Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt

Effect of a dietary intervention to reduce the n-6/n-3 fatty acid ratio in maternal nutrition during pregnancy and lactation on selected maternal and cord blood biomarkers in relation to infant body composition up to 2 years of life

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Table of contents

Summary

Accumulating evidence suggests a contribution of the perinatal environment for later obesity risk. Therefore, prevention approaches starting early in life and the identification of early determinants for obesity are of great importance. Recently, the reduction of the n-6/n-3 fatty acid ratio in maternal nutrition during the perinatal period was proposed as an effective strategy against excessive fat mass growth in the offspring. The INFAT-study (The **i**mpact of **n**utritional **f**atty acids on infant **a**dipose **t**issue development) is the first randomized controlled trial originally designed to investigate the effect of reducing the n-6/n-3 fatty acid ratio in the maternal diet during pregnancy and lactation on infant adipose tissue development. Due to their pro- and anti-inflammatory properties and interaction with transcription factors, dietary fatty acids may also have an impact on maternal or cord blood biomarkers, particularly on parameters of carbohydrate and lipid metabolism and adipokines such as leptin and adiponectin.

Aim of this work was to study the effect of a reduced n-6/n-3 fatty acid ratio in maternal nutrition on selected biomarkers in maternal blood, umbilical cord blood and breast milk and to explore in an observational approach their relationship with offspring weight development and body composition up to 2 years postpartum (pp).

208 healthy pregnant women were allocated to either an intervention group (IG: supplementation with 1200 mg n-3 long chain polyunsaturated fatty acids per day and a dietary counseling to normalize arachidonic acid intake from the 15th week of gestation until 4 months pp) or a control group (CG: general recommendations on a healthy diet during pregnancy). Maternal blood was taken at the 15th and 32nd week of gestation as well as at 6 weeks and 4 months pp. Breast milk was collected at the same time-points pp. The infants were clinically assessed from birth up to 2 years of life with skinfold thickness measurements as the primary outcome.

The intervention did not affect maternal plasma adipokines (leptin, the soluble leptin receptor, and high molecular weight [HMW] adiponectin) and markers of glucose metabolism (insulin and insulin resistance determined by the homeostasis model assessment for insulin resistance [HOMA-IR]) over the course of pregnancy and lactation. However, the increase of maternal serum triglycerides over the course of pregnancy was less pronounced in the IG compared to the CG. Parameters assessed in umbilical cord plasma (leptin and insulin) did not differ

between the IG and CG, but significant gender differences were observed. The adipokines leptin and total adiponectin in breast milk were unaffected by the intervention.

The infants of the IG did not significantly differ from the CG in growth or body composition at the age of 2 years.

Regression analyses accounting for several confounding factors revealed that maternal plasma leptin levels at the 32nd week of gestation were inversely related to infant weight and lean body mass (LBM) up to 2 years pp. Cord plasma leptin was positively related to birth weight, BMI, fat mass and LBM at birth, and inversely associated with weight gain up to 2 years pp. Maternal parameters of carbohydrate and lipid metabolism were largely unrelated to the infant clinical outcomes, whereas maternal plasma HMW adiponectin was positively associated with LBM at birth and body weight at the age of 2 years. Cord plasma insulin correlated positively with birth weight and neonatal fat mass and was inversely associated with weight gain up to 2 years in girls. Leptin in breast milk was mostly unrelated with infant anthropometry, while breast milk adiponectin was positively associated with weight gain and fat mass up to 2 years of life.

In conclusion, both maternal and cord blood parameters were significantly associated with infant anthropometric parameters up to 2 years of age, highlighting the role of the perinatal metabolic environment for early infant growth and body composition. Ongoing follow-up examinations up to 5 years pp will show whether these associations and gender-specific differences persist up to later stages in infancy.

Zusammenfassung

Für die Entstehung von Adipositas scheinen perinatale Einflüsse einen wichtigen Beitrag zu leisten. Deshalb sind frühe Präventionsansätze sowie die Identifizierung von frühen Determinanten des Übergewichtsrisikos von großer Bedeutung. Es gibt Hinweise, dass eine Senkung des n-6/n-3 Fettsäureverhältnisses in der mütterlichen Ernährung während der Schwangerschaft und Stillzeit eine wirksame Strategie gegen die Entstehung von Übergewicht bei den Nachkommen darstellen könnte. Die INFAT-Studie (The **i**mpact of **n**utritional **f**atty acids on infant **a**dipose **t**issue development) untersucht in einem randomisierten kontrollierten Ansatz erstmals den Einfluss einer Reduktion des n-6/n-3-Fettsäurequotienten in der mütterlichen Ernährung während der Schwangerschaft und Stillzeit auf die kindliche Fettgewebsentwicklung. Aufgrund ihrer pro- und anti-inflammatorischen Wirkungen und ihrer Interaktion mit Transkriptionsfaktoren ist außerdem ein Einfluss von Fettsäuren in der Nahrung auf bestimmte mütterliche oder fetale Biomarker im Nabelschnurblut denkbar, insbesondere auf Parameter des Kohlenhydrat- und Lipidstoffwechsels und Adipokine wie Leptin oder Adiponektin.

Ziel dieser Arbeit war es, den Einfluss einer Ernährungsintervention zur Senkung des n-6/n-3 Fettsäureverhältnisses in der mütterlichen Ernährung auf ausgewählte Biomarker im mütterlichen Blut und Nabelschnurblut sowie in der Muttermilch zu untersuchen, und deren Zusammenhang mit der kindlichen Gewichtsentwicklung und Körperzusammensetzung bis zum zweiten Lebensjahr aufzuzeigen.

208 gesunde schwangere Frauen vor der 15. Schwangerschaftswoche (SSW) wurden entweder einer Interventionsgruppe (IG: Supplementierung mit täglich 1200 mg n-3 langkettigen, mehrfach ungesättigten Fettsäuren von der 15. SSW bis zum 4. Monat postpartum [pp] und Ernährungsberatung zur moderaten Einschränkung der Zufuhr von Arachidonsäure) oder einer Kontrollgruppe (KG: allgemeine Empfehlungen zu einer gesunden Ernährung während der Schwangerschaft) zugelost. Blutabnahmen bei den Müttern erfolgten zur 15. und 32. SSW sowie 6 Wochen und 4 Monate pp. Muttermilch wurde ebenfalls 6 Wochen und 4 Monate pp gesammelt. Die Kinder wurden von der Geburt bis zum zweiten Lebensjahr anthropometrisch untersucht mit Hautfaltendickenmessungen als primären Zielparameter.

Die Intervention hatte keinen Einfluss auf die untersuchten Adipokine im mütterlichen Plasma (Leptin, löslicher Leptin-Rezeptor und high molecular weight [HMW] Adiponektin)

sowie auf Parameter des Glucosestoffwechsels (Insulin und Insulinresistenz bestimmt durch homeoastasis model assessment for insulin resistance [HOMA-IR]) im Verlauf der Schwangerschaft und Stillzeit. Jedoch zeigte sich in der IG ein geringerer Anstieg der Triglyceride über die Schwangerschaft im Vergleich zur KG. Die im Nabelschnurplasma untersuchten Parameter (Leptin und Insulin) waren in der IG und KG vergleichbar; es wurden allerdings Unterschiede zwischen den Geschlechtern festgestellt. Die Adipokine in der Muttermilch (Leptin und Gesamt-Adiponektin) blieben ebenfalls von der Intervention unbeeinflusst.

Die Kinder der IG und KG waren hinsichtlich ihrer klinischen Parameter (Wachstum und Körperzusammensetzung) im Alter von 2 Jahren vergleichbar.

Regressionsanalysen unter Berücksichtigung relevanter Kovariaten ergaben eine inverse Beziehung zwischen dem mütterlichen Plasma-Leptinspiegel zur 32. SSW und dem kindlichen Körpergewicht und der fettfreien Körpermasse (FFM) bis zum zweiten Lebensjahr. Leptin im Nabelschnurplasma war positiv mit dem Geburtsgewicht, dem kindlichen BMI, der Fettmasse und FFM zur Geburt und negativ mit der Gewichtszunahme bis zum zweiten Lebensjahr assoziiert. Es zeigten sich weitgehend keine Zusammenhänge zwischen mütterlichen Parametern des Kohlenhydrat- und Fettstoffwechsels und den kindlichen anthropometrischen Variablen. Die HMW Adiponektin-Konzentration im mütterlichen Plasma zur 32. SSW war positiv mit der FFM zur Geburt und dem Körpergewicht zum zweiten Lebensjahr assoziiert. Insulin im Nabelschnurplasma korrelierte positiv mit dem Geburtsgewicht und der neonatalen Fettmasse und war bei den Mädchen negativ mit der Gewichtszunahme bis zum zweiten Lebensjahr assoziiert. Leptin in der Muttermilch zeigte weitgehend keine Assoziation mit den kindlichen Wachstumsparametern, während Adiponektin in der Muttermilch positiv mit der Gewichtszunahme und Körperfettmasse bis zum zweiten Lebensjahr assoziiert war.

Zusammenfassend zeigten sich signifikante Assoziationen sowohl von mütterlichen als auch von fetalen Biomarkern im Nabelschnurblut mit den kindlichen anthropometrischen Parametern bis zum zweiten Lebensjahr, was die Bedeutung des perinatalen metabolischen Milieus für das frühe kindliche Wachstum und die Körperzusammensetzung unterstreicht. Eine Nachbeobachtung der Kinder bis zum fünften Lebensjahr wird darüber Aufschluss geben, inwieweit diese Assoziationen und die beobachteten geschlechts-spezifischen Unterschiede längerfristig bestehen bleiben.

Abbreviations

AA	Arachidonic acid
AdipoQ	Adiponectin
AdipoR	Adiponectin receptor
AgRP	Agouti-related protein
AMPK	Adenosine monophosphate-activated protein kinase
ANCOVA	Analysis of covariance
b	Regression coefficient beta
BMI	Body mass index
C/EBPs	CCAAT/enhancer-binding-proteins
CG	Control group
CI	Confidence interval
CLIA	Chemiluminescence immunoassay
DEXA	Dual-energy X-ray absorptiometry
DHA	Docosahexaenoic acid
ELISA	Enzyme Linked Immunosorbent Assay
EPA	Eicosapentaenoic acid
FFM	fettfreie Körpermasse
FLI	Free leptin index
GDM	Gestational diabetes mellitus
gest.	Gestation
GWG	Gestational weight gain
HAPO	Hyperglycemia and Adverse Pregnancy Outcome
HMW	High molecular weight
HOMA-IR	Homeostasis model assessment for insulin resistance
^{125}I	125-Iodine
IG	Intervention group/Interventionsgruppe
IL-6	Interleukin 6
INFAT	The impact of nutritional fatty acids on infant adipose tissue development
IRS	Insulin receptor substrate
JAK/STAT	Janus Kinase/Signal Transducer and Activator of Transcription
KG	Kontrollgruppe

LBM	Lean body mass
LCPUFAs	Long-chain polyunsaturated fatty acids
LIFA	Ligand-immunofunctional assay
LMM	Linear mixed model
LMW	Low molecular weight
LPL	Lipoprotein lipase
MMW	Mid-molecular weight
mo	Month
NPY	Neuropeptide Y
n.s.	non significant
OB-R	Leptin receptor
PI	Ponderal Index
PI3K	Phosphatidylinositol 3-kinase
POMC	Proopiomelanocortin
pp	postpartum
PPAR	Peroxisome proliferator-activated receptor
RBCs	Red blood cells
RCT	Randomized controlled trial
SD	Standard deviation
SFT	Skinfold thicknesses
sOB-R	Soluble leptin receptor
SREBP-1c	Sterol regulatory element-binding protein 1c
SSW	Schwangerschaftswoche
TG	Triglycerides
TNF-α	Tumor necrosis factor α
US	Ultrasonography
VLDL	Very low density lipoprotein
WC	Waist circumference
wk	Week
α-MSH	Melanocyte-stimulating hormone α

1 Introduction

The rising prevalence of childhood obesity is a growing health concern worldwide (de Onis et al. 2010). Evidence is accumulating that the perinatal period might represent a vulnerable time with the potential to shape the long-term susceptibility of an individual to develop obesity or related metabolic diseases (Gluckman and Hanson 2008). As current attempts to stop the global obesity epidemic have shown little success, there is an urgent need for novel primary prevention approaches. Recently, the dietary fatty acid composition in maternal nutrition during pregnancy and lactation was proposed to be a critical determinant for offspring adipose tissue development. Specifically a reduction of the dietary n-6/n-3 fatty acid ratio was suggested to hold promise as a strategy to limit excessive fat mass growth in the infant (Ailhaud and Guesnet 2004).

The INFAT-Study ("The **i**mpact of **n**utritional **f**atty acids on infant **a**dipose **t**issue development") is the first randomized controlled trial originally designed to address this question (Hauner et al. 2009). In total, 208 healthy pregnant women were enrolled and randomized to either a dietary intervention to decrease the n-6/n-3 fatty acid ratio by a combined approach comprising a dietary supplementation with n-3 LCPUFAs as well as nutritional counseling to reduce the intake of arachidonic acid (AA), or a control group receiving general recommendations on a healthy diet during pregnancy, only. The major goal of the study was to investigate the impact of this early dietary intervention on infant fat mass longitudinally over the first year of life with skinfold thickness measurements as the primary outcome parameter.

Pregnancy is a unique metabolic situation associated with closely interrelated physiological adaptations including insulin resistance, dyslipidemia (Herrera 2000) and a low-grade systemic inflammatory condition originating in adipose tissue and the placenta due to increased production of pro-inflammatory cytokines (Hauguel-de Mouzon and Guerre-Millo 2006). Hence, in view of the well-documented anti-inflammatory (Ruxton et al. 2005) and triglyceride-lowering (Harris 1997) properties of n-3 LCPUFAs, an effect of these fatty acids on maternal parameters of carbohydrate and lipid metabolism is conceivable. Furthermore, fatty acids may influence the expression of adipokines, such as leptin or adiponectin, by interaction with transcription factors or other mechanisms possibly related to fatty acid oxidation, synthesis or storage (Drevon 2005) (**Figure 1**). Animal studies suggest, that the

balance of n-6 to n-3 fatty acids in the maternal diet affects leptin levels and the long-term regulation of several metabolic parameters in the offspring (Korotkova et al. 2002, Korotkova et al. 2005). Therefore, the evaluation of various biomarkers including parameters of glucose and lipid metabolism as well as adipokines over the course of the study in a range of biological samples including maternal blood, umbilical cord blood and breast milk is of great interest.

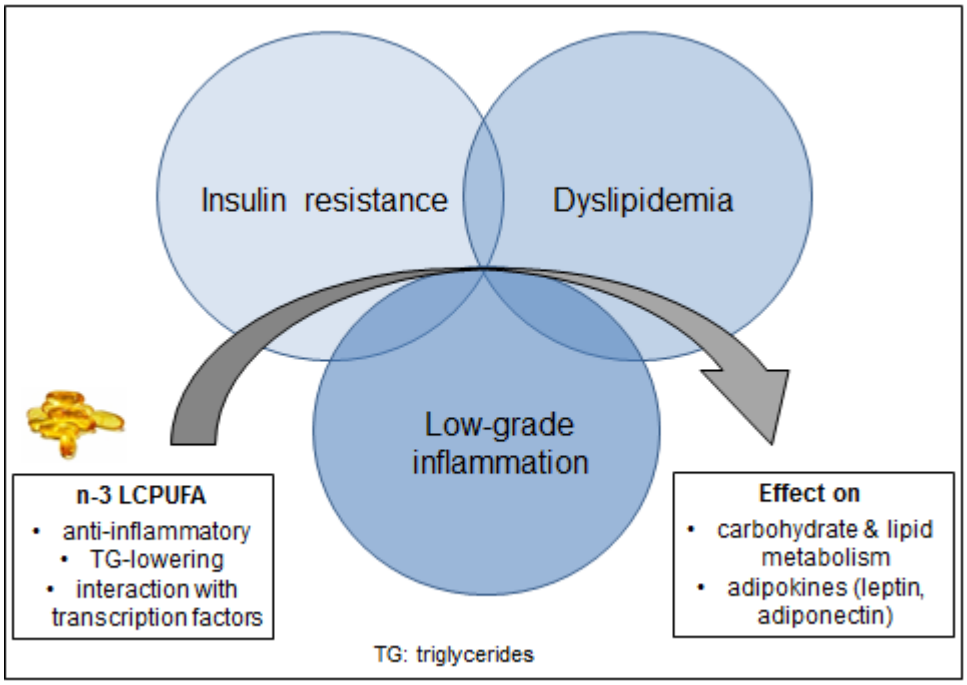

Figure 1 Potential effects of maternal supplementation with n-3 LCPUFAs during pregnancy

Moreover, the combined assessment of biochemical and anthropometric data makes the study particularly suitable to explore potential biomarkers that could predict the risk of early obesity development. Adipokines are among the most promising candidates under study since they are directly produced by adipose tissue and have been implicated in the regulation of energy homeostasis, insulin sensitivity, glucose and lipid metabolism (Hauner 2005).

Recently, both leptin and adiponectin in cord blood and also in breast milk have been shown to be associated with subsequent infant weight gain or body composition, suggesting a role of these proteins in regulating satiety and food intake during early infancy (Ong et al. 1999, Miralles et al. 2006, Weyermann et al. 2007, Doneray et al. 2009, Mantzoros et al. 2009, Woo et al. 2009, Schuster et al. 2011, Woo et al. 2012). Furthermore, maternal blood lipids and insulin sensitivity during pregnancy could act as determinants of fetal growth (Di Cianni et al.

2005, Schaefer-Graf et al. 2008, HAPO-Study-Group 2009) and thus might have implications for later weight gain and body composition, as well. It therefore seems worthwhile to make use of the broad range of available biosamples and the prospective design of the study

1) to investigate the impact of the dietary intervention to reduce the maternal dietary n-6/ n-3 fatty acids ratio on maternal parameters of carbohydrate and lipid metabolism and the adipokines, leptin and adiponectin, over the course of pregnancy and lactation as well as on selected biomarkers in cord blood and breast milk and

2) to describe in an observational approach their relationship with subsequent infant weight development and body composition as assessed by skinfold thickness measurements over the first two years of life.

2 Survey of the literature

2.1 Early determinants of obesity

The global rise in childhood overweight and obesity over the past decades has reached epidemic proportions. Overweight and obese children are likely to remain obese into adulthood and more likely to develop serious chronic diseases such as diabetes and cardiovascular disease at a younger age (WHO 2009). In view of this challenge, there is an urgent need to develop and evaluate novel prevention strategies.

There is now increasing evidence that the perinatal period, i.e. pregnancy and the early postpartum period, represents a critical time window, in which environmental and nutritional influences may determine an individual´s susceptibility for obesity and its sequelae over the life course (Gluckman and Hanson 2008). This concept, that early exposures during periods of high developmental plasticity, even before birth, may impact on lifelong health, is commonly referred to as "fetal" or "developmental programming" (Armitage et al. 2005).

In recent years, multiple perinatal factors which are in large part modifiable have been shown to be associated with subsequent obesity development in the offspring, including maternal pre-pregnancy BMI (Drake and Reynolds 2010, Nelson et al. 2010), gestational weight gain (Oken et al. 2007, Oken et al. 2008b, Wrotniak et al. 2008, Crozier et al. 2010, Schack-Nielsen et al. 2010, von Kries et al. 2010) and smoking during pregnancy (Oken et al. 2008a, Ino 2010). Increasing trends of overweight in women of child-bearing age and excessive gestational weight gain may induce an "intergenerational vicious cycle of obesity" with heavier mothers giving birth to heavier daughters, who are at increased risk to enter their own pregnancy overweight or obese, and thus perpetuating the cycle (Dabelea and Crume 2011).

Among the early postnatal factors affecting later risk for obesity, the mode of infant feeding (breast or formula feeding) is probably the most important determinant (Bergmann et al. 2003). Three meta-analyses of observational studies have shown significant protective effects of breastfeeding on the risk of obesity later in life (Arenz et al. 2004, Harder et al. 2005, Owen et al. 2005b). However, confounding by sociocultural factors and publication bias are likely to have influenced the results (Owen et al. 2005a). Two types of mechanisms are discussed how breast-feeding might confer protection against later obesity development. On the one hand, behavioral aspects could play a role: Compared to bottle feeding, the act of breast-feeding could result in better recognition of satiety signals by the infant, leading to a

better self-regulation of energy intake in the long term. On the other hand, biologically active components in breast milk, like hormones or growth factors, which are absent in infant formula, could be responsible for this effect (Gillman and Mantzoros 2007). Especially the adipokines which are involved in appetite and energy balance such as leptin and adiponectin are currently discussed to provide a mechanistic link by both short- and long-term regulation of the infant metabolism and thereby influencing weight gain in the offspring (Thompson 2012).

2.2 Fatty acids and adipose tissue development

In addition to these meanwhile well recognized perinatal influencing factors, there is some evidence that the maternal diet during pregnancy and lactation has an impact on the development of later obesity in the offspring, which is best supported by animal experiments. These studies favor the possibility, that not only the amount of fat ingested (Armitage et al. 2005), but also the fatty acid composition and specifically the ratio of n-6/n-3 fatty acids in the maternal diet could be a critical determinant for adipose tissue growth in the offspring (Massiera et al. 2003).

2.2.1 Adipose tissue development

There is now compelling evidence that early life stages are critical periods for adipose tissue development in humans. Body fat mass is determined by both, adipocyte number and fat cell size. Increased fat storage in already developed, fully differentiated adipocytes, is thought to be the most important mechanism whereby fat depots increase in adults (Spalding et al. 2008). Earlier reports indicate, that distinct periods in early life, one within the first years of life, and again during pre-puberty, represent sensitive phases of adipocyte formation characterized by high proliferation and differentiation capacity of adipocyte precursor cells (Salans et al. 1973, Sjostrom and William-Olsson 1981, Hauner et al. 1989). The prevailing concept, that the number of adipocytes is likely to be set during childhood and adolescence to define the space for later fat expansion, has recently been confirmed by an elegant study using an isotope technique (Spalding et al. 2008). The same study further described an annual turnover rate of fat cells of about 10 % at all ages and levels of body mass index by analyzing the integrated ^{14}C from nuclear bomb tests performed between 1955 and 1963 in genomic DNA.

To date, the development of white adipose tissue in human fetal life has been poorly studied. First traces of adipose tissue differentiation from specific precursor cells can already be found between the 14th and 16th week of prenatal life in the human fetus. Fat lobules are the earliest structures to be identified in the main fat depots, before typical vacuolated fat cells appear. It has been shown that after the 23rd week of gestation the total number of fat lobules remains approximately constant, whereas thereafter the growth of adipose tissue is determined mainly by an increase in size of the lobules, suggesting this period to be highly susceptible for environmental exposures that could impact on early adipocyte formation (Poissonnet et al. 1983).

2.2.2 *In vitro* and animal studies

Especially *in vitro* and animal studies strongly support the concept that the ratio of n-6/ n-3 fatty acids plays a crucial role in adipogenesis. In particular, it was shown that the n-6 fatty acid AA inhibits cell proliferation and promotes differentiation to adipocytes in the preadipocyte stage mediated through action of its metabolite prostacyclin (Massiera et al. 2003, Ailhaud et al. 2006), whereas the n-3 LCPUFAs DHA and EPA rather counteract this process (Massiera et al. 2003, Azain 2004, Madsen et al. 2005, Ailhaud et al. 2006). Furthermore, the n-3 fatty acids were also shown to act on mature adipocytes in the process of lipid storage and accumulation (Madsen et al. 2005). The underlying mechanisms include effects on the regulation of transcription factors representing key molecules for both, adipocyte differentiation (e.g. PPARγ and C/EBPs) and lipogenesis (e.g. SREBP-1c), which are mediated either by the fatty acid per se or their active metabolites such as prostaglandins (Azain 2004, Madsen et al. 2005).

Apart from the *in vitro* evidence, there is good agreement from animal studies for anti-obesity effects of n-3 LCPUFA supplementation as shown by decreased cellularity of adipose tissue (Flachs et al. 2009) and reduced lipid synthesis (Arai et al. 2009), suggesting a role for n-3 fatty acid in reducing both hyperplasia as well as hypertrophy of growing fat depots.

More recently, attention was shifted towards the potential programming effect of modifying the fatty acid composition in the maternal diet during the gestation and lactation period on adipose tissue growth in the offspring. In a "proof of concept" study, Massiera et al. intriguingly demonstrated in mice, that a maternal diet with a markedly increased n-6/n-3 fatty acid ratio (59:1 vs. 2:1) during pregnancy and the suckling period resulted in higher body weight and fat mass in the pups, which persisted until adulthood (Massiera et al. 2003).

However, a recent systematic review of animal studies investigating the effects of increased n-3 LCPUFA supply during pregnancy and lactation on offspring body composition concluded that there is insufficient evidence to date to definitely evaluate the role of pre- and early postnatal maternal n-3 LCPUFA supplementation for offspring fat mass development (Muhlhausler et al. 2011).

2.2.3 Epidemiological data

Rather indirect evidence for a role of the fatty acid composition for human adipose tissue development can be deduced from epidemiological data, showing that the n-6/n-3 fatty acid ratio in the diet of populations in the industrialized countries has changed considerable towards an increasing dominance of n-6 fatty acids over recent decades. These changes are also reflected in the fatty acid pattern of breast milk, in particular regarding the linoleic/α-linolenic acid ratio. These observations coupled with increasing rates of childhood overweight and obesity over the same time period gave further support to a potential causal relationship between the dietary fatty acid composition and the development of infant adipose tissue (Ailhaud et al. 2006, Muhlhausler and Ailhaud 2013).

2.2.4 Human data – Evidence from randomized controlled trials

Based on this observational evidence and early promising results of animal studies, Ailhaud et al. postulated in 2004 that a reduced dietary n-6/n-3 fatty acid ratio during early critical windows of fat cell development may limit adipose tissue growth and, thereby, may offer a novel strategy for the primary prevention of pediatric obesity (Ailhaud and Guesnet 2004).
This hypothesis subsequently stimulated many researchers to address this question in post-hoc analyses of already established randomized controlled trials originally designed to investigate the effect of maternal supplementation with n-3 LCPUFAs during pregnancy and/or lactation on other outcomes in the offspring, such as neurological development or allergic diseases.
However, according to a recent systematic review by Muhlhausler et al. there is still insufficient evidence to evaluate the effect of perinatal n-3 LCPUFA supplementation on infant body composition (Muhlhausler et al. 2010). When aggregating the findings of available RCTs, the high variability of these studies regarding the timing and duration of the intervention (pregnancy and/or lactation), the dosage of n-3 LCPUFA supplementation, compliance and other methodological aspects has to be considered (Muhlhausler et al. 2010).

