.93 ± 0.44	2.26 ± 0.92
	Control	2.25 ± 0.52	3.05 ± 0.85	2.01 ± 0.42	3.14 ± 1.49	3.19 ± 0.98	4.09 ± 1.24
ratio w6/w3	Intervention	4.76	3.55	2.63	2.04	2.93	3.08
	Control	4.93	3.75	5.27	3.57	4.65	4.65
ratio w6LCP/w3LCP	Intervention	2.24	2.51	1.07	1.32	2.23	2.55
	Control	2.35	2.66	2.34	2.45	3.70	3.95

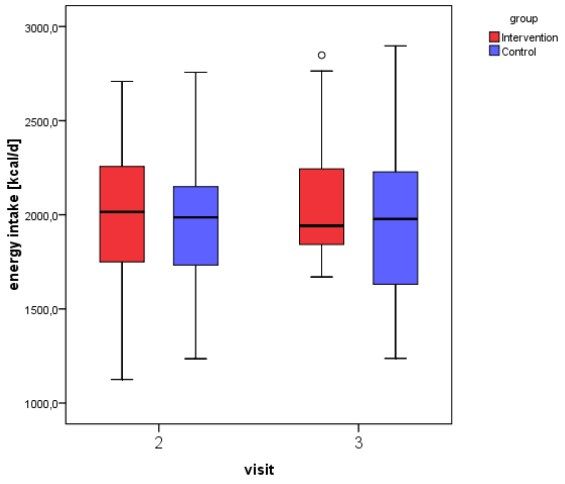
Values for fatty acids are expressed as % of total fatty acids (wt %)

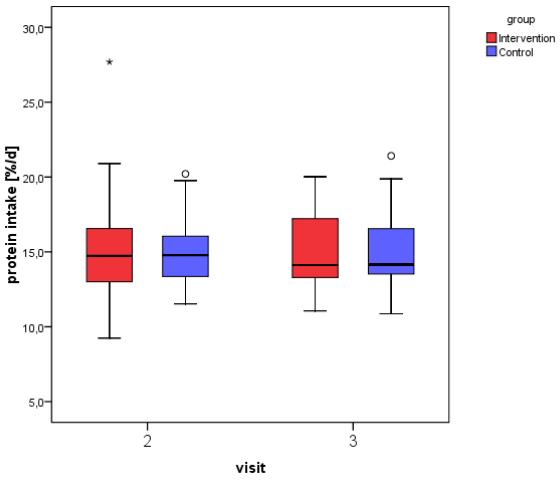
transFA: tC14:1n5; tC16:1n7; tC17:1n7; tC18:1n9/7; ttC18:2n6
SFA: C4:0 - C27:0
MUFA: sum of all cis-FA with one double bond
PUFA: sum of all cis-FA with two or more double bonds
w6: LA + w6LCP
w3: ALA + w3LCP
w6LCP: C20:2n6; DHyLA; AA; C22:2n6; C22:4n6; C22:5n6
w3LCP: C20:3n3; C20:4n3; EPA; C21:5n3; C22:3n3; n3-DPA; DHA

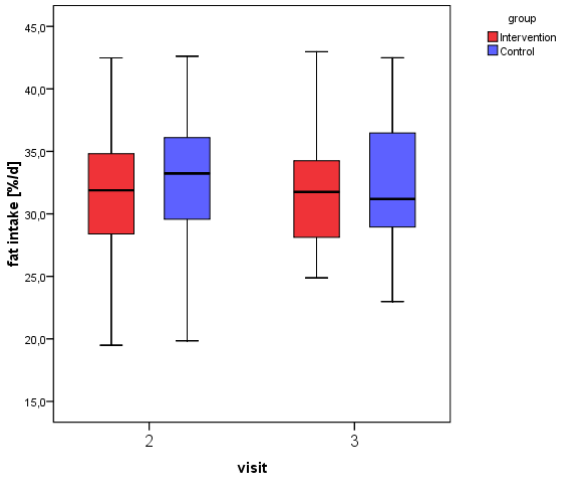
Appendix D: Figures

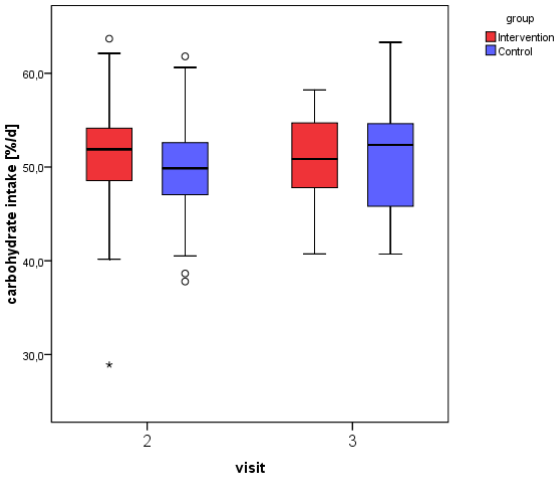
D.1 Dietary intake

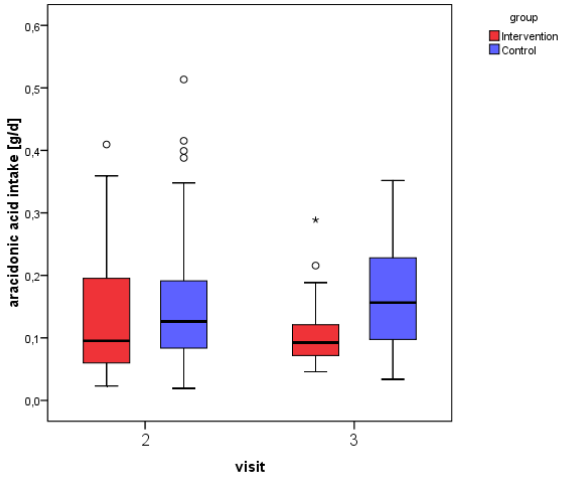
visit **2** (15th wk): n (IG/CG) = 68/70; visit **3** (32nd wk): n = 26/22

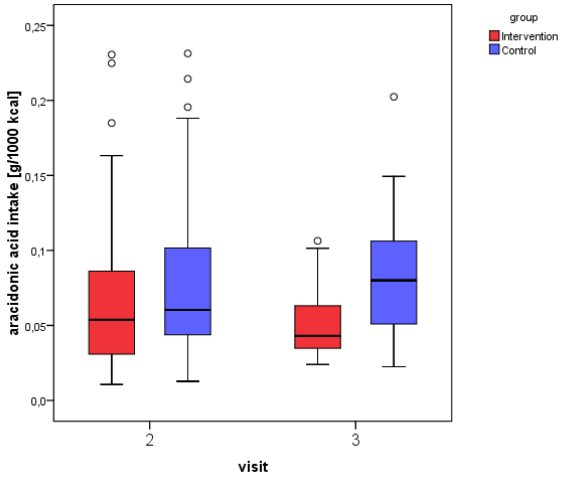








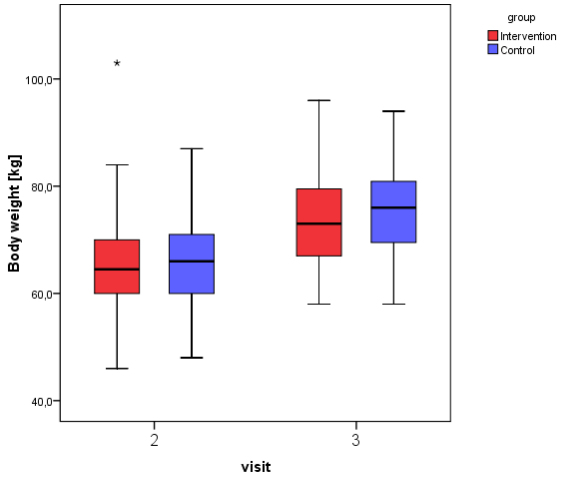


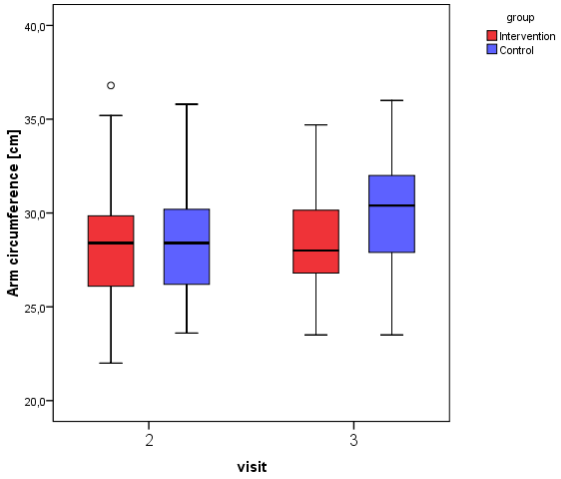


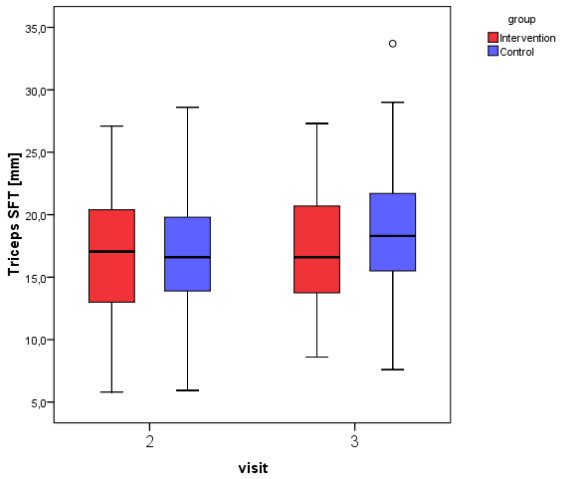
Bottom and top edges of the box are located at 25th and 75th percentiles, center horizontal line is drawn at the median, whiskers mark the maximum and minimum. Outliners are shown as dots, extremes as asterisk.

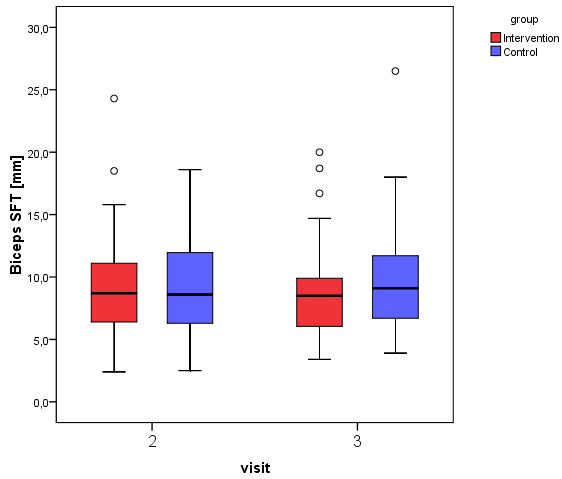
D.2 Maternal anthropometry

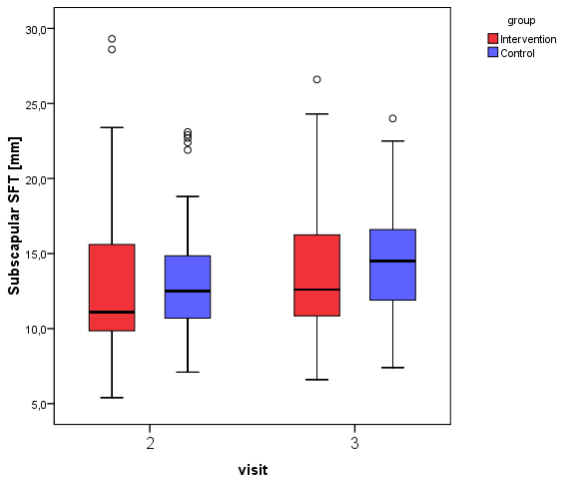
visit **2** (15th wk): n (IG/CG) = 60/63; visit **3** (32nd wk) = 39/45

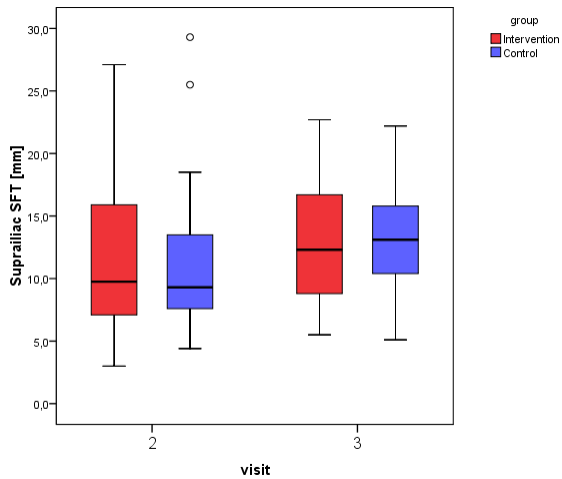












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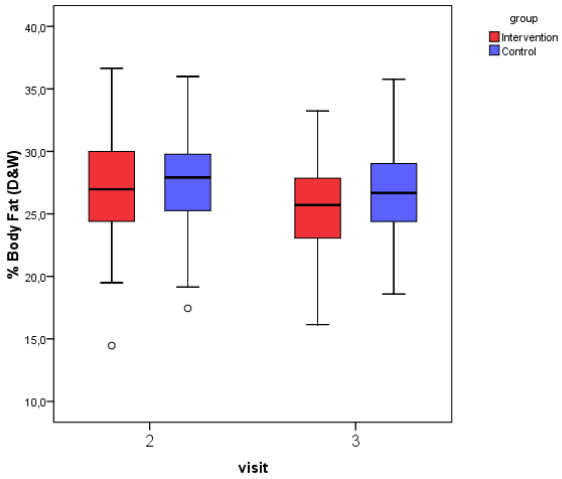
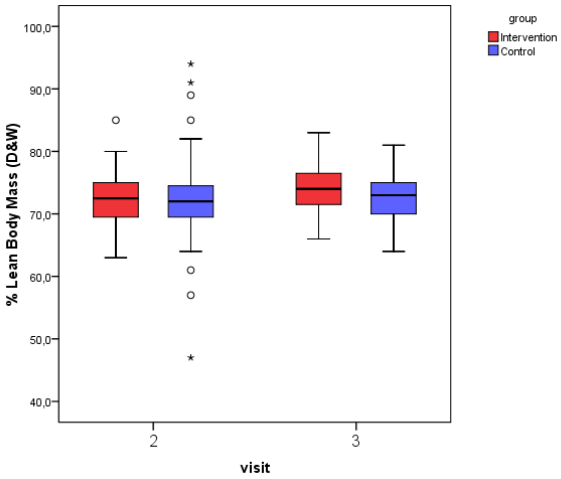
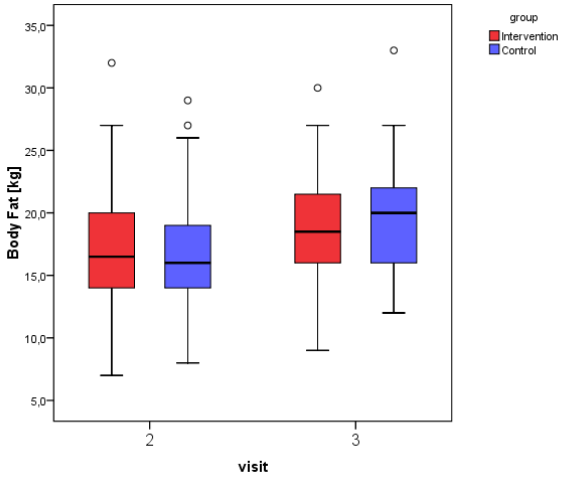
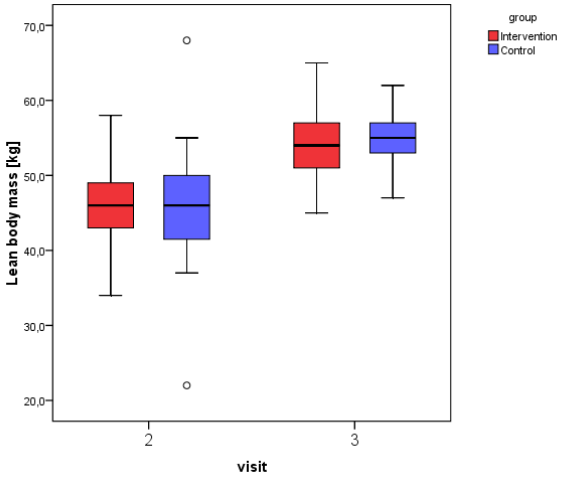




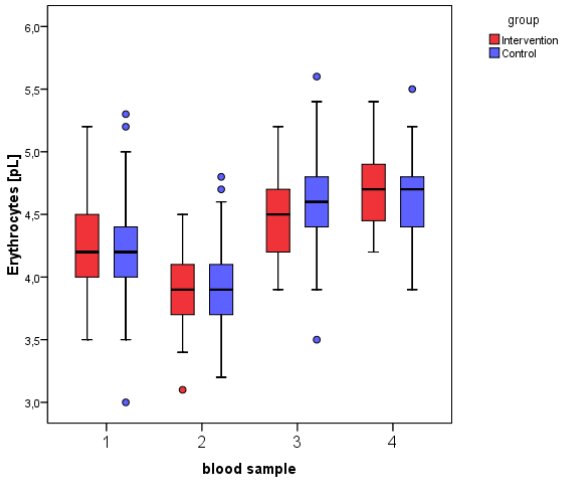


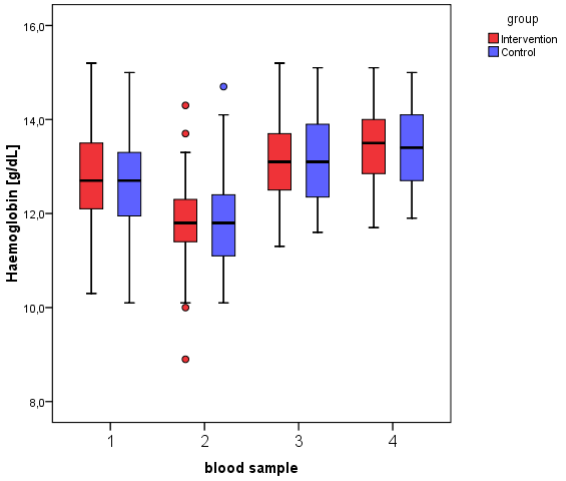


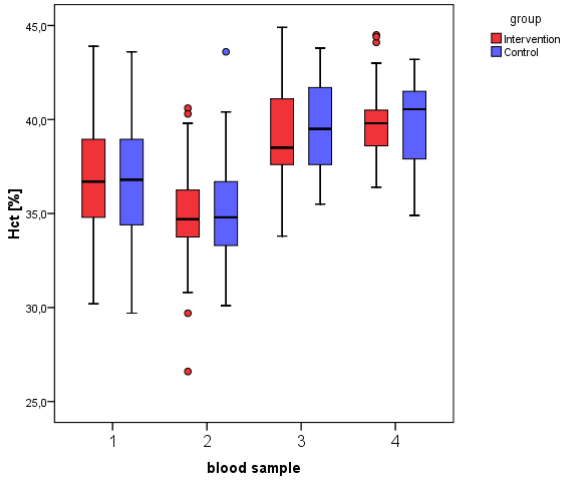
Bottom and top edges of the box are located at 25th and 75th percentiles, center horizontal line is drawn at the median, whiskers mark the maximum and minimum. Outliners are shown as dots, extremes as asterisk.

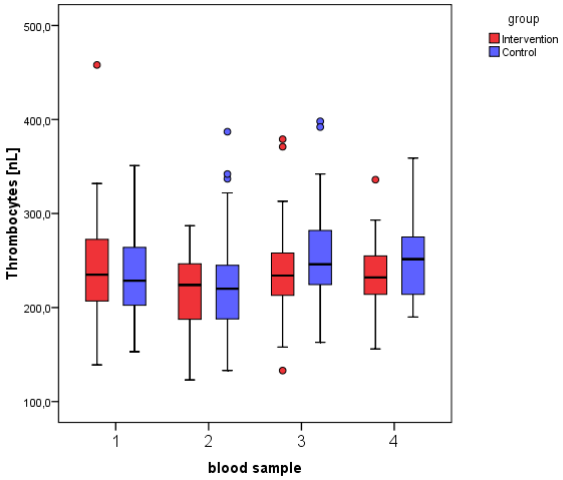
D.3 Maternal biochemical parameters and blood lipids

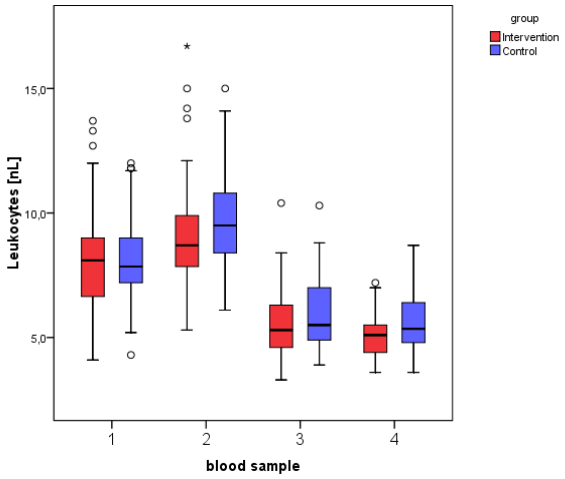
Blood sample (BS) **1** (15th wk): n (IG/SG) = 83/84; BS **2** (32nd wk): n = 59/61; BS **3** (6th wk pp): n = 42/35; BS **4** (16th wk pp): n = 27/22

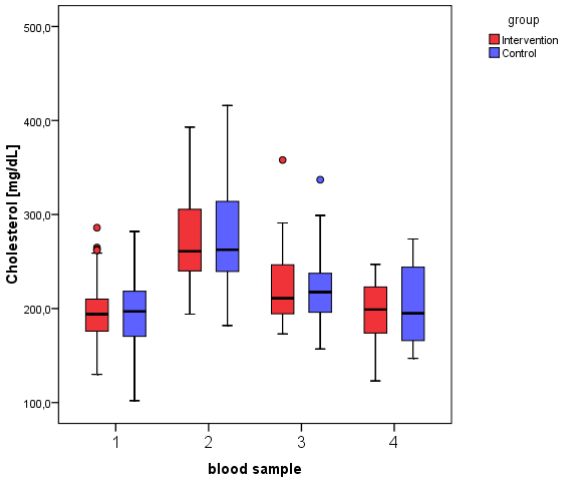




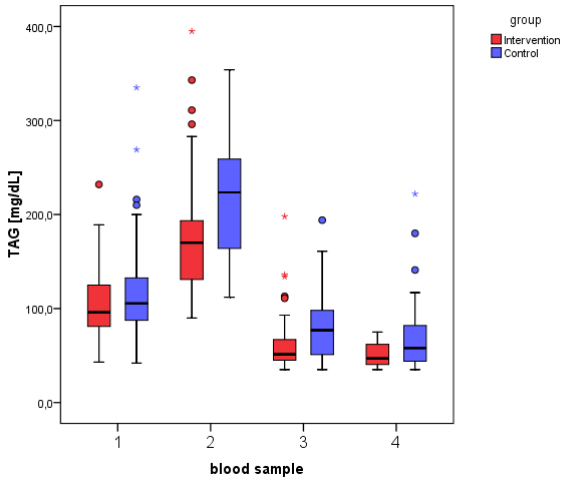


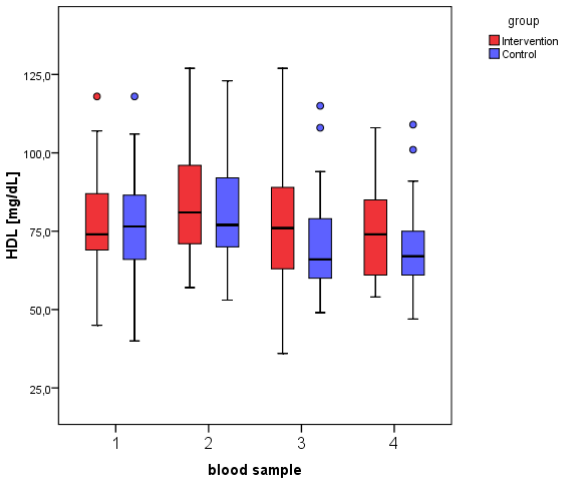


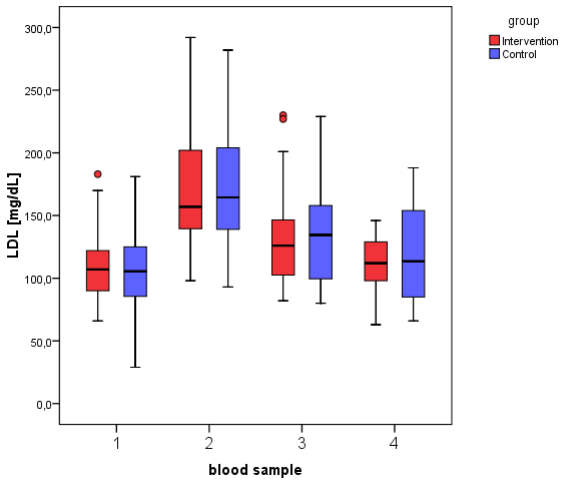


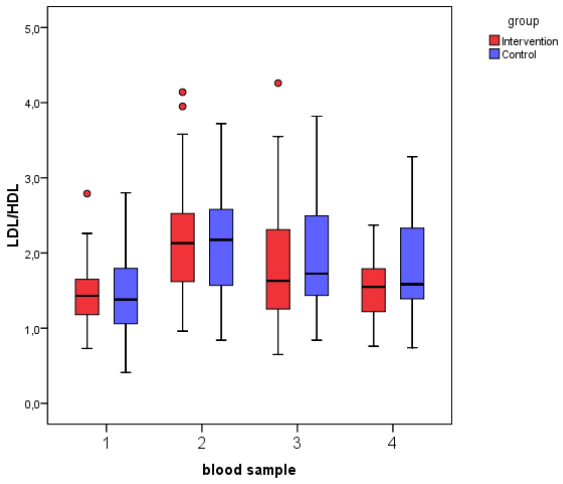


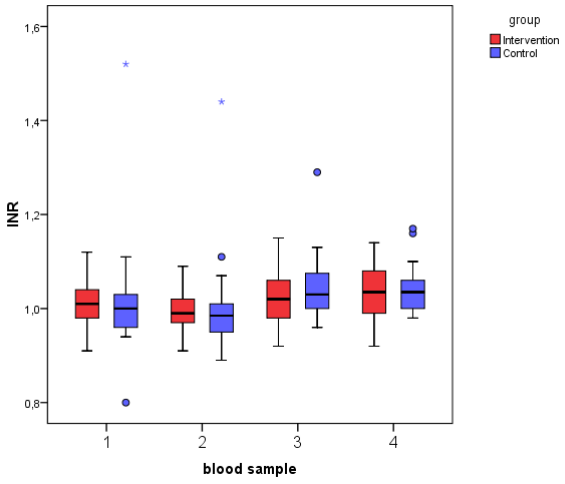
Bottom and top edges of the box are located at 25th and 75th percentiles, center horizontal line is drawn at the median, whiskers mark the maximum and minimum. Outliners are shown as dots, extremes as asterisk.

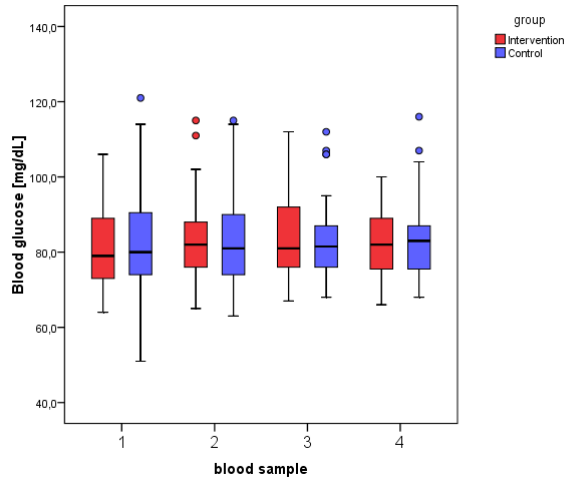








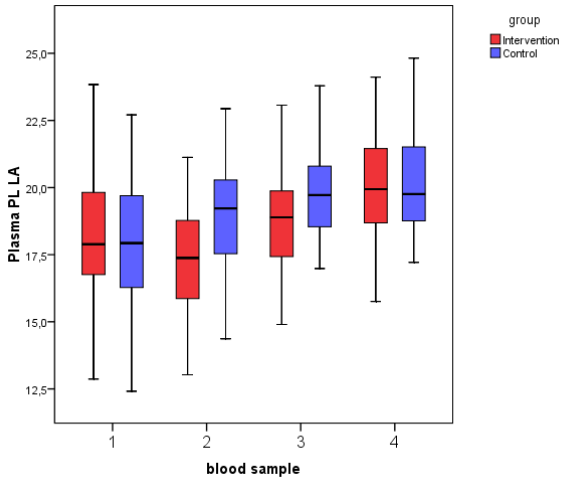


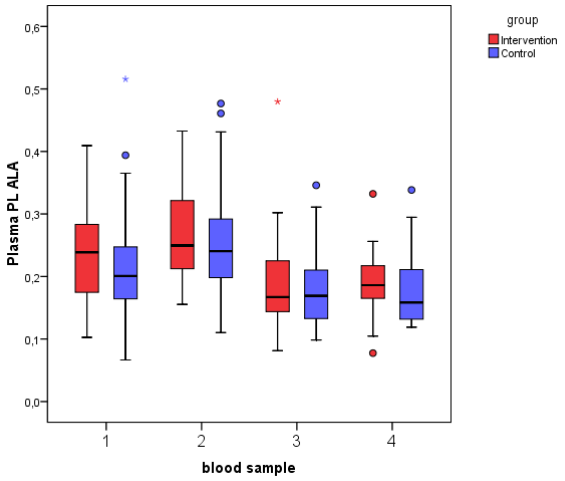


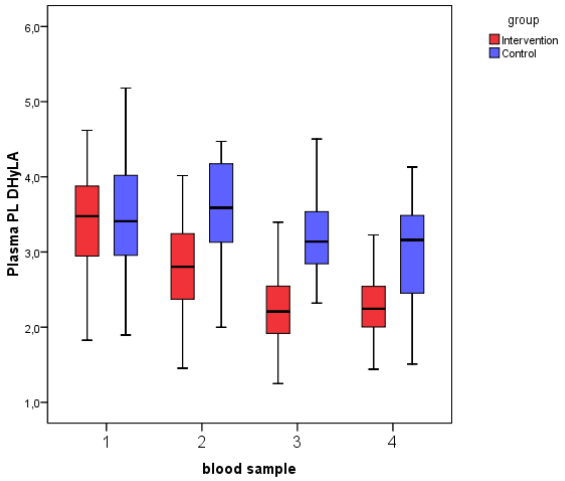
Bottom and top edges of the box are located at 25th and 75th percentiles, center horizontal line is drawn at the median, whiskers mark the maximum and minimum. Outliners are shown as dots, extremes as asterisk.