Of note, most studies relied on rather indirect growth parameters such as BMI or BMI z-scores and in only one study skinfold thickness measurements were performed to determine percentage body fat and, thus, to discriminate between fat and lean body mass (Lauritzen et al. 2005, Asserhoj et al. 2009). Consequently, these inconsistent results do not allow a definite and firm conclusion on the role of n-3 LCPUFAs during the perinatal period by supplementing pregnant women/lactating mothers in determining offspring adiposity development (Muhlhausler et al. 2010, Brunner et al. 2011).

This limited data highlights the need for RCTs involving longitudinal assessments of infant body composition by adequate methods. The INFAT-Study, initiated in 2006, is the first RCT originally designed to investigate the effect of reducing the dietary n-6/n-3 fatty acid ratio in the maternal diet during pregnancy and lactation on infant adipose tissue development as the primary endpoint (Hauner et al. 2009). Importantly, the intervention started early in pregnancy close to the first appearance of adipocytes in the human fetus (Poissonnet et al. 1983) and lasted until 4 months of lactation, resulting in a broad time window of active intervention during the perinatal development. As recently published, the study found no evidence that a reduction of the dietary n-6/n-3 fatty acids ratio during pregnancy and lactation relevantly affects fat mass in the offspring during the first year of life (Hauner et al. 2012). To explore long-term effects of this early intervention on body composition and other outcomes potentially related to an early modification of the dietary fatty acid composition, such as neurological development and atopic diseases, the children are currently followed-up until 5 years of age.

2.3 Metabolic changes during pregnancy

Human pregnancy is accompanied by a range of continuous physiologic adaptations affecting the metabolism of all nutrients in order to ensure appropriate nutrient supply for the developing fetus and to prepare the mother for the demands of lactation (King 2000).

During the first two thirds of pregnancy, the mother is in an anabolic condition, favoring the accumulation of fat depots in maternal tissues as a result of both hyperphagia and enhanced lipid synthesis. In contrast, in the third trimester of gestation, the mother shifts towards a catabolic state allowing an enhanced transfer of nutrients to the fetus to support its rapid growth (Butte 2000, Herrera 2000) (**Figure 2**).

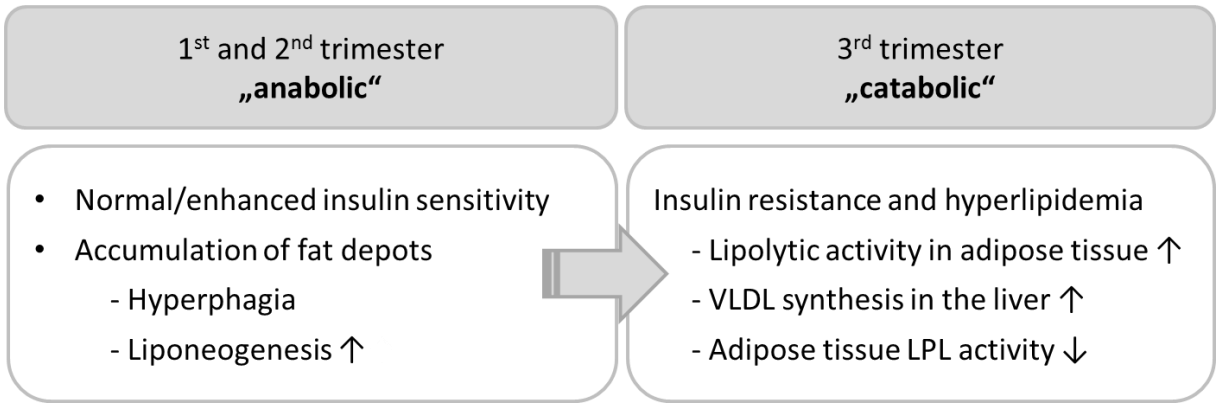

Figure 2 Characteristics of nutrient metabolism by pregnancy trimesters
Data source: Herrera and Ortega (2008)
LPL: lipoprotein lipase; VLDL: very low density lipoproteins

2.3.1 Carbohydrate metabolism

During early pregnancy, basal plasma glucose and insulin levels and hepatic gluconeogenesis do not differ from the nongravid state (Catalano et al. 1991, Catalano et al. 1992, Catalano et al. 1993), and insulin sensitivity is also normal or even enhanced (Herrera 2005). Advancing gestation is characterized by progressive development of insulin resistance as a physiologic adaptation to enhance availability of nutrients across the placenta to meet the increasing requirements of the rapidly developing fetus (Herrera and Ortega 2008). Studies applying the hyperinsulinemic-euglycemic glucose clamp technique and intravenous glucose tolerance testing revealed that insulin action in late normal pregnancy is 50−70 % lower than in non-pregnant women (Catalano et al. 1991, Catalano et al. 1992, Catalano et al. 1993). Although the precise mechanisms are unclear, alterations in the hormonal milieu during pregnancy are likely to contribute to these metabolic changes (Butte 2000). Longitudinal studies over the course of pregnancy show increased basal endogenous glucose production, increased fasting insulin concentration and a reduced ability of insulin to suppress hepatic glucose production with advancing gestation (Catalano et al. 1992, Catalano et al. 1993, Catalano et al. 1999). By the third trimester, the mother tends to develop hypoglycemia whereas fasting insulin is almost twice the concentration of non-pregnant women (Butte 2000, Herrera 2005). The development of hypoglycemia occurs despite enhanced gluconeogenesis and reduced uptake of glucose by maternal tissues due to the progressive decline in insulin sensitivity and is the result of the high rate of placental transfer of glucose, which is the primary energy source for the fetus (Herrera and Ortega 2008).

2.3.2 Lipid metabolism

In parallel to alterations in insulin sensitivity major changes in lipid metabolism occur, which are mainly characterized by an increase of the maternal fat depots in early and mid-pregnancy, followed by an enhanced fat mobilization and hyperlipidemia in late pregnancy.

Maternal fat stores accumulate during the first two thirds of gestation driven by increased insulin sensitivity in adipose tissue present in early pregnancy and increased concentrations of estrogen and progesterone (Butte 2000, Herrera 2005). The enlargement of fat depots ends by the beginning of the last trimester and is the consequence of decreased lipoprotein lipase (LPL) activity in adipose tissue (Alvarez et al. 1996) causing a reduced tissue uptake of circulating triglycerides, and an increased adipose tissue lipolytic activity, especially in the fasting state (Herrera 2005) (**Figure 2**). These metabolic alterations in combination with enhanced VLDL synthesis and release by the liver cause a physiological state of hyperlipidemia, which is mainly accounted for by increases in triglycerides, with smaller rises in phospholipids and cholesterol (Herrera et al. 1988, Alvarez et al. 1996).

Taken together, major physiologic adaptations in nutrient metabolism evolve continuously over the course of pregnancy dependent on hormonal changes, fetal demands and maternal nutrient supply (King 2000).

2.4 Adipose tissue as an endocrine organ

It is now recognized that adipose tissue is far more than an inert lipid-storing organ mobilizing energy in response to fasting or increased energy requirements. Today, it is evident, that adipose tissue is a complex multifunctional organ releasing a variety of molecules, the so called adipokines which are involved in the regulation of many physiological processes including carbohydrate and lipid metabolism, energy homeostasis, inflammatory response, immunity and vascular function (Trayhurn and Wood 2004, Hauner 2005). These factors comprise hormones, cytokines, acute phase proteins and growth factors that exert their biological functions via endocrine, auto- and paracrine mechanisms (Trayhurn and Wood 2004). Thereby, adipose tissue is actively communicating with many other organs and body systems.

White adipose tissue does not only contain mature fat cells, but includes many other cell types from the stromal vascular fraction such as pre-adipocytes, fibroblasts and macrophages, which also contribute to the secretory capacity of adipose tissue (Hauner 2005, Wang et al.

2008). An excess accumulation of fat mass in the obese state has been associated with a dysregulation of the adipose tissue secretory pattern, which is thought to play a role in the development of obesity-related disorders, including insulin resistance, the metabolic syndrome or cardiovascular disesases (Matsuzawa et al. 2003).

2.5 Leptin

2.5.1 Biology of leptin

Leptin, encoded by the *ob*-gene, which was first discovered by Zhang et al. in 1994 (Zhang et al. 1994), is mainly secreted by white adipose tissue, but also in other tissues including the gastro-intestinal tract (Cinti et al. 2000, Sitaraman et al. 2004), placenta (Masuzaki et al. 1997), fetal tissues (Karakosta et al. 2011) and the mammary gland (Smith-Kirwin et al. 1998). Mutant mice lacking leptin, the *ob/ob* mice, are characterized by severe obesity, hyperphagia and insulinemia, but lose weight through exogenous supply with leptin (Halaas et al. 1995, Pelleymounter et al. 1995). These observations highlight the fundamental role of leptin in regulating energy homeostasis and body weight by controlling food intake, satiety and energy expenditure (Friedman and Halaas 1998). At the level of the central nervous system, leptin acts on central effector pathways in the hypothalamus, repressing brain anabolic neural circuits that stimulate eating and inhibit energy expenditure, while simultaneously activating catabolic circuits that inhibit food intake and increase energy expenditure. In the arcuate nucleus, leptin inhibits the orexigenic NPY/AgRP neurons and activates POMC neurons, which stimulate the expression and release of the anorexigenic peptide α-MSH (Schwartz et al. 2000) (**Figure 3**). Thus, leptin is signaling the amount of energy stores to the brain and thereby regulates long-term energy homeostasis through a negative feedback mechanism. Furthermore, leptin has been implicated in the maturation and regulation of the reproductive system. It has been suggested that leptin may serve as an indicator of long-term metabolic fuel availability, signaling the presence of sufficient maternal fat stores to initiate reproduction (Butte 2000).

There is a close correlation between circulating leptin levels and body fat mass (Maffei et al. 1995, Shimizu et al. 1997, Friedman and Halaas 1998). In the overweight/obese state, when leptin levels are high, resistance to the central effects of leptin is assumed, probably due to a reduced transport capacity for leptin through the blood/cerebrospinal-fluid barrier (Caro et al. 1996, Schwartz et al. 1996).

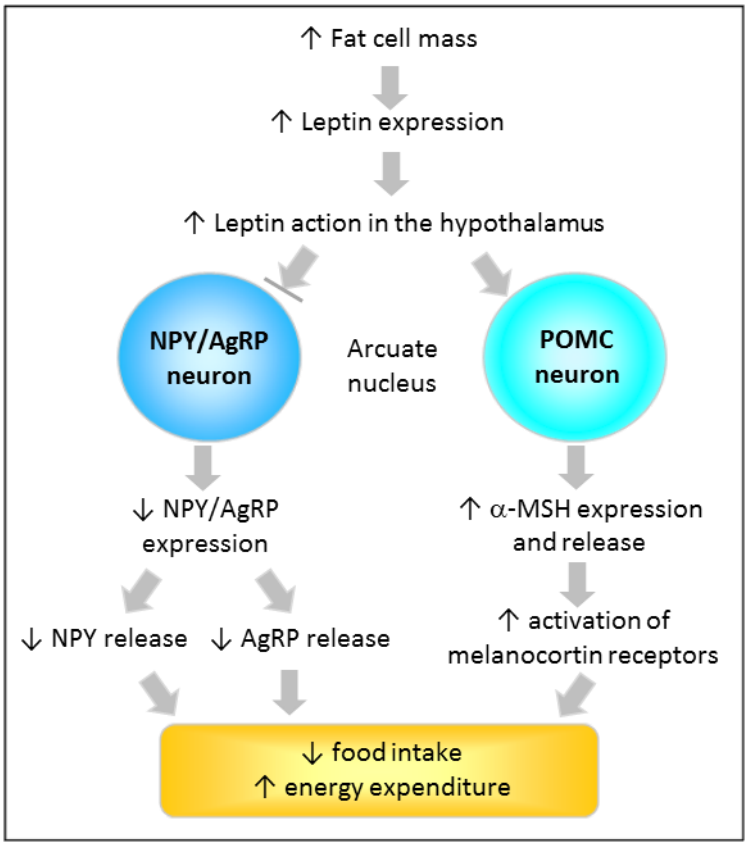

Figure 3 Leptin action in the hypothalamus

Leptin regulates body weight through a negative endocrine feedback loop. Increasing fat cell mass leads to increased leptin expression in adipose tissue and release into the circulation. In the arcuate nucleus, leptin inhibits the orexigenic NPY/AgRP neurons and activates POMC neurons, which stimulate the expression and release of the anorexigenic peptide α-MSH, resulting in decreased food intake and increased energy expenditure [modified from: Schwartz et al. (2000)].

Leptin acts through its receptor (OB-R), a member of the class I cytokine receptor superfamily, that is present in at least six isoforms (OB-R a-f), which share an identical extracellular domain, but can be distinguished by the length of intracellular regions (Hegyi et al. 2004). Only the full-length receptor (OB-Rb), mainly expressed in the hypothalamus, has full signaling capabilities and is able to activate the JAK/STAT pathway, the major pathway used by leptin to exert its effects (Bjorbaek et al. 1997). Besides, other pathways such as mitogen-activated protein kinase (MAPK) and 5`-AMP-activated protein kinase (AMPK) pathway, also play a role in leptin signaling (Hegyi et al. 2004).

The soluble leptin receptor (sOB-R), lacking the transmembrane and intracellular domains, represents the major leptin-binding activity in the human circulation and is suggested to

modulate its biological actions by regulating the ratio of free to bound leptin in the circulation (Lammert et al. 2001).

2.5.2 Leptin in pregnancy and lactation

Numerous studies have shown, that pregnancy represents a state of hyperleptinemia (Butte et al. 1997, Masuzaki et al. 1997, Schubring et al. 1998, Tamura et al. 1998). As in the non-pregnant state, leptin levels correlate with maternal BMI, especially in the first trimester, with weaker correlations found with advancing gestation (Schubring et al. 1998). Longitudinal measurements of leptin over the course of pregnancy have revealed that serum leptin concentrations reach a peak around 22−27 weeks of gestation and then decline slightly by term (Tamura et al. 1998). It has been hypothesized that leptin levels in pregnancy may function as a feedback modulator of substrate supply and adipose tissue status supporting normal progression of pregnancy (Schubring et al. 1998).
The observation that maternal leptin levels significantly decrease after delivery led to the assumption that substantial amounts of leptin are of placental origin. Masuzaki et al. were the first to demonstrate that leptin is synthesized and secreted from the placenta, and thus from non-adipose tissue, both *in vivo* and *in vitro* (Masuzaki et al. 1997). Subsequent studies indicated that the majority of placental leptin is released to the maternal side (Linnemann et al. 2000, Lepercq et al. 2001).

Cord blood leptin levels seem to be independent of maternal leptin concentration (Schubring et al. 1998, Laml et al. 2001), and have consistently been shown to correlate with birth weight, Ponderal Index and newborn fat mass (Clapp and Kiess 1998, Schubring et al. 1999, Karakosta et al. 2011). Furthermore, cord blood leptin levels were found to be inversely associated with early infant weight gain (Ong et al. 1999, Mantzoros et al. 2009) and to predict infant adiposity in early childhood (Mantzoros et al. 2009).

Leptin has also been shown to be present in human breast milk (Casabiell et al. 1997, Houseknecht et al. 1997). It has been suggested that leptin is transferred from the maternal circulation into human milk, but could also be directly expressed in the mammary gland (Casabiell et al. 1997, Smith-Kirwin et al. 1998). In rats, it has been demonstrated that leptin present in maternal milk can be absorbed by the immature stomach of nursing pups and transferred to their circulation (Casabiell et al. 1997, Sanchez et al. 2005), but this has not yet been proven in human studies. Recent studies have reported an association of leptin levels in

human milk with subsequent infant weight gain (Miralles et al. 2006, Doneray et al. 2009, Schuster et al. 2011), suggesting a critical role for leptin in regulating appetite control and food intake.

2.6 Insulin and leptin as adiposity signals

Besides leptin, insulin, the secretory product of the endocrine pancreas, also can be considered as a major "adiposity signal" acting in the central nervous system through a classical endocrine feedback loop. Both hormones are present in the circulation at levels directly proportional to body fat mass and enter the brain in proportion to their plasma levels, where they convey information about the status of body energy stores to the hypothalamus (Schwartz et al. 2000, Niswender and Schwartz 2003) (**Figure 4**). Receptors for both, insulin and leptin, are expressed by hypothalamic neurons involved in the regulation of energy homeostasis (Baskin et al. 1988, Cheung et al. 1997, Baskin et al. 1999), and defective neuronal signaling by either hormone results in hyperphagia, adiposity, hyperinsulinemia and disordered glucose tolerance (Niswender and Schwartz 2003).

Whilst leptin signals primarily via the JAK-STAT pathway, the insulin receptor belongs to the family of tyrosine kinase receptors, which mediate their activity through autophosphorylation of the receptor. Activation of the receptor leads to the recruitment and phosphorylation of insulin receptor substrate (IRS) proteins which results in the activation of a variety of signaling cascades, including phosphatidylinositol 3-kinase (PI3K)-signaling, and further downstream targets (Niswender et al. 2004). Despite diverging signal transduction pathways, the cellular responses to insulin and leptin in the arcuate nucleus, decreased food intake and increased energy expenditure, are similar (Niswender et al. 2004). This physiological overlap might be explained by a cross-talk between both signal transduction cascades, most likely at the level of IRS/PI3K activation, which has been shown to be a crucial step in the regulation of energy homeostasis (Niswender et al. 2001, Zhao et al. 2002, Niswender et al. 2004).

Thus, the central catabolic effects of insulin are in contrast to its well-established peripheral anabolic actions promoting the storage of energy in the form of carbohydrates, protein and fat. It is suggested that, comparable to other physiological systems, the peripheral and central effects of insulin are balanced, to support euglycemia and optimal body composition (Niswender et al. 2004).

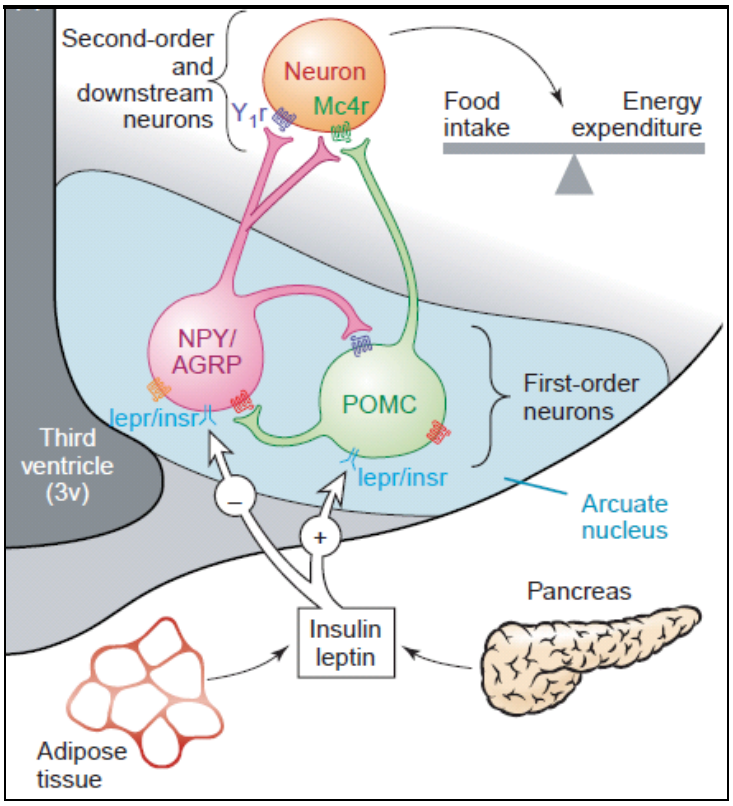

Figure 4 Leptin and insulin as adiposity signals

Neurons in the arcuate nucleus express insulin and leptin receptors and integrate peripheral signals to maintain energy homeostasis. Increased action of both, insulin and leptin in the arcuate nucleus inhibits the NPY/AgRP anabolic pathway and stimulates the POMC catabolic pathway, resulting in reduced food intake and increased energy expenditure (Niswender et al. 2004).

2.7 Adiponectin

2.7.1 Biology of adiponectin

Adiponectin, also known as Acrp 30 (adipocyte complement-related protein of 30 kDa) or AdipoQ, is a 30 kDa protein abundantly and specifically expressed in adipose tissue (Scherer et al. 1995, Hu et al. 1996). Adiponectin is secreted into the circulation in significant amounts, with serum levels in the µg/ml range, accounting for up to 0.05 % of total serum protein (Scherer et al. 1995, Hotta et al. 2000). In the human circulation, it is present as a multimer ranging from trimers and hexamers to high molecular weight (HMW) complexes containing up to 18 subunits (Kadowaki and Yamauchi 2005), which are thought to represent the more physiologically active form of the protein (Kubota et al. 2007).

In contrast to other proteins secreted by adipose tissue, circulating levels of adiponectin are reduced in obesity (Arita et al. 1999) as well as in type 2 diabetes (Hotta et al. 2000), and

weight reduction in obese individuals has been shown to be associated with increasing adiponectin concentrations (Faraj et al. 2003). Furthermore, plasma adiponectin levels have been found to be reduced in patients with cardiovascular disease (Kumada et al. 2003), hypertension (Ouchi et al. 2003), or metabolic syndrome (Trujillo and Scherer 2005), and thus in conditions frequently associated with insulin resistance.

Two adiponectin receptors, AdipoR1 and AdipoR2, have been identified: While AdipoR1 is abundantly expressed in skeletal muscle, AdipoR2 is predominantly expressed in the liver (Yamauchi et al. 2003).

Adiponectin exerts insulin-sensitizing effects by stimulating fatty-acid oxidation and decreasing tissue triglyceride content in the liver and skeletal muscle through activation of AMP-activated protein kinase (AMPK) and PPARα (Kadowaki and Yamauchi 2005). In addition, adiponectin was shown to have anti-atherogenic properties by inhibiting the expression of adhesion molecules and inflammatory cytokines as well as foam cell formation through inhibited expression of scavenger receptors on macrophages (Kadowaki and Yamauchi 2005).
Besides its peripheral actions, adiponectin was also reported to have central effects. It was shown, that adiponectin enhances AMPK activity in the arcuate hypothalamus via its receptor AdipoR1 to stimulate food intake and decrease energy expenditure, indicating a physiological role in the regulation of energy homeostasis inversely to leptin (Kubota et al. 2007).

2.7.2 Adiponectin in pregnancy and lactation

A range of studies in mice (Combs et al. 2003, Kondo et al. 2004) and humans have revealed normal pregnancy as a situation of hypoadiponectinemia (Catalano et al. 2006, O'Sullivan et al. 2006) compared to the non-pregnant state, which was shown to be primarily reflected at the level of HMW adiponectin complexes (Catalano et al. 2006). Longitudinal studies predominantly indicate a decline in adiponectin concentrations with advancing gestation (Asai-Sato et al. 2006, Catalano et al. 2006, Fuglsang et al. 2006), especially by the last trimester, although data are not entirely consistent (Mazaki-Tovi et al. 2007, Mazaki-Tovi et al. 2008). These changes in circulating adiponectin are paralleled by increases in fat mass and reduced insulin sensitivity over the course of pregnancy (Catalano et al. 1991, Catalano et al. 1992, Catalano et al. 1993, Herrera 2000, Catalano et al. 2006), suggesting a mechanistic link

between body fat accretion, insulin resistance and the down-regulation of adiponectin production or secretion, although the causal relationship is still unclear (Catalano et al. 2006).

Both, adiponectin and its receptors have been shown to be expressed in the placenta (Caminos et al. 2005, Chen et al. 2006), suggesting that adiponectin may be involved in the regulation of placental function through autocrine/paracrine mechanisms (Lecke et al. 2011).

High concentrations of adiponectin are reported to be present in cord blood (several fold compared to adult levels) and positive correlations were found with birth weight and newborn adiposity (Sivan et al. 2003, Pardo et al. 2004, Tsai et al. 2004, Inoue et al. 2008). More recently, cord blood adiponectin was reported to be inversely associated with infant weight gain and to predict infant adiposity at the age of 3 years (Mantzoros et al. 2009).

Adiponectin has also been shown to be present in human breast milk (Bronsky et al. 2006, Martin et al. 2006, Weyermann et al. 2006), predominantly in its high molecular weight form (Woo et al. 2009, Newburg et al. 2010). Although transfer to the infant circulation is questionable (Gillman and Mantzoros 2007), associations of breast milk adiponectin with infant growth trajectory and body composition have been reported (Weyermann et al. 2007, Woo et al. 2009, Woo et al. 2012).

3 Aim of the thesis

This thesis is based on data obtained within the framework of the INFAT-study, an open-label randomized controlled trial, originally designed to examine the effect of reducing the maternal dietary n-6/n-3 fatty acid ratio during pregnancy and lactation on adipose tissue development in the offspring.

Pregnancy is characterized by a variety of tightly interrelated physiological alterations including insulin resistance, dyslipidemia and a state of low-grade inflammation. In view of the well-documented anti-inflammatory (Ruxton et al. 2005) and triglyceride-lowering (Harris 1997) properties of n-3 LCPUFAs, an effect of these fatty acids on maternal parameters of carbohydrate and lipid metabolism could be plausible. Furthermore, n-3 LCPUFAs might influence the expression of adipokines by interaction with transcription factors or other mechanisms related to fatty acid metabolism (Drevon 2005). Furthermore, given the increasing evidence that obesity and its comorbidities may have prenatal origins, the investigation of early biomarkers related to later obesity development is of great interest. In this context, parameters implicated in the regulation of energy homeostasis, glucose and lipid metabolism or satiety control, such as insulin or the adipokines, could represent promising candidates.

Aim of this thesis was 1) to test the hypothesis whether an enhanced maternal intake of n-3 LCPUFAs during pregnancy leads to an altered metabolic and adipokine profile in maternal and cord blood samples and 2) to explore in an observational analysis associations of the investigated biomarkers with infant body composition and weight development up to 2 years of life. For this purpose, biosamples collected from different compartments, such as maternal blood, cord blood and breast milk, were utilized.