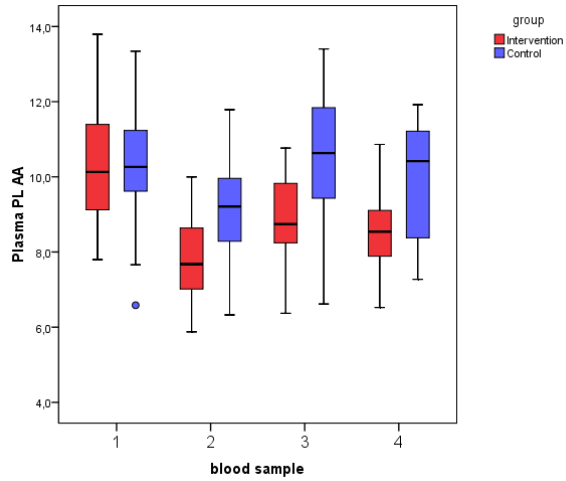
D.4 Maternal plasma PL fatty acid profile

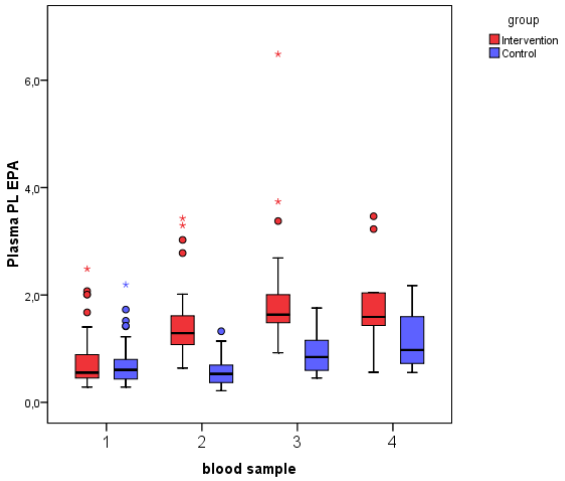
BS **1** (15th wk): n (IG/CG) = 64/63; BS **2** (32nd wk): n = 42/40; BS **3** (6th wk pp): n = 33/24; BS **4** (16th wk pp): n = 16/18

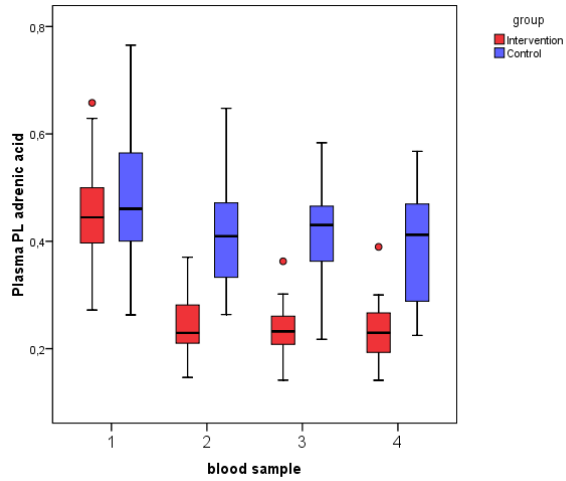




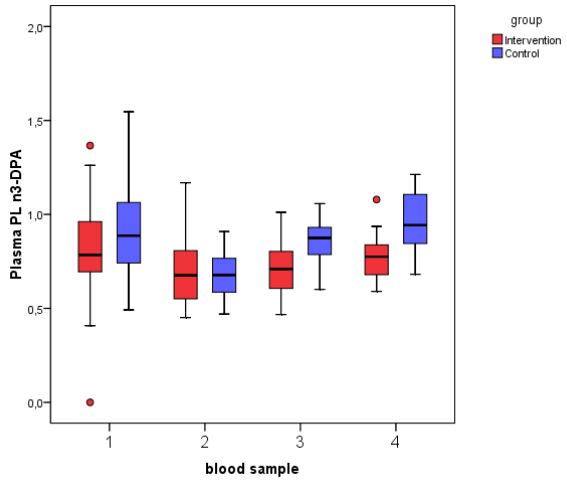


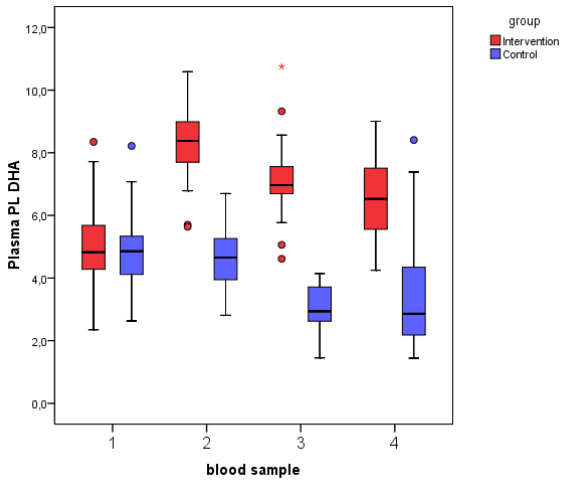


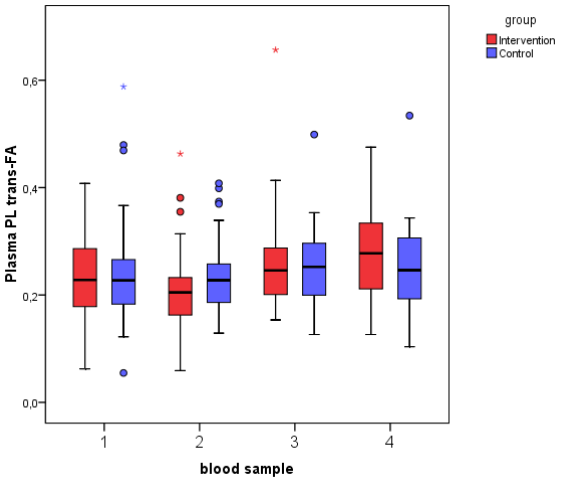


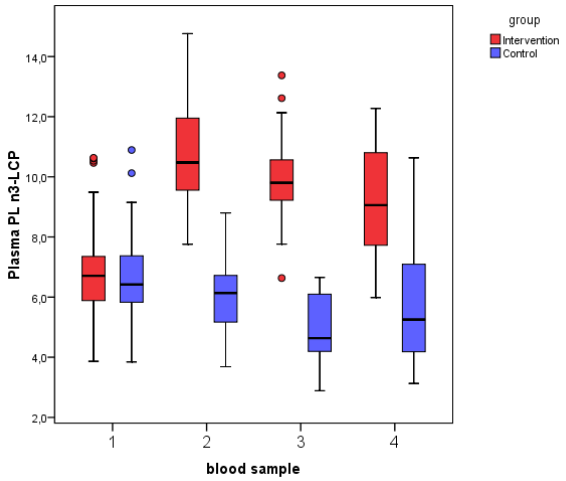


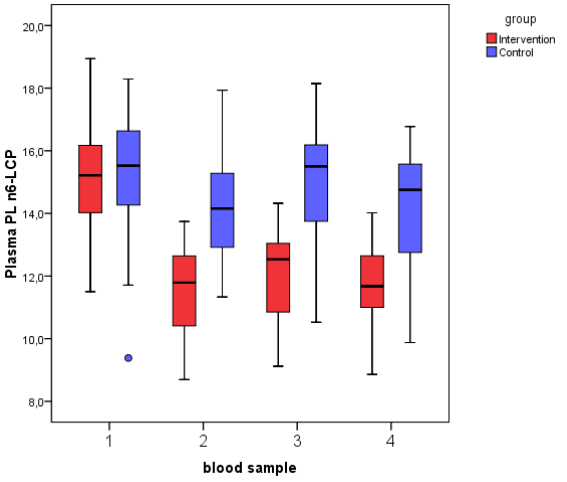
Bottom and top edges of the box are located at 25th and 75th percentiles, center horizontal line is drawn at the median, whiskers mark the maximum and minimum. Outliners are shown as dots, extremes as asterisk.

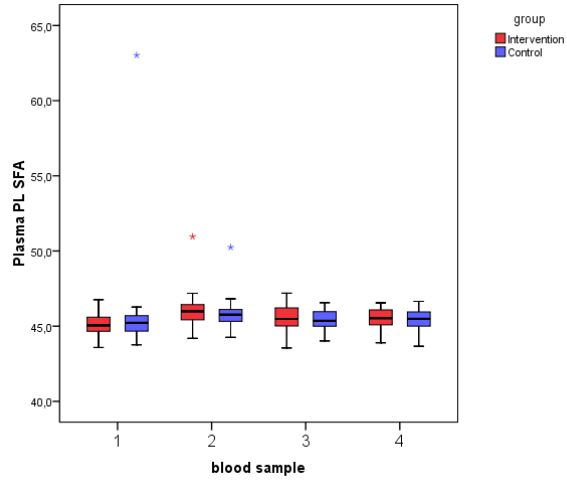




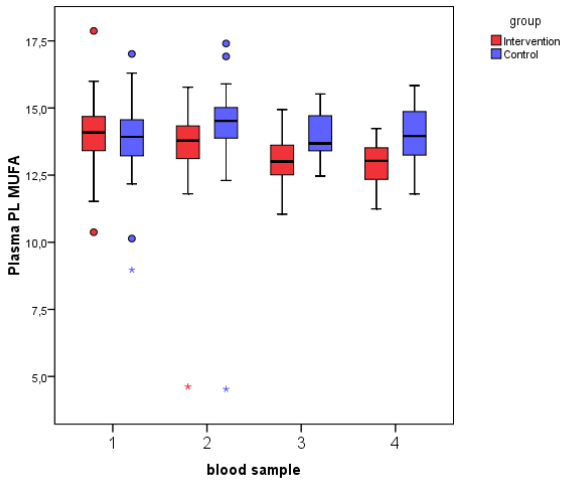


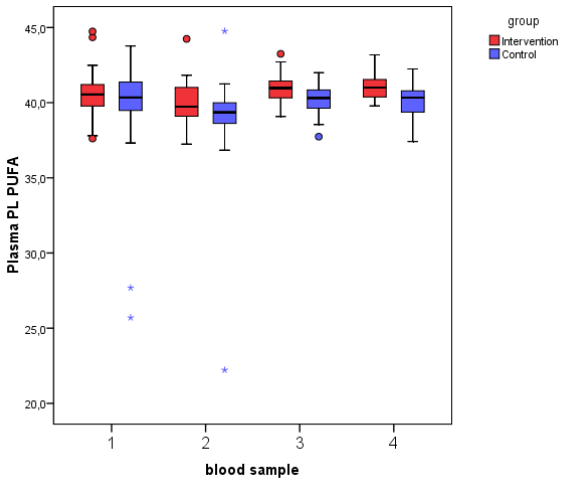


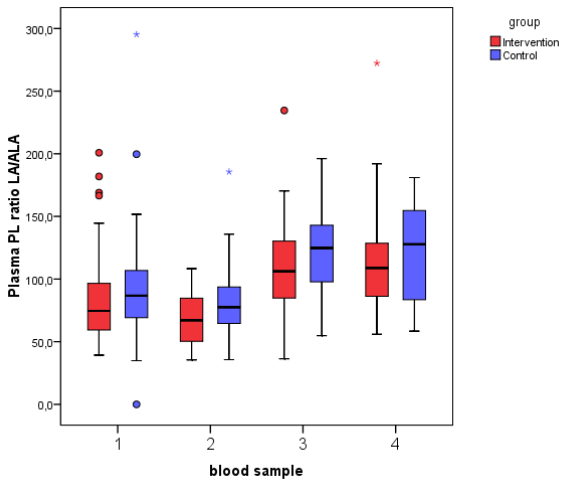


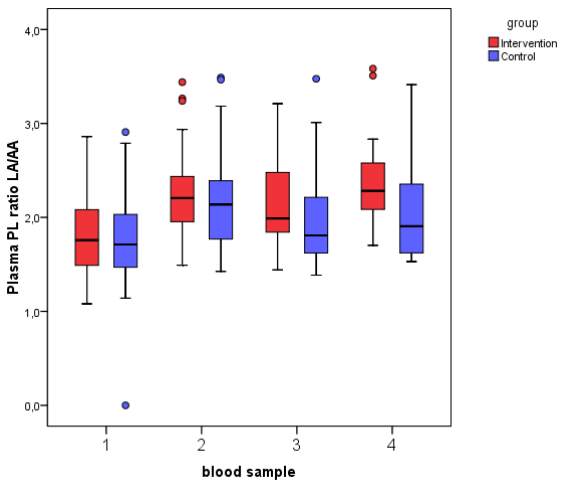


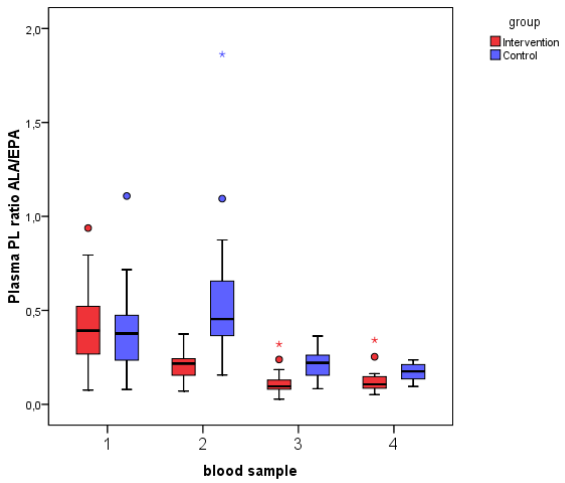
Bottom and top edges of the box are located at 25th and 75th percentiles, center horizontal line is drawn at the median, whiskers mark the maximum and minimum. Outliners are shown as dots, extremes as asterisk.

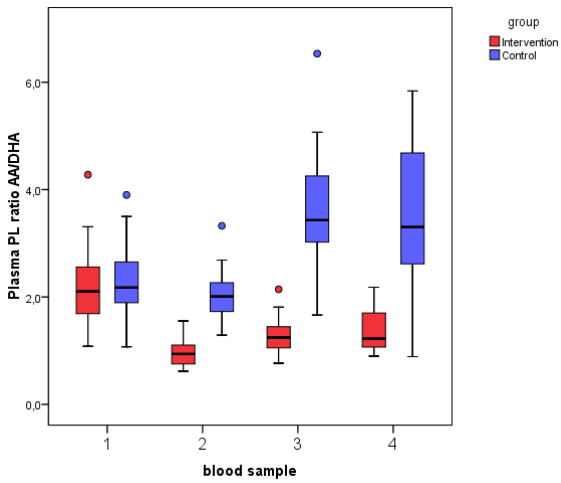








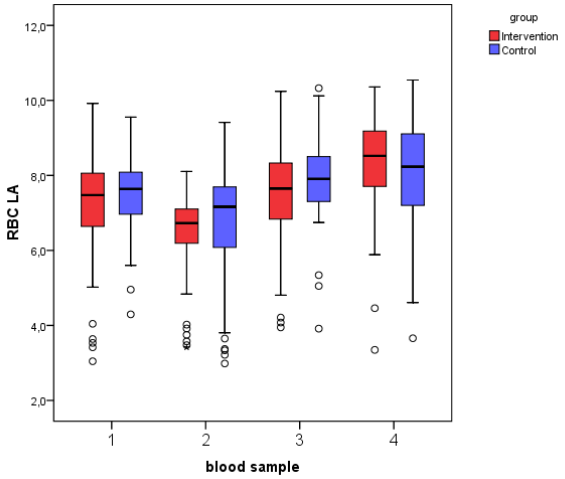


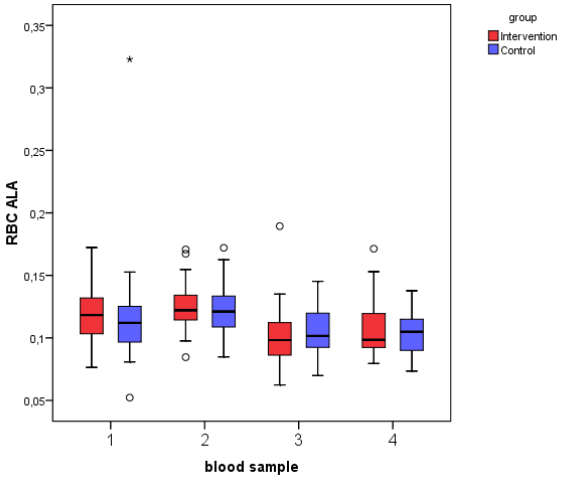


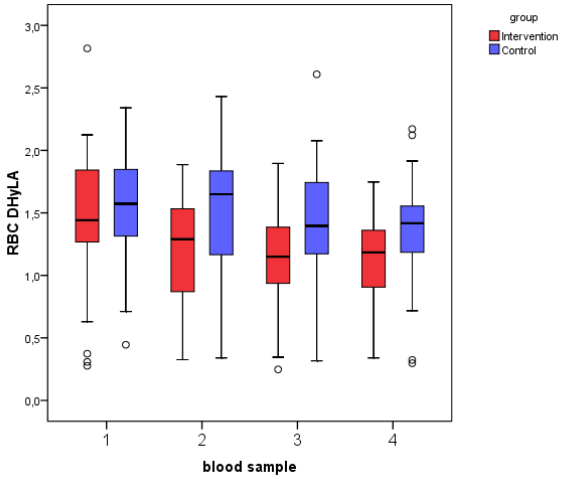
Bottom and top edges of the box are located at 25th and 75th percentiles, center horizontal line is drawn at the median, whiskers mark the maximum and minimum. Outliners are shown as dots, extremes as asterisk.

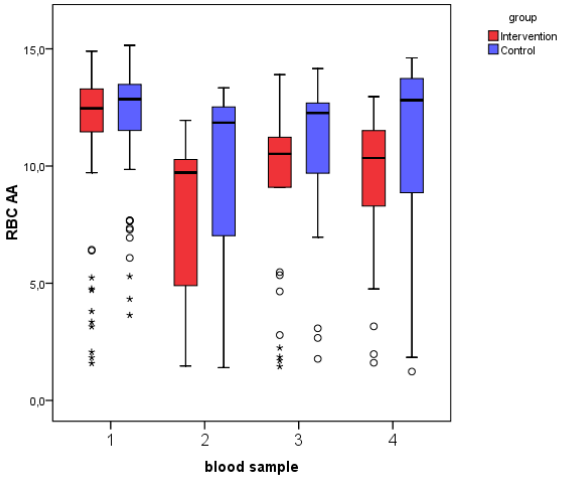
D.5 Maternal red blood cell fatty acid profile

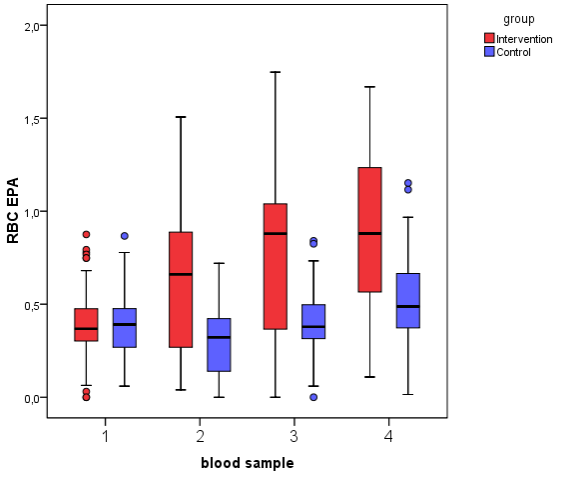
BS **1** (15th wk): n (IG/CG) = 63/62; BS **2** (32nd wk): n = 42/40; BS **3** (6th wk pp): n = 34/25; BS **4** (16th wk pp): n = 10/17

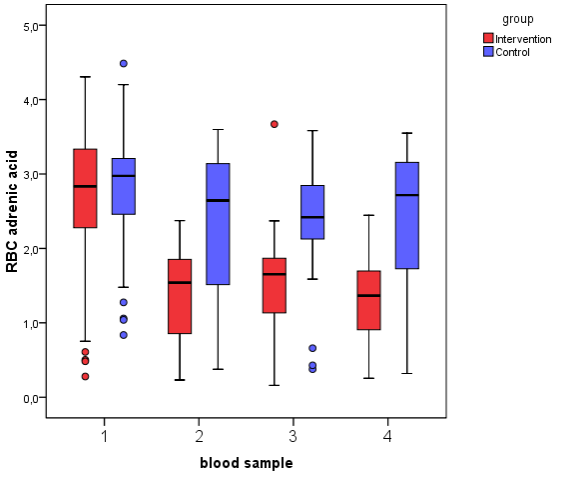




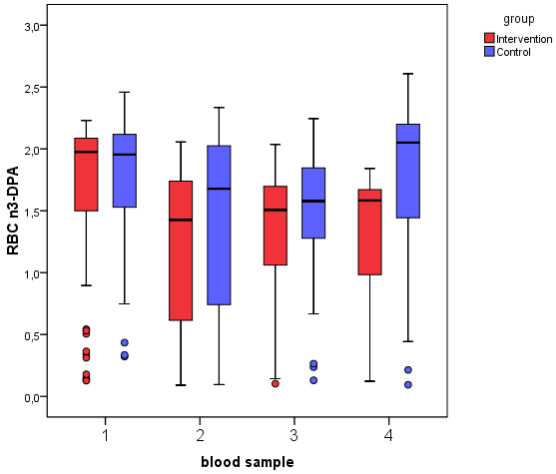


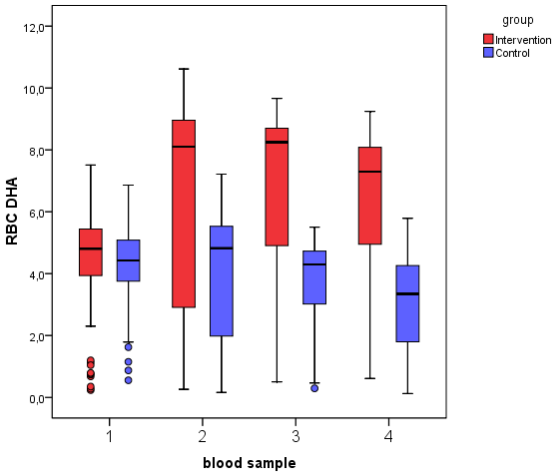


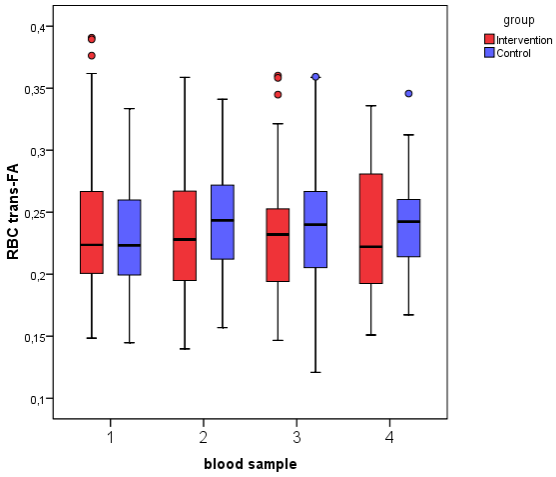


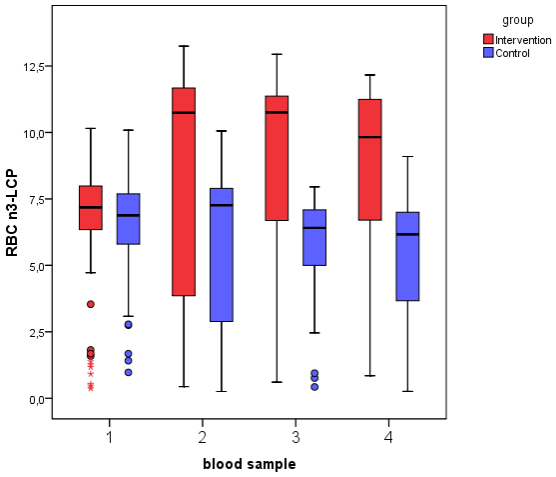


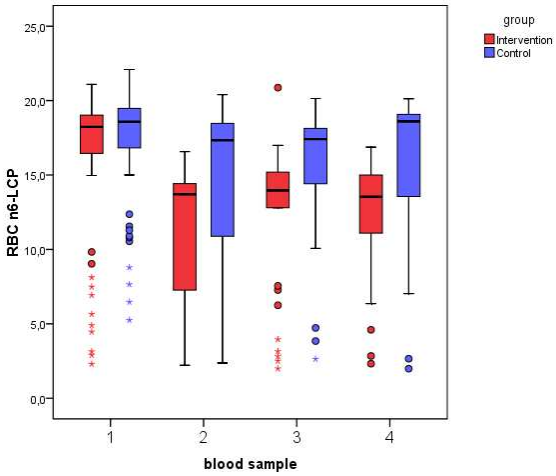
Bottom and top edges of the box are located at 25th and 75th percentiles, center horizontal line is drawn at the median, whiskers mark the maximum and minimum. Outliners are shown as dots, extremes as asterisk.

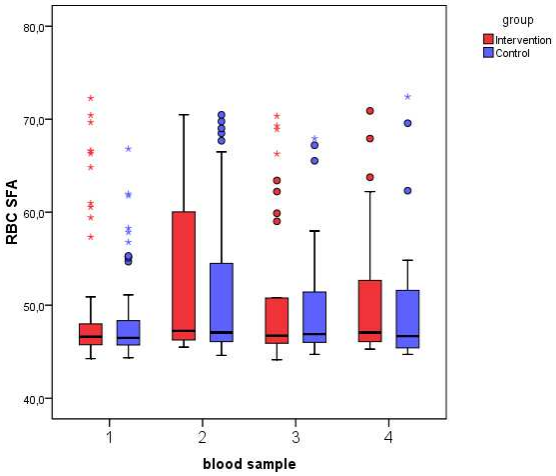




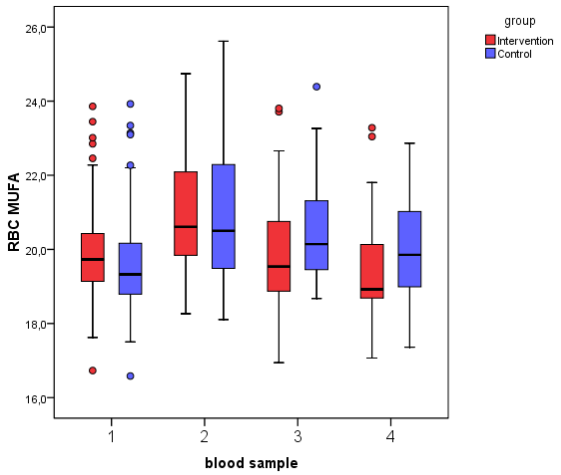


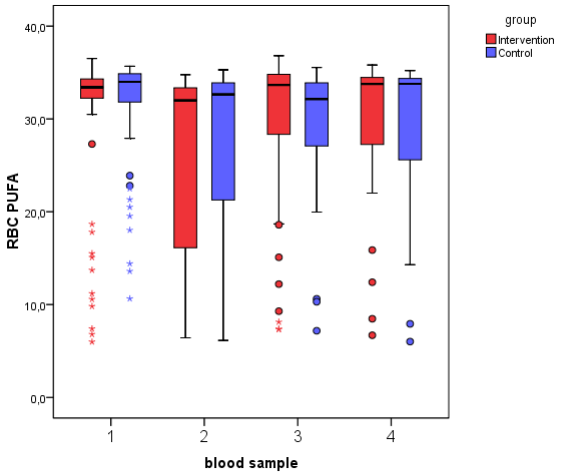


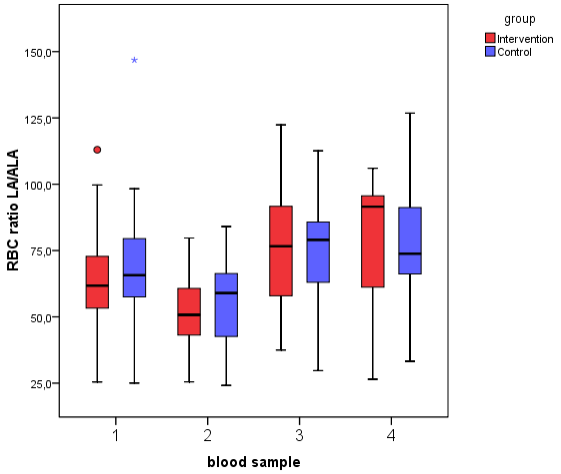


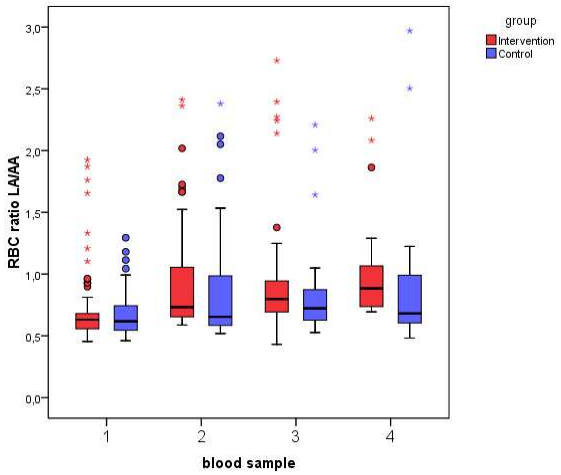


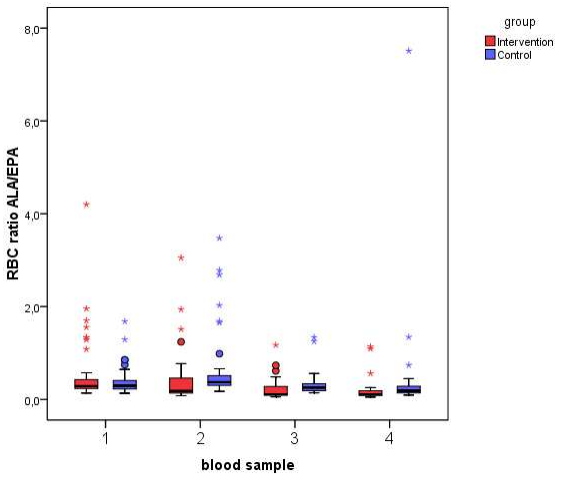
Bottom and top edges of the box are located at 25th and 75th percentiles, center horizontal line is drawn at the median, whiskers mark the maximum and minimum. Outliners are shown as dots, extremes as asterisk.