The present work builds on previous analyses, which were performed in a subsample of the study population, providing preliminary data on the effect of the dietary intervention on maternal plasma and breast milk as well as cord blood adipokines (Schmid 2011). For the present thesis, the measurements of adipokines and other biomarkers in various biosamples were complemented in cooperation with the Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics at the University Hospital of Leipzig.

To add on the previous analysis, particular emphasis was placed on potential associations between maternal or fetal parameters with infant growth, body composition and weight development up to the 2nd year of life.

Specific objectives of this thesis are:

- to investigate whether reducing the n-6/n-3 fatty acid ratio in the maternal diet during pregnancy and lactation has an impact on
  - maternal parameters of carbohydrate and lipid metabolism (insulin, glucose, insulin resistance assessed as HOMA-IR, and triglycerides) over the course of pregnancy and lactation
  - maternal adipokines (leptin axis including leptin, soluble leptin receptor and free leptin index, as well as HMW adiponectin) over the course of pregnancy and lactation
  - selected parameters in cord blood (leptin axis including leptin, soluble leptin receptor and free leptin index; insulin)
  - the adipokines present in breast milk (leptin and total adiponectin)
- to describe the clinical outcomes of the infants up to 2 years of life (growth pattern and infant fat mass assessed by skinfold thickness measurements).
- to explore in an observational analysis the association of the assessed maternal and fetal biomarkers with infant body composition and weight development up to 2 years of life and thus to evaluate their usefulness as potential early biomarkers with prognostic value for subsequent infant adiposity development or weight gain.

4 Study design, subjects and methods

4.1 General description of the study

The INFAT study is an open-label, monocenter, randomized, controlled dietary intervention trial with two parallel groups in pregnant and lactating women and their offspring (Hauner et al. 2009). The study was primarily designed to examine the effect of a reduction in the n-6/n-3 LCPUFA ratio in the diet of pregnant women and breastfeeding mothers on adipose tissue growth in their offspring.

In brief, 208 women were randomly assigned to an intervention group receiving a dietary supplement of 1200 mg n-3 LCPUFAs as fish oil capsules and a concomitant nutritional counseling to normalize the intake of the n-6 fatty acid AA to a moderate level of intake (~90 mg per day) or a control group advised to keep to a healthy diet during pregnancy according to current recommendations. The intervention started in the 15th week of pregnancy and lasted until 4 months postpartum. Infant fat mass was assessed by a combination of methods with skinfold thickness measurements as the primary outcome, complemented by abdominal ultrasonography and, in a subgroup, magnetic resonance imaging. The infants were clinically assessed from birth through the first year of life and are currently followed-up up to 5 years of age.

The study protocol was approved by the ethical committee of the Technical University of Munich (No. 1479/06/2006/2/21) and was registered at clinicaltrials.gov as NCT00362089.

4.2 Primary and secondary endpoints

Fat mass of the infants assessed by skinfold thickness measurements is the primary outcome parameter of the study. Key secondary endpoints include infant growth parameters (weight, height, head circumference, upper arm and waist circumference); the assessment of infant fat mass by further complementary methods, ultrasonography and, in a subgroup, magnetic resonance imaging; the analysis of the fatty acid profile in maternal blood, cord blood, breast milk and infant blood; maternal blood lipid concentrations as well as insulin and adipokines in maternal, fetal and infant biosamples; and the assessment of the dietary intake of the women.

In the ongoing follow-up program up to 5 years of age, long-term effects of the intervention on infant body composition and further health outcomes, such as atopic diseases and neurological development are explored.

For the present analysis, selected biomarkers were determined in maternal blood, cord blood and breast milk samples collected at pre-specified time-points over the course of the study (**Figure 5**). Of the infant clinical data, anthropometric measurements including general growth (weight, height, growth indices, weight gain) and body composition based on skinfold thickness measurements up to 2 years pp were included in the present work.

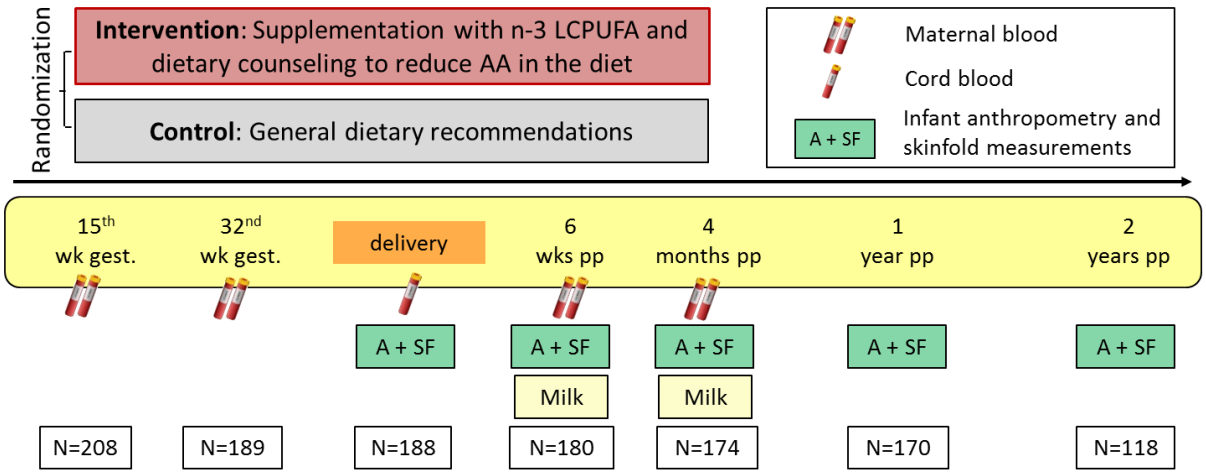

Figure 5 Study design

AA: Arachidonic acid; N: Number of women/infants at the respective time-points

4.3 Recruitment and screening

The recruitment of healthy pregnant women before their 15th week of pregnancy was conducted between July 2006 and May 2009 by support of practice-based gynecologists in the Munich area, directly at the outpatient clinic of the Division of Obstetrics and Perinatal Medicine of the University Hospital Klinikum rechts der Isar, Technische Universität München, Munich, and by advertisements in local newspapers and magazines.

Inclusion and exclusion criteria were as follows (**Table 1**):

Table 1 Inclusion and exclusion criteria

Inclusion	Exclusion
Gestational age ≤ 15th week of gestation	High risk pregnancy (multiple pregnancy, rhesus incompatibility, hepatitis B infection, or parity >4)
Age: between 18 and 43 years	Hypertension
Pre-pregnancy BMI: between 18 and 30 kg/m^2	Chronic diseases (e.g. diabetes) or gastrointestinal disorders accompanied by maldigestion, malaborption or elevated energy and nutritional requirements
Willingness to implement the dietary recommendations	Known metabolic defects (e.g. phenylketonuria)
Sufficient German language skills	Psychiatric diseases
Written informed consent	Hyperemesis gravidarum
	Supplementation with n-3 LCPUFAs before randomization
	Alcohol abuse or smoking

4.4 Study groups

To achieve a sustained reduction of the n-6/n-3 fatty acid ratio in the maternal diet through pregnancy and lactation in the intervention group, a two-component approach was used: The women received a supplement (Marinol D-40^{TM}, Lipid Nutrition, Wormerveer, The Netherlands) containing 1200 mg n-3 LCPUFAs (1020 mg DHA and 180 mg EPA) as well as 9 mg vitamin E as an antioxidant per day from enrollment around the 15th week of gestation until 4 months postpartum. Simultaneously the women obtained a detailed individualized nutritional counseling to normalize the consumption of AA to a moderate amount of intake (~90 mg/day). In contrast, women of the control group received more general recommendations on a healthy diet during pregnancy according to the guidelines of the German Nutrition Society.

4.5 Collection of biosamples

Maternal blood was collected at the 15th week of gestation, 32nd week of gestation and, if the mother was breastfeeding, additionally at 6 weeks and 4 months postpartum after an overnight fast. Immediately after delivery, a blood sample from the umbilical vein was collected in

EDTA-containing tubes. Plasma was separated from red blood cells by centrifugation for 10 min at 2000 x g at 4°C, aliquoted and stored at -80°C until analysis.

Breast milk samples of the lactating mothers were taken at 6 weeks and 4 months pp in a standardized manner after an overnight fast with an electric breast pump. Samples were aliquoted and stored at -80°C until analysis.

4.6 Maternal characteristics and anthropometry

Self-reported maternal pre-pregnancy weight and height were documented. Maternal weight over the course of pregnancy was retrieved from the maternity card. Maternal gestational weight gain (GWG) was calculated as the last measured value at booking minus self-reported weight before pregnancy.

Glucose tolerance status of the mother was defined based on a clinical diagnosis by the woman's gynecologist. The presence of clinically diagnosed gestational diabetes (GDM) was mostly confirmed by a standardized 75 g oral glucose tolerance test. Only in one case the diagnosis was based on elevated glucose at random. If the women had diagnosed GDM, the therapy regimen (diet or insulin treatment) was retrieved from the medical record. As previously reported (Hauner et al. 2012), the rate of GDM was not significantly different between the groups. Ten women of the control group and seven women of the intervention group were diagnosed with GDM. Two women of each group required insulin treatment.

At the 15^{th} and 32^{nd} week of gestation, skinfold thickness measurements were taken at four independent sites (biceps, triceps, subscapular and suprailiacal) by a Holtain skinfold caliper under standardized conditions (Holtain Ltd., Croswell, Crymych, UK). The mean of triplicate measurements per site and time point was used for analysis. Maternal percentage body fat as well as body fat mass [kg] were calculated using equations according to Durnin and Womersley (1974) and van Raaij et al. (1988). For the calculation of body density from skinfold thickness measurements, an equation based on only three skinfolds (triceps, biceps, subscapular) was used, as proposed by Durnin and Womersley (1974).

4.7 Infant anthropometric measurements

For the present analysis, the infant clinical data up to the 2^{nd} year of life were included. The infants were examined at birth or 3−5 days pp, respectively, and at 6 weeks, 4 months, 1 year and 2 years postpartum. Birth weight and length, head circumference, sex and gestational age

of the newborn were collected from the maternal obstetric records, obtained from midwives of the obstetric clinics. At the later time-points anthropometric measurements of the infants were taken at the study centre by trained investigators according to standardized procedures. The weight of the naked infant was measured at the study centre to the nearest 10 g by using a standard infant scale (Babywaage Ultra MBSC-55, myweight®, Erkelenz, Germany). Height was measured with a measuring stick (Säuglingsmessstab seca 207, Seca, Pfaffenweiler, Germany) to the nearest 0.5 cm while the infant was supine with stretched legs. BMI (kg/m^2) and Ponderal Index (kg/m^3) were calculated from these variables.

Longitudinal skinfold thickness measurements were performed in the infants 3-5 days pp in the obstetric clinic or at the family´s home, and later at the defined time-points at the study centre or in home visits. Skinfolds were measured in triplicate under standard conditions with a Holtain caliper (Holtain Ltd., Croswell, Crymych, UK) at the left body axis at four sites: triceps, biceps, subscapular and suprailiac. Percentage body fat was calculated via predictive skinfold equations according to the method of Weststrate and Deurenberg (1989). Regional body fat distribution was assessed by 1) the subscapular-to-triceps skinfold-ratio as an index of central to peripheral fat distribution according Haffner et al. (1987) and 2) the central-to-total skinfolds-ratio according to Weststrate et al. (1989).

If infants could not be clinically examined at 2 years, parents were asked to provide weight, height and head circumference as assessed in the routine paediatric "well-child check-up visit" at 20-24 months.

4.8 Laboratory analysis

The measurement of all biochemical parameters, unless otherwise indicated, was performed at the Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics at the University Hospital Leipzig, Leipzig, Germany (Prof. Dr. Juergen Kratzsch).

4.8.1 Measurement of leptin and sOB-R in plasma

Plasma leptin was measured by ELISA (Mediagnost, Reutlingen, Germany) using an automated processing system (DYNEX Technologies, Chantilly, VA, USA). The concentrations were measured in ng/ml and the lower detection limit for leptin was 0.2 ng/ml. Inter- and intra-assay coefficients of variations were below 5.2 %, respectively.

SOB-R was determined in a subset of maternal and cord blood samples by using an in-house ligand-immunofunctional assay (LIFA) (Lammert et al. 2001, Kratzsch et al. 2002). The intra- and interassay coefficients of variation were below 10.6 %, respectively.
The free leptin index (FLI), being presumed as an index representing the free leptin, was calculated as the ratio of leptin to sOB-R.

4.8.2 Measurement of insulin and glucose in plasma and triglycerides in serum

EDTA plasma insulin was measured by a chemiluminescence immunoassay (CLIA) running via the fully mechanized LIAISON analyzer (Diasorin, Saluggia, Italy). Inter-and intra-assay coefficients of variations were <4 %, respectively.
Fasting glucose from sodium-fluoride plasma and serum triglycerides were determined by an approved external laboratory (Synlab Labordienstleistungen, Munich, Germany) together with routine clinical and safety parameters. Insulin resistance was estimated by the homeostasis model assessment for insulin resistance (HOMA-IR) calculated from fasting glucose and insulin according to Matthews et al. (1985).

4.8.3 Measurement of HMW adiponectin in plasma

HMW adiponectin in maternal plasma samples was determined by ELISA (ALPCO Diagnostics, Salem, USA). This multimeric assay is designed for the quantitative determination of HMW as well as total adiponectin in human serum or plasma and is based on the principle of a sandwich ELISA. To detect HMW adiponectin, plasma samples were pre-treated with a protease which selectively digests low molecular weight (LMW) and mid-molecular weight (MMW) adiponectin. The remaining HMW adiponectin fraction was assayed according to the manufacturer´s instructions.

4.8.4 Measurement of leptin in breast milk

For the analysis of leptin in breast milk three different milk samples in 1 ml tubes were thawed at room temperature and vortexed. The whole milk was sonicated three times for a five second burst by using an ultrasound stick (ultrasonic processor UP100H, 100 W, 30 kHz, 100 % amplitude; Hielscher Ultrasonics GmbH, Teltow, Germany). Skim milk was prepared by centrifugation of whole milk at 14000 rpm for 30 min in an Eppendorf Centrifuge 5415C/D (Eppendorf AG, Hamburg, Germany), to separate milk fat from the liquid phase. A

1 ml injection was used to extract the fat layer and the skim milk sample was used for further analysis.

Leptin concentrations were detected in skim milk by a commercially available human leptin RIA (Mediagnost, Reutlingen, Germany) using ^{125}I-labeled recombinant leptin as a tracer. To increase sensitivity, the high-sensitive protocol of the assay was performed according to the manufacturer's instructions. This included two extra overnight incubation steps and additional use of low concentration standards. 100 µl of skim milk were used for quantification of leptin in breast milk samples. Results were calculated in ng/ml. The lower detection limit for leptin was 0.01 ng/ml.

4.8.5 Measurement of total adiponectin in breast milk

For determination of total adiponectin in breast milk, milk samples were pre-treated as described for leptin measurement (see **4.8.4**) in breast milk and skim milk was used for further analysis.

Total adiponectin concentrations in skim breast milk samples were determined by RIA (Millipore, St. Charles, MO, USA) using ^{125}I-adiponectin as a tracer. A volume of 100 µl was used for the measurements (dilution 1:2). The results were calculated in ng/ml and the lower detection limit for total adiponectin was 2 ng/ml.

4.8.6 Number of samples

The following tables provide an overview of the number of different biosamples (maternal blood, cord blood, breast milk) that were analyzed for the different biochemical parameters (**Table 2–Table 4**).

Table 2 Parameters in maternal plasma/serum over the course of pregnancy and lactation

	15th wk gest.	32nd wk gest.	6 wks pp	4 months pp
Leptin	183 (92/91)	182 (91/91)	82 (41/41)	82 (41/41)
sOB-R	82 (41/41)	82 (41/41)	82 (41/41)	82 (41/41)
FLI	82 (41/41)	82 (41/41)	82 (41/41)	82 (41/41)
Insulin	183 (92/91)	182 (91/91)	82 (41/41)	82 (41/41)
Glucose	188 (101/87)	181 (91/90)	152 (75/77)	123 (59/64)
HOMA-IR	168 (91/77)	175 (87/88)	82 (41/41)	81 (40/41)
Triglycerides	203 (102/101)	187 (94/93)	147 (72/75)	123 (60/63)
HMW adiponectin	182 (91/91)	183 (92/91)	82 (41/41)	82 (41/41)

FLI: free leptin index; gest.: gestation; pp: postpartum; sOB-R: soluble leptin receptor; wk: week
Data are numbers of analyzed samples: total (control group/ intervention group)
All parameters were analyzed from plasma except triglycerides (serum).

Table 3 Parameters in umbilical cord plasma

	Number of samples
Leptin	138 (67/71)
sOB-R	57 (26/31)
FLI	57 (26/31)
Insulin	137 (67/70)

FLI: free leptin index; sOB-R: soluble leptin receptor
Data are numbers of analyzed samples: total (control group/ intervention group)

Table 4 Parameters in breast milk

	6 weeks pp	4 months pp
Leptin	152 (76/76)	120 (57/63)
Total adiponectin	151 (76/75)	120 (57/63)

pp: postpartum
Data are numbers of analyzed samples: total (control group/ intervention group)

4.8.7 Reagents

Leptin ELISA (Mediagnost, Reutlingen Germany)

Cat. # E07

Antibody against leptin (coated plate)

Anti-human leptin antibody

Leptin RIA sensitiv (Mediagnost, Reutlingen, Germany)

Cat. # LEP-R40

Rabbit-anti-human leptin

Anti-rabbit IgG

Insulin CLIA (Diasorin, Saluggia, Italy)

LIAISON® 310360

Suspension of magnetic particles coated with a mouse monoclonal antibody against insulin

Mouse monoclonal antibody against insulin

HMW adiponectin ELISA (ALPCO Diagnostics, Salem, MA, USA)

Cat. # 47-ADPHU-E01

Anti-human adiponectin mouse monoclonal antibody (coated plate)

Biotin-conjugated anti-human adiponectin monoclonal antibody

Total adiponectin RIA (Millipore, St. Charles, MO, USA)

Cat. # HADP-61HK

Rabbit anti-adiponectin antibody

4.9 Statistical analysis

Statistical analyses were performed with the R software package (version 2.8.1; R Foundation for Statistical Computing) and PASW software (version 18.0; SPSS Inc) in collaboration with the Institute for Medical Statistics and Epidemiology, Technische Universität München (Prof. Dr. Kurt Ulm, Dr. Tibor Schuster).

Changes of maternal parameters over time within the groups were assessed by means of the Friedman-Test and Wilcoxon-Tests. Bonferroni correction of p-values was applied to reduce multiple test issue. To determine differences in maternal parameters between the control and the intervention group at the individual time-points, Mann-Whitney-Tests as well as analysis of covariance models (ANCOVA) to adjust for maternal age and pre-pregnancy BMI were employed. Additionally, linear mixed models (LMM) adjusting for maternal age and pre-pregnancy BMI were performed to compare the groups in a longitudinal analysis over all investigated time-points (N=41 women per group).

To determine differences in parameters between the groups in umbilical cord blood, ANCOVA models adjusting for pregnancy duration and Ponderal Index at birth were employed. Differences between the sexes in umbilical cord blood parameters were assessed by Mann-Whitney Test.

To determine correlations between the variables Spearman correlation coefficients or partial correlation coefficients to adjust for potential confounding factors were calculated.

To analyze the impact of maternal (plasma parameters at 32^{nd} week gestation and breast milk parameters at 6 and 4 months pp) and cord blood biomarkers on infant growth parameters and body composition up to 2 years of age, multiple linear regression models adjusting for maternal pre-pregnancy BMI, gestational weight gain, pregnancy duration, group and sex for the data at birth, and additionally for Ponderal Index at birth and breastfeeding status (exclusively breastfed, partially breastfed or formula) for the later time-points were performed. Analyses relating to insulin, HOMA-IR and triglycerides were additionally adjusted for maternal glucose tolerance status (i.e. GDM or normal) during pregnancy. A two-sided p-value ≤ 0.05 was considered statistically significant.

5 Results

5.1 Maternal blood parameters over the course of pregnancy and lactation

5.1.1 Maternal plasma leptin, sOB-R and FLI

Leptin, sOB-R as well as the FLI changed significantly over the course of the study within both groups ($p<0.001$) (**Figure 6–Figure 8**, Appendix **Table A- 1**). Median leptin levels and the FLI increased significantly during pregnancy within both groups ($p<0.001$), whereas the increase in sOB-R was significant in the intervention group, only. All parameters decreased significantly below baseline levels after delivery ($p<0.001$). Both, median leptin and sOB-R concentrations decreased between 6 weeks and 4 months pp in both groups, with a significant change only for sOB-R ($p<0.05$). The median FLI also decreased slightly, but non significantly in the postpartum period (Appendix **Table A- 2**).

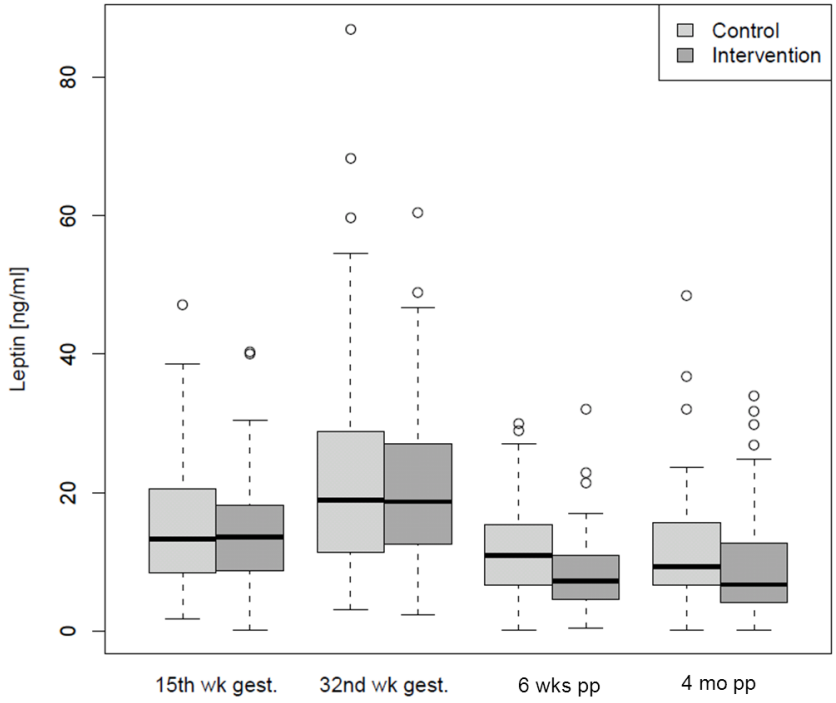

Figure 6 Maternal leptin levels over the course of pregnancy and lactation

Sample sizes (control/intervention): 15th week gestation: N=92/91; 32nd week gestation: N=91/91; 6 weeks pp: N=41/41; 4 months pp: N=41/41

The longitudinal analysis (N=41 per group) revealed a significant group difference in leptin over the course of the study between the groups with lower leptin levels in the intervention compared to the control group (mean difference [95 % CI]: -4.43 [-8.29;-0.52], p=0.027) in the linear mixed model (LMM). After adjustment for maternal age, pre-pregnancy BMI and baseline levels, the difference did not reach significance anymore (adjusted mean difference: -0.74 [-3.04; 1.57], p=0.144). No significant group differences were observed in the LMM for sOB-R (mean difference: 0.64 [-2.56; 3.84], p=0.692) and FLI (mean difference: -0.16 [-0.33; 0.01], p=0.065) (Appendix **Table A- 1**). In addition, there were no significant differences between the groups in these parameters, when the individual time-points were compared separately controlling for maternal age and pre-pregnancy BMI (data not shown).

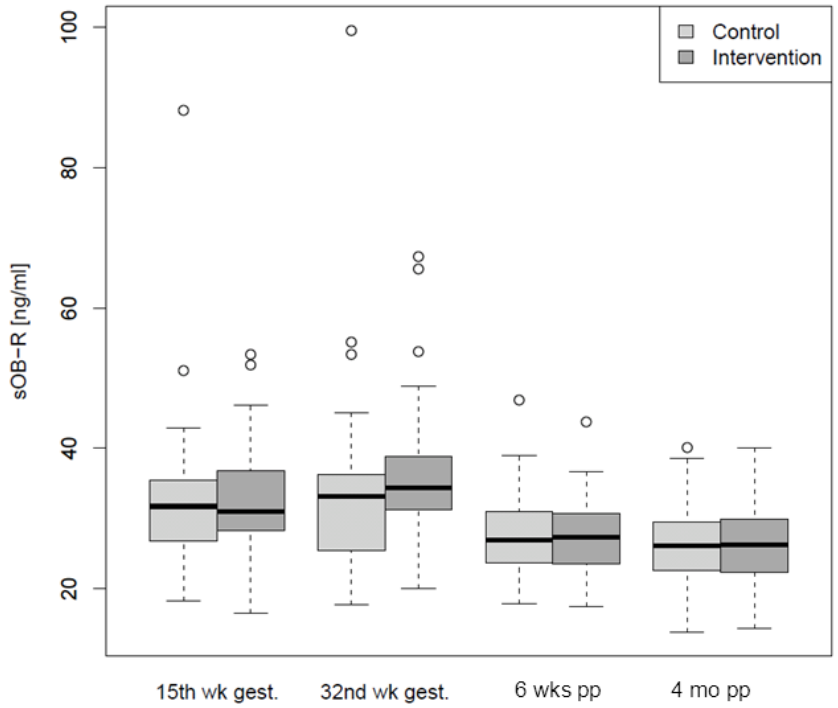

Figure 7 Maternal sOB-R levels over the course of pregnancy and lactation

Sample sizes (control/intervention): N=41/41 for all time-points (Schmid 2011)

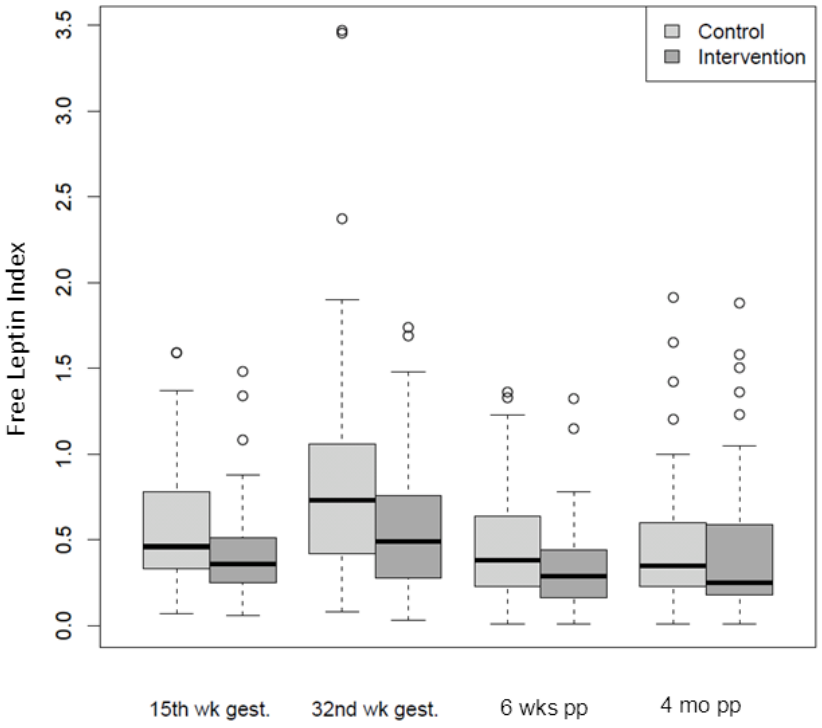

Figure 8 Maternal free leptin index over the course of pregnancy and lactation

Sample sizes (control/intervention): N=41/41for all time-points (Schmid 2011)

5.1.2 Maternal plasma insulin, glucose and HOMA-IR

In both groups, there were significant changes over time in maternal fasting plasma insulin concentration from the 15th week of gestation until 4 months pp ($p<0.001$) (**Figure 9**, Appendix **Table A- 1** and **Table A- 2**). Insulin levels almost doubled between the 15th week and 32nd week of gestation. After delivery the levels dropped below baseline levels at 6 weeks pp, but recovered until 4 months pp. There were no significant differences between the groups at any time-point (Appendix **Table A- 1**). In the longitudinal analysis (linear mixed model) comprising N=41 women per group, women in the intervention group had significantly lower (mean difference: -9.04 [-17.27; -0.82], p=0.043) insulin levels compared to the control group; however, this difference disappeared after adjustment for maternal age, pre-pregnancy BMI and glucose tolerance status (adjusted mean difference: -6.07 [-13.14; 1.01] p=0.097) (Appendix **Table A- 1**).

Fasting glucose concentrations remained relatively constant over the course of pregnancy and lactation with neither significant changes over time within both groups nor significant

differences between the groups (mean difference: -0.69 [-3.66; 2.29], p=0.652) (Appendix **Table A- 1** and **Table A- 2**).

Consequently, HOMA-IR changed significantly over time according to alterations in fasting insulin concentration during pregnancy and lactation (**Figure 10,** Appendix **Table A- 1** and **Table A- 2**). No differences between the groups were observed, neither in the longitudinal model (mean difference: -0.22 [-0.50; 0.06], p=0.123) nor at any single time-point.

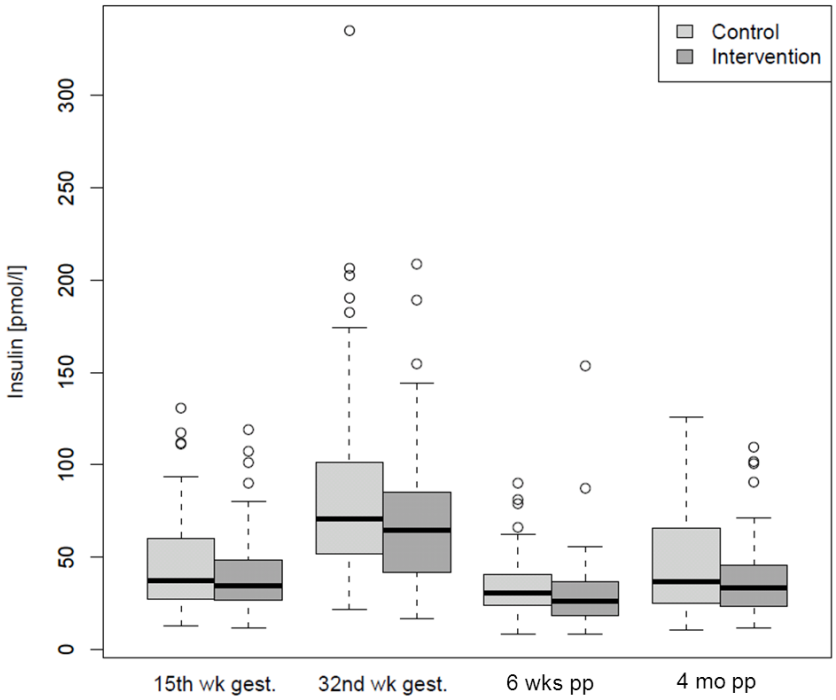

Figure 9 Maternal insulin levels over the course of pregnancy and lactation

Sample sizes (control/intervention): 15th week gestation: N=92/91; 32nd week gestation: N=91/91; 6 weeks pp: N=41/41; 4 months pp: N=41/41

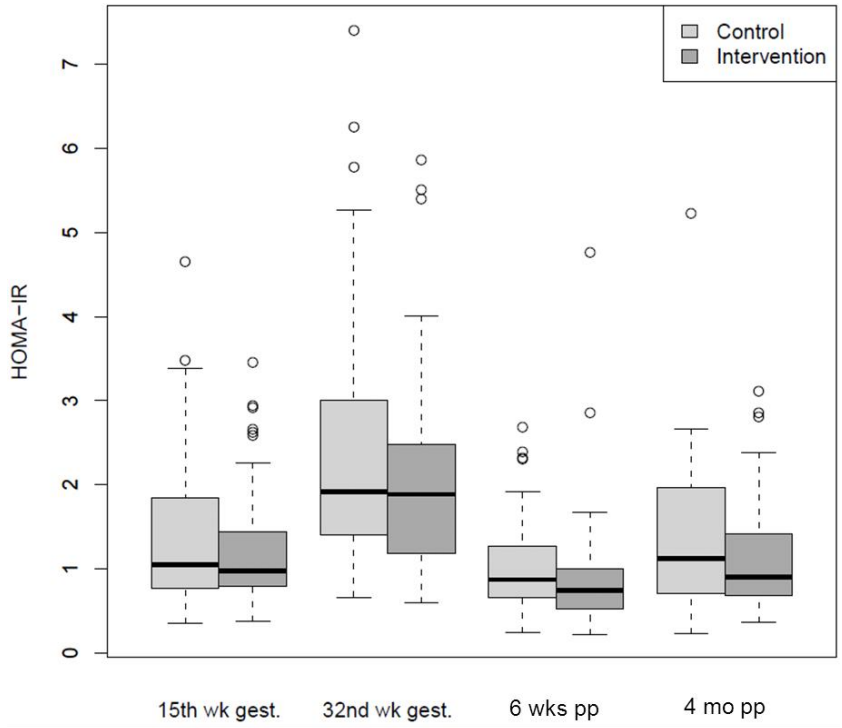

Figure 10 Maternal HOMA-IR over the course of pregnancy and lactation

Sample sizes (control/intervention): 15th week gestation: N=91/77; 32nd week gestation: N=87/88; 6 weeks pp: N=41/41; 4 months pp: N=40/41

5.1.3 Maternal serum triglycerides

Serum triglyceride levels changed significantly over time in both groups (p<0.001) (**Figure 11**, Appendix **Table A- 1** and **Table A- 2**). Over the course of pregnancy, triglyceride levels increased considerably in both groups and decreased markedly after delivery. In the intervention group, triglyceride levels increased significantly less between the 15th and the 32nd week of pregnancy compared to the control group (Δ values: 76.2 ± 53.5 mg/dl in the intervention vs. 103.6 ± 58.1 mg/dl in the control group, p<0.001), resulting in significantly lower triglyceride levels at the 32nd week of gestation in the intervention group (p<0.001). This difference persisted until the lactation period (p<0.01 at 6 weeks pp), albeit no longer significant at 4 months pp after correction for multiple testing (p=0.088). In the longitudinal analysis (LMM), women in the intervention group had significantly lower triglyceride levels compared to the control group (mean difference: -15.44 [-28.04; -28.84], p=0.017), and this difference remained significant in the adjusted analysis (p=0.025) (Appendix **Table A- 1**).

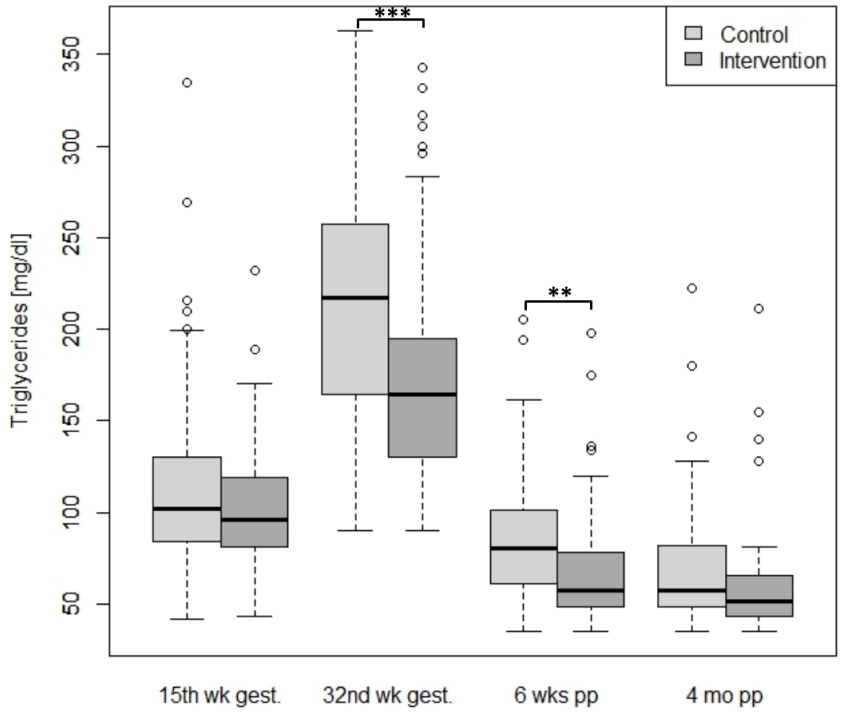

Figure 11 Maternal triglycerides over the course of pregnancy and lactation

Sample sizes (control/intervention): 15th week gestation: N=102/101; 32nd week gestation: N=94/93; 6 weeks pp: N=72/75; 4 months pp: N=60/63
*** p<0.001, ** p<0.01 (control vs. intervention)

5.1.4 Maternal plasma HMW adiponectin

Within both groups, HMW adiponectin levels changed significantly over the course of pregnancy and lactation (p<0.001) (**Figure 12**, Appendix **Table A- 1** and **Table A- 2**). There was a significant decrease in median HMW adiponectin concentration between the 15th week of gestation and the 32nd week of gestation in both groups (p<0.001). After delivery, the levels remained low until 4 months pp in the control group, whereas a slight increase in adiponectin concentration between 6 weeks and 4 months pp was found in the intervention group (p<0.01). No significant differences between the groups could be observed, neither when levels at the individual time-points were compared nor in the longitudinal model (mean difference: -0.32 [-0.68; 0.05], p=0.09) (Appendix **Table A- 1**).

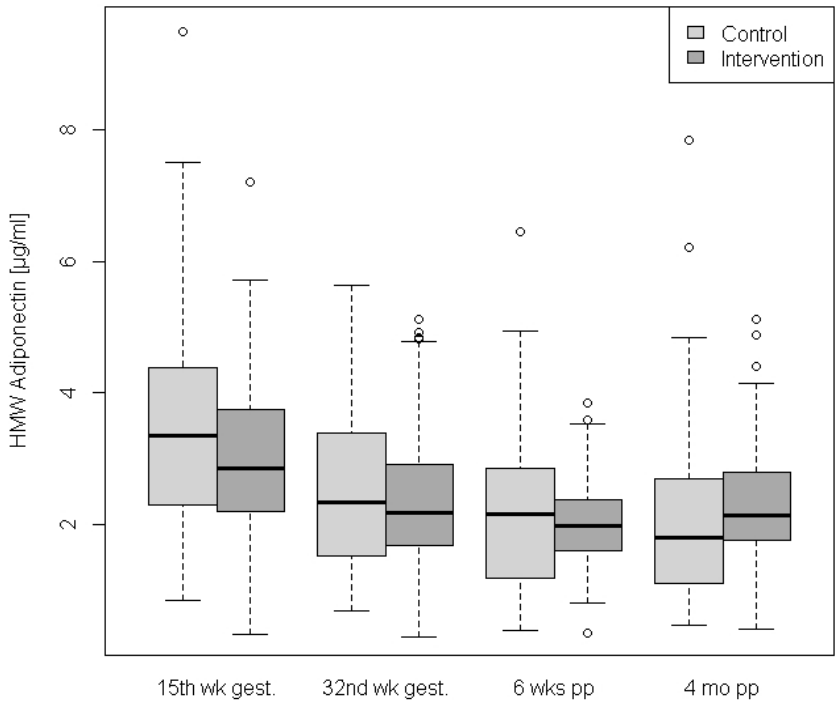

Figure 12 Maternal HMW adiponectin levels over the course of pregnancy and lactation

Sample sizes (control/intervention): 15th week gestation: N=91/91; 32nd week gestation: N=92/91; 6 weeks pp: N=41/41; 4 months pp: N=41/41

5.1.5 Correlations of maternal adipokines with maternal anthropometry

To explore correlations between maternal plasma parameters and maternal anthropometric variables, both groups were combined. As adjustment for group did not change the results, unadjusted values are presented (**Table 5**).

Correlation analyses revealed strong, positive correlations of maternal leptin levels measured at the 15th and 32nd week of gestation with maternal anthropometry at the respective time-points. Maternal leptin at the 15th week of gestation correlated strongly with pre-pregnancy BMI ($p<0.001$) as well as with maternal BMI at the 15th week gestation ($p<0.001$), and also with the sum of four skinfolds ($p<0.001$) and percentage body fat ($p<0.001$) at the 15th week of gestation. Likewise, maternal leptin at the 32nd week of gestation correlated strongly and significantly with maternal BMI ($p<0.001$), the sum of four skinfold thicknesses ($p<0.001$) as well as percentage body fat at the 32nd week of gestation ($p<0.001$). Both, leptin at the 15th and 32nd week of gestation correlated significantly positively with gestational weight gain (GWG), even after adjustment for pre-pregnancy BMI ($p<0.001$).

In contrast to leptin, maternal HMW adiponectin was significantly negatively and less strongly correlated with maternal anthropometric variables both at the 15th and the 32nd week of gestation (**Table 5**). HMW adiponectin at the 32nd week of gestation, but not at the 15th week of gestation, was also significantly negatively correlated with GWG after adjustment for pre-pregnancy BMI (p=0.02).

Table 5 Correlations between maternal plasma adipokines and maternal anthropometry

Variable A	Variable B	N	r[1]	p
15th wk gest. leptin	Pre-pregnancy BMI	183	0.62	**<0.001**
	BMI at 15th wk gest.	183	0.66	**<0.001**
	Sum 4 SFT at 15th wk gest.	119	0.72	**<0.001**
	Body fat at 15th wk gest.	142	0.62	**<0.001**
	Gestational weight gain[2]	177	0.39[3]	**<0.001**
32nd wk gest. leptin	BMI at 32nd wk gest.	178	0.68	**<0.001**
	Sum 4 SFT at 32nd wk gest.	133	0.69	**<0.001**
	Body fat at 32nd wk gest.	143	0.66	**<0.001**
	Gestational weight gain[2]	177	0.50[3]	**<0.001**
15th wk gest. HMW adiponectin	Pre-pregnancy BMI	182	-0.17	**0.02**
	BMI at 15th wk gest.	182	-0.17	**0.02**
	Sum 4 SFT at 15th wk gest.	119	-0.23	**0.01**
	Body fat at 15th wk gest.	141	-0.22	**0.01**
	Gestational weight gain[2]	177	-0.09[3]	0.245
32nd wk gest. HMW adiponectin	BMI at 32nd wk gest.	178	-0.20	**0.01**
	Sum 4 SFT at 32nd wk gest.	133	-0.27	**<0.001**
	Body fat at 32nd wk gest.	143	-0.31	**<0.001**
	Gestational weight gain[2]	178	-0.17[3]	**0.02**

gest.: gestation; SFT: skinfold thicknesses; wk: week
p-values <0.05 are in bold face
[1] Spearman correlation coefficient unless otherwise indicated
[2] calculated as last measured value at booking minus self-reported weight before pregnancy
[3] partial correlation coefficient adjusted for pre-pregnancy BMI

5.1.6 Correlations among the different maternal parameters

The investigated maternal parameters in plasma or serum were highly inter-correlated across all assessed time-points. Here, exemplarily only correlations between the individual parameters at the 32nd week of gestation are reported. Maternal plasma leptin was significantly negatively correlated with plasma HMW adiponectin (r=-0.249, p=0.001), and significantly positively correlated with plasma insulin (r=0.527, p<0.001), HOMA-IR (r=0.555, p<0.001) and serum triglycerides (r=0.155, p=0.037). In contrast, maternal plasma

HMW adiponectin was significantly negatively correlated with plasma insulin (r=-0.281, p<0.001) and HOMA-IR (r=-0.280, p<0.001). Furthermore, there were significant positive correlations between plasma insulin and HOMA-IR (r=0.965, p<0.001), plasma insulin and serum triglycerides (r=0.283, p<0.001), as well as between HOMA-IR and serum triglycerides (r=0.271, p<0.001). These relationships remained significant also after adjustment for maternal pre-pregnancy BMI, age and group (**Table 6**).

Table 6 Correlation matrix of maternal blood parameters at the 32nd week of gestation

	Leptin	**Insulin**	**HOMA-IR**	**TG**	**HMW adiponectin**
Leptin	1 (N=182)	0.298*** (N=182)	0.324*** (N=175)	0.163* (N=181)	-0.175* (N=182)
Insulin	0.298*** (N=182)	1 (N=182)	0.951*** (N=175)	0.173* (N=181)	-0.253** (N=182)
HOMA-IR	0.324*** (N=175)	0.951*** (N=175)	1 (N=175)	0.180* (N=174)	-0.258** (N=175)
TG	0.163* (N=181)	0.173* (N=181)	0.180* (N=174)	1 (N=187)	-0.087 (N=182)
HMW adiponectin	-0.175* (N=182)	-0.253** (N=182)	-0.258** (N=175)	-0.087 (N=182)	1 (N=183)

TG: triglycerides
Data are presented as partial correlation coefficients adjusted for maternal pre-pregnancy BMI, age and group.
* p<0.05; ** p<0.01; *** p<0.001

5.2 Parameters in cord plasma

5.2.1 Cord plasma leptin, sOB-R and FLI

The parameters of the leptin axis in umbilical cord plasma by group and gender are presented in **Table 7**. As pregnancy duration was significantly prolonged in the intervention group resulting in a higher Ponderal Index at birth (Hauner et al. 2012), group comparisons were controlled for these variables. The intervention had no effect on cord plasma leptin (p=0.808), sOB-R (p=0.909) and FLI (p=0.596).

Independent of the group assignment, cord plasma leptin concentrations as well as FLI were significantly higher in girls compared to boys (leptin: p=0.001; FLI: p=0.006). sOB-R concentrations did not differ between the sexes (p=0.947).

Table 7 Leptin, sOB-R and FLI in umbilical cord plasma

	Leptin [ng/ml]	sOB-R [ng/ml]	FLI
by group			
control	10.69 ± 8.75 (67)	14.35 ± 4.26 (26)	0.68 ± 0.56 (26)
intervention	11.61 ± 10.52 (71)	15.01 ± 4.10 (31)	0.89 ± 0.81 (31)
p-value[1]	0.808	0.909	0.596
by gender			
girls	14.01 ± 11.59 (63)	14.67 ± 3.77 (29)	1.05 ± 0.82 (29)
boys	8.77 ± 6.93 (75)	14.75 ± 4.58 (28)	0.54 ± 0.46 (28)
p-value[2]	**0.001**	0.947	**0.006**

FLI: free leptin index [ratio: leptin/sOB-R]
Data are presented as mean ± SD (N).
[1] ANCOVA adjusted for pregnancy duration and Ponderal Index at birth
[2] Mann-Whitney-Test

5.2.2 Cord plasma insulin

Insulin concentrations in umbilical cord plasma by group and gender are shown in **Table 8**. No significant differences between the groups were observed controlling for pregnancy duration and Ponderal Index at birth (p=0.816). The same results were obtained when women with insulin-treated GDM (N=4) were excluded from the analysis (p=0.594, data not shown).

Analysis of the data by gender revealed significantly higher cord plasma insulin levels in girls compared to boys (p=0.037), also after infants of mothers with insulin-treated GDM (p=0.032, data not shown) were excluded.

Table 8 Insulin in umbilical cord plasma [pmol/l]

	N	Mean ± SD	Median [Interquartile Range]	p
by group				
control	67	44.86 ± 39.11	29.00 [37.05]	0.816[1]
intervention	70	42.27 ± 49.94	27.40 [26.87]	
by gender				
girls	63	50.50 ± 52.02	31.90 [37.10]	0.037[2]
boys	74	37.61 ± 36.98	25.85 [24.53]	

[1] ANCOVA adjusted for pregnancy duration and Ponderal Index at birth
[2] Mann-Whitney-Test

5.2.3 Comparison of parameters in maternal plasma with cord plasma

In **Table 9**, the parameters determined in cord plasma are presented in comparison to maternal plasma levels measured at the 32nd week of gestation. Both, leptin and insulin concentrations were significantly higher in maternal compared to umbilical cord plasma ($p<0.001$).

Table 9 Comparison of parameters in maternal plasma with cord plasma

	Maternal plasma (32nd wk gest.)		**Cord plasma**		
	Mean ± SD	N	Mean ± SD	N	p[1]
Leptin [ng/ml]	21.58 ± 13.44	182	11.17 ± 9.68	138	**<0.001**
Insulin [pmol/l]	76.74 ± 43.15	182	43.54 ± 44.83	137	**<0.001**

gest.: gestation; wk: week
p-values <0.05 are in bold face
[1] Mann-Whitney Test

5.2.4 Correlations between parameters in maternal plasma and cord plasma

In **Table 10**, correlations between maternal plasma parameters at 32nd week of gestation and cord plasma parameters as well as correlations between the individual parameters measured in cord blood are shown. Maternal parameters of glucose metabolism at 32nd week of gestation (i.e. fasting insulin, glucose, HOMA-IR) did not significantly correlate with cord blood insulin levels, although there was a tendency towards a slight positive correlation between maternal insulin and fetal insulin ($p=0.057$). Likewise, maternal leptin at 32nd week of gestation did not significantly correlate with cord blood leptin levels. In cord blood, a highly significant positive correlation between leptin and insulin levels was observed ($p<0.001$).

Table 10 Correlation of parameters in maternal plasma at 32nd week of gestation and cord plasma

Variable A	**Variable B**	**N**	**r[1]**	**p**
Maternal 32nd wk gest. insulin	Cord plasma insulin	136	0.164	0.057
Maternal 32nd wk gest. glucose	Cord plasma insulin	132	-0.091	0.301
Maternal 32nd wk gest. HOMA-IR	Cord plasma insulin	131	0.124	0.157
Maternal 32nd wk gest. leptin	Cord plasma leptin	137	-0.048	0.581
Cord plasma leptin	Cord plasma insulin	137	0.350[2]	**<0.001**

gest.: gestation; wk: week
p-values <0.05 are in bold face
[1] Spearman correlation coefficient or partial correlation coefficient if indicated
[2] partial correlation coefficient adjusted for group, sex and gestational age

5.3 Adipokines in breast milk

5.3.1 Leptin and total adiponectin in breast milk over the course of lactation

The levels of the breast milk adipokines, leptin and total adiponectin, over two time-points in the lactation period are shown in **Table 11**. Mean leptin concentrations in breast milk at 6 weeks pp were ~0.19 ng/ml in both groups with a high inter-individual variation. Levels decreased slightly, but non significantly until 4 months pp. No significant differences between the groups were found at any time-point.

The same pattern was seen for total adiponectin in breast milk. Mean levels at 6 weeks pp were 11.33 ng/ml in the control and 13.70 ng/ml in the intervention group, respectively. At 4 months pp, the levels were marginally but non significantly lower. Again, no significant differences between the groups were observed.

Table 11 Leptin and total adiponectin in breast milk

		6 weeks pp			**4 months pp**			
	group	Mean ± SD	N	p^1	Mean ± SD	N	p^1	$p_{\text{over time}}{}^2$
Leptin [ng/ml]	CG	0.19 ± 0.20	76	0.360	0.15 ± 0.13	57	0.164	0.880
	IG	0.19 ± 0.30	75		0.16 ± 0.30	63		0.394
Total adiponectin	CG	11.33 ± 5.39	76	0.616	10.71 ± 5.71	57	>0.99	0.080
[ng/ml]	IG	13.70 ± 10.56	75		11.74 ± 8.71	63		0.175

CG: control group; IG: intervention group; pp: postpartum
[1] p-values obtained from group comparisons by Mann-Whitney-Tests (CG vs. IG) at the different time-points, Bonferroni-corrected for multiple testing (original p-value *2)
[2] Change over time within groups (Wilcoxon-Test)

5.3.2 Comparison of breast milk and maternal plasma adipokine levels

As no differences between the groups were observed, data of both study groups were combined to compare plasma and breast milk adipokine levels. Maternal plasma leptin levels were significantly higher compared to leptin levels in breast milk at both studied time-points ($p<0.001$). The same was seen for plasma HMW adiponectin compared to milk total adiponectin concentrations ($p<0.001$) (**Table 12**).