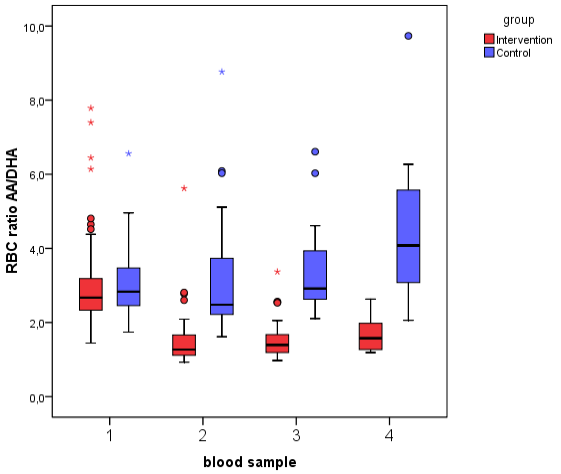








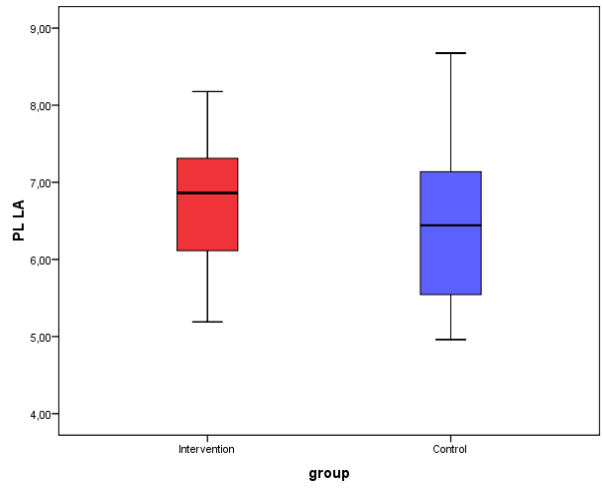


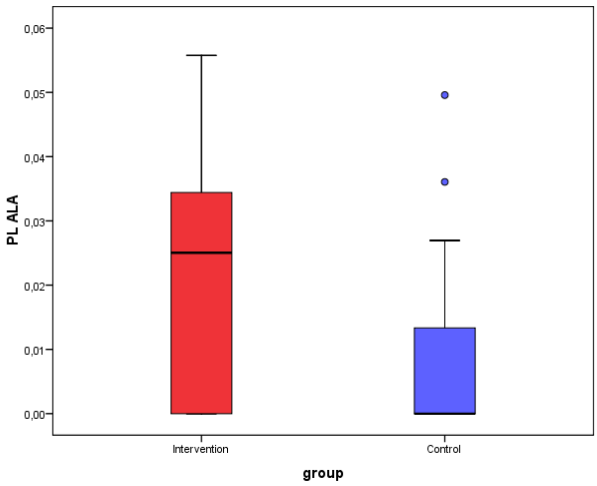


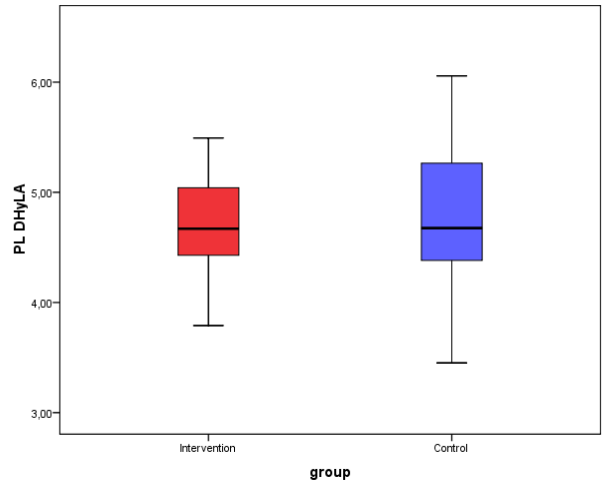
Bottom and top edges of the box are located at 25th and 75th percentiles, center horizontal line is drawn at the median, whiskers mark the maximum and minimum. Outliners are shown as dots, extremes as asterisk.

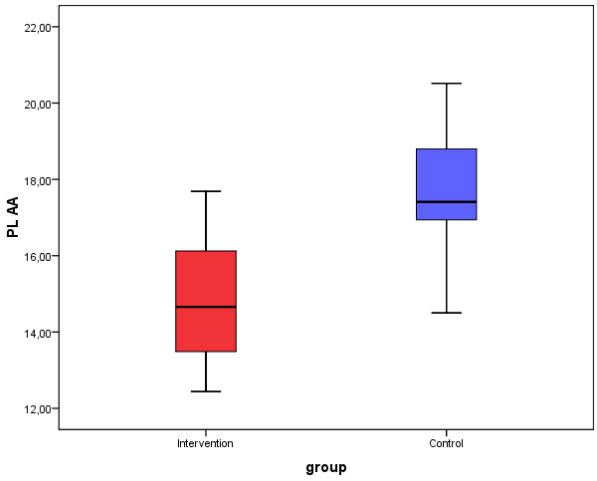
D.6 Cord blood plasma fatty acid profile

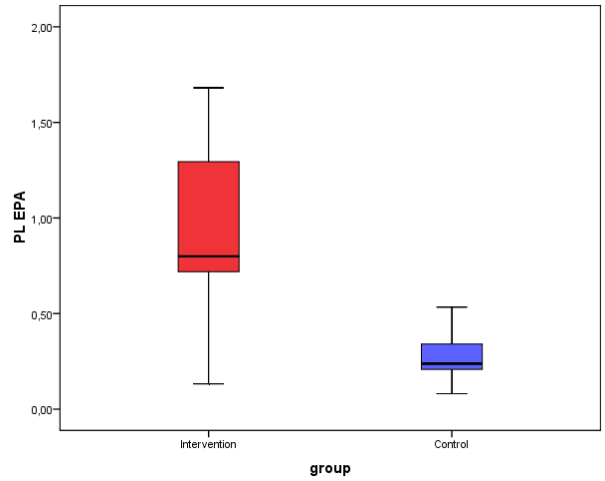
n (IG/CG) = 19/18

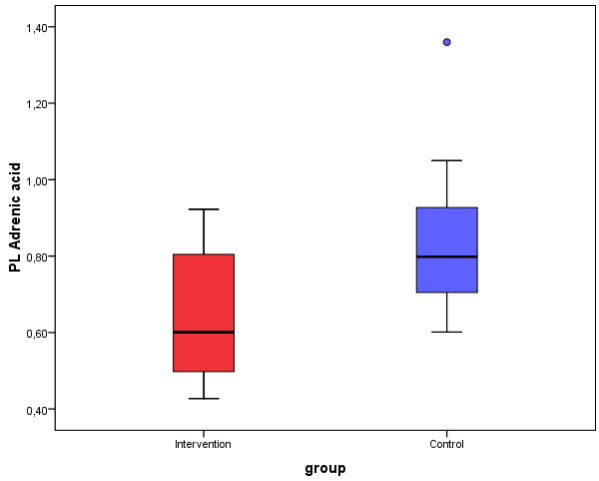




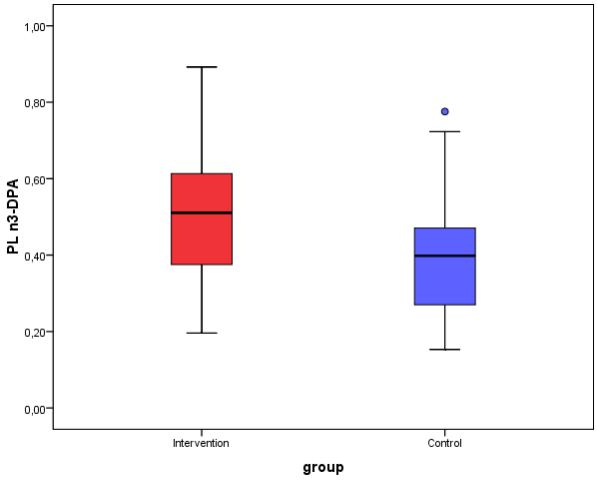


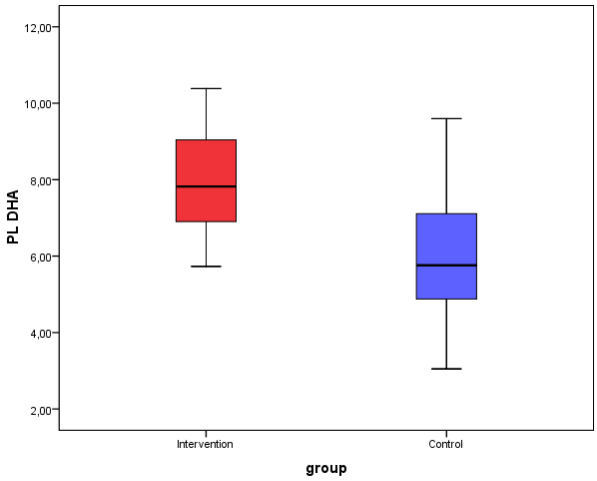


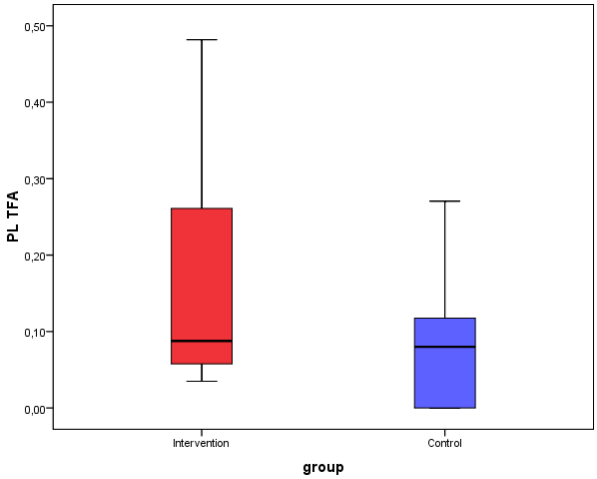


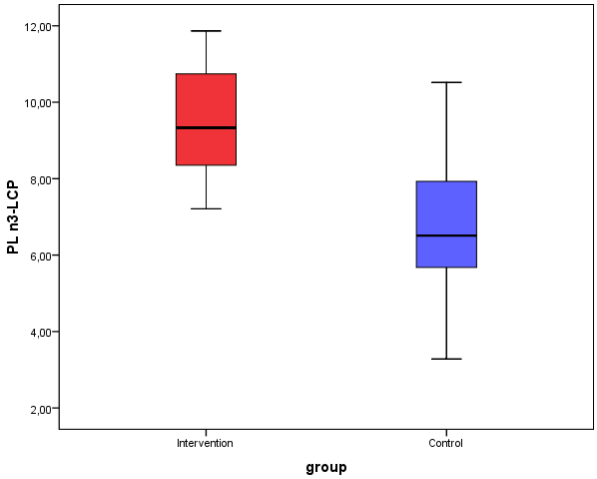


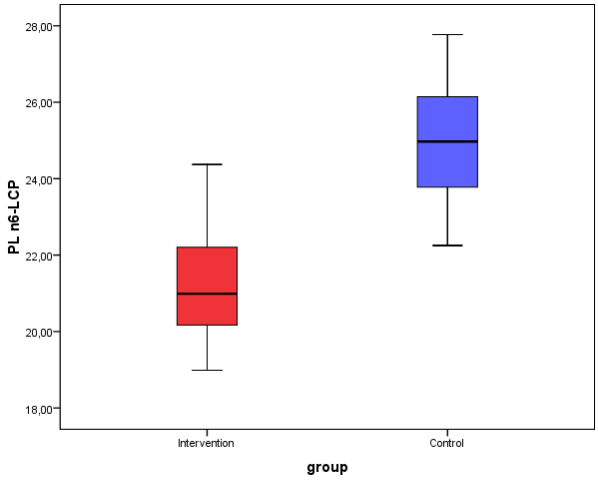
Bottom and top edges of the box are located at 25th and 75th percentiles, center horizontal line is drawn at the median, whiskers mark the maximum and minimum. Outliners are shown as dots, extremes as asterisk.

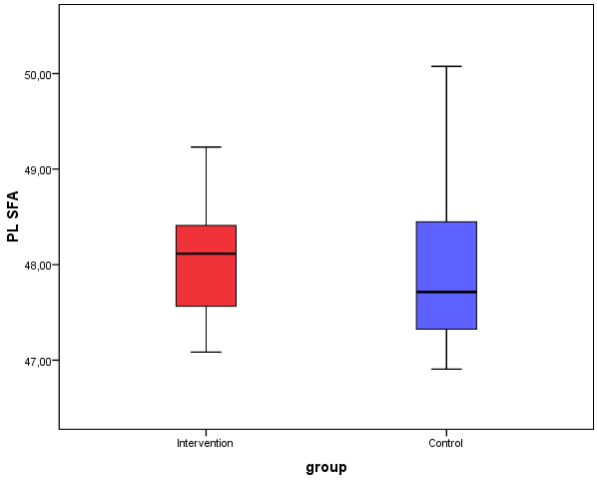




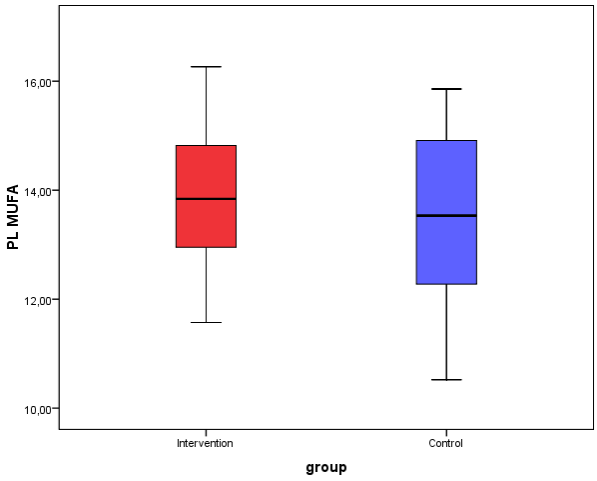


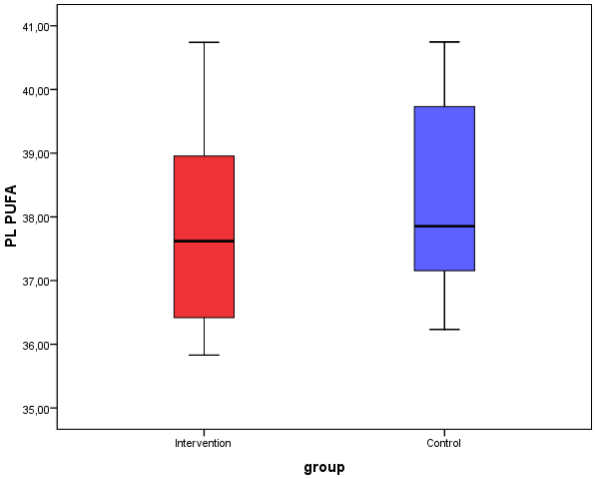


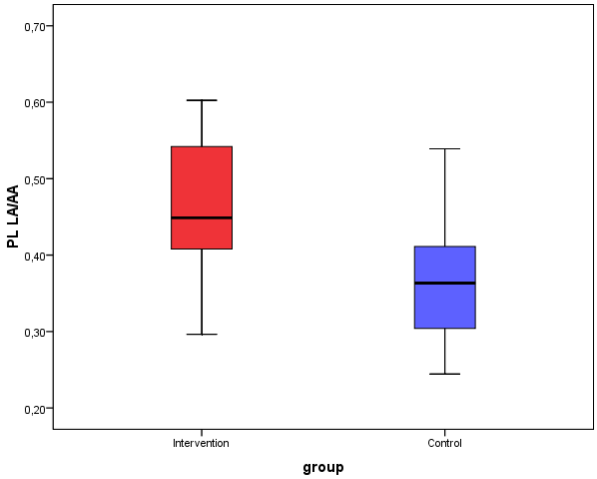


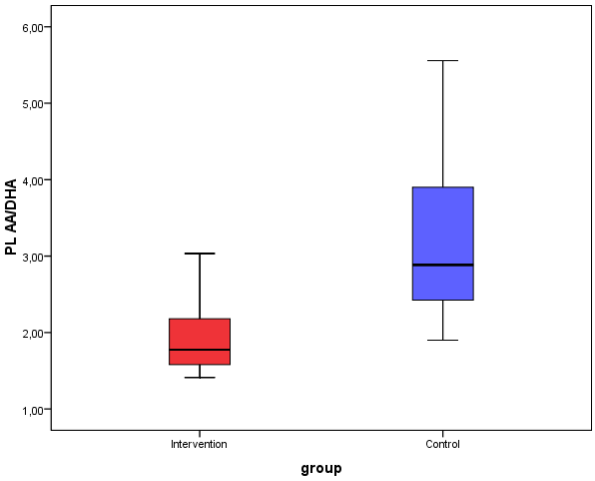


Bottom and top edges of the box are located at 25th and 75th percentiles, center horizontal line is drawn at the median, whiskers mark the maximum and minimum. Outliners are shown as dots, extremes as asterisk.





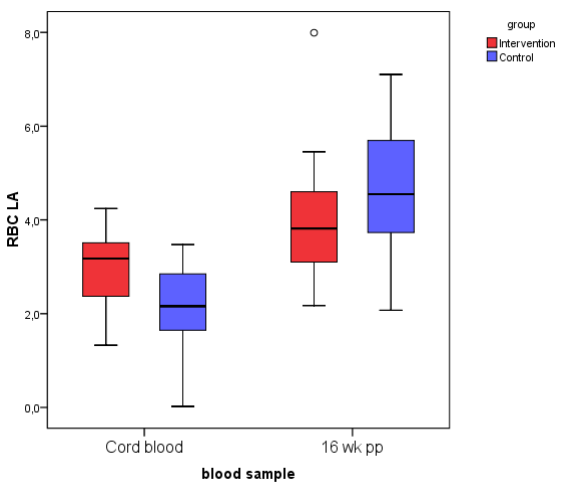


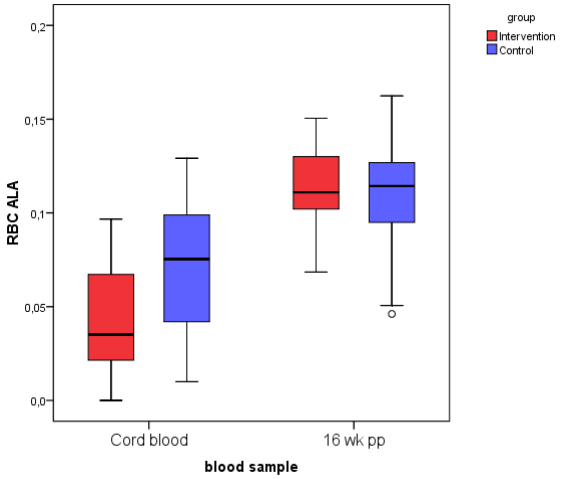


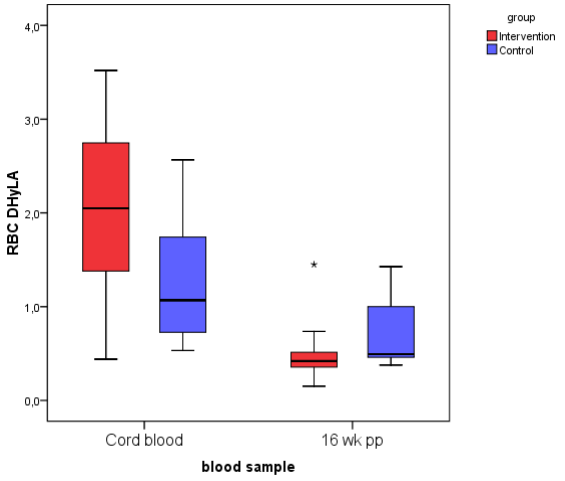
Bottom and top edges of the box are located at 25th and 75th percentiles, center horizontal line is drawn at the median, whiskers mark the maximum and minimum. Outliners are shown as dots, extremes as asterisk.

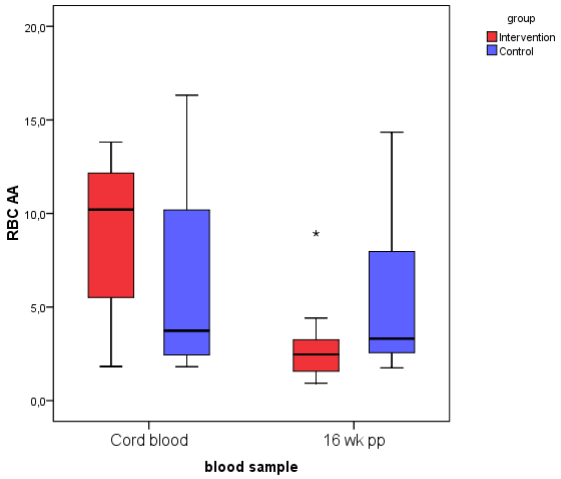
D.7 Infant red blood cell fatty acid profile

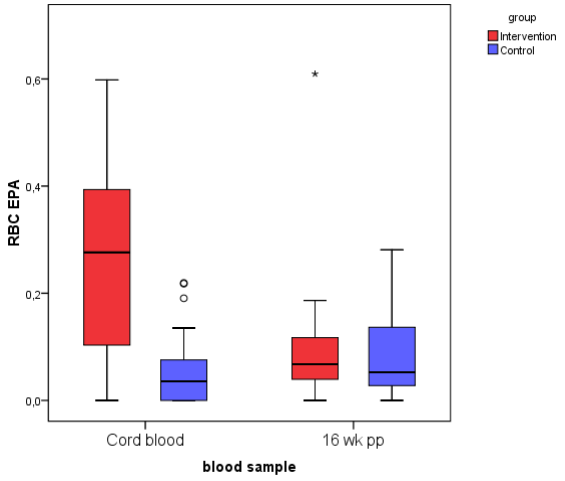
Cord blood n (IG/CG) = 30/28, at 16th wk pp n = 16/14

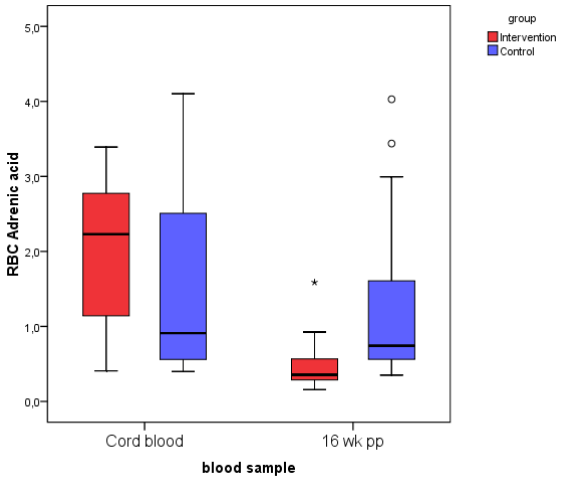




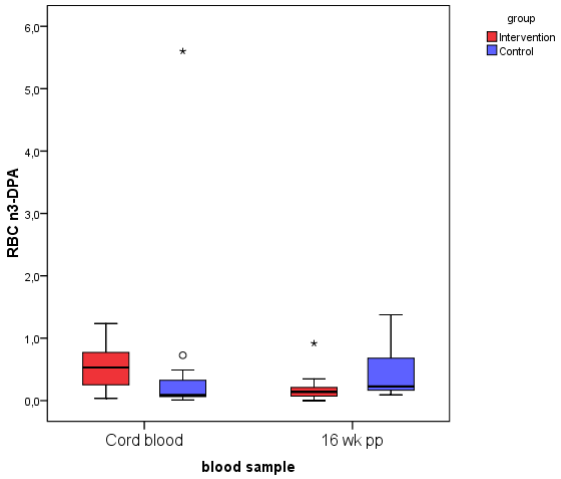


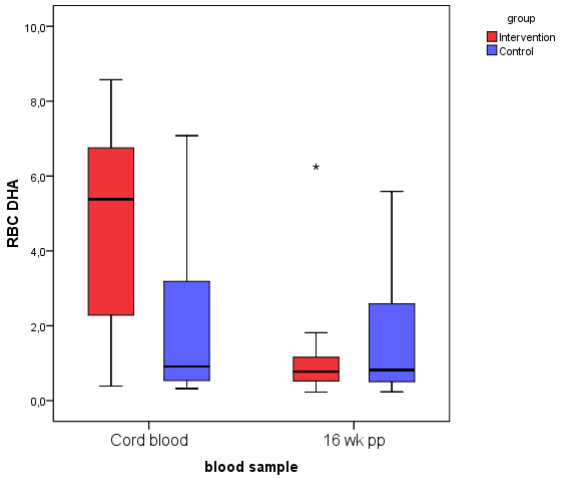


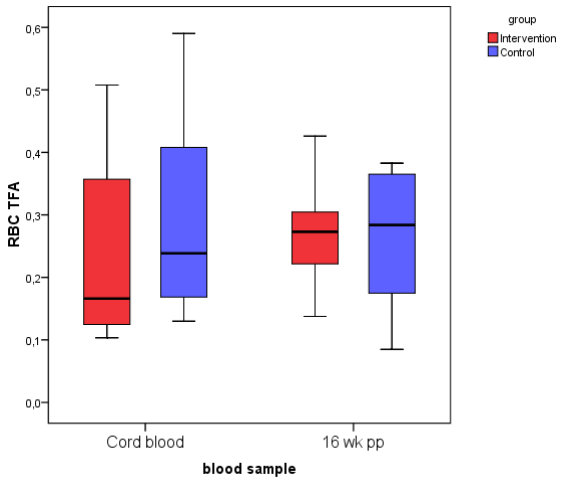


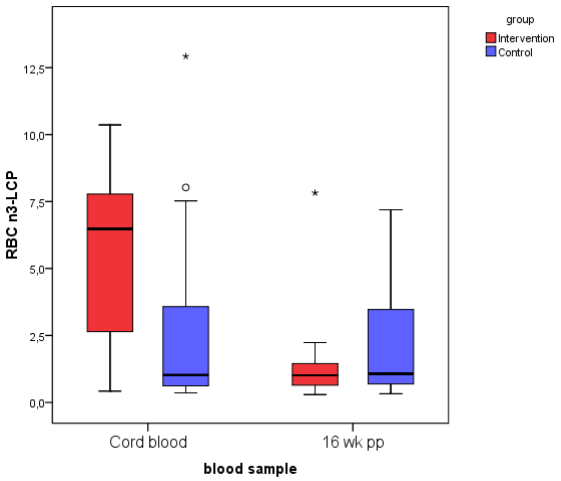


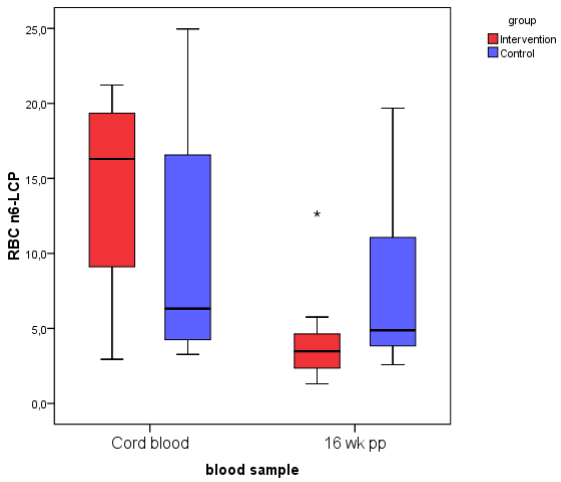
Bottom and top edges of the box are located at 25th and 75th percentiles, center horizontal line is drawn at the median, whiskers mark the maximum and minimum. Outliners are shown as dots, extremes as asterisk.