Table 12 Comparison of maternal plasma and breast milk adipokines during lactation

		Breast milk		Plasma		
		Mean ± SD	N	Mean ± SD	N	p[1]
Leptin	6 weeks pp	0.19 ± 0.25 ng/ml	152	10.44 ± 6.87 ng/ml	82	**<0.001**
	4 months pp	0.16 ± 0.24 ng/ml	120	11.58 ± 9.47 ng/ml	82	**<0.001**
Adiponectin	6 weeks pp	12.51 ± 8.43 ng/ml	151	2.10 ± 1.05 µg/ml	82	**<0.001**
	4 months pp	11.25 ± 10.36 ng/ml	120	2.29 ± 1.33 µg/ml	82	**<0.001**

pp: postpartum
p-values <0.05 are in bold face
[1] Wilcoxon -Test

5.3.3 Correlations between breast milk and maternal plasma adipokine levels

Breast milk adipokine levels correlated strongly with adipokine levels determined in maternal plasma during the lactation period (**Table 13**). Milk leptin measured at 6 weeks pp correlated highly significantly with plasma leptin at 6 weeks pp ($p<0.001$). Likewise, milk total adiponectin determined at 6 weeks pp correlated significantly with plasma HMW adiponectin at 6 weeks pp ($p<0.001$). The same was true for both adipokines at 4 months pp.

Table 13 Correlations between maternal breast milk and plasma adipokines

Variable A	Variable B	N	r[1]	p
6 wks pp milk leptin	6 wks pp plasma leptin	82	0.55	**<0.001**
4 mo pp milk leptin	4 mo pp plasma leptin	80	0.36	**<0.001**
6 wks pp milk total adiponectin	6 wks pp plasma HMW adiponectin	82	0.52	**<0.001**
4 mo pp milk total adiponectin	4 mo pp plasma HMW adiponectin	80	0.54	**<0.001**

mo: month; pp: postpartum; wk: week
p-values <0.05 are in bold face
[1] Spearman correlation coefficient

5.3.4 Correlations of breast milk adipokines with maternal anthropometry

As maternal anthropometry was not assessed in the postpartum period, only correlations between breast milk adipokines with maternal anthropometric parameters before and during pregnancy could be explored.

Milk leptin concentrations, both at 6 weeks and 4 months pp, correlated positively and highly significantly with pre-pregnancy BMI as well as with BMI, the sum of four skinfolds and percentage body fat at the 15th week of gestation (**Table 14**). In contrast, breast milk total adiponectin was not related to any of the maternal anthropometric variables. Correlations of

breast milk adipokines with the women´s anthropometric data at the 32nd week of gestation were similar (data not shown).

Table 14 Correlations of breast milk adipokines with maternal anthropometry

Variable A	Variable B	N	r[1]	p
6 wks pp milk leptin	Pre-pregnancy BMI	147	0.49	**<0.001**
	BMI at 15th wk gest.	152	0.52	**<0.001**
	Sum 4 SFT at 15th wk gest.	98	0.57	**<0.001**
	Body fat at 15th wk gest.	115	0.54	**<0.001**
4 mo pp milk leptin	Pre-pregnancy BMI	118	0.39	**<0.001**
	BMI at 15th wk gest.	120	0.45	**<0.001**
	Sum 4 SFT at 15th wk gest.	79	0.46	**<0.001**
	Body fat at 15th wk gest.	93	0.42	**<0.001**
6 wks pp total milk adiponectin	Pre-pregnancy BMI	148	0	0.800
	BMI at 15th wk gest.	151	-0.01	0.885
	Sum 4 SFT at 15th wk gest.	97	0.03	0.804
	Body fat at 15th wk gest.	114	-0.03	0.782
4 mo pp total milk adiponectin	Pre-pregnancy BMI	118	0.06	0.663
	BMI at 15th wk gest.	120	0	0.999
	Sum 4 SFT at 15th wk gest.	79	0.10	0.381
	Body fat at 15th wk gest.	93	-0.01	0.909

gest.: gestation; mo: month; pp: postpartum; SFT: skinfold thicknesses; wk: week
p-values <0.05 are in bold face
[1] Spearman correlation coefficient

5.4 Infant clinical outcomes at 2 years of age

The clinical data of the infants over the first year of life have been already published (Hauner et al. 2012). Importantly, the participants continuing the study after the 1 year visit did not significantly differ from those lost to follow-up regarding the major socio-demographic and clinical characteristics such as educational level (p=0.234), maternal/ paternal BMI (p= 0.287 and p=0.338, respectively), maternal age (p=0.375), parity (p=0.334), gestational weight gain (p=0.659), pregnancy duration (p=0.757) or mode of infant feeding (p=0.534). In total, 118 infants (control: N=57; intervention: N=61) were clinically assessed at 2 years of age.

The growth pattern of the infants by the age of 2 years is presented in **Table 15**. There were no significant differences between the groups in body weight (control: 12279 ± 1343 g vs. intervention: 12498 ± 1445 g), length (control: 86.8 ± 2.8 cm vs. intervention: 87.1 ± 2.9 cm),

head circumference (control: 48.6 ± 1.3 cm vs. intervention: 48.7 ± 1.3 cm) and indices of growth (BMI: control: 16.3 ± 1.6 kg/m^2 vs. intervention: 16.5 ± 1.3 kg/m^2; Ponderal Index: control: 18.7 ± 2.0 kg/m^3 vs. intervention: 18.9 ± 1.5 kg/m^3), neither in the unadjusted nor in the adjusted model controlling for sex, pregnancy duration, Ponderal Index at birth and mode of infant feeding.

Table 15 Growth pattern and growth indices at 2 years of age

	group	Mean ± SD (N)	Unadjusted mean difference [95% CI][1]	Adjusted mean difference [95% CI][2]
Age infant [months]	CG	24.4 ± 0.6 (57)	0.0 [-0.2; 0.2]	
	IG	24.4 ± 0.7 (61)		
Head circumference [cm]	CG	48.6 ± 1.3 (57)	0.0 [-0.4; 0.5]	0.0 [-0.4; 0.5]
	IG	48.7 ± 1.3 (61)		
Weight [g]	CG	12279 ± 1343 (57)	219 [-291; 729]	159 [-347; 665]
	IG	12498 ± 1445 (61)		
Length [cm]	CG	86.8 ± 2.8 (57)	0.2 [-0.8; 1.3]	0.3 [-0.7; 1.4]
	IG	87.1 ± 2.9 (61)		
Arm circumference [cm]	CG	15.5 ± 1.1 (57)	0.1 [-0.3; 0.5]	0.1 [-0.3; 0.5]
	IG	15.7 ± 1.1 (61)		
Waist [cm]	CG	48.0 ± 2.7 (51)	-0.1 [-0.3; 0.5]	-0.3 [-1.4; 0.9]
	IG	47.8 ± 2.7 (50)		
BMI [kg/m^2]	CG	16.3 ± 1.6 (57)	0.2 [-0.4; 0.7]	0.1 [-0.5; 0.6]
	IG	16.5 ± 1.6 (61)		
Ponderal Index [kg/m^3]	CG	18.7 ± 2.0 (57)	0.1 [-0.5; 0.8]	0.0 [-0.7; 0.6]
	IG	18.9 ± 1.5 (61)		

[1] Student´s t-test
[2] ANCOVA adjusted for sex, pregnancy duration, Ponderal Index at birth and breastfeeding status at 4 months

Similarly, the groups did not significantly differ in the individual skinfold thicknesses (biceps, triceps, subscapular, suprailiacal), the sum of four skinfolds (control: 23.5 ± 3.5 mm vs. intervention: 23.8 ± 3.3 mm), percent body fat calculated from predicted skinfold equations (control: 19.2 ± 2.6 % vs. intervention: 19.2 ± 2.3 %), as well as the absolute amount of fat and lean body mass (fat mass: control: 2347 ± 482 g vs. intervention: 2422 ± 515 g; lean body mass: control: 9926 ± 917 g vs. intervention: 10092 ± 1011 g) (**Table 16**). Furthermore, the subcutaneous fat distribution estimated as the subscapular/triceps skinfold-ratio as well as

trunk-to-total skinfold thicknesses were comparable between the groups. Results were similar for the unadjusted and the adjusted analysis.

Table 16 Skinfold thicknesses, fat mass and subcutaneous fat distribution at 2 years pp

	group	Mean ±SD (N)	Unadjusted mean difference [95% CI][1]	Adjusted mean difference [95% CI][2]
Biceps [mm]	CG	4.8 ± 0.8 (54)	0.0 [-0.3; 0.3]	-0.2 [-0.4; 0.3]
	IG	4.8 ± 0.9 (59)		
Triceps [mm]	CG	8.6 ± 1.6 (54)	0.3 [-0.3; 1.0]	0.4 [-0.3; 1.0]
	IG	8.9 ± 1.8 (58)		
Subscapular [mm]	CG	6.0 ± 1.1 (57)	0.1 [-0.3; 0.5]	0.0 [-0.4; 0.5]
	IG	6.1 ± 1.1 (61)		
Suprailiacal [mm]	CG	4.2 ± 0.9 (55)	-0.2 [-0.5; 0.2]	-0.1 [-0.4; 0.2]
	IG	4.1 ± 0.8 (58)		
Sum 4 SFT [mm]	CG	23.5 ± 3.5 (53)	0.3 [-1.0; 1.6]	0.4 [-1.0; 1.7]
	IG	23.8 ± 3.3 (57)		
Body fat [%]	CG	19.2 ± 2.6 (53)	0.2 [-0.7; 1.1]	0.2 [-0.7; 1.1]
	IG	19.2 ± 2.3 (57)		
Fat mass [g]	CG	2347 ± 482 (53)	75 [-114; 264]	62 [-133; 256]
	IG	2422 ± 515 (57)		
LBM [g]	CG	9926 ± 917 (53)	167 [-195; 533]	101 [-254; 457]
	IG	10092 ± 1011 (57)		
Subscapular/ triceps-Ratio	CG	0.7 ± 0.2 (54)	-0.0 [-0.1; 0.1]	-0.0 [-0.1; 0.0]
	IG	0.7 ± 0.2 (58)		
Trunk-to-total SFT [%][3]	CG	43.1 ± 4.1 (53)	-0.4 [-2.1; 1.2]	-0.5 [-2.2; 1.3]
	IG	42.7 ± 4.6 (57)		

LBM: lean body mass; SFT: skinfold thickness
[1] Student´s t-test
[2] ANCOVA adjusted for sex, pregnancy duration, Ponderal Index at birth and breastfeeding status at 4 months
[3] Trunk-to-total SFTs were calculated as (subscapular + suprailiac)/ sum of 4 SFTs*100

5.5 Association of maternal and cord blood parameters with infant clinical outcomes up to 2 years of age

For the following analyses, both study groups were combined to explore potential relationships between the studied maternal and cord blood biomarkers with the infant growth and body composition outcomes in the whole study population.

5.5.1 Maternal plasma leptin in relation to infant clinical outcomes

Maternal leptin levels in late pregnancy (32nd week of gestation) were significantly inversely related to birth weight (adjusted regression coefficient (b_{adj}) [95 % CI]: -10.37 [-16.34; -4.39] g, p=0.001), placental weight (b_{adj}: -2.31 [-4.01; -0.60] g, p=0.009), Ponderal Index (b_{adj}: -0.04 [-0.07; 0] kg/m^3, p=0.030), BMI (b_{adj}: -0.03 [-0.04; -0.01] kg/m^2, p=0.002), fat mass (b_{adj}: -2.90 [-4.18; -0.01] g, p=0.050) and lean body mass (LBM) (b_{adj}: -7.25 [-11.81; -2.68] g, p=0.002) at birth in the analysis adjusted for maternal pre-pregnancy BMI, gestational weight gain, pregnancy duration, sex and group (**Table 17**). Similarly, maternal leptin at the 32nd week of pregnancy was negatively associated with infant weight (b_{adj}: -13.95 [-22.18; -5.71] g, p=0.001), BMI (b_{adj}: -0.02 [-0.04; 0] kg/m^2, p=0.016), fat mass (b_{adj}: -4.61 [-7.96; -1.26] g, p=0.008) and LBM (b_{adj}: -9.34 [-14.95; -3.72] g, p=0.001) at 6 weeks pp. Importantly, the significant associations with infant weight and LBM persisted up to 2 years of age (b_{adj} for weight: 24.18 [-48.35; 0] g, p=0.050; b_{adj} for LBM: -16.83 [-33.22; -0.44] g, p=0.044).

Table 17 Maternal plasma leptin at 32nd week gestation [ng/ml] in relation to infant growth and body composition outcomes up to 2 years

		Unadjusted analysis		Adjusted analysis[1]	
Outcome variable	N	Beta [95 % CI]	p	Beta [95 % CI]	p
Birth					
Birth weight [g]	179	-6.25 [-11.25; -1.26]	**0.015**	-10.37 [-16.43; -4.39]	**0.001**
Placental weight [g]	136	-1.06 [-2.35; 0.22]	0.104	-2.31 [-4.01; -0.60]	**0.009**
PI [kg/m^3]	179	-0.03 [-0.05; 0]	0.058	-0.04 [-0.07; 0]	**0.030**
BMI [kg/m^2]	179	-0.02 [-0.03; 0]	**0.016**	-0.03 [-0.04; -0.01]	**0.002**
Body fat [%]	164	-0.03 [-0.06; 0.01]	0.106	-0.03 [-0.07; 0.01]	0.205
Fat mass [g]	164	-1.52 [-3.1; 0.07]	0.063	-2.90 [-4.18; -0.01]	**0.050**
LBM [g]	164	-3.72 [-7.67; 0.23]	0.067	-7.25 [-11.81; -2.68]	**0.002**
6 weeks pp					
Weight [g]	174	-5.55 [-12.30; 1.19]	0.108	-13.95 [-22.18; -5.71]	**0.001**
BMI [kg/m^2]	174	-0.01 [-0.02; 0]	0.157	-0.02 [-0.04; 0]	**0.016**
Body fat [%]	174	-0.01 [-0.05; 0.02]	0.476	-0.04 [-0.09; 0]	0.062
Fat mass [g]	174	-1.70 [-4.22; 0.83]	0.189	-4.61 [-7.96; -1.26]	**0.008**
LBM [g]	174	-3.86 [-8.61; 0.90]	0.113	-9.34 [-14.95; -3.72]	**0.001**
4 months pp					
Weight [g]	171	-2.33 [-10.25; 5.59]	0.565	-10.68 [-20.53; -0.83]	**0.035**
BMI [kg/m^2]	171	-0.00 [-0.02; 0.01]	0.728	-0.01 [-0.03; 0.01]	0.247
Body fat [%]	171	0.00 [-0.03; 0.03]	0.896	-0.01 [-0.04; 0.05]	0.754
Fat mass [g]	170	-0.46 [-3.54; 2.62]	0.770	-2.05 [-6.26; 2.17]	0.342
LBM [g]	170	-1.73 [-7.5; 4.04]	0.557	-8.81 [-15.55; -2.06]	**0.011**
1 year pp					
Weight [g]	168	-4.57 [-16.25; 7.11]	0.444	-17.33 [-31.70; -2.95]	**0.019**
BMI [kg/m^2]	168	-0.01 [-0.02; 0.01]	0.498	-0.02 [-0.04; 0]	0.098
Body fat [%]	163	-0.00 [-0.03; 0.03]	0.919	-0.02 [-0.06; 0.02]	0.365
Fat mass [g]	163	-1.39 [-6.33; 3.56]	0.584	-5.92 [-12.35; 0.53]	0.074
LBM [g]	163	-3.00 [-11.22; 5.23]	0.476	-11.20 [-21.02; -1.38]	**0.027**
2 years pp					
Weight [g]	118	-9.67 [-27.66; 8.321]	0.289	-24.18 [-48.35; 0]	**0.050**
BMI [kg/m^2]	118	-0.02 [-0.04; 0]	0.062	-0.02 [-0.05; 0]	0.069
Body fat [%]	110	-0.02 [-0.05; 0.02]	0.310	-0.03 [-0.05; 0.04]	0.885
Fat mass [g]	110	-3.66 [-10.14; 2.82]	0.265	-5.05 [-14.13; 4.03]	0.273
LBM [g]	110	-4.38 [-16.98; 8.22]	0.492	-16.83 [-33.22; -0.44]	**0.044**

LBM: lean body mass; PI: Ponderal Index; pp: postpartum

Data are presented as the unadjusted or adjusted regression coefficient beta (b) along with the 95 % confidence interval; $p \leq 0.05$ are in bold face

[1] Results were adjusted for maternal pre-pregnancy BMI, gestational weight gain, pregnancy duration, group and sex for the data at birth. Beyond birth, results were additionally adjusted for Ponderal Index at birth and breastfeeding status (exclusively/partially breastfed or formula) at 6 weeks or 4 months, respectively.

5.5.2 Cord plasma leptin in relation to infant clinical outcomes

Leptin in cord blood was significantly positively related to birth weight, BMI, the sum of four skinfolds, percentage body fat, fat mass and LBM in the unadjusted model, but also after correction for multiple maternal and child factors (b_{adj} for birth weight: 16.69 [8.81; 24.56] g, p<0.001; b_{adj} for BMI: 0.04 [0.01; 0.06] kg/m^2, p=0.002; b_{adj} for the sum of four skinfolds: 0.11 [0.06; 0.16] mm, p<0.001; b_{adj} for percentage body fat: 0.12 [0.07; 0.17] %, p<0.001; b_{adj} for fat mass: 6.63 [4.06; 9.19] g, p<0.001; b_{adj} for LBM: 9.97 [3.82; 16.12] g, p=0.002) (**Table 18**). Cord blood leptin was also significantly related to placental weight (b_{adj}: 3.10 [1.15; 5.04] g, p=0.002). In addition, cord leptin was significantly positively related to Ponderal Index at 6 weeks pp in the bivariate, but not in the adjusted analysis (Appendix **Table A- 3**).

Table 18 Cord plasma leptin [ng/ml] in relation to infant growth and body composition outcomes at birth and at 2 years

		Unadjusted analysis		**Adjusted analysis[1]**	
Outcome variable	N	Beta [95 % CI]	p	Beta [95 % CI]	p
Birth					
Birth weight [g]	135	18.35 [11.29; 25.41]	**<0.001**	16.69 [8.81; 24.56]	**<0.001**
Placental weight [g]	126	2.71 [0,98; 4.44]	**0.002**	3.10 [1.15; 5.04]	**0.002**
PI [kg/m^3]	135	0.04 [0; 0.08]	0.066	0.05 [0; 0.09]	0.068
BMI [kg/m^2]	135	0.04 [0.02; 0.06]	**<0.001**	0.04 [0.01; 0.06]	**0.002**
Sum 4 SFT [mm]	128	0.09 [0.05; 0.13]	**<0.001**	0.11 [0.06; 0.16]	**<0.001**
Body fat [%]	128	0.10 [0.05; 0.14]	**<0.001**	0.12 [0.07; 0.17]	**<0.001**
Fat mass [g]	128	5.93 [3.72; 8.14]	**<0.001**	6.63 [4.06; 9.19]	**<0.001**
LBM [g]	128	12.38 [6.71; 18.05]	**<0.001**	9.97 [3.82; 16.12]	**0.002**
2 years pp					
Weight [g]	93	-43.70 [-79.13; -8.26]	**0.016**	-49.72 [-88.27; -11.17]	**0.012**
PI [kg/m^3]	93	-0.03 [-0.07; 0.01]	0.116	-0.04 [-0.08; 0]	0.063
BMI [kg/m^2]	93	-0.04 [-0.07; 0]	**0.032**	-0.05 [-0.08; -0.01]	**0.017**
Sum 4 SFT [mm]	90	-0.05 [-0.14; 0.04]	0.305	-0.06 [-0.15; 0.04]	0.255
Body fat [%]	90	-0.03 [-0.09; 0.03]	0.293	-0.04 [-0.11; 0.03]	0.228
Fat mass [g]	90	-12.40 [-25.53; 0.735]	0.064	-14.86 [-29.49; -0.23]	**0.047**
LBM [g]	90	-32.27 [-57.40; -7.15]	**0.012**	-37.22 [-64.06; -10.37]	**0.007**

LBM: lean body mass; PI: Ponderal Index; pp: postpartum; SFT: skinfold thicknesses
Data are presented as the unadjusted or adjusted regression coefficient beta (b) along with the 95 % confidence interval; p≤0.05 are in bold face
[1] Results were adjusted for maternal pre-pregnancy BMI, gestational weight gain, pregnancy duration, group and sex for the data at birth. At 2 years, results were additionally adjusted for Ponderal Index at birth and breastfeeding status (exclusively/partially breastfed or formula) at 4 months.

When the same analysis was done with the anthropometric data at 2 years of age, a different pattern emerged (**Table 18**): Cord blood leptin was negatively associated with weight (b_{adj}: -49.72 [-88.27; -11.17] g, p=0.012), BMI (b_{adj}: -0.05 [-0.08; -0.01] kg/m^2, p=0.017), fat mass (b_{adj}: -14.86 [-29.49; -0.23] g, p=0.047) as well as LBM (b_{adj}: -37.22 [-64.06; -10.37] g, p=0.007) at 2 years. Furthermore, cord blood leptin was found to be inversely related to early body weight gain in the first 4 months (b_{adj}: -14.01 [-25.89; -2.12] g, p=0.021), up to 1 year (b_{adj}: -23.44 [-41.06; -5.82] g, p=0.010) and up to 2 years of age (b_{adj}: -66.50 [-101.86; -31.14] g, p<0.001) (**Table 19**). Additional analysis differentiating between fat mass and LBM revealed a significant negative relationship between cord blood leptin level and both, gain in fat mass (b_{adj}: -22.60 [-37.24; -7.96] g, p=0.003, N=87) and gain in LBM from birth until 2 years pp (b_{adj}: -47.82 [-72.38; -23.25] g, p<0.001, N=87) (**Figure 13**).

Table 19 Cord plasma leptin [ng/ml] in relation to infant body weight gain

		Unadjusted analysis		**Adjusted analysis[1]**	
Outcome variable	N	Beta [95 % CI]	p	Beta [95 % CI]	p
Weight gain [g] (birth – 4 months pp)	132	-20.04 [-31.07; -9.01]	**0.001**	-14.01 [-25.89; -2.12]	**0.021**
Weight gain [g] (birth – 1 year pp)	128	-27.87 [-44.56; -11.19]	**0.001**	-23.44 [-41.06; -5.82]	**0.010**
Weight gain [g] (birth – 2 years pp)	93	-65.24 [-97.26; -33.22]	**<0.001**	-66.50 [-101.86; -31.14]	**<0.001**

pp: postpartum
Data are presented as the unadjusted or adjusted regression coefficient beta (b) along with the 95 % confidence interval; p≤0.05 are in bold face
[1] Results were adjusted for maternal pre-pregnancy BMI, gestational weight gain, pregnancy duration, group, sex, Ponderal Index at birth and breastfeeding status (exclusively/partially breastfed or formula) at 4 months.

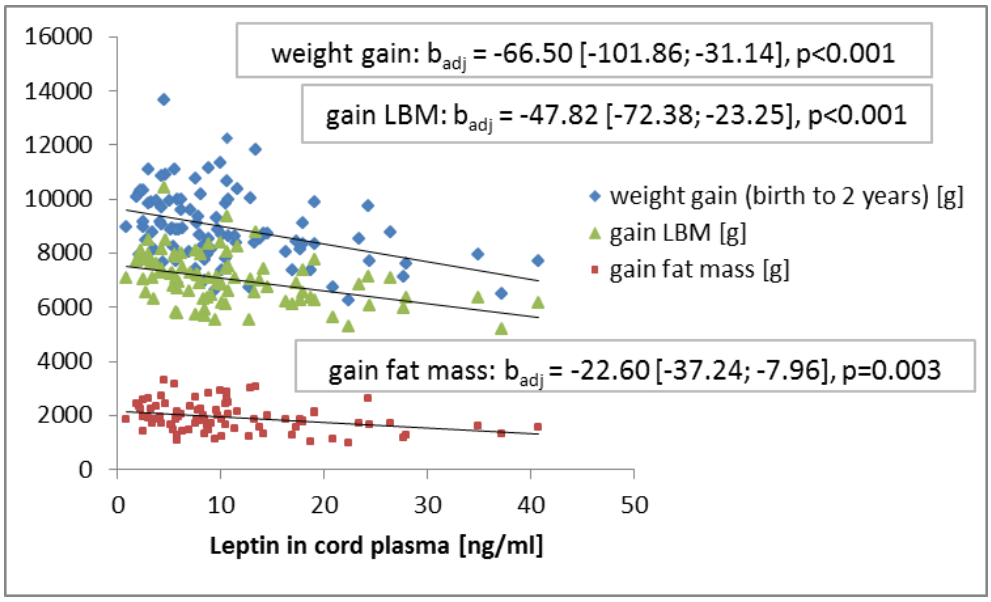

Figure 13 Cord plasma leptin in relation to infant weight gain, gain in fat and lean body mass (LBM) up to 2 years

b_{adj}: regression coefficient beta from linear regression analysis along with the [95 % confidence interval] adjusted for maternal pre-pregnancy BMI, gestational weight gain, glucose tolerance status, pregnancy duration, group, sex, Ponderal Index at birth and breastfeeding status (exclusively/partially breastfed or formula) at 4 months.

5.5.3 Contribution of maternal and cord plasma leptin to the growth and body composition outcomes at birth and 2 years

As both, maternal leptin and cord blood leptin were significantly associated with a range of body composition and growth outcomes at birth and at 2 years, additional analyses to compare the respective contribution of maternal and cord blood leptin for these outcomes were performed. Including maternal and cord blood leptin simultaneously in a single regression model revealed that cord blood leptin consistently explained a higher proportion of the variance of the outcomes at birth (birth weight, placental weight, BMI, fat mass and LBM) as well as at 2 years (weight, fat mass and LBM), both in the unadjusted and the adjusted analysis (**Table 20**).

Table 20 Proportion of the variance in infant body composition and growth outcomes at birth and at 2 years explained by maternal and cord blood leptin[1]

	Maternal leptin		Cord blood leptin	
Outcome variable	unadjusted	adjusted[2]	unadjusted	adjusted[2]
Birth				
Birth weight	0.035	0.036	0.157	0.156
Placental weight	0.020	0.025	0.067	0.062
BMI	0.053	0.056	0.091	0.088
Fat mass	0.012	0.015	0.174	0.170
LBM	0.026	0.026	0.123	0.124
2 years				
Weight	0.012	0.012	0.062	0.062
Fat mass	0.018	0.018	0.039	0.039
LBM	0.010	0.010	0.070	0.070

LBM: lean body mass
[1] Data are fractions of explained variance by maternal and cord blood leptin of the total variance of outcome variables.
[2] Results were adjusted for maternal pre-pregnancy BMI, gestational weight gain, pregnancy duration, group and sex for the data at birth. At 2 years, results were additionally adjusted for Ponderal Index at birth and breastfeeding status (exclusively/partially breastfed or formula) at 4 months.