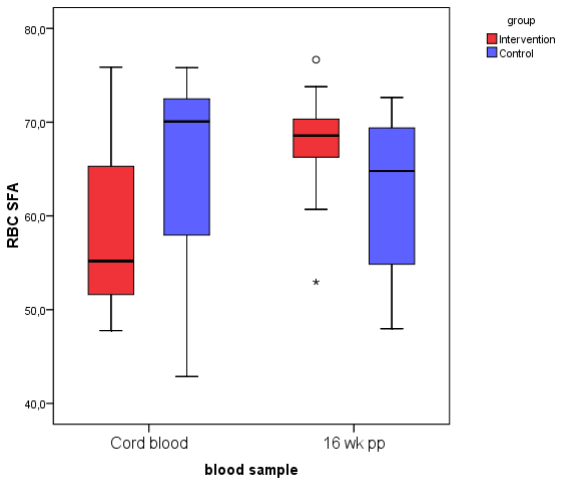




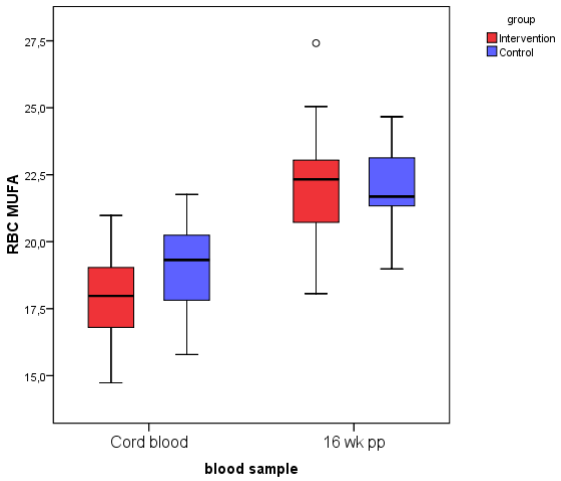


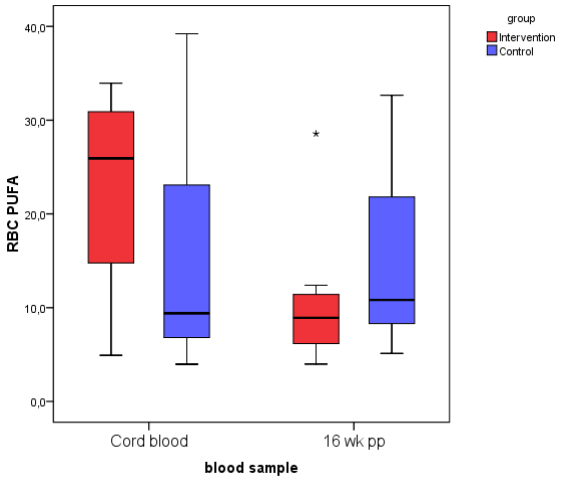


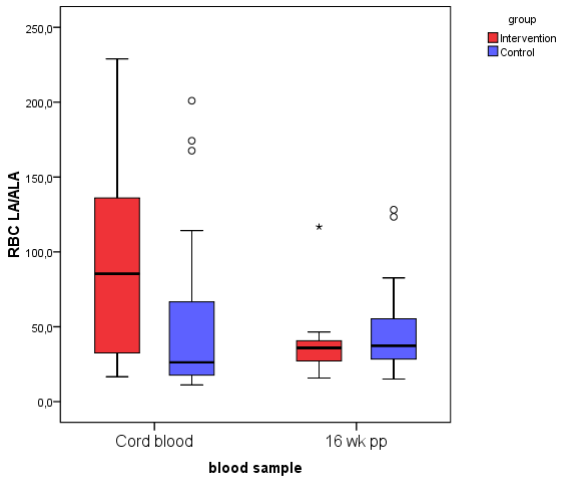


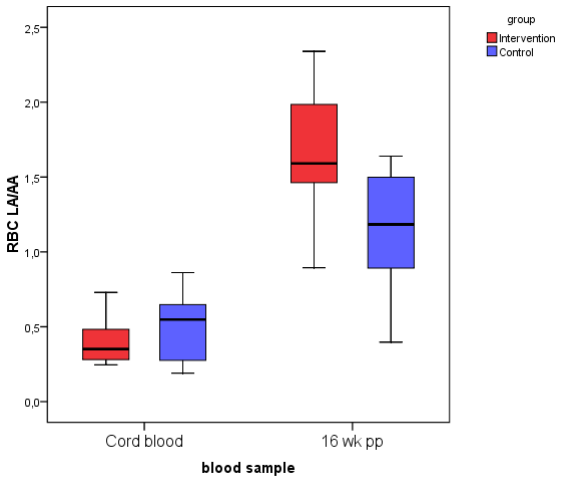


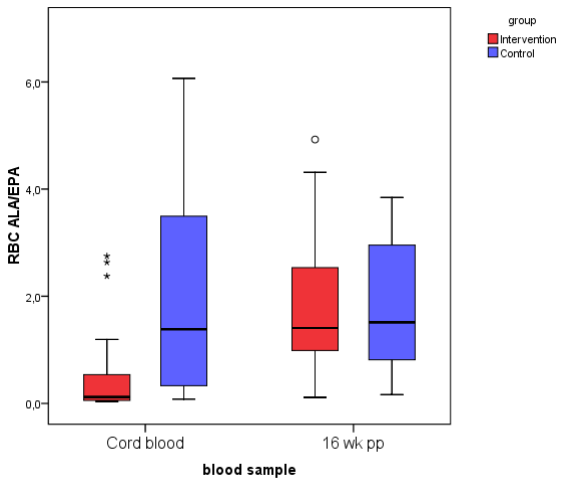
Bottom and top edges of the box are located at 25th and 75th percentiles, center horizontal line is drawn at the median, whiskers mark the maximum and minimum. Outliners are shown as dots, extremes as asterisk.

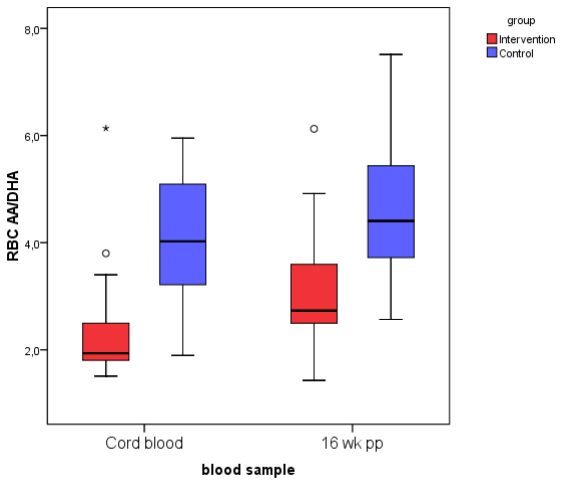








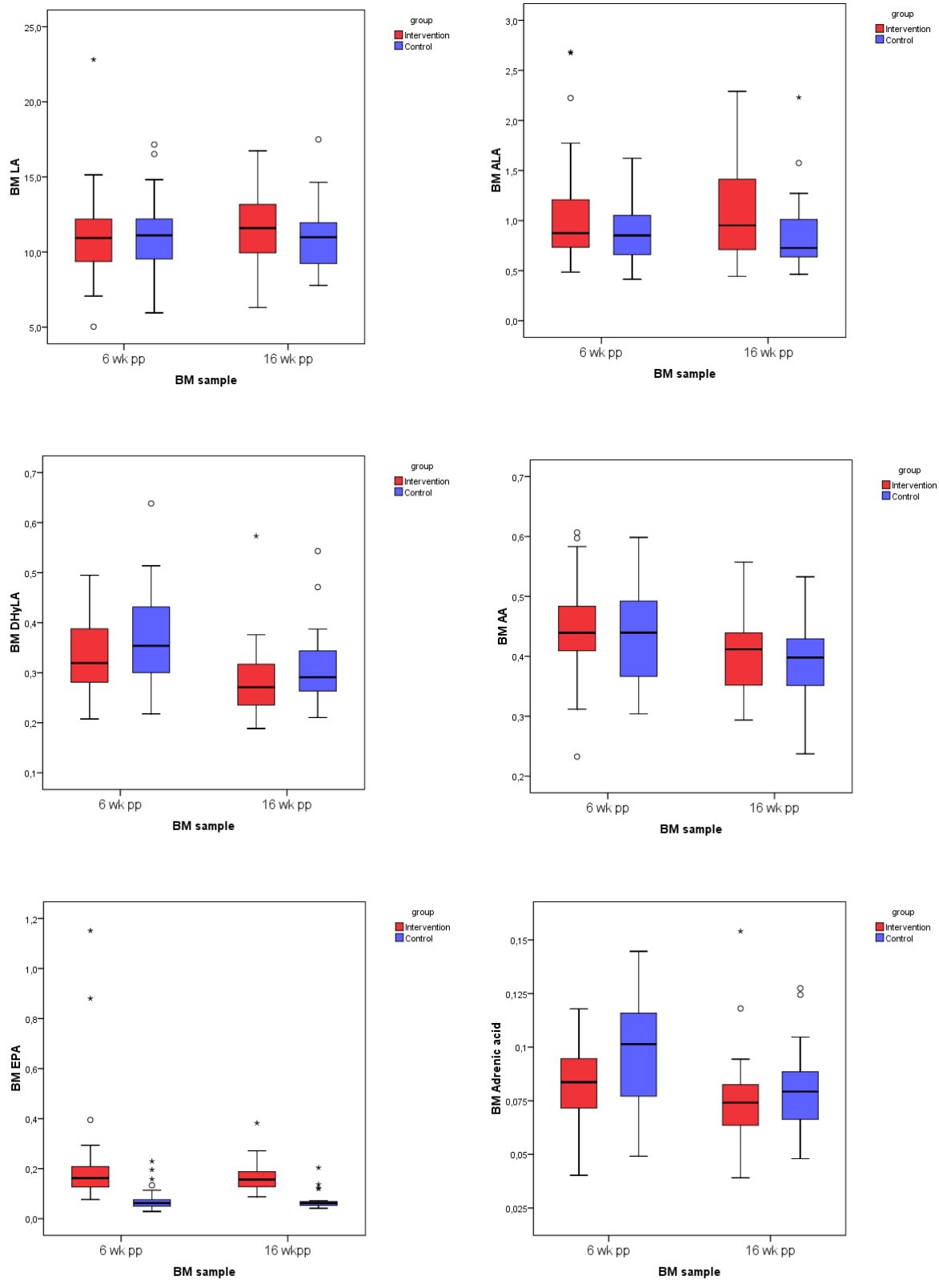




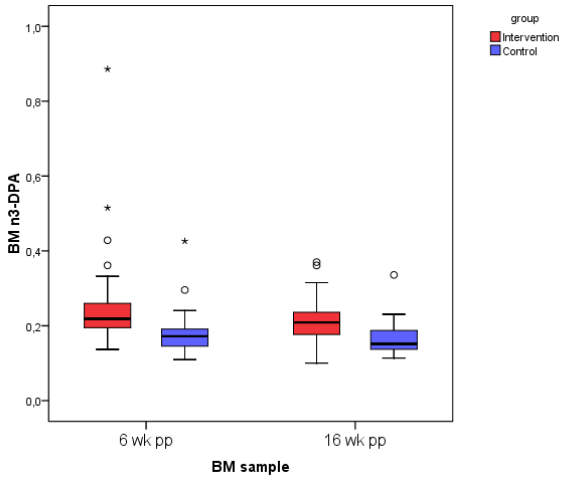
Bottom and top edges of the box are located at 25th and 75th percentiles, center horizontal line is drawn at the median, whiskers mark the maximum and minimum. Outliners are shown as dots, extremes as asterisk.

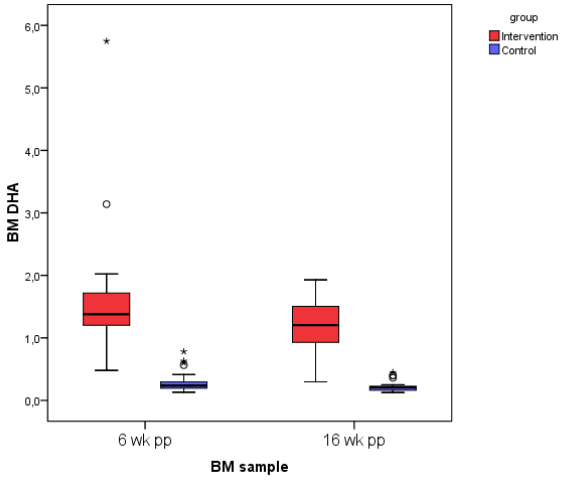
D.8 Breast milk fatty acid profile

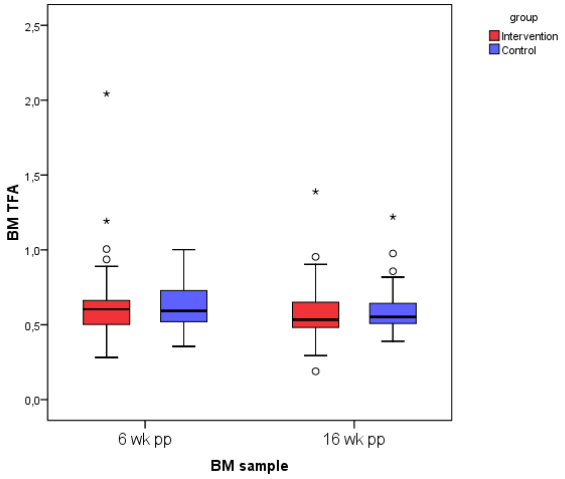
at 6th wk pp n (IG/CG) = 40/36; at 16th wk pp n = 28/22

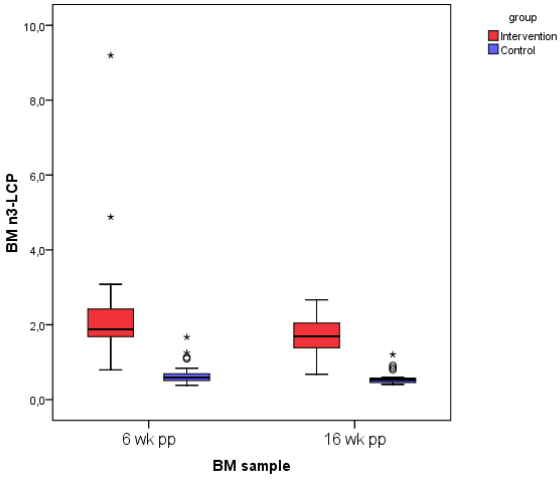


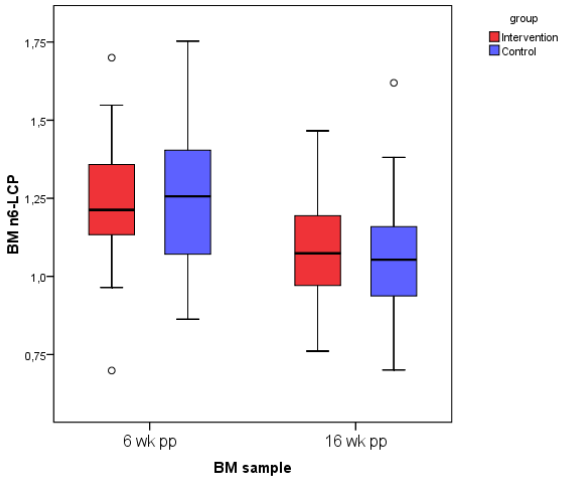
Bottom and top edges of the box are located at 25th and 75th percentiles, center horizontal line is drawn at the median, whiskers mark the maximum and minimum. Outliners are shown as dots, extremes as asterisk.

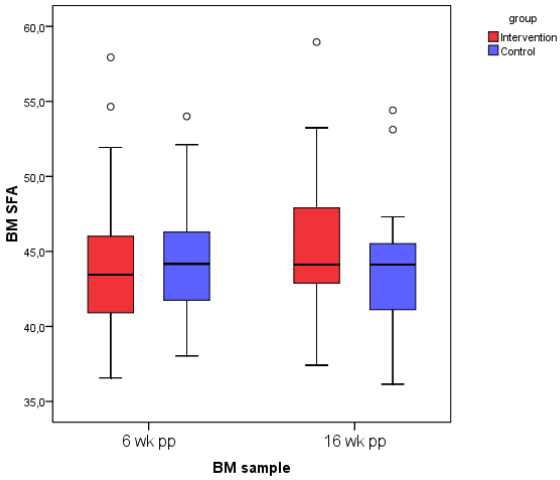




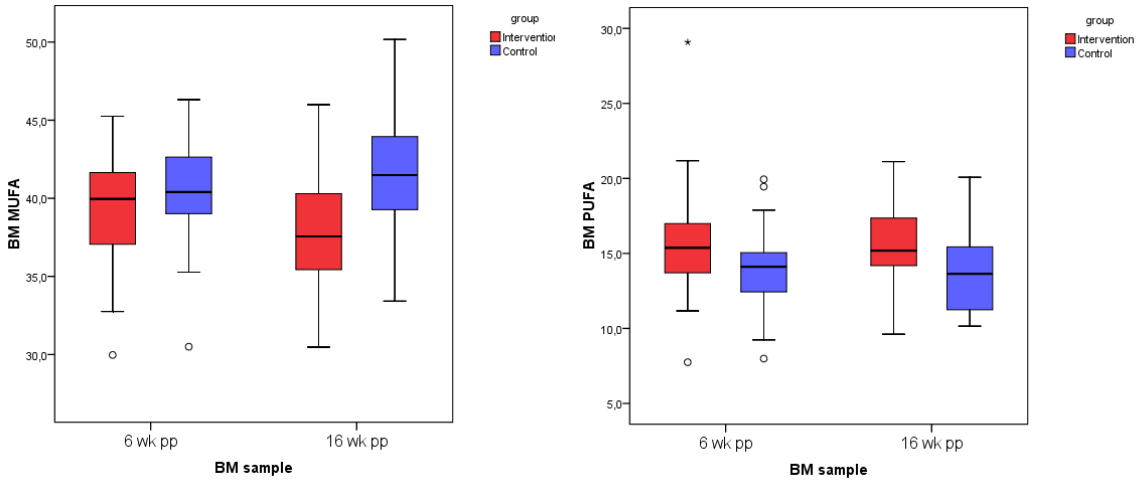


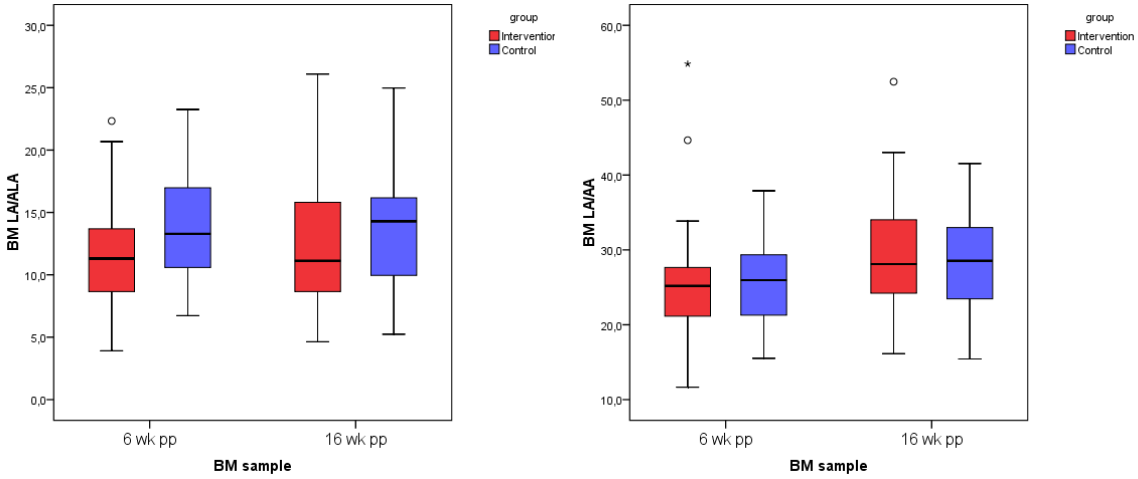


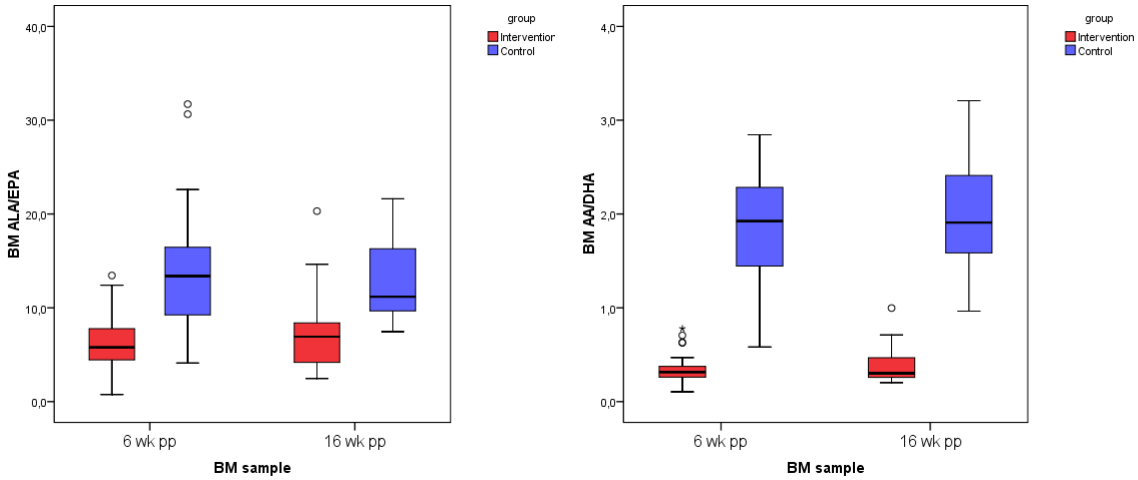




Bottom and top edges of the box are located at 25th and 75th percentiles, center horizontal line is drawn at the median, whiskers mark the maximum and minimum. Outliners are shown as dots, extremes as asterisk.



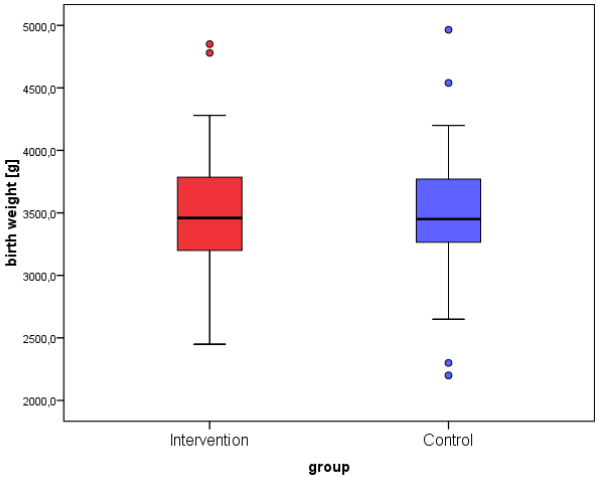


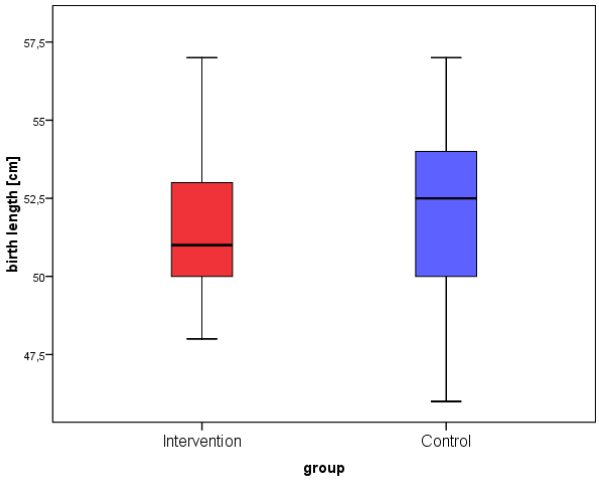


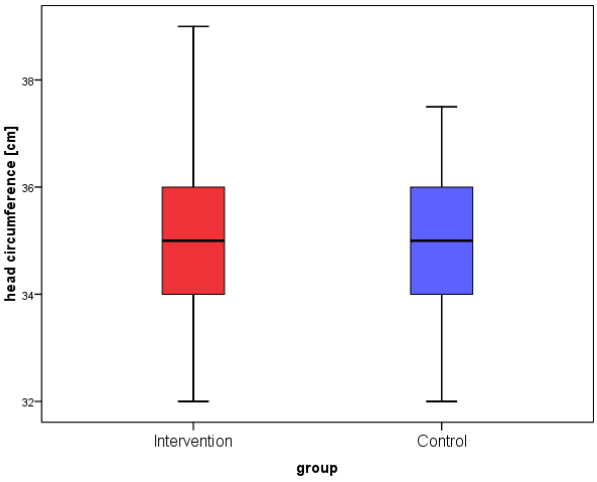
Bottom and top edges of the box are located at 25th and 75th percentiles, center horizontal line is drawn at the median, whiskers mark the maximum and minimum. Outliners are shown as dots, extremes as asterisk.

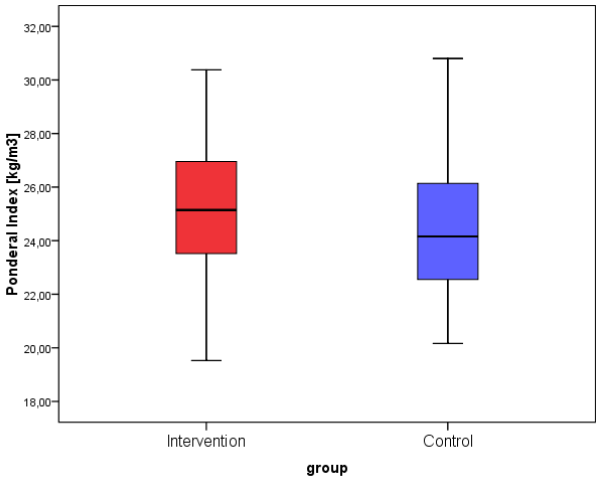
D.9 Birth outcomes

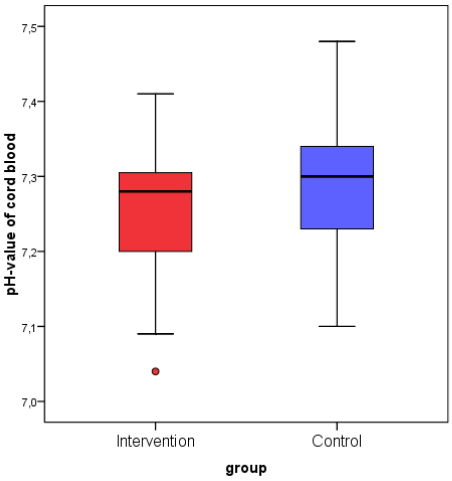
n (IG/SG) = 56/56 (unless otherwise noted)

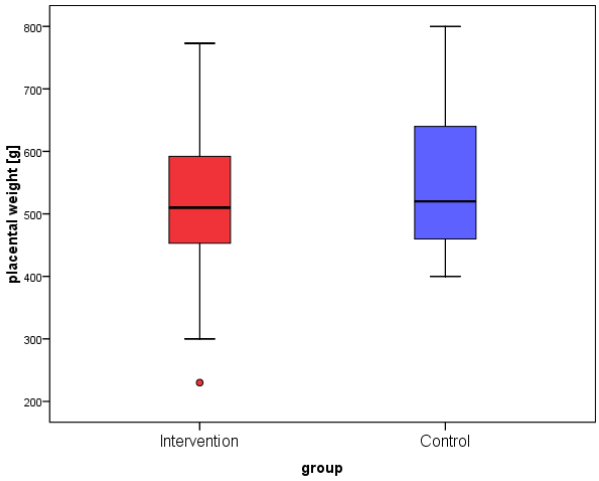










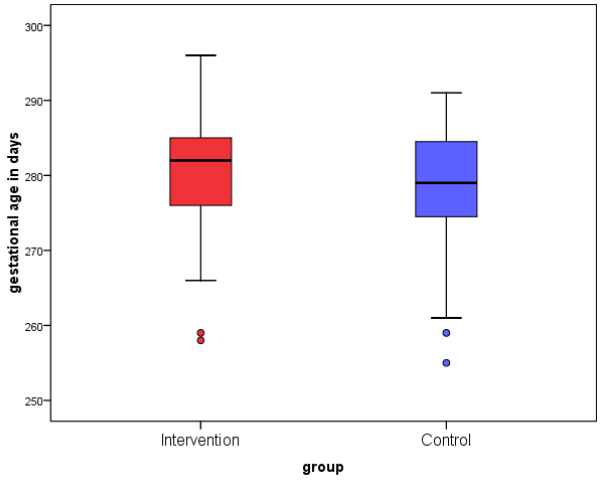


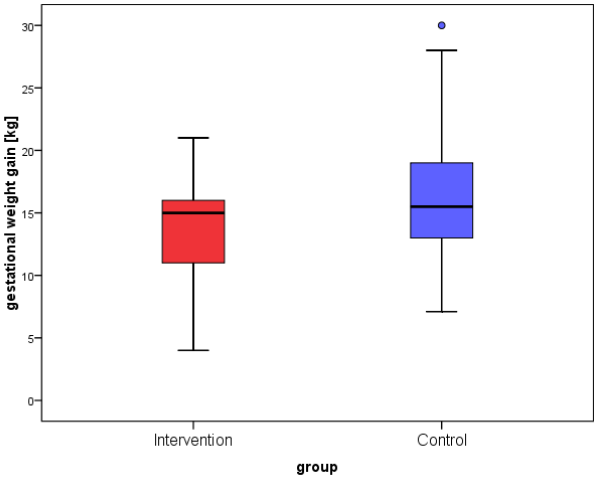
n (IG/SG) = 55/52

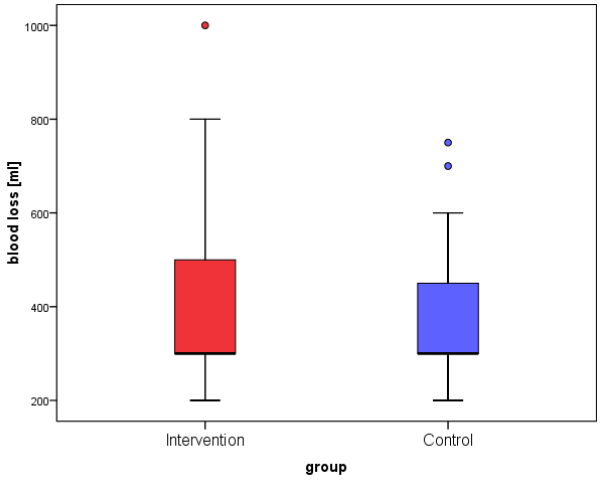
n (IG/SG) = 45/42

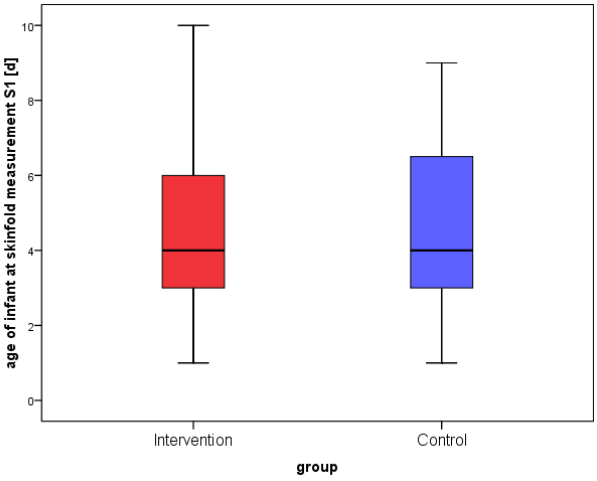
Bottom and top edges of the box are located at 25th and 75th percentiles, center horizontal line is drawn at the median, whiskers mark the maximum and minimum. Outliners are shown as dots, extremes as asterisk.

n (IG/SG) = 50/53





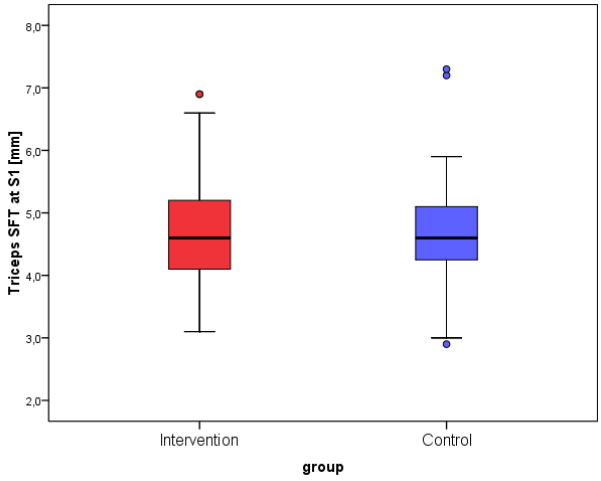


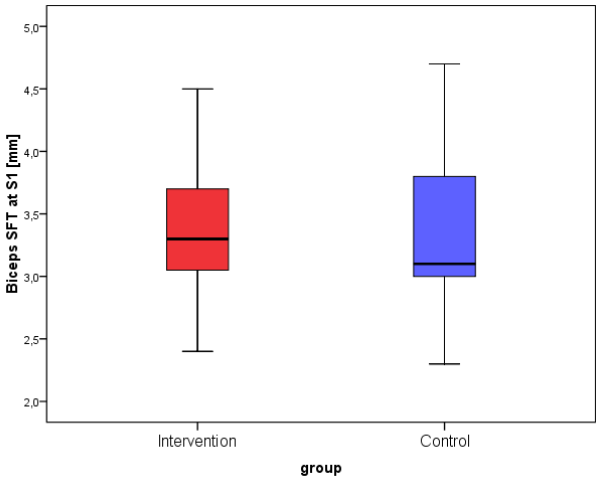


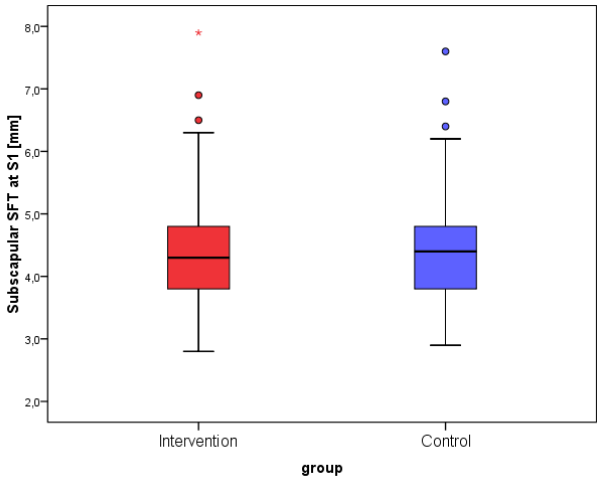
Bottom and top edges of the box are located at 25th and 75th percentiles, center horizontal line is drawn at the median, whiskers mark the maximum and minimum. Outliners are shown as dots, extremes as asterisk.

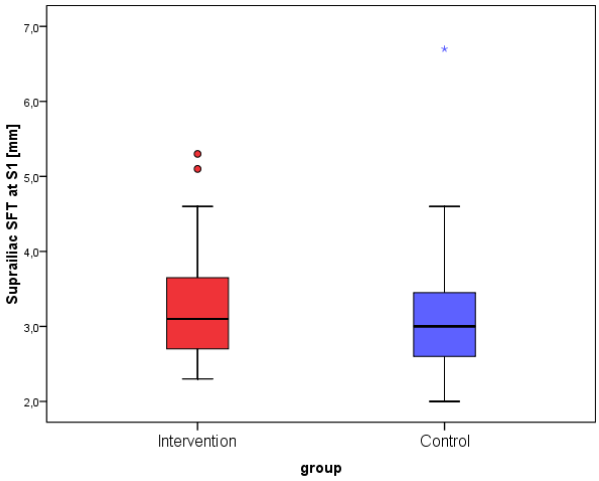
D.10 Infant anthropometry

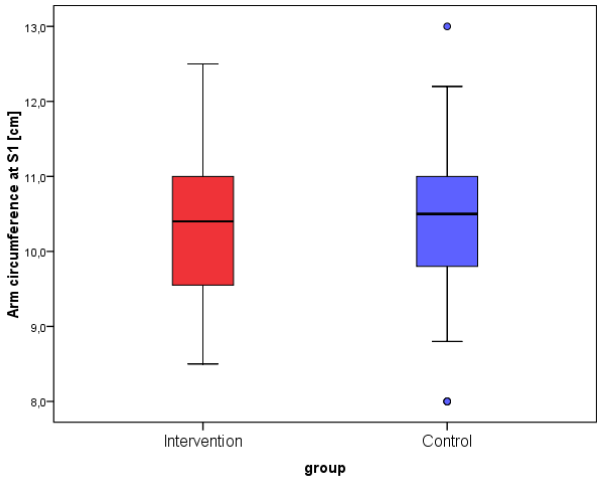
n (IG/CG) = 51/51











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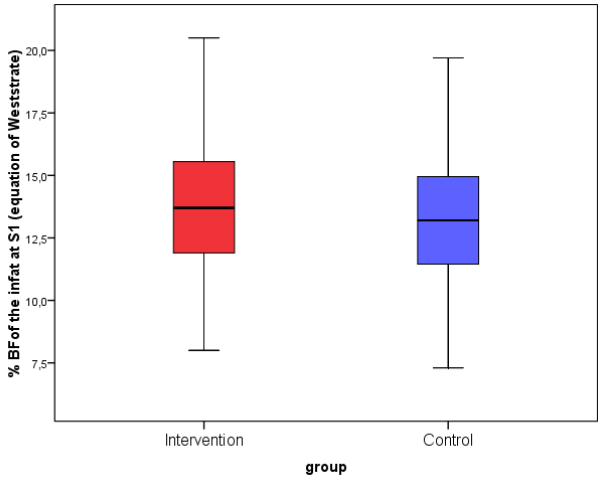

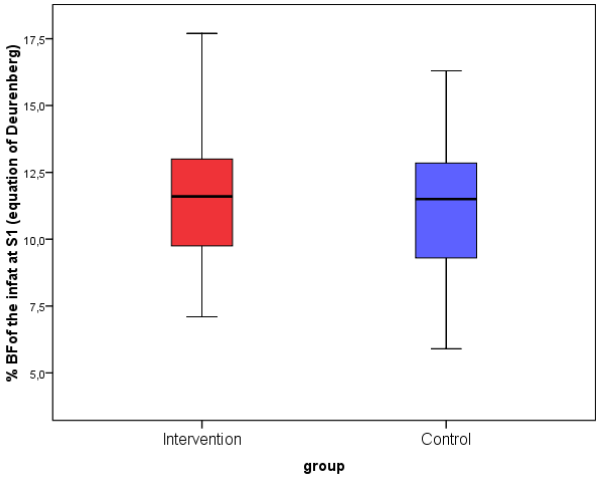

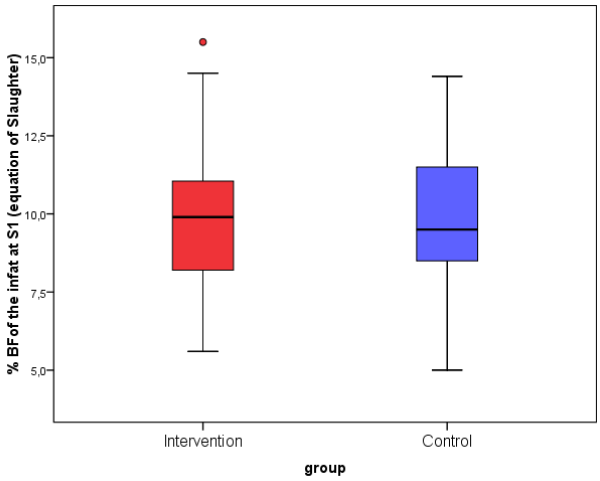

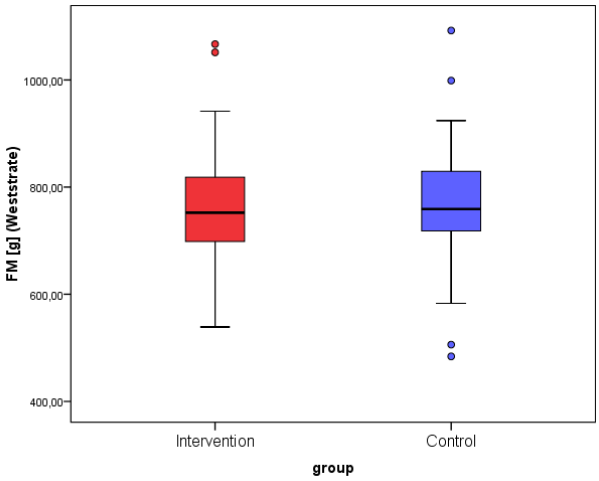

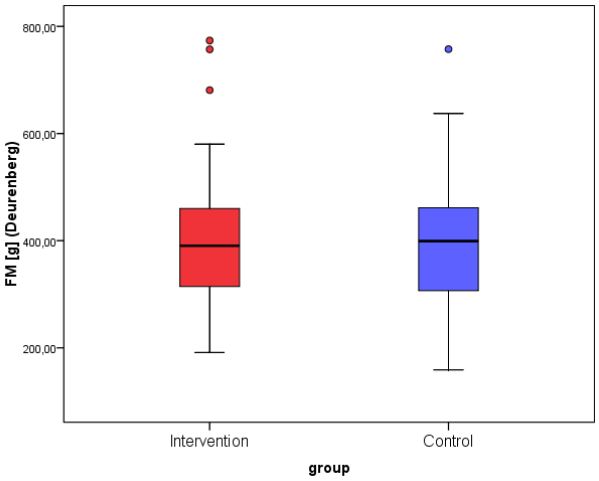

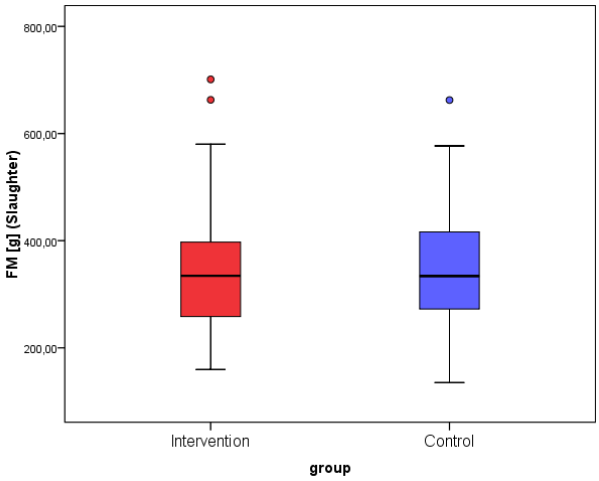

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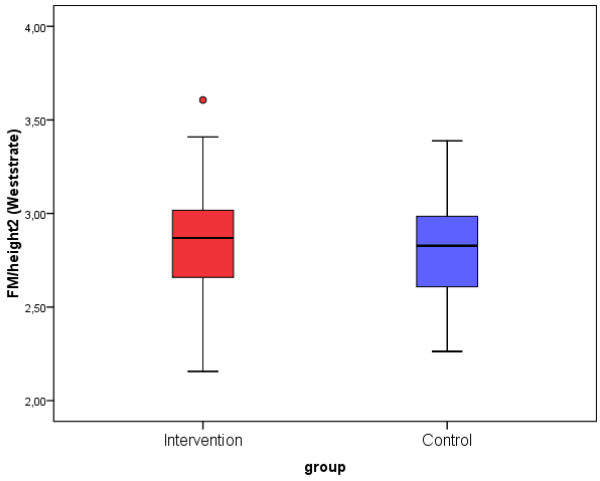

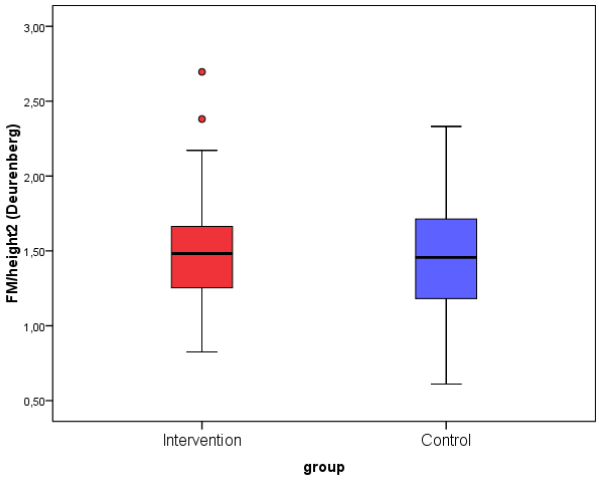

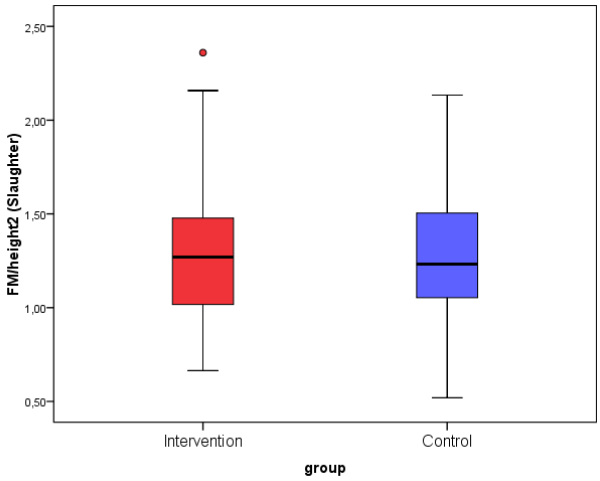

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References

Abrams, B., S. L. Altman and K. E. Pickett (2000). "Pregnancy weight gain: still controversial." Am J Clin Nutr **71**(5 Suppl): 1233S-41S.

Abrams, B. F. and R. K. Laros, Jr. (1986). "Prepregnancy weight, weight gain, and birth weight." Am J Obstet Gynecol **154**(3): 503-9.

Adair, C. D., L. Sanchez-Ramos, D. L. Briones and P. Ogburn, Jr. (1996). "The effect of high dietary n-3 fatty acid supplementation on angiotensin II pressor response in human pregnancy." Am J Obstet Gynecol **175**(3 Pt 1): 688-91.

Ailhaud, G. and P. Guesnet (2004). "Fatty acid composition of fats is an early determinant of childhood obesity: a short review and an opinion." Obes Rev **5**(1): 21-6.

Ailhaud, G., F. Massiera, P. Weill, P. Legrand, J. M. Alessandri and P. Guesnet (2006). "Temporal changes in dietary fats: role of n-6 polyunsaturated fatty acids in excessive adipose tissue development and relationship to obesity." Prog Lipid Res **45**(3): 203-36.

Ailhaud, G. a. H. H. (2004). Development of white adipose tissue Handbook of obesity: Etiology and pathophysiology. A. Bray. New York, Marcel Dekker, Inc.

Al, M. D., G. Hornstra, Y. T. van der Schouw, M. T. Bulstra-Ramakers and H. J. Huisjes (1990). "Biochemical EFA status of mothers and their neonates after normal pregnancy." Early Hum Dev **24**(3): 239-48.

Al, M. D., A. C. van Houwelingen, A. D. Kester, T. H. Hasaart, A. E. de Jong and G. Hornstra (1995). "Maternal essential fatty acid patterns during normal pregnancy and their relationship to the neonatal essential fatty acid status." Br J Nutr **74**(1): 55-68.

Alvarez, J. J., A. Montelongo, A. Iglesias, M. A. Lasuncion and E. Herrera (1996). "Longitudinal study on lipoprotein profile, high density lipoprotein subclass, and postheparin lipases during gestation in women." J Lipid Res **37**(2): 299-308.

Alvino, G., V. Cozzi, T. Radaelli, H. Ortega, E. Herrera and I. Cetin (2008). "Maternal and fetal fatty acid profile in normal and intrauterine growth restriction pregnancies with and without preeclampsia." Pediatr Res **64**(6): 615-20.

Amri, E. Z., G. Ailhaud and P. A. Grimaldi (1994). "Fatty acids as signal transducing molecules: involvement in the differentiation of preadipose to adipose cells." J Lipid Res **35**(5): 930-7.

Anderson, N. K., K. A. Beerman, M. A. McGuire, N. Dasgupta, J. M. Griinari, J. Williams and M. K. McGuire (2005). "Dietary fat type influences total milk fat content in lean women." J Nutr **135**(3): 416-21.

Andreasyan, K., A. L. Ponsonby, T. Dwyer, R. Morley, M. Riley, K. Dear and J. Cochrane (2007). "Higher maternal dietary protein intake in late pregnancy is associated with a lower infant ponderal index at birth." Eur J Clin Nutr **61**(4): 498-508.

Apfelbacher, C. J., A. Loerbroks, J. Cairns, H. Behrendt, J. Ring and U. Kramer (2008). "Predictors of overweight and obesity in five to seven-year-old children in Germany: results from cross-sectional studies." BMC Public Health **8**: 171.

Arbeitskreis-Omega-3 (2002). "Bedeutung und empfehlenswerte Höhe der Zufuhr langkettiger Omega-3-Fettsäuren." Ernährungsumschau **49**: 94-98.

Arterburn, L. M., E. B. Hall and H. Oken (2006). "Distribution, interconversion, and dose response of n-3 fatty acids in humans." Am J Clin Nutr **83**(6 Suppl): 1467S-1476S.

Bahrami, G. and Z. Rahimi (2005). "Fatty acid composition of human milk in Western Iran." Eur J Clin Nutr **59**(4): 494-7.

Baillie, R. A., R. Takada, M. Nakamura and S. D. Clarke (1999). "Coordinate induction of peroxisomal acyl-CoA oxidase and UCP-3 by dietary fish oil: a mechanism for decreased body fat deposition." Prostaglandins Leukot Essent Fatty Acids **60**(5-6): 351-6.

Baird, J., D. Fisher, P. Lucas, J. Kleijnen, H. Roberts and C. Law (2005). "Being big or growing fast: systematic review of size and growth in infancy and later obesity." Bmj **331**(7522): 929.

Balk, E. M., A. H. Lichtenstein, M. Chung, B. Kupelnick, P. Chew and J. Lau (2006). "Effects of omega-3 fatty acids on serum markers of cardiovascular disease risk: a systematic review." Atherosclerosis **189**(1): 19-30.

Balloch, A. J. and M. N. Cauchi (1993). "Reference ranges for haematology parameters in pregnancy derived from patient populations." Clin Lab Haematol **15**(1): 7-14.

Barry, J. M., W. Bartley, J. L. Linzell and D. S. Robinson (1963). "The Uptake from the Blood of Triglyceride Fatty Acids of Chylomicra and Low-Density Lipoproteins by the Mammary Gland of the Goat." Biochem J **89**: 6-11.

Basaran, A. (2009). "Pregnancy-induced hyperlipoproteinemia: review of the literature." Reprod Sci **16**(5): 431-7.

Bauch, A., O. Lindtner, G. B. Mensink and B. Niemann (2006). "Dietary intake and sources of long-chain n-3 PUFAs in German adults." Eur J Clin Nutr **60**(6): 810-2.

Baum, D., R. Q. Beck, L. D. Hammer, J. A. Brasel and M. R. Greenwood (1986). "Adipose tissue thymidine kinase activity in man." Pediatr Res **20**(2): 118-21.

Beermann, C., A. Grenn, M. Möbius, J.J. Schmitt and G. Boehm (2003). "Lipid Class Separation by HPLC of Seed Lipid Compositions from Different Brassica napus L. Varieties." J. Am Oil Chem. Soc **80**: 747-753.

Beermann, C., J. Jelinek, T. Reinecker, A. Hauenschild, G. Boehm and H. U. Klor (2003). "Short term effects of dietary medium-chain fatty acids and n-3 long-chain polyunsaturated fatty acids on the fat metabolism of healthy volunteers." Lipids Health Dis **2**: 10.

Beermann, C., M. Mobius, N. Winterling, J. J. Schmitt and G. Boehm (2005). "sn-position determination of phospholipid-linked fatty acids derived from erythrocytes by liquid chromatography electrospray ionization ion-trap mass spectrometry." Lipids **40**(2): 211-8.

Belfort, M. B., S. L. Rifas-Shiman, J. Rich-Edwards, K. P. Kleinman and M. W. Gillman (2007). "Size at birth, infant growth, and blood pressure at three years of age." J Pediatr **151**(6): 670-4.

Belzung, F., T. Raclot and R. Groscolas (1993). "Fish oil n-3 fatty acids selectively limit the hypertrophy of abdominal fat depots in growing rats fed high-fat diets." Am J Physiol **264**(6 Pt 2): R1111-8.

Benassayag, C., T. M. Mignot, M. Haourigui, C. Civel, J. Hassid, B. Carbonne, E. A. Nunez and F. Ferre (1997). "High polyunsaturated fatty acid, thromboxane A2, and alpha-fetoprotein concentrations at the human feto-maternal interface." J Lipid Res **38**(2): 276-86.

Benassayag, C., V. Rigourd, T. M. Mignot, J. Hassid, M. J. Leroy, B. Robert, C. Civel, G. Grange, E. Dallot, J. Tanguy, E. A. Nunez and F. Ferre (1999). "Does high

polyunsaturated free fatty acid level at the feto-maternal interface alter steroid hormone message during pregnancy?" Prostaglandins Leukot Essent Fatty Acids **60**(5-6): 393-9.

Berghaus, T. M., H. Demmelmair and B. Koletzko (1998). "Fatty acid composition of lipid classes in maternal and cord plasma at birth." Eur J Pediatr **157**(9): 763-8.

Berghaus, T. M., H. Demmelmair and B. Koletzko (2000). "Essential fatty acids and their long-chain polyunsaturated metabolites in maternal and cord plasma triglycerides during late gestation." Biol Neonate **77**(2): 96-100.

Bergmann, R., K. Bergmann, E. Haschke-Becher, R. Richter, J. W. Dudenhausen, D. Barclay and F. Haschke (2007). "Does maternal docosahexaenoic acid supplementation during pregnancy and lactation lower BMI in late infancy?" J Perinat Med **35**: 295-300.

Bergmann, R. L., R. Richter, K. E. Bergmann, A. Plagemann, M. Brauer and J. W. Dudenhausen (2003). "Secular trends in neonatal macrosomia in Berlin: influences of potential determinants." Paediatr Perinat Epidemiol **17**(3): 244-9.

Birch, E. E., D. R. Hoffman, R. Uauy, D. G. Birch and C. Prestidge (1998). "Visual acuity and the essentiality of docosahexaenoic acid and arachidonic acid in the diet of term infants." Pediatr Res **44**(2): 201-9.

Bitman, J., D. Wood, R. Miller, H. Tyrell, C. Reynolds and H. Baxter (1984). "Comparison of the phospholipid composition of breast milk from mothers of term and preterm infants during lactation." Am J Clin Nutr **40**: 1108-1119.

Bligh, E. G. and W. J. Dyer (1959). "A rapid method of total lipid extraction and purification." Can J Biochem Physiol **37**(8): 911-7.

Boersma, E. R., P. J. Offringa, F. A. Muskiet, W. M. Chase and I. J. Simmons (1991). "Vitamin E, lipid fractions, and fatty acid composition of colostrum, transitional milk, and mature milk: an international comparative study." Am J Clin Nutr **53**(5): 1197-204.

Borod, E., R. Atkinson, W. R. Barclay and S. E. Carlson (1999). "Effects of third trimester consumption of eggs high in docosahexaenoic acid on docosahexaenoic acid status and pregnancy." Lipids **34 Suppl**: S231.

Boulton, T. J., M. Dunlop and J. M. Court (1978). "The growth and development of fat cells in infancy." Pediatr Res **12**(9): 908-11.

Bracco, U. (1994). "Effect of triglyceride structure on fat absorption." Am J Clin Nutr **60**(6 Suppl): 1002S-1009S.

Bray, G. A., J. P. DeLany, J. Volaufova, D. W. Harsha and C. Champagne (2002). "Prediction of body fat in 12-y-old African American and white children: evaluation of methods." Am J Clin Nutr **76**(5): 980-90.

Brenna, J. T., N. Salem, Jr., A. J. Sinclair and S. C. Cunnane (2009). "alpha-Linolenic acid supplementation and conversion to n-3 long-chain polyunsaturated fatty acids in humans." Prostaglandins Leukot Essent Fatty Acids **80**(2-3): 85-91.

Brenna, J. T., B. Varamini, R. G. Jensen, D. A. Diersen-Schade, J. A. Boettcher and L. M. Arterburn (2007). "Docosahexaenoic and arachidonic acid concentrations in human breast milk worldwide." Am J Clin Nutr **85**(6): 1457-64.

Brizzi, P., G. Tonolo, F. Esposito, L. Puddu, S. Dessole, M. Maioli and S. Milia (1999). "Lipoprotein metabolism during normal pregnancy." Am J Obstet Gynecol **181**(2): 430-4.

Brossard, N., M. Croset, C. Pachiaudi, J. P. Riou, J. L. Tayot and M. Lagarde (1996). "Retroconversion and metabolism of [13C]22:6n-3 in humans and rats after intake of a single dose of [13C]22:6n-3-triacylglycerols." Am J Clin Nutr **64**(4): 577-86.

Brown, C. E., N. F. Gant, K. Cox, B. Spitz, C. R. Rosenfeld and R. R. Magness (1990). "Low-dose aspirin. II. Relationship of angiotensin II pressor responses, circulating eicosanoids, and pregnancy outcome." Am J Obstet Gynecol **163**(6 Pt 1): 1853-61.

Budowski, P., H. Druckmann, B. Kaplan and P. Merlob (1994). "Mature milk from Israeli mothers is rich in polyunsaturated fatty acids." World Rev Nutr Diet **75**: 105-8.

Bühling, K. J. and W. Friedmann (2004). Intensivkurs Gynäkologie und Geburtshilfe. München, Jena, Urban & Fischer.

Burdge, G. C., A. E. Jones and S. A. Wootton (2002). "Eicosapentaenoic and docosapentaenoic acids are the principal products of alpha-linolenic acid metabolism in young men*." Br J Nutr **88**(4): 355-63.

Burdge, G. C. and S. A. Wootton (2002). "Conversion of alpha-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women." Br J Nutr **88**(4): 411-20.

Burdi, A. R., C. M. Poissonnet, S. M. Garn, M. Lavelle, M. D. Sabet and P. Bridges (1985). "Adipose tissue growth patterns during human gestation: a histometric comparison of buccal and gluteal fat depots." Int J Obes **9**(4): 247-56.

Butte, N. F. (2000). "Carbohydrate and lipid metabolism in pregnancy: normal compared with gestational diabetes mellitus." Am J Clin Nutr **71**(5 Suppl): 1256S-61S.

Butte, N. F. (2005). "Energy requirements during pregnancy and consequences of deviations from requirement on fetal outcome." Nestle Nutr Workshop Ser Pediatr Program **55**: 49-67; discussion 67-71.

Butte, N. F., K. J. Ellis, W. W. Wong, J. M. Hopkinson and E. O. Smith (2003). "Composition of gestational weight gain impacts maternal fat retention and infant birth weight." Am J Obstet Gynecol **189**(5): 1423-32.

Butte, N. F., J. M. Hopkinson, W. W. Wong, E. O. Smith and K. J. Ellis (2000). "Body composition during the first 2 years of life: an updated reference." Pediatr Res **47**(5): 578-85.

Bygdeman, M., K. Gemzell, C. Gottlieb and M. L. Swahn (1991). "Uterine contractility and interaction between prostaglandins and antiprogestins. Clinical implications." Ann N Y Acad Sci **626**: 561-7.

Campbell, D. M., M. H. Hall, D. J. Barker, J. Cross, A. W. Shiell and K. M. Godfrey (1996). "Diet in pregnancy and the offspring's blood pressure 40 years later." Br J Obstet Gynaecol **103**(3): 273-80.

Campbell, F. M., P. G. Bush, J. H. Veerkamp and A. K. Dutta-Roy (1998). "Detection and cellular localization of plasma membrane-associated and cytoplasmic fatty acid-binding proteins in human placenta." Placenta **19**(5-6): 409-15.

Campbell, F. M. and A. K. Dutta-Roy (1995). "Plasma membrane fatty acid-binding protein (FABPpm) is exclusively located in the maternal facing membranes of the human placenta." FEBS Lett **375**(3): 227-30.

Campbell, F. M., M. J. Gordon and A. K. Dutta-Roy (1998). "Placental membrane fatty acid-binding protein preferentially binds arachidonic and docosahexaenoic acids." Life Sci **63**(4): 235-40.

Carlson, S. E., R. J. Cooke, S. H. Werkman and E. A. Tolley (1992). "First year growth of preterm infants fed standard compared to marine oil n-3 supplemented formula." Lipids **27**(11): 901-7.

Carlson, S. E., S. H. Werkman, J. M. Peeples, R. J. Cooke and E. A. Tolley (1993). "Arachidonic acid status correlates with first year growth in preterm infants." Proc Natl Acad Sci U S A **90**(3): 1073-7.

Carlson, S. E., S. H. Werkman and E. A. Tolley (1996). "Effect of long-chain n-3 fatty acid supplementation on visual acuity and growth of preterm infants with and without bronchopulmonary dysplasia." Am J Clin Nutr **63**(5): 687-97.

Carnielli, V. P., M. Simonato, G. Verlato, I. Luijendijk, M. De Curtis, P. J. Sauer and P. E. Cogo (2007). "Synthesis of long-chain polyunsaturated fatty acids in preterm newborns fed formula with long-chain polyunsaturated fatty acids." Am J Clin Nutr **86**(5): 1323-30.

Carnielli, V. P., D. J. Wattimena, I. H. Luijendijk, A. Boerlage, H. J. Degenhart and P. J. Sauer (1996). "The very low birth weight premature infant is capable of synthesizing arachidonic and docosahexaenoic acids from linoleic and linolenic acids." Pediatr Res **40**(1): 169-74.

Catalano, P. M. and H. M. Ehrenberg (2006). "The short- and long-term implications of maternal obesity on the mother and her offspring." Bjog **113**(10): 1126-33.

Catalano, P. M., L. Presley, J. Minium and S. Hauguel-de Mouzon (2009). "Fetuses of obese mothers develop insulin resistance in utero." Diabetes Care **32**(6): 1076-80.