5.5.4 Maternal plasma insulin and HOMA-IR in relation to infant clinical outcomes

Maternal fasting insulin levels and HOMA-IR at the 32nd week of gestation were found to be largely unrelated to the infant growth and body composition outcomes up to 2 years pp. The only significant finding was, that HOMA-IR was inversely associated with lean body mass at birth (b_{adj}: -54.94 [-99.23; -10.64] g, p=0.016) in the analysis controlling for maternal pre-pregnancy BMI, gestational weight gain, maternal glucose tolerance status, pregnancy duration, group and sex (Appendix **Table A- 4**).

5.5.5 Cord plasma insulin in relation to infant clinical outcomes

Cord blood insulin was significantly related to birth weight (b: 1.68 [0.03; 3.33] g, p=0.048), the sum of 4 skinfolds (b: 0.02 [0.01; 0.02] mm, p=0.001), percentage body fat (b: 0.02 [0.01; 0.03] %, p=0.001) as well as absolute amount of fat mass (b: 0.81 [0.31; 1.31] g, p=0.002) at birth. These relationships remained significant after adjustment for maternal pre-pregnancy BMI, gestational weight gain, maternal glucose tolerance status, pregnancy duration, group and sex (**Table 21**). No significant associations were found for cord blood insulin with the

infant growth and body composition data beyond birth up to the first years of life (Appendix **Table A- 5**).

In contrast, cord blood insulin was significantly negatively related to weight, BMI, fat and lean body mass at 2 years pp as well as with weight gain from birth up to 2 years of age (**Table 21**). However, in the adjusted model controlling for maternal pre-pregnancy BMI, gestational weight gain, maternal glucose tolerance status, pregnancy duration, group, sex, Ponderal Index at birth and mode of infant feeding at 4 months pp, only the relationship with weight gain remained significant (b_{adj}: -6.90 [-13.13; -0.67] g, p=0.030) (**Table 21**). Testing for sex interaction revealed no significant interaction for girls vs. boys within this association (p=0.710). Nevertheless, the relationship of cord blood insulin with body weight gain turned out to be much stronger in girls compared to boys (b_{adj} for girls: -7.63 [-15.29; 0.03] g, p=0.051, N=39; b_{adj} for boys: -2.82 [-15.76; 10.11] g, p=0.662, N=54) (**Figure 14**).

Table 21 Cord plasma insulin [pmol/l] in relation to infant growth and body composition outcomes at birth and at 2 years

		Unadjusted analysis		**Adjusted analysis[1]**	
Outcome variable	N	Beta [95 % CI]	p	Beta [95 % CI]	p
Birth					
Birth weight [g]	137	1.68 [0.03; 3.33]	**0.048**	1.87 [0.27; 3.47]	**0.023**
PI [kg/m^3]	137	-0.00 [-0.01; 0.01]	0.875	-0.00 [-0.01; 0.01]	0.803
BMI [kg/m^2]	137	0.00 [0; 0.01]	0.434	0.00 [-0.00; 0.01]	0.423
Sum 4 SFT [mm]	130	0.02 [0.01; 0.02]	**0.001**	0.02 [0.01; 0.03]	**0.003**
Body fat [%]	130	0.02 [0.01; 0.03]	**0.001**	0.02 [0.01; 0.03]	**0.002**
Fat mass [g]	130	0.81 [0.31; 1.31]	**0.002**	0.80 [0.28; 1.32]	**0.003**
LBM [g]	130	0.89 [-0.39; 2.17]	0.176	1.01 [-0.21; 2.24]	0.105
2 years pp					
Weight [g]	93	-7.60 [-13.79; -1.41]	**0.017**	-5.50 [-12.12; 1.13]	0.103
PI [kg/m^3]	93	-0.01 [-0.01; 0.00]	0.064	-0.01 [-0.01; 0.00]	0.211
BMI [kg/m^2]	93	-0.01 [-0.01; -0.00]	**0.021**	-0.01 [-0.01; 0.00]	0.115
Sum 4 SFT [mm]	90	-0.01 [-0.01; 0.00)]	0.064	-0.01 [-0.03; 0.01]	0.226
Body fat [%]	90	-0.01 [-0.02; 0.01]	0.269	-0.01 [-0.02; 0.01]	0.249
Fat mass [g]	90	-2.36 [-4.65; -0.07]	**0.043**	-2.02 [-4.48; 0.43]	0.105
LBM [g]	90	-5.60 [-10.00; -1.21]	**0.013**	-3.80 [-8.45; 0.85]	0.108
Weight gain [g] (birth – 2 years pp)	93	-8.66 [-14.46; -2.85]	**0.004**	-6.90 [-13.13; -0.67]	**0.030**

LBM: lean body mass; PI: Ponderal Index; pp: postpartum; SFT: skinfold thicknesses
Data are presented as the unadjusted or adjusted regression coefficient beta (b) along with the 95 % confidence interval; p≤0.05 are in bold face
[1] Results were adjusted for maternal pre-pregnancy BMI, gestational weight gain, glucose tolerance status, pregnancy duration, group and sex for the data at birth. At 2 years, results were additionally adjusted for Ponderal Index at birth and breastfeeding status (exclusively/partially breastfed or formula) at 4 months.

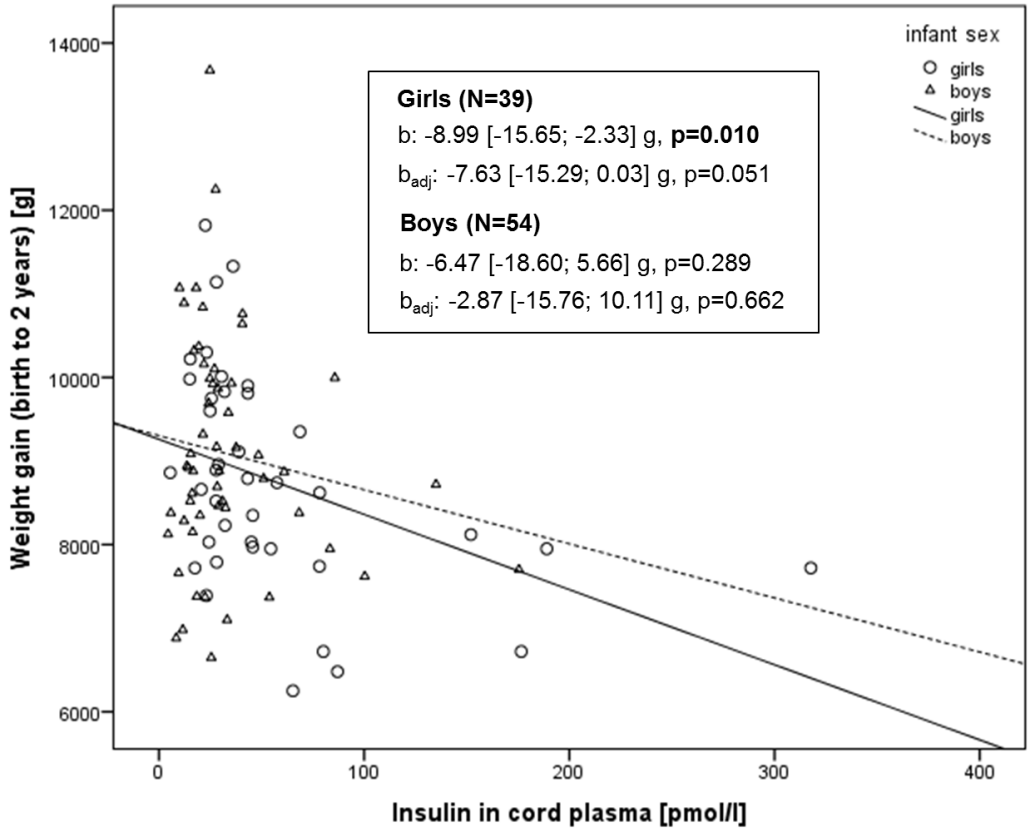

Figure 14 Cord plasma insulin in relation to infant weight gain up to 2 years by gender

b: regression coefficient beta from linear regression analysis along with the [95 % confidence interval]; b_{adj}: data adjusted for maternal pre-pregnancy BMI, gestational weight gain, glucose tolerance status, pregnancy duration, group, sex, Ponderal Index at birth and breastfeeding status (exclusively/partially breastfed or formula) at 4 months.
* $p \leq 0.05$

5.5.6 Maternal serum triglyceride levels in relation to infant clinical outcomes

With regard to maternal triglyceride levels, we investigated not only associations with absolute triglyceride levels at 32nd week of gestation, but also the relationship of the change in triglyceride levels (Δ TG) between the 15th and the 32nd week of gestation with the infant clinical outcomes up to 2 years of life. No significant associations were found for absolute triglyceride levels. However, Δ TG during pregnancy was weakly, but significantly associated with infant Ponderal Index at 4 months pp (b_{adj}: 0.01 [0; 0.01] kg/m^3, p=0.020), but not with any of the other growth or body composition outcomes up to 2 years of life (Appendix **Table A- 6**).

5.5.7 Maternal plasma HMW adiponectin in relation to infant clinical outcomes

Maternal HMW adiponectin at the 32nd week of gestation was significantly positively associated with LBM at birth, both in the unadjusted and the adjusted model (b_{adj}: 44.92 [1.74; 88.10] g, p=0.04) (**Table 22**). However, there were no other significant relationships with the infant anthropometric parameters within the first years of life (Appendix **Table A-7**).

At 2 years of age, significant positive relationships were found between maternal HMW adiponectin in late pregnancy and infant weight (p=0.04), the sum of 4 skinfolds (p=0.04), percent body fat (p=0.03) and body fat mass (p=0.04) in the unadjusted analysis. However, only the association with body weight persisted in the adjusted analysis controlling for multiple confounding factors (b_{adj}: 198.34 [16.49; 380.19] g, p=0.03) (**Table 22**).

Table 22 Maternal plasma HMW adiponectin [µg/ml] at the 32nd week of gestation in relation to infant growth and body composition outcomes at birth and at 2 years

		Unadjusted analysis		**Adjusted analysis[1]**	
Outcome variable	N	Beta [95 % CI]	p	Beta [95 % CI]	p
Birth					
Weight [g]	183	55.51 [-3.74; 114.77]	0.07	45.36 [-10.86; 101.58]	0.12
PI [kg/m^3]	183	0.12 [-0.18; 0.42]	0.44	0.11 [-0.21; 0.42]	0.51
BMI [kg/m^2]	183	0.11 [-0.05; 0.26]	0.17	0.09 [-0.06; 0.24]	0.23
Sum 4 SFT [mm]	168	-0.02 [-0.37; 0.33]	0.91	-0.01 [-0.38; 0.35]	0.94
Body fat [%]	168	-0.06 [0.43; 0.30]	0.73	-0.06 [-0.44; 0.31]	0.75
Fat mass [g]	168	7.15 [-11.80; 26.18]	0.46	6.92 [-12.63; 26.47]	0.49
LBM [g]	167	49.48 [2.62; 96.34]	**0.04**	44.92 [1.74; 88.10]	**0.04**
2 years pp[2]					
Weight [g]	169	189.61 [10.56; 368.66]	**0.04**	198.34 [16.49; 380.19]	**0.03**
PI [kg/m^3]	169	0.10 [-0.13; 0.33]	0.40	0.08 [0.17; 0.32]	0.53
BMI [kg/m^2]	169	0.14 [-0.04; 0.33]	0.13	0.14 [-0.06; 0.33]	0.17
Sum 4 SFT [mm]	110	0.58 [0.03; 1.13]	**0.04**	0.54 [-0.05; 10.14]	0.08
Body fat [%]	110	0.42 [0.05; 0.80]	**0.03**	0.39 [-0.02; 0.80]	0.06
Fat mass [g]	110	88.16 [7.43; 168.89]	**0.04**	85.07 [-3.52; 173.66]	0.06
LBM [g]	110	125.02 [-32.94; 282.97]	0.12	133.12 [-31.59; 297.84]	0.12
Weight gain (birth ─ 2 years pp)	169	120.21 [-48.42; 288.83]	0.16	149.15 [-25.36; 323.67]	0.10

LBM: lean body mass; PI: Ponderal Index; pp: postpartum; SFT: skinfold thicknesses
Data are presented as the unadjusted or adjusted regression coefficient beta (b) along with the 95 % confidence interval; p≤0.05 are in bold face
[1] Results were adjusted for maternal pre-pregnancy BMI, gestational weight gain, pregnancy duration, group and sex for the data at birth. Beyond birth, results were additionally adjusted for Ponderal Index at birth and breastfeeding status (exclusively/partially breastfed or formula) at 6 weeks or 4 months, respectively.
[2] Infants, for whom only data from the well-child visit at 2 years were available, were also included.

5.5.8 Adipokines in breast milk in relation to infant clinical outcomes

Breast milk was collected from lactating mothers at two time-points during the lactation period (6 weeks and 4 months pp). Adipokines measured in breast milk at both time-points were related to infant growth and body composition up to 2 years pp. Of the infants included in the analysis (i.e. whose mothers donated breast milk), 85 % were exclusively and 15 % were partially breastfed until 6 weeks pp; 83 % and 17 %, respectively, were exclusively or partially breastfed until 4 months pp.

Breast milk leptin

Milk leptin at 6 weeks pp was not related to any of the infant clinical outcomes from 6 weeks until 2 years pp (Appendix **Table A- 8**). In contrast, milk leptin concentration at 4 months pp was significantly negatively associated with infant weight (b_{adj}: -635.54 [-1194.86; -76.22] g, p=0.03), BMI (b_{adj}: -1.20 [-2.37; -0.02] kg/m^2, p=0.05) as well as lean body mass (b_{adj}: -425.90 [-797.10; -54.69] g, p=0.03) at the age of 4 months in the model corrected for maternal pre-pregnancy BMI, gestational weight gain, pregnancy duration, infant sex, Ponderal Index at birth and mode of infant feeding (**Table 23**). Although milk leptin at 4 months was also significantly negatively associated with the subscapular-to-triceps skinfold-ratio at 1 year in the unadjusted model (b: -0.14 [-0.27; -0.01], p=0.034, N=116), significance was lost in the adjusted analysis (data not shown). No significant relationships were found with regard to infant growth or body composition in the longer-term observation up to 2 years pp (Appendix **Table A- 9**).

Table 23 Breast milk leptin [ng/ml] at 4 months pp in relation to infant growth and body composition outcomes at 4 months pp

		Unadjusted analysis		Adjusted analysis[1]	
Outcome variable	N	Beta [95 % CI]	p	Beta [95 % CI]	p
4 months pp					
Weight [g]	119	-188.85 [-743.83; 366.13]	0.51	-635.54 [-1194.86; -76.22]	**0.03**
PI [kg/m^3]	119	-1.16 [-2.91; 0.59]	0.20	-1.58 [-3.64; 0.49]	0.14
BMI [kg/m^2]	119	-0.63 [-1.69; 0.42]	0.24	-1.20 [-2.37; 0.02]	**0.05**
Sum 4 SFT [mm]	120	-2.02 [-5.25; 1.2]	0.22	-1.70 [-5.42; 2.02]	0.37
Body fat [%]	120	-1.31 [-3.44; 0.81]	0.23	-1.03 [-3.48; 1.42]	0.41
Body fat [g]	119	-130.76 [-349.84; 88.32]	0.24	-209.63 [-459.63; 40.38]	0.10
LBM [g]	119	-58.05 [-454.63; 338.53]	0.78	-425.90 [-797.1; 54.69]	**0.03**

LBM: lean body mass; PI: Ponderal Index; pp: postpartum; SFT: skinfold thicknesses
Data are presented as the unadjusted or adjusted regression coefficient beta (b) along with the 95 % confidence interval; p≤0.05 are in bold face
[1] Results were adjusted for maternal pre-pregnancy BMI, gestational weight gain, pregnancy duration, group, sex, Ponderal Index at birth and breastfeeding status (exclusively/partially breastfed or formula) at 4 months.

Breast milk total adiponectin

Regression analyses of total milk adiponectin at 6 weeks pp with the infant clinical data revealed predominantly inverse relationships with infant anthropometric outcomes at earlier time-points (up to 4 months pp), albeit mostly non significant, but positive relationships with later infant outcomes at 1 and 2 years pp (**Table 24**): In the fully adjusted analysis, significantly inverse relations were found for milk adiponectin at 6 weeks with infant weight (b_{adj}: -13.30 [-26.67; 0.06] g, p=0.05) and lean body mass at 4 months (b_{adj}: -11.46 [-20.56; -2.36] g, p=0.01). In contrast, the associations were positive for the sum of 4 skinfolds (b_{adj}: 0.09 [0.01; 0.18] mm, p=0.04), percentage body fat (b_{adj}: 0.06 [0; 0.12] %, p=0.04) and fat mass (b_{adj}: 0.01 [0.2; 17.43] g, p=0.01) at 1 year of age as well as with infant weight gain up to 1 year (b_{adj}: 23.50 [0.91; 46.08] g, p=0.04) and up to 2 years pp (b_{adj}: 31.05 [-0.31; 62.42] g, p=0.05).

Likewise, milk adiponectin at 4 months was significantly positively related to infant fat mass expressed as the sum of 4 skinfolds (b_{adj}: 0.14 [0.02; 0.25] mm, p=0.01) (**Figure 15**), percentage body fat (b_{adj}: 0.10 [0.02; 0.18] %, p=0.02), and absolute body fat (b_{adj}: 17.36 [0.37; 34.36] g, p=0.05) at 2 years as well as to weight gain up to 2 years (b_{adj}: 29.38 [2.66; 55.44] g, p=0.03) even after adjustment for multiple confounding factors (**Figure 16**, Appendix **Table A- 10**). However, no significant associations were found with regional fat patterning expressed as the central-to-peripheral skinfold-ratio as well as the trunk-to-total skinfold-ratio (data not shown).

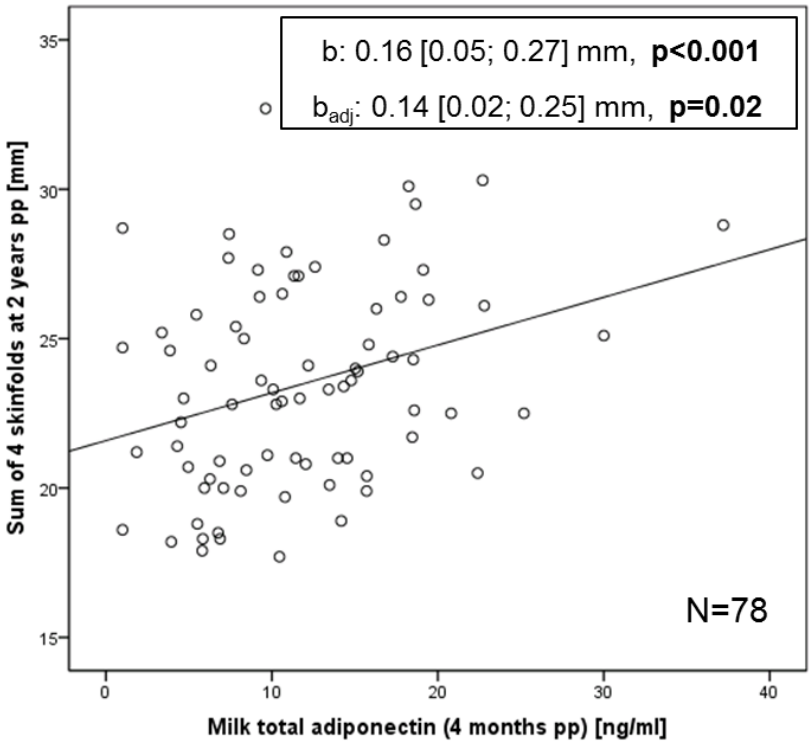

Figure 15 Breast milk total adiponectin at 4 months pp in relation to infants´ sum of 4 skinfolds at 2 years pp

b: regression coefficient beta from linear regression analysis along with the [95 % confidence interval]; b_{adj}: data adjusted for maternal pre-pregnancy BMI, gestational weight gain, pregnancy duration, group, sex, Ponderal Index at birth and breastfeeding status (exclusively/partially breastfed or formula) at 4 months.

Table 24 Breast milk total adiponectin [ng/ml] at 6 weeks pp in relation to infant growth and body composition outcomes up to 2 years pp

		Unadjusted analysis		**Adjusted analysis[1]**	
Outcome variable	N	Beta [95 % CI]	p	Beta [95 % CI]	p
6 weeks pp					
Weight [g]	151	-0.24 [-12.04; 11.55]	0.97	-4.66 [-18.8; 9.49]	0.52
PI [kg/m^3]	151	-0.05 [-0.1; 0]	**0.04**	-0.06 [-0.13; 0]	0.07
BMI [kg/m^2]	151	-0.02 [-0.04; 0.01]	0.15	-0.03 [-0.06; 0.01]	0.11
Sum 4 SFT [mm]	151	-0.00 [-0.08; 0.07]	0.90	-0.02 [-0.12; 0.08]	0.73
Body fat [%]	151	-0.01 [-0.06; 0.05]	0.82	-0.02 [-0.09; 0.06]	0.65
Fat mass [g]	151	-0.18 [-4.57; 4.21]	0.94	-1.51 [-7.26; 4.24]	0.61
LBM [g]	151	-0.06 [-8.35; 8.22]	0.99	-3.14 [-12.72; 6.43]	0.52
4 months pp					
Weight [g]	147	-8.61 [-22.37; 5.15]	0.22	-13.30 [-26.67; 0.06]	**0.05**
PI [kg/m^3]	147	-0.01 [-0.06; 0.03]	0.49	-0.02 [-0.07; 0.02]	0.32
BMI [kg/m^2]	147	-0.01 [-0.04; 0.01]	0.26	-0.02 [-0.05; 0]	0.10
Sum 4 SFT [mm]	147	0.02 [-0.06; 0.1]	0.67	0.02 [-0.07; 0.11]	0.63
Body fat [%]	147	0.01 [-0.04; 0.06]	0.70	0.01 [-0.05; 0.07]	0.68
Fat mass [g]	146	-1.32 [-6.76; 4.11]	0.63	-2.19 [-7.97; 3.58]	0.46
LBM [g]	146	-7.59 [-17.53; 2.34]	0.14	-11.46 [-20.56; -2.36]	**0.01**
Weight gain (6 wks ─ 4 mo pp)	147	-8.47 [-17.08; 0.13]	0.06	-8.59 [-19.52; 2.35]	0.13
1 year pp					
Weight [g]	145	21.11 [0.97; 41.25]	**0.04**	11.16 [-8.65; 30.97]	0.27
PI [kg/m^3]	145	0.02 [-0.02; 0.06]	0.30	0.01 [-0.03; 0.05]	0.72
BMI [kg/m^2]	145	0.02 [0; 0.05]	0.10	0.01 [-0.02; 0.04]	0.44
Sum 4 SFT [mm]	140	0.09 [0.01; 0.17]	**0.03**	0.09 [0.01; 0.18]	**0.04**
Body fat [%]	140	0.06 [0.01; 0.12]	**0.03**	0.06 [0; 0.12]	**0.04**
Fat mass [g]	140	10.76 [2.48; 19.04]	**0.01**	8.82 [0.2; 17.43]	**0.05**
LBM [g]	140	11.67 [-2.44; 25.78]	0.11	4.04 [-9.35; 17.42]	0.56
Weight gain (6 wks ─ 1 year pp)	145	21.51 [4.11; 38.9]	**0.02**	23.50 [0.91; 46.08]	**0.04**
2 years pp[2]					
Weight [g]	144	26.85 [0.58; 53.11]	**0.05**	26.55 [-7.68; 60.79]	0.13
PI [kg/m^3]	144	0.00 [-0.03; 0.04]	0.91	0.01 [-0.03; 0.06]	0.63
BMI [kg/m^2]	144	0.01 [-0.01; 0.04]	0.35	0.02 [-0.02; 0.05]	0.31
Sum 4 SFT [mm]	96	0.10 [0; 0.21]	**0.05**	0.09 [-0.01; 0.2]	0.10
Body fat [%]	96	0.07 [0; 0.14]	**0.05**	0.06 [-0.01; 0.14]	0.10
Fat mass [g]	96	14.68 [-0.67; 30.02]	0.06	12.75 [-3.74; 29.25]	0.13
LBM [g]	96	15.22 [-14.32; 44.76]	0.32	13.12 [-16.91; 43.16]	0.39
Weight gain (6 wks ─ 2 years pp)	144	27.01 [3.34; 50.69]	**0.03**	31.05 [-0.31; 62.42]	**0.05**

LBM: lean body mass; mo: month; PI: Ponderal Index; pp: postpartum; SFT: skinfold thicknesses; wk: week
Data are presented as the unadjusted or adjusted regression coefficient beta (b) along with the 95 % confidence interval; p≤0.05 are in bold face

[1] Results were adjusted for maternal pre-pregnancy BMI, gestational weight gain, pregnancy duration, group, sex, Ponderal Index at birth and breastfeeding status (exclusively/partially breastfed or formula) at 4 months.

[2] Infants, for whom only data from the well-child visit at 2 years were available, were also included.

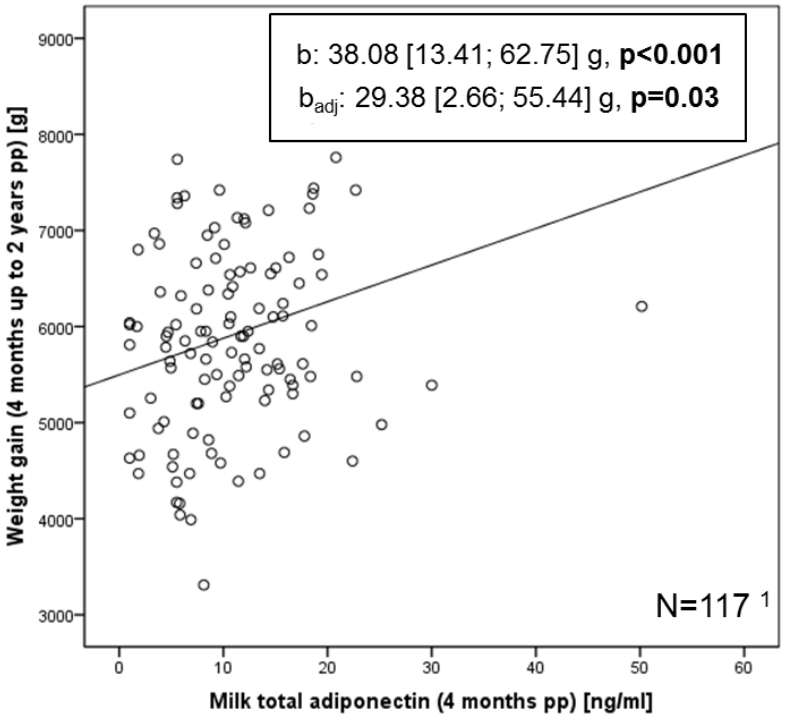

Figure 16 Breast milk total adiponectin at 4 months pp in relation to infant weight gain up to 2 years pp

b: regression coefficient beta from linear regression analysis along with the [95 % confidence interval]; b_{adj}: data adjusted for maternal pre-pregnancy BMI, gestational weight gain, pregnancy duration, group, sex, Ponderal Index at birth and breastfeeding status (exclusively/partially breastfed or formula) at 4 months.

[1] Infants, for whom only data from the well-child visit at 2 years were available, were also included.

6 Discussion

The present study investigated the effect of reducing the dietary n-6/n-3 LCPUFA ratio during pregnancy and lactation on selected biomarkers in maternal blood, cord blood and breast milk and, irrespective of the intervention, their relationship with infant body composition from birth up to 2 years of age.

6.1 Maternal leptin, sOB-R and FLI over the course of pregnancy and lactation

Longitudinal analysis

In the present study, maternal leptin levels increased significantly during pregnancy followed by a decline below baseline levels after delivery. The observed changes over time are in accordance with findings from other longitudinal studies over the course of gestation and/or lactation (Butte et al. 1997, Schubring et al. 1998, Tamura et al. 1998). Elevated leptin levels compared to the non-pregnant state can already be found in the first trimester, before a significant increase in body weight becomes apparent, thus providing evidence that factors other than increased maternal adiposity modulate the circulating levels (Henson and Castracane 2006). Butte et al. followed pregnant women from late pregnancy (~36 weeks) until 6 months postpartum and reported that serum leptin concentration per unit fat mass was higher in pregnancy compared to the postpartum period. By linear regression it was shown that the relation (i.e. slope) between circulating leptin and fat mass did not differ between pregnancy and postpartum, but the intercept markedly changed resulting in an upward shift of the regression line, thus also implying that factors other than fat mass regulate leptin expression during pregnancy (Butte et al. 1997). Subsequent studies demonstrated that the placenta produces substantial amounts of leptin thereby contributing to the rise in leptin in the maternal circulation (Masuzaki et al. 1997, Sagawa et al. 2002). In vitro perfusion studies with human placentas have shown that approximately 98 % of placental leptin is released into the maternal circulation, whereas only 1−2 % enters the fetal circulation (Linnemann et al. 2000). After delivery, maternal leptin levels decrease dramatically, further supporting the role of the placenta as an important source for leptin production in pregnancy (Masuzaki et al. 1997, Sagawa et al. 2002). Conversely, studies in women with singleton and multiple pregnancies revealed, that leptin levels were not directly related to placental mass, but rather

maternal adiposity was the determining factor, suggesting that the hormonal milieu of pregnancy may upregulate leptin synthesis in maternal adipose tissue (Henson and Castracane 2006). Taken together, the exact mechanisms leading to the hyperleptinemia in pregnancy are not completely understood, but seem to be a result of both, increasing maternal fat stores and/or upregulation of leptin expression in adipose tissue as well as synthesis by the placenta. Although the physiological significance of hyperleptinemia during pregnancy is not entirely clear, it might be a critical factor for the normal progression of pregnancy (Schubring et al. 1998). The expression of both leptin and its receptors in the placenta might be suggestive of autocrine/paracrine mechanisms acting to maintain placental endocrine function (Henson et al. 1998, Henson and Castracane 2006). Leptin may also be involved in fetal development and growth, including bone formation and pulmonary development (Henson and Castracane 2006). With regard to potential functions for the mother, high leptin levels especially towards the end of pregnancy might represent a physiological state of central leptin-resistance as the elevated leptin levels are not accompanied by a reduction in food intake (Popovic and Casanueva 2002), similar to the situation in obese individuals. The marked decrease of leptin levels postpartum has been suggested to provide a stimulus for increased energy uptake allowing for the increasing energy requirements of lactation (Schubring et al. 1998).

Whilst increasing leptin concentrations during pregnancy are well-documented (Helland et al. 1998a, Schubring et al. 1998), data on changes in sOB-R over the course of pregnancy and lactation are less consistent (Lewandowski et al. 1999, Nuamah et al. 2003, Krizova et al. 2004). In our study, there was an increase in maternal sOB-R concentration during pregnancy, followed by a significant decrease after delivery and a further decrease during the lactation period. The finding that increasing leptin goes along with increasing sOB-R levels until week 32 of gestation is interesting as it is in contrast to non-pregnant women with increasing BMI (Chan et al. 2002). A similar situation to our data was described in mice where a 20-to 40-fold increase of leptin levels during pregnancy was accompanied by up to 40-fold increased sOb-R serum concentration (Gavrilova et al. 1997). The authors of this work assumed that high levels of leptin binding protein (sOb-R) predominantly act inhibitory thereby causing leptin resistance (Gavrilova et al. 1997). This might be a physiological adaptation as the end of gestation is an advantageous time for increased food intake and metabolic efficiency in preparation for birth and nursing (Gavrilova et al. 1997). In turn, the decrease of leptin and

sOB-R levels after delivery could reflect the regression of maternal body weight and fat mass and restored leptin sensitivity.

Effect of the intervention

We found no relevant effect of the intervention to reduce the dietary n-6/n-3 fatty acid ratio, on maternal levels of leptin, sOB-R or the FLI. To date, there is little information to what extent dietary factors, and especially a change in the dietary n-6/n-3 fatty acid ratio, influence circulating leptin levels during pregnancy and lactation. Our results are in accordance with findings from Helland et al., the only study comparable to the design and question of our study (Helland et al. 1998a). In this randomized controlled double-blind trial the women either received 10ml cod liver oil rich in n-3 LCPUFAs or the same amount of corn oil from ~18 weeks of gestation until 3 months after delivery. No differences between the groups were found in maternal plasma leptin measured at 18 weeks and 35 weeks of gestation and in cord blood, either. Cummings et al. achieved similar results in an animal study: Rats were supplemented with either fish oil or EPA or received a control diet, but also no differences in plasma leptin concentrations between the groups were observed (Cummings et al. 2010).
The effect of n-3 LCPUFAs on circulating leptin levels might also be dependent on health status as well as dosing, timing and duration of n-3 LCPUFA supplementation. It is conceivable that n-3 LCPUFAs might affect leptin levels via weight loss, as found in a supplementation study with obese prepubertal and pubertal children (Lopez-Alarcon et al. 2011). As pregnancy represents an exceptional physiological situation associated with weight gain and hyperleptinemia, effects of n-3 LCPUFAs could be different compared to non-pregnant individuals. In view of the high compliance of the women in implementing the dietary intervention in our study, as confirmed by repeated analysis of the fatty acid pattern in maternal red blood cells (Hauner et al. 2012), our results suggest that an enhanced intake of n-3 LCPUFAs in this dose range during pregnancy and lactation is unlikely to relevantly affect maternal or cord blood leptin levels.

Correlations of leptin with maternal anthropometry

We found highly significant correlations of maternal plasma leptin with pre-pregnancy BMI and with both, BMI and fat mass at the 15^{th} and 32^{nd} week of gestation, which has also been shown in previous studies (Butte et al. 1997, Masuzaki et al. 1997, Helland et al. 1998a, Schubring et al. 1998, Tamura et al. 1998). While in our study the correlations were similar at

both investigated time-points during pregnancy, Schubring et al. reported decreasing correlation coefficients between leptin and maternal BMI with advancing gestation, suggesting that the regulation of leptin levels and/or the biologic effects mediated by leptin might differ depending on the phase of gestation (Schubring et al. 1998). Moreover, the fact that weight changes during pregnancy are not only brought about by an increase in fat mass, but also by the expansion of the extracellular fluid and other tissues, could in part explain this observation (Schubring et al. 1997).

Moreover, our data showed a significant positive correlation between maternal leptin levels and gestational weight gain (GWG), independent of maternal pre-pregnancy BMI, which is in agreement with other reports (Butte et al. 1997, Stein et al. 1998, Laml et al. 2001). This finding deserves some attention, as women with higher weight status - and thus higher leptin levels - would commonly be expected to gain less weight during pregnancy. It was suggested that high rates of GWG may occur as a result of reduced sensitivity to the actions of leptin which may lead to an uncoupling of feeding behavior (Schubring et al. 1998). Furthermore, other factors potentially involved in the release of leptin, such as insulin, with its known anabolic and anti-lipolytic actions, or other pregnancy hormones influencing insulin resistance could provide a possible explanation for this association (Stein et al. 1998).

In addition to the association with GWG, the studies of Butte et al. (1997) and Stein et al. (1998) further demonstrated a positive relationship of maternal leptin levels with weight retention after delivery. Unfortunately, we were unable to verify this in our study, as maternal weight was only monitored over the course of pregnancy and not postpartum.

6.2 Leptin, sOB-R and FLI in cord blood

Comparable to the results on maternal parameters of the leptin axis, there were no differences in leptin and sOB-R concentrations as well as the FLI in cord blood between the groups, which is compatible with findings from the n-3 LCPUFA supplementation study of Helland et al. (1998a). Consistent with other studies (Helland et al. 1998a, Schubring et al. 1998, Schulz et al. 2000), we also observed no correlation between maternal and cord blood leptin levels, although contrary reports also exist (Tamura et al. 1998). Thus, our data support the independence of maternal and fetal leptin levels corresponding to the concept of the “two-compartment model” of feto-placental leptin regulation (Laml et al. 2001).

Furthermore, we could confirm previous findings reporting a gender-difference in fetal leptin concentrations (Tome et al. 1997, Helland et al. 1998a, Ong et al. 1999, Yang and Kim 2000, Yang et al. 2002). In contrast, no differences between the sexes were observed for sOB-R which supports data suggesting sOB-R to be independent of gender from birth up to the adolescent age (Kratzsch et al. 2002, Kratzsch et al. 2005). Provided that cord blood leptin represents a reliable index of fetal fat mass (Clapp and Kiess 1998, Schubring et al. 1999), one could speculate, that the higher levels in females compared to males could be ascribed to differences in the amount of fat mass between the sexes at birth. However, analysis by gender did not reveal differences in the sum of four skinfolds, body fat percentage and fat mass between newborn boys and girls in our population. This observation suggests that other factors apart from neonatal body composition are responsible for the sexual dimorphism in cord blood leptin concentration and that leptin production by fetal adipose tissue and/or placenta might be differentially regulated between the sexes. Several explanations for this gender difference are discussed in the literature, e.g. gender-specific genes affecting insulin sensitivity or hormonal factors (Wilkin and Murphy 2006, Karakosta et al. 2011). As boys display significantly higher testosterone levels compared to girls in umbilical cord blood according to a recent meta-analysis (Barry et al. 2011) and testosterone was shown to suppress leptin both at the mRNA and protein level (Wabitsch et al. 1997), gender-specific differences in testosterone level could be a possible explanation for the difference in fetal leptin between the sexes.

6.3 Maternal insulin, glucose and HOMA-IR over the course of pregnancy and lactation

Longitudinal analysis

Within both groups, fasting insulin levels and HOMA-IR showed a significant increase during pregnancy, followed by a decrease below baseline levels after delivery and recovering levels close to the values at study entry by 4 months pp. Overall, these changes reflect the physiological adaptations during pregnancy characterized by decreased insulin sensitivity compensated by an increase in insulin secretion especially towards the end of pregnancy (Butte 2000, Herrera 2000, Herrera and Ortega 2008). In opposite to the marked changes in fasting insulin and HOMA-IR, basal glucose levels remained rather stable over the course of

the study, which is in contrast to reports showing a tendency towards hypoglycemia in late pregnancy (Butte 2000, Herrera 2005).

Effect of the intervention

We found no significant effect of the intervention on longitudinal changes of maternal fasting glucose, insulin and HOMA-IR over the course of pregnancy and lactation. At present, little is known on the potential role of dietary factors in modulating glucose homeostasis in pregnancy. However, there is some evidence from animal research and limited human studies in non-pregnant subjects that n-3 LCPUFAs might be associated with improved insulin action (Fedor and Kelley 2009). Potential underlying mechanisms for an effect of n-3 fatty acids on insulin sensitivity have been suggested to be related to direct effects on adipose tissue function, such as a PPARγ-dependent increase in plasma adiponectin or through their anti-inflammatory actions (Kalupahana et al. 2011). Nevertheless, the results from intervention trials in healthy or overweight/obese volunteers (Egert et al. 2008, Ramel et al. 2008) and patients with impaired glucose tolerance (Fasching et al. 1991) or type 2 diabetes (Montori et al. 2000) remain inconclusive.

Human data on the role of n-3 fatty acids in glucose metabolism during pregnancy are mostly limited to epidemiological studies, which have yielded inconsistent results (Wang et al. 2000, Bo et al. 2001, Radesky et al. 2008). A recent large RCT explored the role of n-3 LCPUFAs during pregnancy in influencing the risk of developing gestational diabetes, but found no protective effect of maternal supplementation with n-3 LCPUFAs on the incidence of gestational diabetes (Zhou et al. 2012). Our results are in agreement with this report, suggesting that an enhanced supply of n-3 LCPUFAs is rather unlikely to relevantly affect glucose metabolism or insulin resistance during pregnancy or postpartum.

6.4 Insulin in cord blood

Comparable to maternal insulin concentrations, the intervention had no impact on insulin levels measured in cord blood. In contrast, a smaller previous study with 47 mother-infant pairs in which pregnant women were randomly assigned to consume a DHA-containing functional food providing ~200 mg/d DHA or placebo bar from 24 weeks gestation until delivery reported lower cord plasma insulin levels along with a reduced Ponderal Index at birth, potentially indicating beneficial effects of prenatal DHA supply for infant body

composition and insulin sensitivity (Courville et al. 2011). However, given our results based on a greater power and higher n-3 LCPUFA doses, a clinically meaningful effect of an enhanced maternal n-3 LCPUFA intake in the prenatal period on cord blood insulin concentrations seems rather unlikely.

Irrespective of the group assignment, girls displayed significantly higher cord plasma insulin levels compared to boys in the present study. This in accordance with other data (Shields et al. 2007, Regnault et al. 2011), although findings are not entirely consistent across the literature (Godfrey et al. 1996, Ong et al. 1999, Ong et al. 2000, Tsai et al. 2004).

In view of the lower birth weights but higher cord blood insulin levels in girls, it was suggested that girls might be inherently more insulin-resistant in utero and around birth compared to boys (Shields et al. 2007). This "gender insulin hypothesis" proposes that gender-specific genes affecting insulin sensitivity might account for the gender difference in birth weight (Wilkin and Murphy 2006).

6.5 Maternal HMW adiponectin over the course of pregnancy and lactation

Longitudinal analysis

Several human studies have demonstrated decreased adiponectin levels during normal pregnancy compared to the non-pregnant state (Asai-Sato et al. 2006, Catalano et al. 2006, Fuglsang et al. 2006, O'Sullivan et al. 2006). In the present study, maternal plasma HMW adiponectin levels were found to decrease significantly over the course of pregnancy and to remain low during the lactation period until 4 months pp. This finding is basically also reflected in previous longitudinal studies (Asai-Sato et al. 2006, Catalano et al. 2006, Fuglsang et al. 2006) although these predominantly reported data on total adiponectin concentrations. With regard to changes of circulating adiponectin levels over the course of pregnancy different temporal patterns have been observed: Asai-Sato et al. reported a gradual decline of adiponectin concentrations with advancing gestation (Asai-Sato et al. 2006), whereas Fuglsang et al. described an initial increase of adiponectin levels with a peak at mid-pregnancy followed by a subsequent continuous decline until late pregnancy (Fuglsang et al. 2006). Catalano et al. provided also data on the different adiponectin multimers (Catalano et al. 2006) and reported, that both HMW and LMW adiponectin multimers decline with advancing gestation, although the change was significant only for HMW adiponectin in late

pregnancy compared with pre-gravid values. Overall the hypoadiponectinemia in pregnancy was found to be reflected by a lower amount of HMW adiponectin and also by the ratio of high to low molecular weight multimers (Catalano et al. 2006). Moreover, cross-sectional studies comparing women at different stages of pregnancy reported significantly lower adiponectin levels in late compared to early pregnancy (Cseh et al. 2004, Nien et al. 2007), although the findings are not entirely consistent (Mazaki-Tovi et al. 2007, Mazaki-Tovi et al. 2008).

The underlying mechanisms leading to the decrease in circulating adiponectin concentration during pregnancy are still poorly understood; however there are some potential explanations which are currently discussed: First, the enlargement of adipose tissue stores during gestation might provide a possible explanation for the decrease in plasma adiponectin. According to a study by Catalano et al. the hypoadiponectinemia in pregnancy was reflected by a 2.5-fold decrease in white adipose tissue adiponectin mRNA expression accompanied by a 25 % increase in fat mass (Catalano et al. 2006). Furthermore, there is a significantly inverse correlation between adiponectin levels in pregnancy and both maternal BMI and fat mass (Cseh et al. 2004, Verhaeghe et al. 2005, Catalano et al. 2006, Fuglsang et al. 2006, O'Sullivan et al. 2006), which was also demonstrated by our data. Thus, it is conceivable that the accretion of adipose tissue stores might be acting as a negative feedback signal for adiponectin production and/or secretion by a yet unidentified mechanism (Nien et al. 2007).
Besides its correlation with maternal BMI and fat mass, we found a weak, but significantly inverse correlation between HMW adiponectin measured in the last trimester, and weight gain during pregnancy. This is in contrast to other reports, showing no correlation between adiponectin concentration and pregnancy weight gain (Retnakaran et al. 2004, Hilakivi-Clarke et al. 2012), and thus should be further investigated.

Moreover, it has been suggested that adiponectin is related to alterations in maternal carbohydrate metabolism characterized by progressive insulin resistance especially in the last trimester as an adaptation to meet the energy requirements of the growing fetus (Herrera 2000). Such a relationship is also supported by our data showing a significant inverse correlation between circulating adiponectin and markers of insulin sensitivity. We found adiponectin levels to be significantly negatively correlated with fasting insulin concentration and HOMA-IR which is in agreement with other studies (Catalano et al. 2006, O'Sullivan et al. 2006, Vitoratos et al. 2008). However, whether the impaired insulin action is the cause or

consequence of hypoadiponectinemia remains to be elucidated (Catalano et al. 2006). In contrast, there are several studies, which could not find an association between adiponectin and measures of insulin sensitivity during gestation (Lappas et al. 2005, McLachlan et al. 2006, Ritterath et al. 2010). According to findings of Ritterath et al. the decline in adiponectin levels in pregnancy seems to be more closely associated with changes in lipid metabolism such as triglyceride levels (Ritterath et al. 2010), than with increasing insulin resistance during pregnancy. However, such a correlation with blood lipids could not be established in our study.

Furthermore, the low-grade systemic inflammation evolving in pregnancy, associated with an enhanced production of cytokines, such as TNF-α and IL-6 from adipose tissue and the placenta (Hauguel-de Mouzon and Guerre-Millo 2006), has been implicated in the decrease of circulating adiponectin concentrations observed in pregnancy (Catalano et al. 2006).

Another explanation for the decreasing adiponectin levels over the course of pregnancy could be a suppressive effect of the lactogenic hormone prolactin (Asai-Sato et al. 2006), which is known to increase gradually during gestation. Such an effect of prolactin was also suggested to operate in the lactation period, when adiponectin levels remain low (Fuglsang et al. 2006) as also shown by our data or even further decrease as reported by others (Asai-Sato et al. 2006).
The further reduction in maternal adiponectin after delivery might also be suggestive of a partial contribution of the placenta to adiponectin production, which could be conceivable as both adiponectin and its receptors were found to be expressed by the placenta (Caminos et al. 2005, Lappas et al. 2005). However, since we and others did not see a significant change of adiponectin levels in the postpartum period compared to late pregnancy, one might suggest that during pregnancy the majority of circulating adiponectin is produced and released from the extra-placental adipose tissue (Vitoratos et al. 2008).

Effect of the intervention

In the present study, the intervention to reduce the dietary n-6/n-3 fatty acid ratio did not affect maternal plasma HMW adiponectin concentration at any time-point during pregnancy or lactation. Nonetheless, there is some evidence from both, animal and human studies, that consumption of either fish or fish oil supplements can increase circulating adiponectin levels

(Flachs et al. 2006, Krebs et al. 2006, Neschen et al. 2006, Lara et al. 2007, Guebre-Egziabher et al. 2008), an effect which might be mediated by the potential of n-3 LCPUFAs to activate PPARγ in adipose tissue (Semple et al. 2006). However, available data from human intervention studies are inconsistent, comprised to non-pregnant subjects and mostly focused on total adiponectin concentrations. Several studies involving healthy normal-weight subjects found increases in total adiponectin levels following fish consumption (Lara et al. 2007, Guebre-Egziabher et al. 2008, Kondo et al. 2010). Other studies achieved similar results after supplementation with fish oil in overweight or obese individuals (Krebs et al. 2006, Sneddon et al. 2008, Gammelmark et al. 2012). By contrast, a study by Ramel et al. studying the effect of varying amounts of n-3 LCPUFAs by providing either lean fish, fatty fish, fish oil capsules or a control supplement to overweight and obese young subjects found a reduction of adiponectin levels in all groups, with no significant difference between the groups (Ramel et al. 2008). In turn, in the study of Kratz et al. consumption of n-3 PUFA from plant and marine sources at levels of 3.5 % of dietary energy compared to a control diet did not have a significant effect on plasma total and HMW adiponectin in overweight or moderately obese healthy men over the course of a 14 week period (Kratz et al. 2008), which is compatible with our findings. Another recent study compared the short-term effects of fish vs. fish oil consumption over a period of 4 weeks on total and HMW adiponectin levels in overweight and obese adults and found different changes in adiponectin concentration between the groups. Whereas HMW adiponectin slightly increased in the "fish" group, a significant decrease was observed in the "fish oil supplement" group, indicating that the impact of fish intake on circulating levels may not be mediated by their content in n-3 LCPUFAs alone (Neale et al. 2012).

In conclusion, our results do not support a significant effect of an enhanced n-3 LCPUFA intake on circulating adiponectin levels during pregnancy despite some indications in the literature. However, in general the ability to make comparisons between previous studies and our own study might be limited due to the different approaches especially with regard to the studied population and the various kinds of dietary interventions used. Overall, based on the current literature, the relation of n-3 LCPUFAs to circulating adiponectin and its different multimers remains to be clarified and warrants further study.

6.6 Maternal triglycerides over the course of pregnancy and lactation

In both groups, serum triglyceride levels increased remarkably over the course of pregnancy to decrease even below baseline levels in the lactation period, reflecting the physiological metabolic adaptations in response to pregnancy. Marked changes in lipid metabolism characterized by hyperlipidemia, mainly corresponding to increases in the triglyceride fraction, are a physiological phenomenon of normal pregnancy (Herrera 2005). Serum triglyceride levels have been reported to rise continuously from around 8−10 weeks of gestation reaching a two- to threefold increase until term followed by a considerable decrease postpartum (Desoye et al. 1987, Butte 2000, King 2000).

The increasing triglyceride levels over the course of pregnancy are the result of several metabolic adaptations occurring under the influence of gestational hormones, and are also mediated by the increase in insulin resistance especially in the last trimenon of pregnancy. These include increased adipose tissue lipolytic activity, enhanced hepatic VLDL production and decreased removal from the circulation as a consequence of reduced adipose tissue lipoprotein lipase (LPL) activity (Herrera and Ortega 2008).

We observed a less pronounced increase in serum trigylceride levels in the intervention compared to the control group between the 15th week and the 32nd week of gestation. The resulting difference in triglyceride concentration between the groups was still apparent at 4 months pp, albeit no longer significant after correction for multiple testing. Our results are in agreement with the study of Helland et al., in which pregnant women were allocated to receive either 10 ml cod liver oil (n-3 PUFAs) or corn oil (n-6 PUFAs) daily from 17−19 weeks of gestation until three months after delivery (Helland et al. 2006). In contrast, another study reported no effect of supplementing pregnant women with n-3 LCPUFAs compared to olive oil starting from the 20th week of pregnancy until delivery on maternal lipid concentrations (Barden et al. 2006).

The triglyceride lowering potential of n-3 LCPUFAs is well established in non-pregnant subjects (Harris 1997). It has been consistently reported that this effect occurs in a dose-dependent manner and proportional to baseline concentrations (Balk et al. 2006, Harris et al. 2008). The precise underlying mechanisms, how n-3 LCPUFAs lower triglyceride levels are not fully understood; there is however convincing evidence that these fatty acids can both decrease hepatic VLDL triglyceride synthesis and release and enhance peripheral triglyceride

clearance from chylomicrons and VLDL particles due to increased LPL activity (Harris et al. 2008). Some of these effects might be mediated by peroxisome proliferator-activated receptors (PPARs), which have been shown to be activated by n-3 LCPUFAs (Sampath and Ntambi 2005). Thus, our results of a less pronounced increase in maternal triglyceride levels over the course of pregnancy are compatible with the well studied effects of n-3 LCPUFAs on blood lipids in the non-pregnant population.

In conclusion, our results can be regarded as a further indicator of the women´s compliance in implementing the dietary intervention resulting in an effectively reduced n-6/n-3 fatty acid ratio in the maternal diet which was previously shown by analysis of the fatty acid composition in various compartments (Hauner et al. 2012).