Catalano, P. M., A. Thomas, L. Huston-Presley and S. B. Amini (2003). "Increased fetal adiposity: a very sensitive marker of abnormal in utero development." Am J Obstet Gynecol **189**(6): 1698-704.

Catalano, P. M., A. J. Thomas, D. A. Avallone and S. B. Amini (1995). "Anthropometric estimation of neonatal body composition." Am J Obstet Gynecol **173**(4): 1176-81.

Catalano, P. M., E. D. Tyzbir, S. R. Allen, J. H. McBean and T. L. McAuliffe (1992). "Evaluation of fetal growth by estimation of neonatal body composition." Obstet Gynecol **79**(1): 46-50.

Cedergren, M. (2006). "Effects of gestational weight gain and body mass index on obstetric outcome in Sweden." Int J Gynaecol Obstet **93**(3): 269-74.

Cedergren, M. I. (2007). "Optimal gestational weight gain for body mass index categories." Obstet Gynecol **110**(4): 759-64.

Cetin, I. and B. Koletzko (2008). "Long-chain omega-3 fatty acid supply in pregnancy and lactation." Curr Opin Clin Nutr Metab Care **11**(3): 297-302.

Chambaz, J., D. Ravel, M. C. Manier, D. Pepin, N. Mulliez and G. Bereziat (1985). "Essential fatty acids interconversion in the human fetal liver." Biol Neonate **47**(3): 136-40.

Chardigny, J. M., R. L. Wolff, E. Mager, J. L. Sebedio, L. Martine and P. Juaneda (1995). "Trans mono- and polyunsaturated fatty acids in human milk." Eur J Clin Nutr **49**(7): 523-31.

Chen, Z. Y., G. Pelletier, R. Hollywood and W. M. Ratnayake (1995). "Trans fatty acid isomers in Canadian human milk." Lipids **30**(1): 15-21.

Chomtho, S., J. C. Wells, J. E. Williams, A. Lucas and M. S. Fewtrell (2008). "Associations between birth weight and later body composition: evidence from the 4-component model." Am J Clin Nutr **88**(4): 1040-8.

Christie, W. W. (1995). "Separation of Lipid Classes from Plant Tissues by High-Performance Liquid Chromatography on Chemically Bonded Stationary Phases." J. High. Resolut. Chromatogr. **18**: 97-100.

Clandinin, M. T., J. E. Chappell, T. Heim, P. R. Swyer and G. W. Chance (1981). "Fatty acid utilization in perinatal de novo synthesis of tissues." Early Hum Dev **5**(4): 355-66.

Clandinin, M. T., J. E. Chappell, S. Leong, T. Heim, P. R. Swyer and G. W. Chance (1980). "Intrauterine fatty acid accretion rates in human brain: implications for fatty acid requirements." Early Hum Dev **4**(2): 121-9.

Clark, K. J., M. Makrides, M. A. Neumann and R. A. Gibson (1992). "Determination of the optimal ratio of linoleic acid to alpha-linolenic acid in infant formulas." J Pediatr **120**(4 Pt 2): S151-8.

Clarke, S. D. (2000). "Polyunsaturated fatty acid regulation of gene transcription: a mechanism to improve energy balance and insulin resistance." Br J Nutr **83 Suppl 1**: S59-66.

Cnattingius, S., R. Bergstrom, L. Lipworth and M. S. Kramer (1998). "Prepregnancy weight and the risk of adverse pregnancy outcomes." N Engl J Med **338**(3): 147-52.

Cogswell, M. E. and R. Yip (1995). "The influence of fetal and maternal factors on the distribution of birthweight." Semin Perinatol **19**(3): 222-40.

Connor, W. E., R. Lowensohn and L. Hatcher (1996). "Increased docosahexaenoic acid levels in human newborn infants by administration of sardines and fish oil during pregnancy." Lipids **31 Suppl**: S183-7.

Conquer, J. A. and B. J. Holub (1997). "Dietary docosahexaenoic acid as a source of eicosapentaenoic acid in vegetarians and omnivores." Lipids **32**(3): 341-5.

Conquer, J. A. and B. J. Holub (1998). "Effect of supplementation with different doses of DHA on the levels of circulating DHA as non-esterified fatty acid in subjects of Asian Indian background." J Lipid Res **39**(2): 286-92.

Cook, H. W. and E. A. Emken (1990). "Geometric and positional fatty acid isomers interact differently with desaturation and elongation of linoleic and linolenic acids in cultured glioma cells." Biochem Cell Biol **68**(3): 653-60.

Couet, C., J. Delarue, P. Ritz, J. M. Antoine and F. Lamisse (1997). "Effect of dietary fish oil on body fat mass and basal fat oxidation in healthy adults." Int J Obes Relat Metab Disord **21**(8): 637-43.

Crane, J. M., J. White, P. Murphy, L. Burrage and D. Hutchens (2009). "The effect of gestational weight gain by body mass index on maternal and neonatal outcomes." J Obstet Gynaecol Can **31**(1): 28-35.

Crawford, M. A., A. G. Hassam and G. Williams (1976). "Essential fatty acids and fetal brain growth." Lancet **1**(7957): 452-3.

Cunningham, F. G., K. Cox and N. F. Gant (1975). "Further observations on the nature of pressor responsivity to angiotensin II in human pregnancy." Obstet Gynecol **46**(5): 581-3.

D'Almeida, A., J. P. Carter, A. Anatol and C. Prost (1992). "Effects of a combination of evening primrose oil (gamma linolenic acid) and fish oil (eicosapentaenoic + docahexaenoic acid) versus magnesium, and versus placebo in preventing pre-eclampsia." Women Health **19**(2-3): 117-31.

DACH (2000). Referenzwerte für die Nährstoffzufuhr. Frankfurt am Main, Umschau/Braus.

de Arcos, F., C. Castelo-Branco, E. Casals, C. Sanllehy and V. Cararach (1998). "Normal and gestational diabetic pregnancies. Lipids, lipoproteins and apolipoproteins." J Reprod Med **43**(2): 144-8.

De Vriese, S. R., M. Dhont and A. B. Christophe (2003). "FA composition of cholesteryl esters and phospholipids in maternal plasma during pregnancy and at delivery and in cord plasma at birth." Lipids **38**(1): 1-7.

Decsi, T., I. Burus, S. Molnar, H. Minda and V. Veitl (2001). "Inverse association between trans isomeric and long-chain polyunsaturated fatty acids in cord blood lipids of full-term infants." Am J Clin Nutr **74**(3): 364-8.

Decsi, T. and B. Koletzko (2005). "N-3 fatty acids and pregnancy outcomes." Curr Opin Clin Nutr Metab Care **8**(2): 161-6.

Deheeger, M. and M. F. Rolland-Cachera (2004). "[Longitudinal study of anthropometric measurements in Parisian children aged ten months to 18 years]." Arch Pediatr **11**(9): 1139-44.

DeLany, J. P., M. M. Windhauser, C. M. Champagne and G. A. Bray (2000). "Differential oxidation of individual dietary fatty acids in humans." Am J Clin Nutr **72**(4): 905-11.

Demmelmair, H., M. Baumheuer, B. Koletzko, K. Dokoupil and G. Kratl (1998). "Metabolism of U13C-labeled linoleic acid in lactating women." J Lipid Res **39**(7): 1389-96.

Demmelmair, H., U. von Schenck, E. Behrendt, T. Sauerwald and B. Koletzko (1995). "Estimation of arachidonic acid synthesis in full term neonates using natural variation of 13C content." J Pediatr Gastroenterol Nutr **21**(1): 31-6.

Dennison, B. A., L. S. Edmunds, H. H. Stratton and R. M. Pruzek (2006). "Rapid infant weight gain predicts childhood overweight." Obesity (Silver Spring) **14**(3): 491-9.

Denomme, J., K. D. Stark and B. J. Holub (2005). "Directly quantitated dietary (n-3) fatty acid intakes of pregnant Canadian women are lower than current dietary recommendations." J Nutr **135**(2): 206-11.

Desoye, G., M. O. Schweditsch, K. P. Pfeiffer, R. Zechner and G. M. Kostner (1987). "Correlation of hormones with lipid and lipoprotein levels during normal pregnancy and postpartum." J Clin Endocrinol Metab **64**(4): 704-12.

Deurenberg, P., J. J. Pieters and J. G. Hautvast (1990). "The assessment of the body fat percentage by skinfold thickness measurements in childhood and young adolescence." Br J Nutr **63**(2): 293-303.

DeVader, S. R., H. L. Neeley, T. D. Myles and T. L. Leet (2007). "Evaluation of gestational weight gain guidelines for women with normal prepregnancy body mass index." Obstet Gynecol **110**(4): 745-51.

DGE (2004). Ernährungsbericht 2004, Deutsche Gesellschaft für Ernährung.

Dhall, K. and R. Bagga (1995). "Maternal determinants of birth weight of north Indian babies." Indian J Pediatr **62**(3): 333-44.

Di Stasi, D., R. Bernasconi, R. Marchioli, R. M. Marfisi, G. Rossi, G. Tognoni and M. T. Tacconi (2004). "Early modifications of fatty acid composition in plasma phospholipids, platelets and mononucleates of healthy volunteers after low doses of n-3 polyunsaturated fatty acids." Eur J Clin Pharmacol **60**(3): 183-90.

Djian, P., A. K. Roncari and C. H. Hollenberg (1983). "Influence of anatomic site and age on the replication and differentiation of rat adipocyte precursors in culture." J Clin Invest **72**(4): 1200-8.

Domingo, J. L. (2007). "Omega-3 fatty acids and the benefits of fish consumption: is all that glitters gold?" Environ Int **33**(7): 993-8.

Donahue, S. M., S. L. Rifas-Shiman, S. F. Olsen, D. R. Gold, M. W. Gillman and E. Oken (2009). "Associations of maternal prenatal dietary intake of n-3 and n-6 fatty acids with maternal and umbilical cord blood levels." Prostaglandins Leukot Essent Fatty Acids **80**(5-6): 289-96.

Dörner, G. (1975). Perinatal hormone levels and brain organization. Anatomical neuroendocrinology. W. Stumpf and L. Grant. Basel, Karger**:** 245-252.

Drews, U. (1993). Taschenatlas der Embryologie. Stuttgart, Thieme.

Dunstan, J. A., T. A. Mori, A. Barden, L. J. Beilin, P. G. Holt, P. C. Calder, A. L. Taylor and S. L. Prescott (2004). "Effects of n-3 polyunsaturated fatty acid supplementation in pregnancy on maternal and fetal erythrocyte fatty acid composition." Eur J Clin Nutr **58**(3): 429-37.

Durmus, B., D. O. Mook-Kanamori, S. Holzhauer, A. Hofman, E. M. van der Beek, G. Boehm, E. A. Steegers and V. W. Jaddoe (2009). "Growth in fetal life and infancy is associated with abdominal adiposity at the age of 2 years. The Generation R Study." Clin Endocrinol (Oxf).

Durnin, J. V. (1987). "Energy requirements of pregnancy: an integration of the longitudinal data from the five-country study." Lancet **2**(8568): 1131-3.

Durnin, J. V. and J. Womersley (1974). "Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years." Br J Nutr **32**(1): 77-97.

Durnwald, C., L. Huston-Presley, S. Amini and P. Catalano (2004). "Evaluation of body composition of large-for-gestational-age infants of women with gestational diabetes mellitus compared with women with normal glucose tolerance levels." Am J Obstet Gynecol **191**(3): 804-8.

Duttaroy, A. K. (2004). "Fetal growth and development: roles of fatty acid transport proteins and nuclear transcription factors in human placenta." Indian J Exp Biol **42**(8): 747-57.

Duttaroy, A. K. (2009). "Transport of fatty acids across the human placenta: a review." Prog Lipid Res **48**(1): 52-61.

Ehrenberg, H. M., B. M. Mercer and P. M. Catalano (2004). "The influence of obesity and diabetes on the prevalence of macrosomia." Am J Obstet Gynecol **191**(3): 964-8.

Ekelund, U., K. K. Ong, Y. Linne, M. Neovius, S. Brage, D. B. Dunger, N. J. Wareham and S. Rossner (2007). "Association of weight gain in infancy and early childhood with metabolic risk in young adults." J Clin Endocrinol Metab **92**(1): 98-103.

Elberg, J., J. R. McDuffie, N. G. Sebring, C. Salaita, M. Keil, D. Robotham, J. C. Reynolds and J. A. Yanovski (2004). "Comparison of methods to assess change in children's body composition." Am J Clin Nutr **80**(1): 64-9.

Elia, M., P. Betts, D. M. Jackson and J. Mulligan (2007). "Fetal programming of body dimensions and percentage body fat measured in prepubertal children with a 4-component model of body composition, dual-energy X-ray absorptiometry, deuterium dilution, densitometry, and skinfold thicknesses." Am J Clin Nutr **86**(3): 618-24.

Elias, S. L. and S. M. Innis (2001). "Infant plasma trans, n-6, and n-3 fatty acids and conjugated linoleic acids are related to maternal plasma fatty acids, length of gestation, and birth weight and length." Am J Clin Nutr **73**(4): 807-14.

Emken, E. A., R. O. Adlof and R. M. Gulley (1994). "Dietary linoleic acid influences desaturation and acylation of deuterium-labeled linoleic and linolenic acids in young adult males." Biochim Biophys Acta **1213**(3): 277-88.

Emken, E. A., R. O. Adlof, D. L. Hachey, C. Garza, M. R. Thomas and L. Brown-Booth (1989). "Incorporation of deuterium-labeled fatty acids into human milk, plasma, and lipoprotein phospholipids and cholesteryl esters." J Lipid Res **30**(3): 395-402.

Evans, R. M., G. D. Barish and Y. X. Wang (2004). "PPARs and the complex journey to obesity." Nat Med **10**(4): 355-61.

Everett, R. B., R. J. Worley, P. C. MacDonald and N. F. Gant (1978). "Effect of prostaglandin synthetase inhibitors on pressor response to angiotensin II in human pregnancy." J Clin Endocrinol Metab **46**(6): 1007-10.

Fall, C. H., C. Osmond, D. J. Barker, P. M. Clark, C. N. Hales, Y. Stirling and T. W. Meade (1995). "Fetal and infant growth and cardiovascular risk factors in women." Bmj **310**(6977): 428-32.

Fewtrell, M. S., K. Kennedy, A. Singhal, R. M. Martin, A. Ness, M. Hadders-Algra, B. Koletzko and A. Lucas (2008). "How much loss to follow-up is acceptable in long-term randomised trials and prospective studies?" Arch Dis Child **93**(6): 458-61.

Fidler, N. and B. Koletzko (2000). "The fatty acid composition of human colostrum." Eur J Nutr **39**(1): 31-7.

Fidler, N., T. Sauerwald, A. Pohl, H. Demmelmair and B. Koletzko (2000). "Docosahexaenoic acid transfer into human milk after dietary supplementation: a randomized clinical trial." J Lipid Res **41**(9): 1376-83.

Finley, D. A., B. Lonnerdal, K. G. Dewey and L. E. Grivetti (1985). "Breast milk composition: fat content and fatty acid composition in vegetarians and non-vegetarians." Am J Clin Nutr **41**(4): 787-800.

Fisch, R. O., M. K. Bilek and R. Ulstrom (1975). "Obesity and leanness at birth and their relationship to body habitus in later childhood." Pediatrics **56**(4): 521-8.

Fleith, M. and M. T. Clandinin (2005). "Dietary PUFA for preterm and term infants: review of clinical studies." Crit Rev Food Sci Nutr **45**(3): 205-29.

Fliesler, S. J. and R. E. Anderson (1983). "Chemistry and metabolism of lipids in the vertebrate retina." Prog Lipid Res **22**(2): 79-131.

Fomon, S. J., F. Haschke, E. E. Ziegler and S. E. Nelson (1982). "Body composition of reference children from birth to age 10 years." Am J Clin Nutr **35**(5 Suppl): 1169-75.

Fomon, S. J. and S. E. Nelson (2002). "Body composition of the male and female reference infants." Annu Rev Nutr **22**: 1-17.

Forsum, E., M. Lof, H. Olausson and E. Olhager (2006). "Maternal body composition in relation to infant birth weight and subcutaneous adipose tissue." Br J Nutr **96**(2): 408-14.

Forsum, E., A. Sadurskis and J. Wager (1988). "Resting metabolic rate and body composition of healthy Swedish women during pregnancy." Am J Clin Nutr **47**(6): 942-7.

Forsum, E., A. Sadurskis and J. Wager (1989). "Estimation of body fat in healthy Swedish women during pregnancy and lactation." Am J Clin Nutr **50**(3): 465-73.

Francois, C. A., S. L. Connor, R. C. Wander and W. E. Connor (1998). "Acute effects of dietary fatty acids on the fatty acids of human milk." Am J Clin Nutr **67**(2): 301-8.

Freinkel, N. (1980). "Banting Lecture 1980. Of pregnancy and progeny." Diabetes **29**(12): 1023-35.

Friedman, Z., A. Danon, E. L. Lamberth, Jr. and W. J. Mann (1978). "Cord blood fatty acid composition in infants and in their mothers during the third trimester." J Pediatr **92**(3): 461-6.

Gaillard, D., R. Negrel, M. Lagarde and G. Ailhaud (1989). "Requirement and role of arachidonic acid in the differentiation of pre-adipose cells." Biochem J **257**(2): 389-97.

Gale, C. R., C. N. Martyn, S. Kellingray, R. Eastell and C. Cooper (2001). "Intrauterine programming of adult body composition." J Clin Endocrinol Metab **86**(1): 267-72.

Galli, C., E. Agradi, A. Petroni and A. Socini (1980). "Modulation of prostaglandin production in tissues by dietary essential fatty acids." Acta Med Scand Suppl **642**: 171-9.

Gant, N. F., S. Chand, P. J. Whalley and P. C. MacDonald (1974). "The nature of pressor responsiveness to angiotensin II in human pregnancy." Obstet Gynecol **43**(6): 854.

Gant, N. F., P. J. Whalley, R. B. Everett, R. J. Worley and P. C. MacDonald (1987). "Control of vascular reactivity in pregnancy." Am J Kidney Dis **9**(4): 303-7.

Genzel-Boroviczeny, O., J. Wahle and B. Koletzko (1997). "Fatty acid composition of human milk during the 1st month after term and preterm delivery." Eur J Pediatr **156**(2): 142-7.

Geppert, J., V. Kraft, H. Demmelmair and B. Koletzko (2006). "Microalgal docosahexaenoic acid decreases plasma triacylglycerol in normolipidaemic vegetarians: a randomised trial." Br J Nutr **95**(4): 779-86.

Gere, A., E. A. Bernolak, O. Gaal, P. Cholnoky and I. Ory (1983). "Fatty acid composition of human milk and milk-based formulas in Hungary." Acta Paediatr Hung **24**(1): 53-61.

Gerster, H. (1998). "Can adults adequately convert alpha-linolenic acid (18:3n-3) to eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3)?" Int J Vitam Nutr Res **68**(3): 159-73.

Ghebremeskel, K., M. A. Crawford, C. Lowy, Y. Min, B. Thomas, I. Golfetto, D. Bitsanis and K. Costeloe (2000). "Arachidonic and docosahexaenoic acids are strongly associated in maternal and neonatal blood." Eur J Clin Nutr **54**(1): 50-6.

Gibson, R. A., M. A. Neumann and M. Makrides (1997). "Effect of increasing breast milk docosahexaenoic acid on plasma and erythrocyte phospholipid fatty acids and neural indices of exclusively breast fed infants." Eur J Clin Nutr **51**(9): 578-84.

Gillman, M. W. (2008). "The first months of life: a critical period for development of obesity." Am J Clin Nutr **87**(6): 1587-9.

Gillman, M. W., S. Rifas-Shiman, C. S. Berkey, A. E. Field and G. A. Colditz (2003). "Maternal gestational diabetes, birth weight, and adolescent obesity." Pediatrics **111**(3): e221-6.

Gillman, M. W., S. L. Rifas-Shiman, K. Kleinman, E. Oken, J. W. Rich-Edwards and E. M. Taveras (2008). "Developmental origins of childhood overweight: potential public health impact." Obesity (Silver Spring) **16**(7): 1651-6.

Godfrey, K. and D. J. Barker (2003). Fetal, infant and childhood growth and adult health. Nutrition in early life. J. Morgan and J. Dickerson. Chichester, Wiley & Sons.

Godfrey, K., S. Robinson, D. J. Barker, C. Osmond and V. Cox (1996). "Maternal nutrition in early and late pregnancy in relation to placental and fetal growth." Bmj **312**(7028): 410-4.

Godfrey, K. M., D. J. Barker, S. Robinson and C. Osmond (1997). "Maternal birthweight and diet in pregnancy in relation to the infant's thinness at birth." Br J Obstet Gynaecol **104**(6): 663-7.

Grandjean, P., K. S. Bjerve, P. Weihe and U. Steuerwald (2001). "Birthweight in a fishing community: significance of essential fatty acids and marine food contaminants." Int J Epidemiol **30**(6): 1272-8.

Gregoire, F. M., C. M. Smas and H. S. Sul (1998). "Understanding adipocyte differentiation." Physiol Rev **78**(3): 783-809.

Groh-Wargo, S., J. Jacobs, N. Auestad, D. L. O'Connor, J. J. Moore and E. Lerner (2005). "Body composition in preterm infants who are fed long-chain polyunsaturated fatty acids: a prospective, randomized, controlled trial." Pediatr Res **57**(5 Pt 1): 712-8.

Gude, N. M., C. T. Roberts, B. Kalionis and R. G. King (2004). "Growth and function of the normal human placenta." Thromb Res **114**(5-6): 397-407.

Guihard-Costa, A. M., E. Papiernik and S. Kolb (2004). "Maternal predictors of subcutaneous fat in the term newborn." Acta Paediatr **93**(3): 346-9.

Guillaume, M., L. Lapidus, F. Beckers, A. Lambert and P. Bjorntorp (1995). "Familial trends of obesity through three generations: the Belgian-Luxembourg child study." Int J Obes Relat Metab Disord **19 Suppl 3**: S5-9.

Hachey, D. L., M. R. Thomas, E. A. Emken, C. Garza, L. Brown-Booth, R. O. Adlof and P. D. Klein (1987). "Human lactation: maternal transfer of dietary triglycerides labeled with stable isotopes." J Lipid Res **28**(10): 1185-92.

Hadders-Algra, M. (2008). "Prenatal long-chain polyunsaturated fatty acid status: the importance of a balanced intake of docosahexaenoic acid and arachidonic acid." J Perinat Med **36**(2): 101-9.

Hager, A., L. Sjostrm, B. Arvidsson, P. Bjorntorp and U. Smith (1977). "Body fat and adipose tissue cellularity in infants: a longitudinal study." Metabolism **26**(6): 607-14.

Haggarty, P. (2002). "Placental regulation of fatty acid delivery and its effect on fetal growth--a review." Placenta **23 Suppl A**: S28-38.

Haggarty, P. (2004). "Effect of placental function on fatty acid requirements during pregnancy." Eur J Clin Nutr **58**(12): 1559-70.

Haggarty, P., J. Ashton, M. Joynson, D. R. Abramovich and K. Page (1999). "Effect of maternal polyunsaturated fatty acid concentration on transport by the human placenta." Biol Neonate **75**(6): 350-9.

Halldorsson, T. I., H. M. Meltzer, I. Thorsdottir, V. Knudsen and S. F. Olsen (2007). "Is high consumption of fatty fish during pregnancy a risk factor for fetal growth retardation? A study of 44,824 Danish pregnant women." Am J Epidemiol **166**(6): 687-96.

Hamosh, M. and N. Salem, Jr. (1998). "Long-chain polyunsaturated fatty acids." Biol Neonate **74**(2): 106-20.

Hanebutt, F. L., H. Demmelmair, B. Schiessl, E. Larque and B. Koletzko (2008). "Long-chain polyunsaturated fatty acid (LC-PUFA) transfer across the placenta." Clin Nutr **27**(5): 685-93.

Harrington, T. A., E. L. Thomas, G. Frost, N. Modi and J. D. Bell (2004). "Distribution of adipose tissue in the newborn." Pediatr Res **55**(3): 437-41.

Harris, W. S. (1997). "n-3 fatty acids and serum lipoproteins: human studies." Am J Clin Nutr **65**(5 Suppl): 1645S-1654S.

Harris, W. S., W. E. Connor and S. Lindsey (1984). "Will dietary omega-3 fatty acids change the composition of human milk?" Am J Clin Nutr **40**(4): 780-5.

Harrison, G. G., R. S. Branson and Y. E. Vaucher (1983). "Association of maternal smoking with body composition of the newborn." Am J Clin Nutr **38**(5): 757-62.

Harzer, G., M. Haug, I. Dieterich and P. R. Gentner (1983). "Changing patterns of human milk lipids in the course of the lactation and during the day." Am J Clin Nutr **37**(4): 612-21.

Hauner, H., M. Wabitsch and E. Pfeiffer (1989). Proliferation and differentiation of adipose tissue derived stromal-vascular cells from children at different ages. First European Congress on Obesity, Libbey, London.

Hauner, H., C. Vollhardt, K. T. Schneider, A. Zimmermann, T. Schuster and U. Amann-Gassner (2009). "The impact of nutritional fatty acids during pregnancy and lactation on early human adipose tissue development. Rationale and design of the INFAT study." Ann Nutr Metab **54**(2): 97-103.

Hausman, D. B., H. M. McCloskey and R. J. Martin (1991). "Maternal dietary fat type influences the growth and fatty acid composition of newborn and weanling rats." J Nutr **121**(12): 1917-23.

Hay, W. W., Jr. (1994). "Placental transport of nutrients to the fetus." Horm Res **42**(4-5): 215-22.

Heird, W. C. and A. Lapillonne (2005). "The role of essential fatty acids in development." Annu Rev Nutr **25**: 549-71.

Helland, I. B., K. Saarem, O. D. Saugstad and C. A. Drevon (1998). "Fatty acid composition in maternal milk and plasma during supplementation with cod liver oil." Eur J Clin Nutr **52**(11): 839-45.

Helland, I. B., O. D. Saugstad, L. Smith, K. Saarem, K. Solvoll, T. Ganes and C. A. Drevon (2001). "Similar effects on infants of n-3 and n-6 fatty acids supplementation to pregnant and lactating women." Pediatrics **108**(5): E82.

Henderson, R. A., R. G. Jensen, C. J. Lammi-Keefe, A. M. Ferris and K. R. Dardick (1992). "Effect of fish oil on the fatty acid composition of human milk and maternal and infant erythrocytes." Lipids **27**(11): 863-9.

Herrera, E. (2002). "Lipid metabolism in pregnancy and its consequences in the fetus and newborn." Endocrine **19**(1): 43-55.

Herrera, E., E. Amusquivar, I. Lopez-Soldado and H. Ortega (2006). "Maternal lipid metabolism and placental lipid transfer." Horm Res **65 Suppl 3**: 59-64.

Herrera, E., H. Ortega, G. Alvino, N. Giovannini, E. Amusquivar and I. Cetin (2004). "Relationship between plasma fatty acid profile and antioxidant vitamins during normal pregnancy." Eur J Clin Nutr **58**(9): 1231-8.

Hibbeln, J. R., J. M. Davis, C. Steer, P. Emmett, I. Rogers, C. Williams and J. Golding (2007). "Maternal seafood consumption in pregnancy and neurodevelopmental outcomes in childhood (ALSPAC study): an observational cohort study." Lancet **369**(9561): 578-85.

Hibbeln, J. R., L. R. Nieminen, T. L. Blasbalg, J. A. Riggs and W. E. Lands (2006). "Healthy intakes of n-3 and n-6 fatty acids: estimations considering worldwide diversity." Am J Clin Nutr **83**(6 Suppl): 1483S-1493S.

Highman, T. J., J. E. Friedman, L. P. Huston, W. W. Wong and P. M. Catalano (1998). "Longitudinal changes in maternal serum leptin concentrations, body composition, and resting metabolic rate in pregnancy." Am J Obstet Gynecol **178**(5): 1010-5.

Hill, J. O., J. C. Peters, D. Lin, F. Yakubu, H. Greene and L. Swift (1993). "Lipid accumulation and body fat distribution is influenced by type of dietary fat fed to rats." Int J Obes Relat Metab Disord **17**(4): 223-36.

Hillier, T. A., K. L. Pedula, M. M. Schmidt, J. A. Mullen, M. A. Charles and D. J. Pettitt (2007). "Childhood obesity and metabolic imprinting: the ongoing effects of maternal hyperglycemia." Diabetes Care **30**(9): 2287-92.

Hoffman, D. R., E. E. Birch, D. G. Birch, R. Uauy, Y. S. Castaneda, M. G. Lapus and D. H. Wheaton (2000). "Impact of early dietary intake and blood lipid composition of long-chain polyunsaturated fatty acids on later visual development." J Pediatr Gastroenterol Nutr **31**(5): 540-53.

Hoggard, N., J. Crabtree, S. Allstaff, D. R. Abramovich and P. Haggarty (2001). "Leptin secretion to both the maternal and fetal circulation in the ex vivo perfused human term placenta." Placenta **22**(4): 347-52.

Hornstra, G. (2000). "Essential fatty acids in mothers and their neonates." Am J Clin Nutr **71**(5 Suppl): 1262S-9S.

Hussein, N., E. Ah-Sing, P. Wilkinson, C. Leach, B. A. Griffin and D. J. Millward (2005). "Long-chain conversion of [13C]linoleic acid and alpha-linolenic acid in response to marked changes in their dietary intake in men." J Lipid Res **46**(2): 269-80.

Hytten, F. and **I. Leitch** (1971). The physiology of human pregnancy. Oxford, UK, Blackwell Scientific Publications.

Idota, T., M. Sakurai, M. Sugawara, Y. Matuoka, Y. Ishiyama, Y. Murakami, H. Moriguchi, M. Takuchi, K. Shimoda and Y. Asai (1991). "The latest survey for the composition of milk obtained from Japanese mothers. Part II. Changes of fatty acid composition, phospholipid and cholesterol contents during lactation." Jpn J Pediatr Gastroenterol Nutr **5**: 159-173.

Innis, S. M. (2005). "Essential fatty acid transfer and fetal development." Placenta **26 Suppl A**: S70-5.

Innis, S. M., D. H. Adamkin, R. T. Hall, S. C. Kalhan, C. Lair, M. Lim, D. C. Stevens, P. F. Twist, D. A. Diersen-Schade, C. L. Harris, K. L. Merkel and J. W. Hansen (2002). "Docosahexaenoic acid and arachidonic acid enhance growth with no adverse effects in preterm infants fed formula." J Pediatr **140**(5): 547-54.

Innis, S. M., N. Auestad and J. S. Siegman (1996). "Blood lipid docosahexaenoic and arachidonic acid in term gestation infants fed formulas with high docosahexaenoic acid, low eicosapentaenoic acid fish oil." Lipids **31**(6): 617-25.

Innis, S. M. and S. L. Elias (2003). "Intakes of essential n-6 and n-3 polyunsaturated fatty acids among pregnant Canadian women." Am J Clin Nutr **77**(2): 473-8.

Innis, S. M. and R. W. Friesen (2008). "Essential n-3 fatty acids in pregnant women and early visual acuity maturation in term infants." Am J Clin Nutr **87**(3): 548-57.

Insull, W., T. Hirsch, T. James and E. Ahren (1959). "The fatty acids of human milk. I. Alterations produced by manipulation of caloric balance and exchange of dietary fats." J Clin Invest **38**: 443-450.

IOM (1990). Nutrition During Pregnancy. Washington, DC, National Academy Press.

IOM (2009). Weight gain during pregnancy: Reexamining the Guidelines. Washington, D.C., The National Academies Press.

Javadi, M., H. Everts, R. Hovenier, S. Kocsis, A. E. Lankhorst, A. G. Lemmens, J. T. Schonewille, A. H. Terpstra and A. C. Beynen (2004). "The effect of six different C18 fatty acids on body fat and energy metabolism in mice." Br J Nutr **92**(3): 391-9.

Jensen, C. L. (2006). "Effects of n-3 fatty acids during pregnancy and lactation." Am J Clin Nutr **83**(6 Suppl): 1452S-1457S.

Jensen, C. L., H. Chen, J. K. Fraley, R. E. Anderson and W. C. Heird (1996). "Biochemical effects of dietary linoleic/alpha-linolenic acid ratio in term infants." Lipids **31**(1): 107-13.

Jensen, C. L., M. Maude, R. E. Anderson and W. C. Heird (2000). "Effect of docosahexaenoic acid supplementation of lactating women on the fatty acid composition of breast milk lipids and maternal and infant plasma phospholipids." Am J Clin Nutr **71**(1 Suppl): 292S-9S.

Jensen, C. L., T. C. Prager, Y. Zou, J. K. Fraley, M. Maude, R. E. Anderson and W. C. Heird (1999). "Effects of maternal docosahexaenoic acid supplementation on visual function and growth of breast-fed term infants." Lipids **34 Suppl**: S225.

Jensen, C. L., R. G. Voigt, T. C. Prager, Y. L. Zou, J. K. Fraley, J. C. Rozelle, M. R. Turcich, A. M. Llorente, R. E. Anderson and W. C. Heird (2005). "Effects of maternal docosahexaenoic acid intake on visual function and neurodevelopment in breastfed term infants." Am J Clin Nutr **82**(1): 125-32.

Jensen, R. G. (1999). "Lipids in human milk." Lipids **34**(12): 1243-71.

Jones, P. J. and D. A. Schoeller (1988). "Polyunsaturated:saturated ratio of diet fat influences energy substrate utilization in the human." Metabolism **37**(2): 145-51.

Judge, M. P., O. Harel and C. J. Lammi-Keefe (2007). "A docosahexaenoic acid-functional food during pregnancy benefits infant visual acuity at four but not six months of age." Lipids **42**(2): 117-22.

Jump, D. B. (2002). "Dietary polyunsaturated fatty acids and regulation of gene transcription." Curr Opin Lipidol **13**(2): 155-64.

Jump, D. B., D. Botolin, Y. Wang, J. Xu, O. Demeure and B. Christian (2008). "Docosahexaenoic acid (DHA) and hepatic gene transcription." Chem Phys Lipids **153**(1): 3-13.

Katan, M. B., J. P. Deslypere, A. P. van Birgelen, M. Penders and M. Zegwaard (1997). "Kinetics of the incorporation of dietary fatty acids into serum cholesteryl esters, erythrocyte membranes, and adipose tissue: an 18-month controlled study." J Lipid Res **38**(10): 2012-22.

Kensara, O. A., S. A. Wootton, D. I. Phillips, M. Patel, A. A. Jackson and M. Elia (2005). "Fetal programming of body composition: relation between birth weight and body composition measured with dual-energy X-ray absorptiometry and anthropometric methods in older Englishmen." Am J Clin Nutr **82**(5): 980-7.

Kiel, D. W., E. A. Dodson, R. Artal, T. K. Boehmer and T. L. Leet (2007). "Gestational weight gain and pregnancy outcomes in obese women: how much is enough?" Obstet Gynecol **110**(4): 752-8.

Kimura, R. E. (1989). "Fatty acid metabolism in the fetus." Semin Perinatol **13**(3): 202-10.

King, J. C. (2000). "Physiology of pregnancy and nutrient metabolism." Am J Clin Nutr **71**(5 Suppl): 1218S-25S.

Kirchengast, S. and B. Hartmann (1998). "Maternal prepregnancy weight status and pregnancy weight gain as major determinants for newborn weight and size." Ann Hum Biol **25**(1): 17-28.

Kirkland, J. L., C. H. Hollenberg and W. S. Gillon (1990). "Age, anatomic site, and the replication and differentiation of adipocyte precursors." Am J Physiol **258**(2 Pt 1): C206-10.

Klauss, S. (2001). Overview: Biological Significance of Fat and Adipose Tissues. Adipose Tissues. S. Klauss. Georgetown, Texas, USA, Eurekah.com / Landes Bioscience**:** 1-10.

Knittle, J. L., F. Ginsberg-Fellner and R. E. Brown (1977). "Adipose tissue development in man." Am J Clin Nutr **30**(5): 762-6.

Knittle, J. L., K. Timmers, F. Ginsberg-Fellner, R. E. Brown and D. P. Katz (1979). "The growth of adipose tissue in children and adolescents. Cross-sectional and longitudinal studies of adipose cell number and size." J Clin Invest **63**(2): 239-46.

Knopp, R. H., R. O. Bergelin, P. W. Wahl, C. E. Walden, M. Chapman and S. Irvine (1982). "Population-based lipoprotein lipid reference values for pregnant women compared to nonpregnant women classified by sex hormone usage." Am J Obstet Gynecol **143**(6): 626-37.

Knopp, R. H., M. R. Warth, D. Charles, M. Childs, J. R. Li, H. Mabuchi and M. I. Van Allen (1986). "Lipoprotein metabolism in pregnancy, fat transport to the fetus, and the effects of diabetes." Biol Neonate **50**(6): 297-317.

Knudsen, V. K., I. M. Orozova-Bekkevold, T. B. Mikkelsen, S. Wolff and S. F. Olsen (2008). "Major dietary patterns in pregnancy and fetal growth." Eur J Clin Nutr **62**(4): 463-70.

Koletzko, B. (1992). "Trans fatty acids may impair biosynthesis of long-chain polyunsaturates and growth in man." Acta Paediatr **81**(4): 302-6.

Koletzko, B. (2008). "Prenatal supply of docosahexaenoic acid (DHA): should we be worried?" J Perinat Med **36**(3): 265-7; author reply 268-9.

Koletzko, B. and M. Braun (1991). "Arachidonic acid and early human growth: is there a relation?" Ann Nutr Metab **35**(3): 128-31.

Koletzko, B., I. Cetin and J. T. Brenna (2007). "Dietary fat intakes for pregnant and lactating women." Br J Nutr **98**(5): 873-7.

Koletzko, B. and T. Decsi (1997). "Metabolic aspects of trans fatty acids." Clin Nutr **16**(5): 229-37.

Koletzko, B., E. Lien, C. Agostoni, H. Bohles, C. Campoy, I. Cetin, T. Decsi, J. W. Dudenhausen, C. Dupont, S. Forsyth, I. Hoesli, W. Holzgreve, A. Lapillonne, G. Putet, N. J. Secher, M. Symonds, H. Szajewska, P. Willatts and R. Uauy (2008). "The roles of long-chain polyunsaturated fatty acids in pregnancy, lactation and infancy: review of current knowledge and consensus recommendations." J Perinat Med **36**(1): 5-14.

Koletzko, B., I. Thiel and P. O. Abiodun (1992). "The fatty acid composition of human milk in Europe and Africa." J Pediatr **120**(4 Pt 2): S62-70.

Koo, W. W., J. C. Walters and E. M. Hockman (2004). "Body composition in neonates: relationship between measured and derived anthropometry with dual-energy X-ray absorptiometry measurements." Pediatr Res **56**(5): 694-700.

Korotkova, M., B. Gabrielsson, M. Lonn, L. A. Hanson and B. Strandvik (2002). "Leptin levels in rat offspring are modified by the ratio of linoleic to alpha-linolenic acid in the maternal diet." J Lipid Res **43**(10): 1743-9.

Kramer, M. S. (1993). "Effects of energy and protein intakes on pregnancy outcome: an overview of the research evidence from controlled clinical trials." Am J Clin Nutr **58**(5): 627-35.

Kramer, M. S. and R. Kakuma (2003). "Energy and protein intake in pregnancy." Cochrane Database Syst Rev(4): CD000032.

Kratz, M., H. S. Callahan, P. Y. Yang, C. C. Matthys and D. S. Weigle (2009). "Dietary n-3-polyunsaturated fatty acids and energy balance in overweight or moderately obese men and women: a randomized controlled trial." Nutr Metab (Lond) **6**: 24.

Krauss-Etschmann, S., R. Shadid, C. Campoy, E. Hoster, H. Demmelmair, M. Jimenez, A. Gil, M. Rivero, B. Veszpremi, T. Decsi and B. V. Koletzko (2007). "Effects of fish-oil and folate supplementation of pregnant women on maternal and fetal plasma concentrations of docosahexaenoic acid and eicosapentaenoic acid: a European randomized multicenter trial." Am J Clin Nutr **85**(5): 1392-400.

Krebs, J. D., L. M. Browning, N. K. McLean, J. L. Rothwell, G. D. Mishra, C. S. Moore and S. A. Jebb (2006). "Additive benefits of long-chain n-3 polyunsaturated fatty acids and weight-loss in the management of cardiovascular disease risk in overweight hyperinsulinaemic women." Int J Obes (Lond) **30**(10): 1535-44.

Kris-Etherton, P. M., J. A. Grieger and T. D. Etherton (2009). "Dietary reference intakes for DHA and EPA." Prostaglandins Leukot Essent Fatty Acids.

Kris-Etherton, P. M., D. S. Taylor, S. Yu-Poth, P. Huth, K. Moriarty, V. Fishell, R. L. Hargrove, G. Zhao and T. D. Etherton (2000). "Polyunsaturated fatty acids in the food chain in the United States." Am J Clin Nutr **71**(1 Suppl): 179S-88S.

Kurth, B. M. and A. Schaffrath Rosario (2007). "[The prevalence of overweight and obese children and adolescents living in Germany. Results of the German Health Interview and Examination Survey for Children and Adolescents (KiGGS)]." Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz **50**(5-6): 736-43.

Labayen, I., L. A. Moreno, M. G. Blay, V. A. Blay, M. I. Mesana, M. Gonzalez-Gross, G. Bueno, A. Sarria and M. Bueno (2006). "Early programming of body composition and fat distribution in adolescents." J Nutr **136**(1): 147-52.

Lagiou, P., R. M. Tamimi, L. A. Mucci, H. O. Adami, C. C. Hsieh and D. Trichopoulos (2004). "Diet during pregnancy in relation to maternal weight gain and birth size." Eur J Clin Nutr **58**(2): 231-7.

Laidlaw, M. and B. J. Holub (2003). "Effects of supplementation with fish oil-derived n-3 fatty acids and gamma-linolenic acid on circulating plasma lipids and fatty acid profiles in women." Am J Clin Nutr **77**(1): 37-42.

Lain, K. Y. and P. M. Catalano (2007). "Metabolic changes in pregnancy." Clin Obstet Gynecol **50**(4): 938-48.

Lakin, V., P. Haggarty, D. R. Abramovich, J. Ashton, C. F. Moffat, G. McNeill, P. J. Danielian and D. Grubb (1998). "Dietary intake and tissue concentration of fatty acids in omnivore, vegetarian and diabetic pregnancy." Prostaglandins Leukot Essent Fatty Acids **59**(3): 209-20.

Langley-Evans, S. C. (2008). "Nutritional programming of disease: unravelling the mechanism." J Anat.

Langley-Evans, S. C., G. J. Phillips and A. A. Jackson (1994). "In utero exposure to maternal low protein diets induces hypertension in weanling rats, independently of maternal blood pressure changes." Clin Nutr **13**(5): 319-24.

Lapillonne, A., S. D. Clarke and W. C. Heird (2003). "Plausible mechanisms for effects of long-chain polyunsaturated fatty acids on growth." J Pediatr **143**(4 Suppl): S9-16.

Larque, E., H. Demmelmair, B. Berger, U. Hasbargen and B. Koletzko (2003). "In vivo investigation of the placental transfer of (13)C-labeled fatty acids in humans." J Lipid Res **44**(1): 49-55.

Larque, E., S. Krauss-Etschmann, C. Campoy, D. Hartl, J. Linde, M. Klingler, H. Demmelmair, A. Cano, A. Gil, B. Bondy and B. Koletzko (2006). "Docosahexaenoic acid supply in pregnancy affects placental expression of fatty acid transport proteins." Am J Clin Nutr **84**(4): 853-61.

Lauritzen, L., H. S. Hansen, M. H. Jorgensen and K. F. Michaelsen (2001). "The essentiality of long chain n-3 fatty acids in relation to development and function of the brain and retina." Prog Lipid Res **40**(1-2): 1-94.

Lauritzen, L., C. Hoppe, E. M. Straarup and K. F. Michaelsen (2005). "Maternal fish oil supplementation in lactation and growth during the first 2.5 years of life." Pediatr Res **58**(2): 235-42.

Lauritzen, L., M. H. Jorgensen, T. B. Mikkelsen, M. Skovgaard, E. M. Straarup, S. F. Olsen, C. E. Hoy and K. F. Michaelsen (2004). "Maternal fish oil supplementation in lactation: effect on visual acuity and n-3 fatty acid content of infant erythrocytes." Lipids **39**(3): 195-206.

Lederman, S. A., A. Paxton, S. B. Heymsfield, J. Wang, J. Thornton and R. N. Pierson, Jr. (1997). "Body fat and water changes during pregnancy in women with different body weight and weight gain." Obstet Gynecol **90**(4 Pt 1): 483-8.

Lederman, S. A., A. Paxton, S. B. Heymsfield, J. Wang, J. Thornton and R. N. Pierson, Jr. (1999). "Maternal body fat and water during pregnancy: do they raise infant birth weight?" Am J Obstet Gynecol **180**(1 Pt 1): 235-40.

Lepage, G. and C. C. Roy (1984). "Improved recovery of fatty acid through direct transesterification without prior extraction or purification." J Lipid Res **25**(12): 1391-6.

Lesser, K. B. and M. W. Carpenter (1994). "Metabolic changes associated with normal pregnancy and pregnancy complicated by diabetes mellitus." Semin Perinatol **18**(5): 399-406.

Li, H., A. D. Stein, H. X. Barnhart, U. Ramakrishnan and R. Martorell (2003). "Associations between prenatal and postnatal growth and adult body size and composition." Am J Clin Nutr **77**(6): 1498-505.

Lien, E. L. (2009). "Toxicology and safety of DHA." Prostaglandins Leukot Essent Fatty Acids.

Lippi, G., A. Albiero, M. Montagnana, G. L. Salvagno, S. Scevarolli, M. Franchi and G. C. Guidi (2007). "Lipid and lipoprotein profile in physiological pregnancy." Clin Lab **53**(3-4): 173-7.

Loos, R. J., G. Beunen, R. Fagard, C. Derom and R. Vlietinck (2001). "Birth weight and body composition in young adult men--a prospective twin study." Int J Obes Relat Metab Disord **25**(10): 1537-45.

Loos, R. J., G. Beunen, R. Fagard, C. Derom and R. Vlietinck (2002). "Birth weight and body composition in young women: a prospective twin study." Am J Clin Nutr **75**(4): 676-82.

Loosemore, E. D., M. P. Judge and C. J. Lammi-Keefe (2004). "Dietary intake of essential and long-chain polyunsaturated fatty acids in pregnancy." Lipids **39**(5): 421-4.

Löschl, L. (1996). Anthropometrie der Schwangeren. Institut für Ernährungswissenschaften. Wien, Universität Wien. **Magister:** 46-52.

Lucas, A. (1991). "Programming by early nutrition in man." Ciba Found Symp **156**: 38-50; discussion 50-5.

Lundstrom, V. and K. Green (1978). "Endogenous levels of prostaglandin F2alpha and its main metabolites in plasma and endometrium of normal and dysmenorrheic women." Am J Obstet Gynecol **130**(6): 640-6.

Mace, K., Y. Shahkhalili, O. Aprikian and S. Stan (2006). "Dietary fat and fat types as early determinants of childhood obesity: a reappraisal." Int J Obes **30**(S4): S50-S57.

MacLeod, S. and J. L. Kiely (1988). "The effects of maternal age and parity on birthweight: a population-based study in New York City." Int J Gynaecol Obstet **26**(1): 11-9.

Madsen, L., A. C. Rustan, H. Vaagenes, K. Berge, E. Dyroy and R. K. Berge (1999). "Eicosapentaenoic and docosahexaenoic acid affect mitochondrial and peroxisomal fatty acid oxidation in relation to substrate preference." Lipids **34**(9): 951-63.

Makrides, M., L. Duley and S. F. Olsen (2006). "Marine oil, and other prostaglandin precursor, supplementation for pregnancy uncomplicated by pre-eclampsia or intrauterine growth restriction." Cochrane Database Syst Rev **3**: CD003402.

Makrides, M., M. A. Neumann and R. A. Gibson (1996). "Effect of maternal docosahexaenoic acid (DHA) supplementation on breast milk composition." Eur J Clin Nutr **50**(6): 352-7.

Makrides, M., M. A. Neumann, K. Simmer and R. A. Gibson (1995). "Erythrocyte fatty acids of term infants fed either breast milk, standard formula, or formula supplemented with long-chain polyunsaturates." Lipids **30**(10): 941-8.

Malcolm, C. A., D. L. McCulloch, C. Montgomery, A. Shepherd and L. T. Weaver (2003). "Maternal docosahexaenoic acid supplementation during pregnancy and visual evoked potential development in term infants: a double blind, prospective, randomised trial." Arch Dis Child Fetal Neonatal Ed **88**(5): F383-90.

Martin, J. C., P. Bougnoux, J. M. Antoine, M. Lanson and C. Couet (1993). "Triacylglycerol structure of human colostrum and mature milk." Lipids **28**(7): 637-43.

Martinez, M. and I. Mougan (1998). "Fatty acid composition of human brain phospholipids during normal development." J Neurochem **71**(6): 2528-33.

Massiera, F., P. Saint-Marc, J. Seydoux, T. Murata, T. Kobayashi, S. Narumiya, P. Guesnet, E. Z. Amri, R. Negrel and G. Ailhaud (2003). "Arachidonic acid and prostacyclin signaling promote adipose tissue development: a human health concern?" J Lipid Res **44**(2): 271-9.

Mathews, F., L. Youngman and A. Neil (2004). "Maternal circulating nutrient concentrations in pregnancy: implications for birth and placental weights of term infants." Am J Clin Nutr **79**(1): 103-10.

Mathews, F., P. Yudkin and A. Neil (1999). "Influence of maternal nutrition on outcome of pregnancy: prospective cohort study." Bmj **319**(7206): 339-43.

Max-Rubner-Institut (2008). Nationale Verzehrsstudie II Karlsruhe, Max Rubner-Institut, Bundesforschungsinstitut für Ernährung und Lebensmittel.

Mazurkiewicz, J. C., G. F. Watts, F. G. Warburton, B. M. Slavin, C. Lowy and E. Koukkou (1994). "Serum lipids, lipoproteins and apolipoproteins in pregnant non-diabetic patients." J Clin Pathol **47**(8): 728-31.

McBride, O. W. and E. D. Korn (1964). "The uptake of doubly labeled chylomicrons by guinea pig mammary gland and liver." J Lipid Res **5**(3): 459-67.

McLaren, D. S. (1987). "A fresh look at some perinatal growth and nutritional standards." World Rev Nutr Diet **49**: 87-120.

Mellies, M. J., T. T. Ishikawa, P. S. Gartside, K. Burton, J. MacGee, K. Allen, P. M. Steiner, D. Brady and C. J. Glueck (1979). "Effects of varying maternal dietary fatty acids in lactating women and their infants." Am J Clin Nutr **32**(2): 299-303.

Meyer, B. J., N. J. Mann, J. L. Lewis, G. C. Milligan, A. J. Sinclair and P. R. Howe (2003). "Dietary intakes and food sources of omega-6 and omega-3 polyunsaturated fatty acids." Lipids **38**(4): 391-8.

Milnes, M., R. Roberts and L. Guillette (2002). "Effects of incubation temperature and estrogen exposure on aromatase activity in the brain and gonads of embryonic alligators." Environ Health Perspect **110**: 393-396.

Minda, H., E. Larque, B. Koletzko and T. Decsi (2002). "Systematic review of fatty acid composition of plasma phospholipids of venous cord blood in full-term infants." Eur J Nutr **41**(3): 125-31.

Monteiro, P. O. and C. G. Victora (2005). "Rapid growth in infancy and childhood and obesity in later life--a systematic review." Obes Rev **6**(2): 143-54.

Montelongo, A., M. A. Lasuncion, L. F. Pallardo and E. Herrera (1992). "Longitudinal study of plasma lipoproteins and hormones during pregnancy in normal and diabetic women." Diabetes **41**(12): 1651-9.

Montgomery, C., B. K. Speake, A. Cameron, N. Sattar and L. T. Weaver (2003). "Maternal docosahexaenoic acid supplementation and fetal accretion." Br J Nutr **90**(1): 135-45.

Moreira, P., C. Padez, I. Mourao-Carvalhal and V. Rosado (2007). "Maternal weight gain during pregnancy and overweight in Portuguese children." Int J Obes (Lond) **31**(4): 608-14.

Murphy, M. J., B. S. Metcalf, A. N. Jeffery, L. D. Voss and T. J. Wilkin (2006). "Does lean rather than fat mass provide the link between birth weight, BMI, and metabolic risk? EarlyBird 23." Pediatr Diabetes **7**(4): 211-4.

Muskiet, F. A., S. A. van Goor, R. S. Kuipers, F. V. Velzing-Aarts, E. N. Smit, H. Bouwstra, D. A. Dijck-Brouwer, E. R. Boersma and M. Hadders-Algra (2006).

"Long-chain polyunsaturated fatty acids in maternal and infant nutrition." Prostaglandins Leukot Essent Fatty Acids **75**(3): 135-44.

Naoum, H. G., R. C. De Chazal, B. M. Eaton and S. F. Contractor (1987). "Characterization and specificity of lipoprotein binding to term human placental membranes." Biochim Biophys Acta **902**(2): 193-9.

Negrel, R., D. Gaillard and G. Ailhaud (1989). "Prostacyclin as a potent effector of adipose-cell differentiation." Biochem J **257**(2): 399-405.

Nelson, G. J., P. C. Schmidt, G. L. Bartolini, D. S. Kelley and D. Kyle (1997). "The effect of dietary docosahexaenoic acid on plasma lipoproteins and tissue fatty acid composition in humans." Lipids **32**(11): 1137-46.

Nestel, P. J. (2000). "Fish oil and cardiovascular disease: lipids and arterial function." Am J Clin Nutr **71**(1 Suppl): 228S-31S.

Neuringer, M. and W. E. Connor (1986). "n-3 fatty acids in the brain and retina: evidence for their essentiality." Nutr Rev **44**(9): 285-94.

Nohr, E. A., M. Vaeth, J. L. Baker, T. Sorensen, J. Olsen and K. M. Rasmussen (2008). "Combined associations of prepregnancy body mass index and gestational weight gain with the outcome of pregnancy." Am J Clin Nutr **87**(6): 1750-9.

Ogunleye, A., A. T. Fakoya, S. Niizeki, H. Tojo, I. Sasajima, M. Kobayashi, S. Tateishi and K. Yamaguchi (1991). "Fatty acid composition of breast milk from Nigerian and Japanese women." J Nutr Sci Vitaminol (Tokyo) **37**(4): 435-42.

Oken, E. and M. W. Gillman (2003). "Fetal origins of obesity." Obes Res **11**(4): 496-506.

Oken, E., E. B. Levitan and M. W. Gillman (2008). Maternal smoking during pregnancy and child overweight: systematic review and meta-analysis. Int J Obes (Lond). **32:** 201-10.

Oken, E., S. L. Rifas-Shiman, A. E. Field, A. L. Frazier and M. W. Gillman (2008). "Maternal gestational weight gain and offspring weight in adolescence." Obstet Gynecol **112**(5): 999-1006.

Oken, E., R. O. Wright, K. P. Kleinman, D. Bellinger, C. J. Amarasiriwardena, H. Hu, J. W. Rich-Edwards and M. W. Gillman (2005). "Maternal fish consumption, hair mercury, and infant cognition in a U.S. Cohort." Environ Health Perspect **113**(10): 1376-80.

Okereke, N. C., L. Huston-Presley, S. B. Amini, S. Kalhan and P. M. Catalano (2004). "Longitudinal changes in energy expenditure and body composition in obese women with normal and impaired glucose tolerance." Am J Physiol Endocrinol Metab **287**(3): E472-9.

Okun, N., A. Verma, B. F. Mitchell and G. Flowerdew (1997). "Relative importance of maternal constitutional factors and glucose intolerance of pregnancy in the development of newborn macrosomia." J Matern Fetal Med **6**(5): 285-90.

Olhager, E. and E. Forsum (2006). "Assessment of total body fat using the skinfold technique in full-term and preterm infants." Acta Paediatr **95**(1): 21-8.

Olsen, S. F., H. S. Hansen, N. J. Secher, B. Jensen and B. Sandstrom (1995). "Gestation length and birth weight in relation to intake of marine n-3 fatty acids." Br J Nutr **73**(3): 397-404.

Olsen, S. F., H. S. Hansen, T. I. Sorensen, B. Jensen, N. J. Secher, S. Sommer and L. B. Knudsen (1986). "Intake of marine fat, rich in (n-3)-polyunsaturated fatty acids, may increase birthweight by prolonging gestation." Lancet **2**(8503): 367-9.

Olsen, S. F. and H. D. Joensen (1985). "High liveborn birth weights in the Faroes: a comparison between birth weights in the Faroes and in Denmark." J Epidemiol Community Health **39**(1): 27-32.

Olsen, S. F., M. L. Osterdal, J. D. Salvig, T. Weber, A. Tabor and N. J. Secher (2007). "Duration of pregnancy in relation to fish oil supplementation and habitual fish intake: a randomised clinical trial with fish oil." Eur J Clin Nutr **61**(8): 976-85.

Olsen, S. F., J. D. Sorensen, N. J. Secher, M. Hedegaard, T. B. Henriksen, H. S. Hansen and A. Grant (1992). "Randomised controlled trial of effect of fish-oil supplementation on pregnancy duration." Lancet **339**(8800): 1003-7.

Ong, K. K. and D. B. Dunger (2004). "Birth weight, infant growth and insulin resistance." Eur J Endocrinol **151 Suppl 3**: U131-9.

Ong, K. K. and R. J. Loos (2006). "Rapid infancy weight gain and subsequent obesity: systematic reviews and hopeful suggestions." Acta Paediatr **95**(8): 904-8.

Onwude, J. L., R. J. Lilford, H. Hjartardottir, A. Staines and D. Tuffnell (1995). "A randomised double blind placebo controlled trial of fish oil in high risk pregnancy." Br J Obstet Gynaecol **102**(2): 95-100.

Otto, S. J., A. C. van Houwelingen and G. Hornstra (2000). "The effect of supplementation with docosahexaenoic and arachidonic acid derived from single cell oils on plasma and erythrocyte fatty acids of pregnant women in the second trimester." Prostaglandins Leukot Essent Fatty Acids **63**(5): 323-8.

Pankiewicz, E., A. Cretti, E. Ronin-Walknowska, M. B. Czeszynska, H. Konefal and G. Hnatyszyn (2007). "Maternal adipose tissue, maternal and cord blood essential fatty acids and their long-chain polyunsaturated derivatives composition after elective caesarean section." Early Hum Dev **83**(7): 459-64.

Parrish, C. C., D. A. Pathy, J. G. Parkes and A. Angel (1991). "Dietary fish oils modify adipocyte structure and function." J Cell Physiol **148**(3): 493-502.

Parsons, T. J., C. Power, S. Logan and C. D. Summerbell (1999). "Childhood predictors of adult obesity: a systematic review." Int J Obes Relat Metab Disord **23 Suppl 8**: S1-107.

Parsons, T. J., C. Power and O. Manor (2001). "Fetal and early life growth and body mass index from birth to early adulthood in 1958 British cohort: longitudinal study." Bmj **323**(7325): 1331-5.

Pawlosky, R. J., J. R. Hibbeln, Y. Lin, S. Goodson, P. Riggs, N. Sebring, G. L. Brown and N. Salem, Jr. (2003). "Effects of beef- and fish-based diets on the kinetics of n-3 fatty acid metabolism in human subjects." Am J Clin Nutr **77**(3): 565-72.

Pawlosky, R. J., J. R. Hibbeln, J. A. Novotny and N. Salem, Jr. (2001). "Physiological compartmental analysis of alpha-linolenic acid metabolism in adult humans." J Lipid Res **42**(8): 1257-65.

Paxton, A., S. A. Lederman, S. B. Heymsfield, J. Wang, J. C. Thornton and R. N. Pierson, Jr. (1998). "Anthropometric equations for studying body fat in pregnant women." Am J Clin Nutr **67**(1): 104-10.

Percy, P., A. Percy, G. Vilbergsson and J. E. Mansson (1996). "Polyunsaturated fatty acid accretion in first- and second-trimester human fetal brain: lack of correlation with levels in paired placental samples." Biochem Mol Med **59**(1): 38-43.

Piechota, W. and A. Staszewski (1992). "Reference ranges of lipids and apolipoproteins in pregnancy." Eur J Obstet Gynecol Reprod Biol **45**(1): 27-35.

Poisson, J. P., R. P. Dupuy, P. Sarda, B. Descomps, M. Narce, D. Rieu and A. Crastes de Paulet (1993). "Evidence that liver microsomes of human neonates desaturate essential fatty acids." Biochim Biophys Acta **1167**(2): 109-13.

Poissonnet, C. M., A. R. Burdi and F. L. Bookstein (1983). "Growth and development of human adipose tissue during early gestation." Early Hum Dev **8**(1): 1-11.

Precht, D. and J. Molkentin (1999). "C18:1, C18:2 and C18:3 trans and cis fatty acid isomers including conjugated cis delta 9, trans delta 11 linoleic acid (CLA) as well as total fat composition of German human milk lipids." Nahrung **43**(4): 233-44.

Prentice, A. M. and G. R. Goldberg (2000). "Energy adaptations in human pregnancy: limits and long-term consequences." Am J Clin Nutr **71**(5 Suppl): 1226S-32S.

Prentice, A. M., C. J. Spaaij, G. R. Goldberg, S. D. Poppitt, J. M. van Raaij, M. Totton, D. Swann and A. E. Black (1996). "Energy requirements of pregnant and lactating women." Eur J Clin Nutr **50 Suppl 1**: S82-110; discussion S10-1.

Price, P. T., C. M. Nelson and S. D. Clarke (2000). "Omega-3 polyunsaturated fatty acid regulation of gene expression." Curr Opin Lipidol **11**(1): 3-7.

Prins, J. B. and S. O'Rahilly (1997). "Regulation of adipose cell number in man." Clin Sci (Lond) **92**(1): 3-11.

Radesky, J. S., E. Oken, S. L. Rifas-Shiman, K. P. Kleinman, J. W. Rich-Edwards and M. W. Gillman (2008). "Diet during early pregnancy and development of gestational diabetes." Paediatr Perinat Epidemiol **22**(1): 47-59.

Ramel, A., M. T. Jonsdottir and I. Thorsdottir (2009). "Consumption of cod and weight loss in young overweight and obese adults on an energy reduced diet for 8-weeks." Nutr Metab Cardiovasc Dis.

Rasmussen, F. and M. Johansson (1998). "The relation of weight, length and ponderal index at birth to body mass index and overweight among 18-year-old males in Sweden." Eur J Epidemiol **14**(4): 373-80.

Ravelli, A. C., J. H. van der Meulen, R. P. Michels, C. Osmond, D. J. Barker, C. N. Hales and O. P. Bleker (1998). "Glucose tolerance in adults after prenatal exposure to famine." Lancet **351**(9097): 173-7.

Reddy, S., T. A. Sanders and O. Obeid (1994). "The influence of maternal vegetarian diet on essential fatty acid status of the newborn." Eur J Clin Nutr **48**(5): 358-68.

Reginato, M. J., S. L. Krakow, S. T. Bailey and M. A. Lazar (1998). "Prostaglandins promote and block adipogenesis through opposing effects on peroxisome proliferator-activated receptor gamma." J Biol Chem **273**(4): 1855-8.

Reilly, J. J., J. Armstrong, A. R. Dorosty, P. M. Emmett, A. Ness, I. Rogers, C. Steer and A. Sherriff (2005). "Early life risk factors for obesity in childhood: cohort study." Bmj **330**(7504): 1357.

Reilly, J. J., J. Wilson and J. V. Durnin (1995). "Determination of body composition from skinfold thickness: a validation study." Arch Dis Child **73**(4): 305-10.

Rice, G. E., M. H. Wong, W. Farrugia and K. F. Scott (1998). "Contribution of type II phospholipase A2 to in vitro phospholipase A2 enzymatic activity in human term placenta." J Endocrinol **157**(1): 25-31.

Rich-Edwards, J. W., M. J. Stampfer, J. E. Manson, B. Rosner, S. E. Hankinson, G. A. Colditz, W. C. Willett and C. H. Hennekens (1997). "Birth weight and risk of cardiovascular disease in a cohort of women followed up since 1976." Bmj **315**(7105): 396-400.

Roche, A. F. (1981). "The adipocyte-number hypothesis." Child Dev **52**(1): 31-43.

Rodriguez, A., P. Sarda, C. Nessmann, P. Boulot, C. L. Leger and B. Descomps (1998). "Delta6- and delta5-desaturase activities in the human fetal liver: kinetic aspects." J Lipid Res **39**(9): 1825-32.

Rodriguez, G., M. P. Samper, P. Ventura, L. A. Moreno, J. L. Olivares and J. M. Perez-Gonzalez (2004). "Gender differences in newborn subcutaneous fat distribution." Eur J Pediatr **163**(8): 457-61.

Rogers, I. (2003). "The influence of birthweight and intrauterine environment on adiposity and fat distribution in later life." Int J Obes Relat Metab Disord **27**(7): 755-77.

Rogers, I. S., A. R. Ness, C. D. Steer, J. C. Wells, P. M. Emmett, J. R. Reilly, J. Tobias and G. D. Smith (2006). "Associations of size at birth and dual-energy X-ray absorptiometry measures of lean and fat mass at 9 to 10 y of age." Am J Clin Nutr **84**(4): 739-47.

Roseboom, T. J., J. H. van der Meulen, C. Osmond, D. J. Barker, A. C. Ravelli and O. P. Bleker (2000). "Plasma lipid profiles in adults after prenatal exposure to the Dutch famine." Am J Clin Nutr **72**(5): 1101-6.

Roseboom, T. J., J. H. van der Meulen, C. Osmond, D. J. Barker, A. C. Ravelli, J. M. Schroeder-Tanka, G. A. van Montfrans, R. P. Michels and O. P. Bleker (2000). "Coronary heart disease after prenatal exposure to the Dutch famine, 1944-45." Heart **84**(6): 595-8.

Roseboom, T. J., J. H. van der Meulen, A. C. Ravelli, G. A. van Montfrans, C. Osmond, D. J. Barker and O. P. Bleker (1999). "Blood pressure in adults after prenatal exposure to famine." J Hypertens **17**(3): 325-30.

Rosenfeld, E., A. Beyerlein, M. Hadders-Algra, K. Kennedy, A. Singhal, M. Fewtrell, A. Lucas, B. Koletzko and R. von Kries (2009). "IPD meta-analysis shows no effect of LC-PUFA supplementation on infant growth at 18 months." Acta Paediatr **98**(1): 91-7.

Rossner, S. and A. Ohlin (1990). "Maternal body weight and relation to birth weight." Acta Obstet Gynecol Scand **69**(6): 475-8.

Rump, P. and G. Hornstra (2001). "Relation between arachidonic acid and docosahexaenoic acid in maternal and neonatal blood." Eur J Clin Nutr **55**(10): 916-7.

Rump, P., R. P. Mensink, A. D. Kester and G. Hornstra (2001). "Essential fatty acid composition of plasma phospholipids and birth weight: a study in term neonates." Am J Clin Nutr **73**(4): 797-806.

Ruyle, M., W. E. Connor, G. J. Anderson and R. I. Lowensohn (1990). "Placental transfer of essential fatty acids in humans: venous-arterial difference for docosahexaenoic acid in fetal umbilical erythrocytes." Proc Natl Acad Sci U S A **87**(20): 7902-6.

Ruzickova, J., M. Rossmeisl, T. Prazak, P. Flachs, J. Sponarova, M. Veck, E. Tvrzicka, M. Bryhn and J. Kopecky (2004). "Omega-3 PUFA of marine origin limit diet-induced obesity in mice by reducing cellularity of adipose tissue." Lipids **39**(12): 1177-85.

Ryan, A. S., M. A. Keske, J. P. Hoffman and E. B. Nelson (2009). "Clinical overview of algal-docosahexaenoic acid: effects on triglyceride levels and other cardiovascular risk factors." Am J Ther **16**(2): 183-92.

Sachdev, H. S., C. H. Fall, C. Osmond, R. Lakshmy, S. K. Dey Biswas, S. D. Leary, K. S. Reddy, D. J. Barker and S. K. Bhargava (2005). "Anthropometric indicators of body composition in young adults: relation to size at birth and serial measurements of body mass index in childhood in the New Delhi birth cohort." Am J Clin Nutr **82**(2): 456-66.

Sadurskis, A., N. Kabir, J. Wager and E. Forsum (1988). "Energy metabolism, body composition, and milk production in healthy Swedish women during lactation." Am J Clin Nutr **48**(1): 44-9.

Salem, N., Jr., B. Wegher, P. Mena and R. Uauy (1996). "Arachidonic and docosahexaenoic acids are biosynthesized from their 18-carbon precursors in human infants." Proc Natl Acad Sci U S A **93**(1): 49-54.

Sanders, T. A. (2009). "DHA status of vegetarians." Prostaglandins Leukot Essent Fatty Acids.

Sanders, T. A., F. R. Ellis and J. W. Dickerson (1978). "Studies of vegans: the fatty acid composition of plasma choline phosphoglycerides, erythrocytes, adipose tissue, and breast milk, and some indicators of susceptibility to ischemic heart disease in vegans and omnivore controls." Am J Clin Nutr **31**(5): 805-13.

Sanders, T. A. and S. Reddy (1992). "The influence of a vegetarian diet on the fatty acid composition of human milk and the essential fatty acid status of the infant." J Pediatr **120**(4 Pt 2): S71-7.

Sanjurjo, P., J. I. Ruiz-Sanz, P. Jimeno, L. Aldamiz-Echevarria, L. Aquino, R. Matorras, J. Esteban and M. Banque (2004). "Supplementation with docosahexaenoic acid in the last trimester of pregnancy: maternal-fetal biochemical findings." J Perinat Med **32**(2): 132-6.

Sastry, P. S. (1985). "Lipids of nervous tissue: composition and metabolism." Prog Lipid Res **24**(2): 69-176.

Sattar, N., I. A. Greer, J. Louden, G. Lindsay, M. McConnell, J. Shepherd and C. J. Packard (1997). "Lipoprotein subfraction changes in normal pregnancy: threshold effect of plasma triglyceride on appearance of small, dense low density lipoprotein." J Clin Endocrinol Metab **82**(8): 2483-91.

Sauerwald, T. U., D. L. Hachey, C. L. Jensen, H. Chen, R. E. Anderson and W. C. Heird (1996). "Effect of dietary alpha-linolenic acid intake on incorporation of docosahexaenoic and arachidonic acids into plasma phospholipids of term infants." Lipids **31 Suppl**: S131-5.

Sauerwald, T. U., D. L. Hachey, C. L. Jensen, H. Chen, R. E. Anderson and W. C. Heird (1997). "Intermediates in endogenous synthesis of C22:6 omega 3 and C20:4 omega 6 by term and preterm infants." Pediatr Res **41**(2): 183-7.

Sayer, A. A., H. E. Syddall, E. M. Dennison, H. J. Gilbody, S. L. Duggleby, C. Cooper, D. J. Barker and D. I. Phillips (2004). "Birth weight, weight at 1 y of age, and body composition in older men: findings from the Hertfordshire Cohort Study." Am J Clin Nutr **80**(1): 199-203.

Schaefer-Graf, U. M., J. Pawliczak, D. Passow, R. Hartmann, R. Rossi, C. Buhrer, T. Harder, A. Plagemann, K. Vetter and O. Kordonouri (2005). "Birth weight and parental BMI predict overweight in children from mothers with gestational diabetes." Diabetes Care **28**(7): 1745-50.

Schiessl, B., A. Beyerlein, N. Lack and R. von Kries (2009). "Temporal trends in pregnancy weight gain and birth weight in Bavaria 2000-2007: slightly decreasing birth weight with increasing weight gain in pregnancy." J Perinat Med.

Schmelzle, H. R. and C. Fusch (2002). "Body fat in neonates and young infants: validation of skinfold thickness versus dual-energy X-ray absorptiometry." Am J Clin Nutr **76**(5): 1096-100.

Schneider, H., P. Husslein and K. T. M. Schneider (2004). Die Geburtshilfe. Berlin, Heidelberg, New York, Springer.

Schutz, Y., J. P. Flatt and E. Jequier (1989). "Failure of dietary fat intake to promote fat oxidation: a factor favoring the development of obesity." Am J Clin Nutr **50**(2): 307-14.

Schutz, Y., A. Tremblay, R. L. Weinsier and K. M. Nelson (1992). "Role of fat oxidation in the long-term stabilization of body weight in obese women." Am J Clin Nutr **55**(3): 670-4.

Secher, N. J. and S. F. Olsen (1990). "Fish-oil and pre-eclampsia." Br J Obstet Gynaecol **97**(12): 1077-9.

Sellmayer, A. and B. Koletzko (1999). "Long-chain polyunsaturated fatty acids and eicosanoids in infants--physiological and pathophysiological aspects and open questions." Lipids **34**(2): 199-205.

Sewell, M. F., L. Huston-Presley, D. M. Super and P. Catalano (2006). "Increased neonatal fat mass, not lean body mass, is associated with maternal obesity." Am J Obstet Gynecol **195**(4): 1100-3.

Simmer, K., S. K. Patole and S. C. Rao (2008). "Longchain polyunsaturated fatty acid supplementation in infants born at term." Cochrane Database Syst Rev(1): CD000376.

Simmer, K., S. M. Schulzke and S. Patole (2008). "Longchain polyunsaturated fatty acid supplementation in preterm infants." Cochrane Database Syst Rev(1): CD000375.

Singhal, A., J. Wells, T. J. Cole, M. Fewtrell and A. Lucas (2003). "Programming of lean body mass: a link between birth weight, obesity, and cardiovascular disease?" Am J Clin Nutr **77**(3): 726-30.

Slaughter, M. H., T. G. Lohman, R. A. Boileau, C. A. Horswill, R. J. Stillman, M. D. Van Loan and D. A. Bemben (1988). "Skinfold equations for estimation of body fatness in children and youth." Hum Biol **60**(5): 709-23.

Smit, E. N., M. Koopmann, E. R. Boersma and F. A. Muskiet (2000). "Effect of supplementation of arachidonic acid (AA) or a combination of AA plus docosahexaenoic acid on breastmilk fatty acid composition." Prostaglandins Leukot Essent Fatty Acids **62**(6): 335-40.

Smit, E. N., I. A. Martini, H. Mulder, E. R. Boersma and F. A. Muskiet (2002). "Estimated biological variation of the mature human milk fatty acid composition." Prostaglandins Leukot Essent Fatty Acids **66**(5-6): 549-55.

Smuts, C. M., E. Borod, J. M. Peeples and S. E. Carlson (2003). "High-DHA eggs: feasibility as a means to enhance circulating DHA in mother and infant." Lipids **38**(4): 407-14.

Smuts, C. M., M. Huang, D. Mundy, T. Plasse, S. Major and S. E. Carlson (2003). "A randomized trial of docosahexaenoic acid supplementation during the third trimester of pregnancy." Obstet Gynecol **101**(3): 469-79.

Snow, M. (1989). Effects of genome on fetal size at birth. Fetal growth, Proceedings of the 20th Study Group. F. Sharp, R. Fraser and R. Milner. London, Royal College of Obstetricans and Gynecologists**:** 1-11.

Sohlstrom, A. and E. Forsum (1997). "Changes in total body fat during the human reproductive cycle as assessed by magnetic resonance imaging, body water dilution, and skinfold thickness: a comparison of methods." Am J Clin Nutr **66**(6): 1315-22.

Soltani, H. and R. B. Fraser (2000). "A longitudinal study of maternal anthropometric changes in normal weight, overweight and obese women during pregnancy and postpartum." Br J Nutr **84**(1): 95-101.

Sorensen, H. T., S. Sabroe, K. J. Rothman, M. Gillman, P. Fischer and T. I. Sorensen (1997). "Relation between weight and length at birth and body mass index in young adulthood: cohort study." Bmj **315**(7116): 1137.

Sorensen, J. D., S. F. Olsen, A. K. Pedersen, J. Boris, N. J. Secher and G. A. FitzGerald (1993). "Effects of fish oil supplementation in the third trimester of pregnancy on prostacyclin and thromboxane production." Am J Obstet Gynecol **168**(3 Pt 1): 915-22.

Spalding, K. L., E. Arner, P. O. Westermark, S. Bernard, B. A. Buchholz, O. Bergmann, L. Blomqvist, J. Hoffstedt, E. Naslund, T. Britton, H. Concha, M. Hassan, M. Ryden, J. Frisen and P. Arner (2008). "Dynamics of fat cell turnover in humans." Nature **453**(7196): 783-7.

Specker, B. L., H. E. Wey and D. Miller (1987). "Differences in fatty acid composition of human milk in vegetarian and nonvegetarian women: long-term effect of diet." J Pediatr Gastroenterol Nutr **6**(5): 764-8.

Steinhart, H. a. J. F. (1997). "Contents of trans fatty acids (TFA) in German foods and estimation of daily intake." Fett/Lipid **99**: 314-318.

Stettler, N., V. A. Stallings, A. B. Troxel, J. Zhao, R. Schinnar, S. E. Nelson, E. E. Ziegler and B. L. Strom (2005). "Weight gain in the first week of life and overweight in adulthood: a cohort study of European American subjects fed infant formula." Circulation **111**(15): 1897-903.

Stettler, N., B. S. Zemel, S. Kumanyika and V. A. Stallings (2002). "Infant weight gain and childhood overweight status in a multicenter, cohort study." Pediatrics **109**(2): 194-9.

Stewart, F., V. A. Rodie, J. E. Ramsay, I. A. Greer, D. J. Freeman and B. J. Meyer (2007). "Longitudinal assessment of erythrocyte fatty acid composition throughout pregnancy and post partum." Lipids **42**(4): 335-44.

Storlien, L. H., A. J. Hulbert and P. L. Else (1998). "Polyunsaturated fatty acids, membrane function and metabolic diseases such as diabetes and obesity." Curr Opin Clin Nutr Metab Care **1**(6): 559-63.

Stotland, N. E., Y. W. Cheng, L. M. Hopkins and A. B. Caughey (2006). "Gestational weight gain and adverse neonatal outcome among term infants." Obstet Gynecol **108**(3 Pt 1): 635-43.

Szajewska, H., A. Horvath and B. Koletzko (2006). "Effect of n-3 long-chain polyunsaturated fatty acid supplementation of women with low-risk pregnancies on pregnancy outcomes and growth measures at birth: a meta-analysis of randomized controlled trials." Am J Clin Nutr **83**(6): 1337-44.

Szitanyi, P., B. Koletzko, A. Mydlilova and H. Demmelmair (1999). "Metabolism of 13C-labeled linoleic acid in newborn infants during the first week of life." Pediatr Res **45**(5 Pt 1): 669-73.

Taveras, E. M., S. L. Rifas-Shiman, M. B. Belfort, K. P. Kleinman, E. Oken and M. W. Gillman (2009). "Weight status in the first 6 months of life and obesity at 3 years of age." Pediatrics **123**(4): 1177-83.

Thame, M., C. Osmond, F. Bennett, R. Wilks and T. Forrester (2004). "Fetal growth is directly related to maternal anthropometry and placental volume." Eur J Clin Nutr **58**(6): 894-900.

Thame, M., H. Trotman, C. Osmond, H. Fletcher and M. Antoine (2007). "Body composition in pregnancies of adolescents and mature women and the relationship to birth anthropometry." Eur J Clin Nutr **61**(1): 47-53.

Theobald, H. E., P. J. Chowienczyk, R. Whittall, S. E. Humphries and T. A. Sanders (2004). "LDL cholesterol-raising effect of low-dose docosahexaenoic acid in middle-aged men and women." Am J Clin Nutr **79**(4): 558-63.

Thews, G., E. Mutschler and E. Vaupel (1999). Anatomie, Physiologie, Pathophysiologie des Menschen. Stuttgart, Wissenschaftliche Verlags-Gesellschaft.

Thomas, B., K. Ghebremeskel, C. Lowy, M. Crawford and B. Offley-Shore (2006). "Nutrient intake of women with and without gestational diabetes with a specific focus on fatty acids." Nutrition **22**(3): 230-6.

Tofail, F., I. Kabir, J. D. Hamadani, F. Chowdhury, S. Yesmin, F. Mehreen and S. N. Huda (2006). "Supplementation of fish-oil and soy-oil during pregnancy and psychomotor development of infants." J Health Popul Nutr **24**(1): 48-56.

Toschke, A. M., J. Vignerova, L. Lhotska, K. Osancova, B. Koletzko and R. Von Kries (2002). "Overweight and obesity in 6- to 14-year-old Czech children in 1991: protective effect of breast-feeding." J Pediatr **141**(6): 764-9.

Uauy, R., P. Mena, B. Wegher, S. Nieto and N. Salem, Jr. (2000). "Long chain polyunsaturated fatty acid formation in neonates: effect of gestational age and intrauterine growth." Pediatr Res **47**(1): 127-35.

Uauy, R., P. Peirano, D. Hoffman, P. Mena, D. Birch and E. Birch (1996). "Role of essential fatty acids in the function of the developing nervous system." Lipids **31 Suppl**: S167-76.

Uauy, R. D., D. G. Birch, E. E. Birch, J. E. Tyson and D. R. Hoffman (1990). "Effect of dietary omega-3 fatty acids on retinal function of very-low-birth-weight neonates." Pediatr Res **28**(5): 485-92.

van der Schouw, Y. T., M. D. Al, G. Hornstra, M. T. Bulstra-Ramakers and H. J. Huisjes (1991). "Fatty acid composition of serum lipids of mothers and their babies after normal and hypertensive pregnancies." Prostaglandins Leukot Essent Fatty Acids **44**(4): 247-52.

van Eijsden, M., G. Hornstra, M. F. van der Wal, T. G. Vrijkotte and G. J. Bonsel (2008). "Maternal n-3, n-6, and trans fatty acid profile early in pregnancy and term birth weight: a prospective cohort study." Am J Clin Nutr **87**(4): 887-95.

van Goor, S. A., D. A. Dijck-Brouwer, M. Hadders-Algra, B. Doornbos, J. J. Erwich, A. Schaafsma and F. A. Muskiet (2009). "Human milk arachidonic acid and docosahexaenoic acid contents increase following supplementation during pregnancy and lactation." Prostaglandins Leukot Essent Fatty Acids **80**(1): 65-9.

van Houwelingen, A. C., J. D. Sorensen, G. Hornstra, M. M. Simonis, J. Boris, S. F. Olsen and N. J. Secher (1995). "Essential fatty acid status in neonates after fish-oil supplementation during late pregnancy." Br J Nutr **74**(5): 723-31.

van Raaij, J. M., M. E. Peek, S. H. Vermaat-Miedema, C. M. Schonk and J. G. Hautvast (1988). "New equations for estimating body fat mass in pregnancy from body density or total body water." Am J Clin Nutr **48**(1): 24-9.

van Raaij, J. M., C. M. Schonk, S. H. Vermaat-Miedema, M. E. Peek and J. G. Hautvast (1989). "Body fat mass and basal metabolic rate in Dutch women before, during, and after pregnancy: a reappraisal of energy cost of pregnancy." Am J Clin Nutr **49**(5): 765-72.

van Stiphout, W. A., A. Hofman and A. M. de Bruijn (1987). "Serum lipids in young women before, during, and after pregnancy." Am J Epidemiol **126**(5): 922-8.

Vanderhoof, J., S. Gross and T. Hegyi (2000). "A multicenter long-term safety and efficacy trial of preterm formula supplemented with long-chain polyunsaturated fatty acids." J Pediatr Gastroenterol Nutr **31**(2): 121-7.

Vassaux, G., D. Gaillard, G. Ailhaud and R. Negrel (1992). "Prostacyclin is a specific effector of adipose cell differentiation. Its dual role as a cAMP- and Ca(2+)-elevating agent." J Biol Chem **267**(16): 11092-7.

Velzing-Aarts, F. V., F. R. van der Klis, F. P. van der Dijs, C. M. van Beusekom, H. Landman, J. J. Capello and F. A. Muskiet (2001). "Effect of three low-dose fish oil supplements, administered during pregnancy, on neonatal long-chain polyunsaturated fatty acid status at birth." Prostaglandins Leukot Essent Fatty Acids **65**(1): 51-7.

Vidgren, H. M., J. J. Agren, U. Schwab, T. Rissanen, O. Hanninen and M. I. Uusitupa (1997). "Incorporation of n-3 fatty acids into plasma lipid fractions, and erythrocyte

membranes and platelets during dietary supplementation with fish, fish oil, and docosahexaenoic acid-rich oil among healthy young men." Lipids **32**(7): 697-705.

Viswanathan, M., A. M. Siega-Riz, M. K. Moos, A. Deierlein, S. Mumford, J. Knaack, P. Thieda, L. J. Lux and K. N. Lohr (2008). "Outcomes of maternal weight gain." Evid Rep Technol Assess (Full Rep)(168): 1-223.

Vlaardingerbroek, H. and G. Hornstra (2004). "Essential fatty acids in erythrocyte phospholipids during pregnancy and at delivery in mothers and their neonates: comparison with plasma phospholipids." Prostaglandins Leukot Essent Fatty Acids **71**(6): 363-74.

Voigt, M., K. T. Schneider and K. Jahrig (1996). "[Analysis of a 1992 birth sample in Germany. 1: New percentile values of the body weight of newborn infants]." Geburtshilfe Frauenheilkd **56**(10): 550-8.

Vuori, E., K. Kiuru, S. M. Makinen, P. Vayrynen, R. Kara and P. Kuitunen (1982). "Maternal diet and fatty acid pattern of breast milk." Acta Paediatr Scand **71**(6): 959-63.

Wabitsch, M. (2000). "The acquisition of obesity: insights from cellular and genetic research." Proc Nutr Soc **59**(2): 325-30.

Wang, H., L. H. Storlien and X. F. Huang (2002). "Effects of dietary fat types on body fatness, leptin, and ARC leptin receptor, NPY, and AgRP mRNA expression." Am J Physiol Endocrinol Metab **282**(6): E1352-9.

Waterman, I. J., N. Emmison and A. K. Dutta-Roy (1998). "Characterisation of triacylglycerol hydrolase activities in human placenta." Biochim Biophys Acta **1394**(2-3): 169-76.

Wells, J. C., S. Chomtho and M. S. Fewtrell (2007). "Programming of body composition by early growth and nutrition." Proc Nutr Soc **66**(3): 423-34.

Wells, J. C., N. J. Fuller, O. Dewit, M. S. Fewtrell, M. Elia and T. J. Cole (1999). "Four-component model of body composition in children: density and hydration of fat-free mass and comparison with simpler models." Am J Clin Nutr **69**(5): 904-12.

Wells, J. C., P. C. Hallal, A. Wright, A. Singhal and C. G. Victora (2005). "Fetal, infant and childhood growth: relationships with body composition in Brazilian boys aged 9 years." Int J Obes (Lond) **29**(10): 1192-8.

Weseler, A. R., C. E. Dirix, M. J. Bruins and G. Hornstra (2008). "Dietary arachidonic acid dose-dependently increases the arachidonic acid concentration in human milk." J Nutr **138**(11): 2190-7.

Weststrate, J. A. and P. Deurenberg (1989). "Body composition in children: proposal for a method for calculating body fat percentage from total body density or skinfold-thickness measurements." Am J Clin Nutr **50**(5): 1104-15.

Whitaker, R. C. (2004). "Predicting preschooler obesity at birth: the role of maternal obesity in early pregnancy." Pediatrics **114**(1): e29-36.

WHO (1993). Fats and oils in human nutrition. Report of a Joint Expert Consultation Food and Agricultural Organization of the United Nations.

WHO (1995). "Maternal anthropometry and pregnancy outcomes. A WHO Collaborative Study." Bull World Health Organ **73 Suppl**: 1-98.

Widdowson, E. M. (1950). "Chemical composition of newly born mammals." Nature **166**(4224): 626-8.

Wijendran, V., R. B. Bendel, S. C. Couch, E. H. Philipson, K. Thomsen, X. Zhang and C. J. Lammi-Keefe (1999). "Maternal plasma phospholipid polyunsaturated fatty acids

in pregnancy with and without gestational diabetes mellitus: relations with maternal factors." Am J Clin Nutr **70**(1): 53-61.

Winter, C. H., E. B. Hoving and F. A. Muskiet (1993). "Fatty acid composition of human milk triglyceride species. Possible consequences for optimal structures of infant formula triglycerides." J Chromatogr **616**(1): 9-24.

Winzer, C., O. Wagner, A. Festa, B. Schneider, M. Roden, D. Bancher-Todesca, G. Pacini, T. Funahashi and A. Kautzky-Willer (2004). "Plasma adiponectin, insulin sensitivity, and subclinical inflammation in women with prior gestational diabetes mellitus." Diabetes Care **27**(7): 1721-7.

Woltil, H. A., C. M. van Beusekom, A. Schaafsma, F. A. Muskiet and A. Okken (1998). "Long-chain polyunsaturated fatty acid status and early growth of low birth weight infants." Eur J Pediatr **157**(2): 146-52.

Wrotniak, B. H., J. Shults, S. Butts and N. Stettler (2008). "Gestational weight gain and risk of overweight in the offspring at age 7 y in a multicenter, multiethnic cohort study." Am J Clin Nutr **87**(6): 1818-24.

Wu, Z., Y. Xie, N. L. Bucher and S. R. Farmer (1995). "Conditional ectopic expression of C/EBP beta in NIH-3T3 cells induces PPAR gamma and stimulates adipogenesis." Genes Dev **9**(19): 2350-63.

Young, C., T. Hikita, S. Kaneko, Y. Shimizu, S. Hanaka, T. Abe, H. Shimasaki, R. Ikeda, Y. Miyazawa and A. Nakajima (1997). "Fatty acid compositions of colostrum, cord blood, maternal blood and major infant formulas in Japan." Acta Paediatr Jpn **39**(3): 299-304.

Yuhas, R., K. Pramuk and E. L. Lien (2006). "Human milk fatty acid composition from nine countries varies most in DHA." Lipids **41**(9): 851-8.

Zaren, B., G. Lindmark and M. Gebre-Medhin (1996). "Maternal smoking and body composition of the newborn." Acta Paediatr **85**(2): 213-9.

Zhou, W. and J. Olsen (1997). "Gestational weight gain as a predictor of birth and placenta weight according to pre-pregnancy body mass index." Acta Obstet Gynecol Scand **76**(4): 300-7.

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