6.7 Leptin and total adiponectin in breast milk

Milk leptin

In the present study, mean leptin levels averaged 0.19 ng/ml in both groups with a high inter-individual variation, which is in accordance with previous studies reporting on leptin levels in skim milk (Lonnerdal and Havel 2000, Uysal et al. 2002, Bronsky et al. 2006, Savino et al. 2010, Schuster et al. 2011). Although milk leptin is suggested to be associated with the fat globules in breast milk, skim milk has been used for analysis, as lipids were shown to interfere with the leptin assay (Houseknecht et al. 1997, Smith-Kirwin et al. 1998, Lonnerdal and Havel 2000, Eilers et al. 2011). To achieve reliable results, preparation steps for separating leptin from milk fat globules and removing the lipid fraction afterwards, i.e. sonication of milk samples and subsequent centrifugation prior to analysis, were performed, as also described in other studies (Savino et al. 2010, Schuster et al. 2011).

With regard to changes of leptin concentrations in breast milk over the course of lactation, the available data are conflicting. Studies comparing leptin levels of colostrum, transitional and/or mature milk reported either a significant decrease (Ilcol et al. 2006, Eilers et al. 2011), increase (Doneray et al. 2009) or no difference (Aydin et al. 2008) between the different stages of lactation. Whereas a cross-sectional analysis of breast milk samples from 1−180 days of lactation suggested decreasing leptin concentration in breast milk over time (Ilcol et al. 2006), a recent longitudinal study reported relatively constant leptin levels from the first week after delivery until 6 months postpartum (Schuster et al. 2011). The latter finding is

consistent with our study showing no significant change over time in milk leptin concentration between 6 weeks and 4 months pp. Differences in the sample collection procedure and analytical method might partially explain these discrepant findings.

Correlations with maternal anthropometry

In the present study, leptin in breast milk correlated significantly positively with maternal pre-pregnancy BMI as well as with BMI and fat mass assessed during pregnancy. A correlation of breast milk leptin with pre-gravid BMI has been previously reported in other studies (Weyermann et al. 2007, Eilers et al. 2011). Furthermore, numerous studies (Houseknecht et al. 1997, Uysal et al. 2002, Miralles et al. 2006, Schuster et al. 2011, Fields and Demerath 2012, Schueler et al. 2013), even though not all (Ucar et al. 2000, Dundar et al. 2005), demonstrated a positive correlation of milk leptin with maternal BMI over the course of the lactation period, a finding which could not be directly verified in our study as maternal anthropometry was not assessed after delivery. Similar to our data, previous studies reported a correlation between milk leptin and maternal triceps skinfold thickness (Houseknecht et al. 1997) or maternal fat mass assessed by dual-energy X-ray absorptiometry (DEXA) (Schueler et al. 2013). In contrast, in another small study, no correlation of breast milk leptin with maternal total body fat percentage assessed by skinfold thickness measurements could be observed (Ucar et al. 2000). Nevertheless, our data and other previous reports strongly suggest, that the concentration of leptin in human breast milk reflects maternal adiposity as it is well established for plasma leptin (Houseknecht et al. 1997, Schuster et al. 2011).

Moreover, a positive correlation of milk and plasma leptin was observed in our and numerous earlier studies with significantly lower concentrations in breast milk compared to plasma (Houseknecht et al. 1997, Ucar et al. 2000, Bielicki et al. 2004, Ilcol et al. 2006, Miralles et al. 2006, Weyermann et al. 2006, Aydin et al. 2008, Schuster et al. 2011). This might indicate a barrier-conditioned transfer of leptin from the blood to breast milk (Casabiell et al. 1997, Schuster et al. 2011). As another source, epithelial cells in the mammary gland secreting leptin were suggested to directly contribute to the amount of leptin present in breast milk (Smith-Kirwin et al. 1998, Bonnet et al. 2002).

Milk total adiponectin

Breast milk total adiponectin concentrations ranged from 1 to 76 ng/ml in our cohort with a mean concentration of roughly 12 ng/ml in both groups, indicating a remarkable variation among individuals. On the whole, these levels are consistent with those reported in previous studies (Dundar et al. 2005, Bronsky et al. 2006, Martin et al. 2006, Weyermann et al. 2006, Cesur et al. 2012, Ozarda et al. 2012, Savino et al. 2012).

With regard to longitudinal changes in breast milk adiponectin concentration over the course of lactation available data are inconsistent. We found a slight but non significant decrease in total adiponectin concentration in breast milk between 6 weeks and 4 months pp, which is in line with findings of a recent small study showing no difference in breast milk adiponectin levels between the first and 4^{th} month of lactation (Cesur et al. 2012). In contrast, other studies found decreasing milk adiponectin concentrations over the course of lactation (Martin et al. 2006, Woo et al. 2009, Ley et al. 2012, Savino et al. 2012), whilst a cross-sectional study reported a positive relationship with duration of lactation (Ozarda et al. 2012). In another longitudinal study of 70 women a trend towards decreasing breast milk adiponectin levels over the first 3 months of lactation was observed followed by an increase up to 1 year pp which was proposed to be a result of longer intervals between breastfeeding due to the introduction of complementary feeding (Bronsky et al. 2006). Differences in study design (longitudinal vs. cross-sectional), the method and timing of milk collection, assay method or ethnic background of the study population (Martin et al. 2006, Weyermann et al. 2007) might provide an explanation for the discrepancies in these findings. Furthermore, the concentration of adiponectin in breast milk may vary according to hormonal and inflammatory status of breast-feeding women (Ozarda et al. 2012) or obstetrical variables such as nulliparity or gestational age (Ley et al. 2012).

Correlation with maternal anthropometry

In contrast to milk leptin, total adiponectin in breast milk did not correlate with any of the maternal anthropometric parameters such as pre-pregnancy BMI or BMI and skinfold thickness measures during pregnancy in the present study. This is consistent with previous studies reporting no correlation of milk adiponectin with pre-gravid BMI (Dundar et al. 2005, Weyermann et al. 2007, Ley et al. 2012). Nevertheless, there are other reports showing adiponectin in breast milk to be positively correlated with maternal weight before pregnancy

(Bronsky et al. 2006) and maternal post-pregnancy BMI (Martin et al. 2006). However, none of the mentioned previous studies has investigated the relation of milk adiponectin with measures of maternal fat mass, such as skinfold thickness measurements.
With regard to the positive relationship between breast milk adiponectin and maternal weight status or BMI reported in some studies, which is in contrast to the inverse correlation between adiponectin in plasma and adiposity, prolactin has been proposed as a functional link to explain this rather counterintuitive finding (Martin et al. 2006): Adiponectin secretion was shown to be negatively regulated by prolactin (Combs et al. 2003, Nilsson et al. 2005) and prolactin in turn is reduced in obesity (Kumar et al. 1993, Kopelman 2000). Therefore, it was suggested, that the suppression of adiponectin production by prolactin might be less pronounced in women with a higher weight status, resulting in higher adiponectin concentrations locally in breast adipose tissue and enhanced subsequent secretion into human milk (Martin et al. 2006).

In contrast to maternal plasma, where we analyzed the HMW form of adiponectin, total adiponectin was determined in breast milk samples. Nevertheless, the comparison of adiponectin between both compartments is warranted, as adiponectin in human breast milk was shown to be almost entirely present in its HMW form (Woo et al. 2009). We found HMW adiponectin levels in plasma to be strongly correlated with the concentrations of total adiponectin in breast milk with significantly lower levels present in breast milk compared to plasma. This observation is similar to several (Weyermann et al. 2006, Savino et al. 2012, Woo et al. 2012), albeit not all previous studies (Dundar et al. 2005, Cesur et al. 2012, Ozarda et al. 2012), analyzing total adiponectin in both compartments. This might be suggestive of a barrier-conditioned transfer of adiponectin from the bloodstream into breast milk (Bronsky et al. 2006). Alternatively, it is conceivable that adiponectin, as other peptides in human milk, could originate directly from mammary tissue (Newburg et al. 2010, Cesur et al. 2012).

Effect of the intervention on breast milk adipokines

We did not observe a significant effect of the intervention on the breast milk adipokines leptin or total adiponectin. This is in accordance with data from an experimental study in rats published by Korotkova et al. in which dams were fed isocaloric diets differing only in their lipid composition (Korotkova et al. 2002). They reported that milk leptin levels in dams did not significantly differ between groups which have received either a diet enriched in n-6

PUFA, n-3 PUFA or both for the last 10 days of gestation and throughout lactation. However, no studies are available at present addressing the effect of a dietary modification of the n-6/n-3 fatty acid ratio on milk adipokine concentrations in humans.

It is well established that an enhanced supply of n-3 fatty acids during pregnancy and/or lactation can modify both, the maternal plasma and breast milk fatty acid profile (Makrides et al. 1996, Helland et al. 1998b, Lauritzen et al. 2004, Dunstan et al. 2007, Bergmann et al. 2008, Much et al. 2013, Much et al. accepted 2013). Furthermore, we know that concentrations of adipokines in breast milk are closely linked to the respective levels in maternal plasma, albeit the concentrations in breast milk are substantially lower compared to plasma, as shown by previous studies and our own (Houseknecht et al. 1997, Weyermann et al. 2006, Schuster et al. 2011, Woo et al. 2012). Therefore, one could assume that the effect of the intervention on breast milk adipokines would parallel the effect on maternal plasma adipokines, which was also nil as already discussed in the previous sections.

6.8 Infant clinical outcomes at 2 years of age

We could not observe any significant differences in infant growth or fat mass assessed by skinfold thickness measurements between the groups at 2 years of age. Thus, the data at 2 years seem to reflect the same picture as shown over the first year of life, as published previously (Hauner et al. 2012). Despite considerable loss to follow-up, selection bias is unlikely as the most relevant clinical and socio-demographic characteristics did not significantly differ between the participants who continued the follow-up examinations and the ones who dropped out. Therefore, the initial randomization can be considered to be still valid after 2 years.

As previously reviewed by Muhlhausler et al., evidence from RCTs investigating the effect of maternal n-3 LCPUFA supplementation during pregnancy/lactation on infant body composition is uncertain due to mixed results with regard to the direction of the effect, but also owing to the large variation of the previous studies concerning the study design, such as the dose and timing of the intervention (pregnancy and/or lactation), compliance and the age at assessment (Lauritzen et al. 2005, Bergmann et al. 2007, Helland et al. 2008, Asserhoj et al. 2009, Muhlhausler et al. 2010). **Figure 17** provides an overview of available data from RCTs addressing the effect of maternal n-3 LCPUFA supplementation on offspring body composition, inclusive of trials that have been published after the review of Muhlhausler et al.

in 2010 (Campoy et al. 2011, Escolano-Margarit et al. 2011, Rytter et al. 2011, Bergmann et al. 2012).

Among the previous studies investigating the potential role of perinatal maternal n-3 LCPUFA supplementation on offspring body composition, two reported data on children in the same age range as the present analysis. Bergmann et al. compared growth of infants whose mothers either did or did not receive a supplement of 200 mg DHA from 21 weeks gestation until 3 months of lactation and observed significantly lower weight and BMI at 21 months pp in the infants of the DHA group (Bergmann et al. 2007). However, re-assessment of the children at 6 years of age did not reveal significant differences in any anthropometric variable (weight, height, BMI, sum of skinfolds), although a later increase of BMI z-scores, was observed in the longitudinal analysis over the 6 years in the DHA vs. the control group potentially indicating a delayed "adiposity rebound" (Bergmann et al. 2012). In contrast, Lauritzen et al. reported higher waist circumference and BMI in children of mothers having received a supplement containing 1.5 g n-3 LCPUFAs from birth up to 4 months postpartum compared to a control group (olive oil) at 2.5 years of age, but could not detect an effect on percentage fat mass as assessed by skinfold thickness measurements (Lauritzen et al. 2005). Again, re-assessment of the children at 7 years did not show significant differences between the groups with regard to BMI z-scores or skinfold measurements (Asserhoj et al. 2009). In view of these conflicting results, the replication of our previous null findings over the first year of life (Hauner et al. 2012) challenges once more the hypothesis that an early dietary reduction of the n-6/n-3 fatty acid ratio might have the potential to limit early adipose tissue growth.

Apart from RCTs, there are also some studies using an observational approach, which have addressed the role of maternal fatty acids in offspring body composition. Data from the US Project Viva Cohort showed an inverse association of cord blood, but not maternal DHA and EPA concentrations, with obesity risk and fat mass assessed as the sum of subscapular and triceps skinfold thickness at 3 years (Donahue et al. 2011). Moreover, a higher ratio of cord blood n-6/n-3 LCPUFAs was associated with a higher fat mass and odds of obesity in the children.

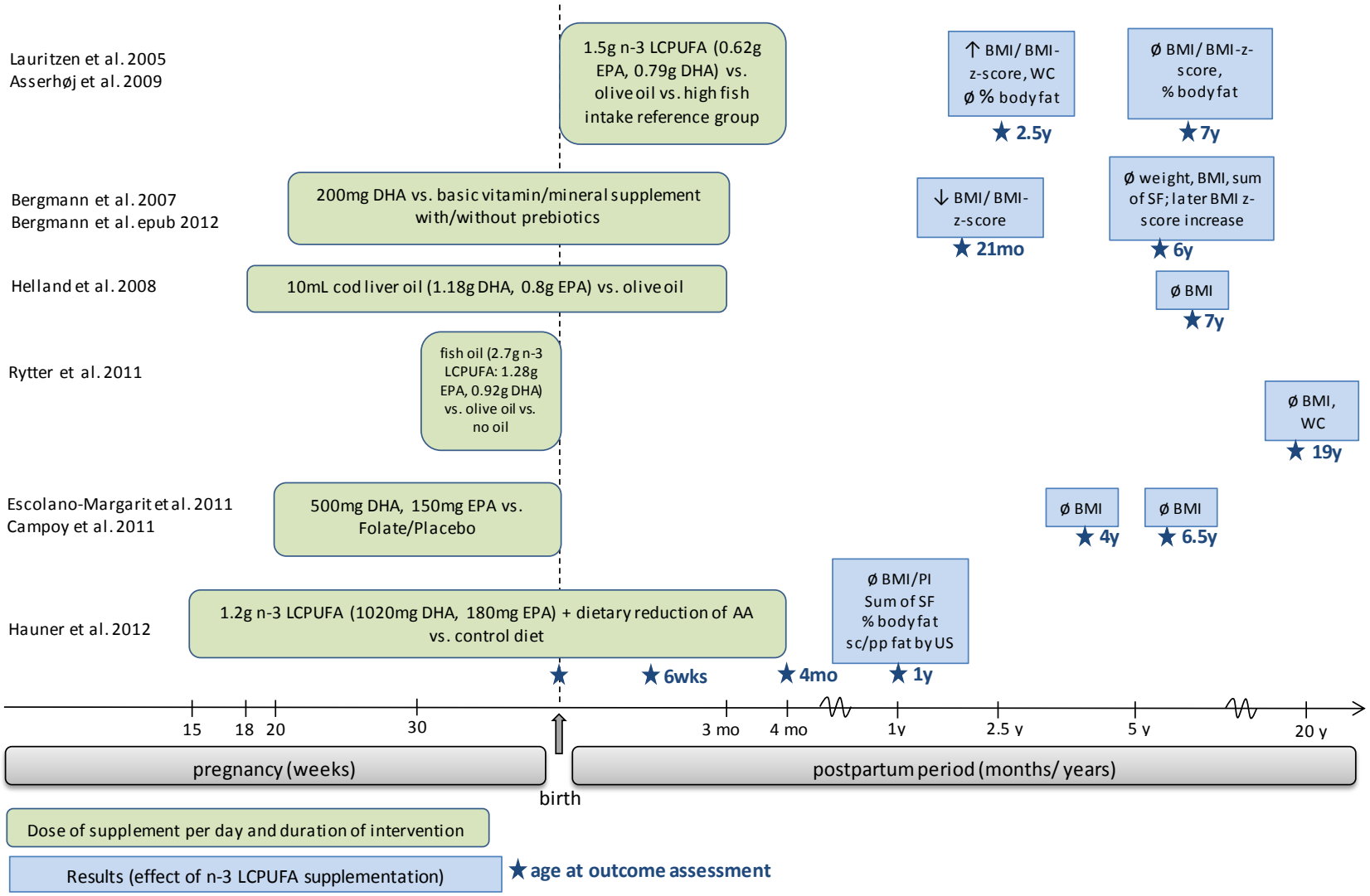

Figure 17 Overview of available RCTs investigating the effect of maternal n-3 LCPUFAs on offspring body composition (Hauner et al. accepted 2013)
(AA: arachidonic acid, PI: Ponderal Index, pp: preperitoneal, sc: subcutaneous, US: ultrasonography, WC: waist circumference, ø: no difference between groups)

In contrast, an observational analysis of our own data up to the first year of life revealed no consistent relationship of either the maternal or cord blood n-6/n-3 fatty acid ratio in red blood cells (RBCs) with infant adiposity (Much et al. 2013). However, in the same study, a significantly inverse relationship between maternal AA and n-6 LCPUFAs in RBCs at the 32nd week of gestation and both, infant BMI and Ponderal Index at 1 year pp, but not with fat mass, was found.

Interestingly, according to a recent analysis from a large British cohort, maternal plasma n-6 PUFA concentrations at 34 weeks gestation positively predicted offspring fat mass measured by DEXA at 4 and 6 years, whereas maternal n-3 PUFA were not consistently associated with offspring body composition (Moon et al. 2013). These results thus might suggest, that reducing the maternal n-6 PUFA intake might be a more effective approach in the prevention of offspring adiposity compared with an enhanced prenatal n-3 PUFA supply.

It seems important, that the present study and also the trials mentioned before only provide data on offspring body composition over a limited period in infancy or childhood and do not allow a final conclusion on long-term adiposity development. Nevertheless, given the potential tracking of obesity from infancy/childhood up to adulthood (Baird et al. 2005) and the widely accepted view of early life representing a critical period of adipose tissue development where the number of adipocytes is likely to be determined (Spalding et al. 2008), these data might serve as a suitable indicator for subsequent adiposity development at later stages. The only study to date with a follow-up period up to adolescence/early adulthood was recently published and found no difference in BMI, waist circumference and selected biochemical parameters between offspring of mothers supplemented with fish oil compared to the control group at the age of 19 years, clearly arguing against a long-term effect of fish oil supplementation during pregnancy on offspring adiposity in adolescence (Rytter et al. 2011). It is however noteworthy, that the intervention (2.7 g n-3 LCPUFAs daily) during this study was restricted to the 3rd trimester of pregnancy which corresponds to the shortest supplementation period compared with all other previous RCTs.

Therefore, long-term follow-up results of other studies involving a longer duration of exposure during the perinatal period, ideally covering both, the prenatal and postnatal phase, have to be awaited. To date, no other study reported data at puberty or beyond which seems to be an important period particularly with regard to the emerging sexual dimorphism in fat distribution (Wells 2007).

6.9 Association of maternal and cord blood parameters with infant clinical outcomes up to 2 years of age

In the observational analysis of the study, associations of both, maternal and cord blood parameters, with the infant anthropometric variables from birth up to 2 years pp were explored.

6.9.1 Maternal and cord blood leptin in relation to infant clinical outcomes

Maternal leptin in late pregnancy was found to be negatively related to birth weight, growth indices (BMI and Ponderal Index), and both, fat and lean body mass at birth. This is consistent with two previous studies showing an inverse association between leptin levels in pregnancy and birth weight (Verhaeghe et al. 2006, Retnakaran et al. 2012). Notably, in both studies, leptin emerged as the most important maternal metabolic parameter associated with birth weight. Another study reported higher maternal leptin levels measured directly after delivery to be associated with lower birth weight (Laml et al. 2001). On the contrary, other investigators could not find correlations between maternal leptin during pregnancy or shortly after delivery with newborn weight (Butte et al. 1997, Schubring et al. 1997, Helland et al. 1998a, Tamura et al. 1998). However, comparable to our results, an inverse correlation with placental weight was reported by Schubring et al. (1997), indicating that maternal leptin might indeed act as a negative growth regulator for the feto-placental unit.
The inverse relationship between maternal leptin levels and multiple growth parameters at the time of delivery in our study might suggest a role for leptin during pregnancy in signaling maternal energy stores, thereby modulating maternal satiety and energy intake through a negative feedback mechanism which may consequently regulate substrate supply and availability for the fetus. Interestingly, the inverse associations with infant weight and lean body mass persisted up to 2 years of age, independent of maternal BMI and other relevant confounders, suggesting long-term implications of circulating maternal leptin, potentially of placental origin, for infant growth. However, compared to cord blood leptin, the contribution of maternal leptin to infant body composition outcomes was rather weak.

In contrast to maternal leptin, leptin in cord blood was found to be strongly positively correlated with anthropometric measures at birth, including birth weight, BMI, and body fat as previously shown by other studies (Clapp and Kiess 1998, Schubring et al. 1999, Karakosta et al. 2011). Interestingly we found that cord blood leptin was also significantly related with

lean body mass at birth, which does not favor the possibility that cord leptin simply reflects fetal adiposity, but instead is involved in the regulation of intrauterine growth in general.
Of note, cord leptin was significantly negatively related with weight, BMI, Ponderal Index, fat and lean body mass in the offspring at 2 years of age indicating that a lower cord blood leptin level predicts greater weight gain and an increase in body weight, as suggested from findings of the US Project Viva Cohort (Mantzoros et al. 2009). This observation may be explained by the well-known function of this satiety-regulating hormone. Low leptin levels at birth may activate hypothalamic, neuroendocrine mechanisms that may result in increased appetite and food intake and thereby greater body weight at the age of 2 years. Thus, our data suggest, that early infancy represents a period highly sensitive for the anorexigenic effects of leptin. This is in agreement with animal data, indicating that sensitivity to the central effects of leptin is particularly high in young, immature rodents from birth, but markedly decreases with aging (Qian et al. 1998). However, as we observed no relationship with the sum of four skinfolds or percentage body fat, but with both, absolute fat mass and lean body mass, it is not unequivocally evident from our data whether cord blood leptin is a predictor for infant adiposity or merely overall somatic growth.
Like previous studies in term and preterm infants (Ong et al. 1999, Fonseca et al. 2004, Mantzoros et al. 2009, Parker et al. 2011) we here show a negative association between cord blood leptin and early infant weight gain which was still detectable at 2 years of age. This might have implications for later health as rapid weight gain in early infancy has been associated with metabolic risk factors and determinants of cardiovascular disease and type 2 diabetes in adolescence/early adulthood (Ekelund et al. 2007, Leunissen et al. 2009). Importantly, we add further to previous findings as we were able to differentiate between gain in fat mass vs. gain in lean body mass complementary to data on weight development alone. Interestingly, these additional analyses revealed that the relationship with weight gain seems to be driven by both, fat and lean body mass.

A very recent analysis from the Project Viva Cohort investigated associations of both, maternal and cord blood leptin with child adiposity at age 3 and 7 years (Boeke et al. 2013). In this study, higher maternal leptin concentrations at 26-28 weeks gestation were associated with lower BMI z-scores and waist circumference at 3 years and lower BMI z-scores at 7 years. Cord blood leptin was also found to be inversely associated with adiposity at 3 years, measured as BMI z-scores, waist circumference and skinfold thicknesses, as well as with BMI

z-scores at 7 years. In addition, data on children`s fat mass at 7 years measured by DEXA were available, but no consistent associations with maternal or cord blood leptin were found. Moreover, leptin levels in the 3 year old children were shown to be associated with greater weight gain and adiposity up to 7 years of age, suggesting that leptin resistance may emerge around the age of 3 years following a period of high leptin sensitivity around birth and in early infancy (Boeke et al. 2013).

In conclusion, in the present analysis, both maternal and cord blood leptin were significantly associated with infant growth and body composition outcomes at birth and up to 2 years of age. Overall, leptin in cord blood emerged as the stronger contributing factor compared with maternal leptin. The negative relationship of cord blood leptin with infant weight gain up to 2 years pp, through gain in both, fat and lean mass, suggests that fetal leptin could have long-term effects on regulating energy balance in early infancy and affects both, adipose and lean tissues.

6.9.2 Maternal insulin and HOMA-IR in relation to infant outcomes

In the present study, maternal variables of glucose metabolism were largely unrelated to the infant clinical outcomes, except that higher maternal HOMA-IR at 32nd weeks gestation was associated with lower lean body mass at birth. Although our data showed no explicit relationship with fat mass, this finding might be to some extent compatible with data showing increased body fat in newborns from mothers with GDM compared to women with normal glucose tolerance (Catalano et al. 2003) and the direct association of maternal glucose homeostasis with neonatal adiposity across the full range of maternal glycemia, and not restricted to manifest diabetes (HAPO-Study-Group 2009). However, in the follow-up of the latter study (Hyperglycemia and Adverse Pregnancy Outcome, HAPO-study) no such relationship could be found with adiposity at 2 years of age within a group of non-diabetic pregnant women (Pettitt et al. 2010), which is comparable to our data.

In another recent study, maternal insulin resistance during pregnancy, irrespective of glucose tolerance status, emerged as a significant independent predictor of infant weight gain and adiposity at 1 year of age measured by skinfold thicknesses (Hamilton et al. 2010). However, these findings might not be directly comparable to our study due to differing methodological approaches. It could thus be plausible, that measuring insulin resistance only in the fasting state as HOMA-IR is not sensitive enough to detect associations with infant adiposity or

weight gain, compared to a more complex assessment of insulin resistance under a glucose challenge.

In conclusion, maternal fasting insulin and HOMA-IR in the last trimester of pregnancy were largely unrelated to infant anthropometric variables from birth through the 2nd year of life indicating not to be major determinants for infant weight development and body composition.

6.9.3 Cord blood insulin in relation to infant outcomes

In contrast to maternal insulin and HOMA-IR, insulin in cord blood was found to be strongly positively correlated with birth weight and measures of newborn fat mass, which is consistent with previous findings (Godfrey et al. 1996, Ong et al. 1999, Ong et al. 2000, Tsai et al. 2004, Shields et al. 2007). These associations persisted even after adjustment for various maternal and child confounders suggesting a direct and independent influence of fetal insulin on adipose tissue growth in utero.

The major finding of our analyses up to the 2nd year of life was, that cord blood insulin was inversely associated with weight gain up to 2 years, even after multiple adjustments. This association with weight gain was stronger and significant only in girls, which is compatible with recent findings from Regnault et al. reporting cord blood C-peptide as a proxy for fetal insulin to be inversely associated with infant weight development over the 1st year of life in girls (Regnault et al. 2011). Slower weight gain has also been reported in infants born to mothers with gestational diabetes, although no sex-specific analyses are available here (Parker et al. 2011). Our data and previous findings thus might suggest a programming effect of fetal insulin exposure for early weight gain, for which girls might be more susceptible than boys (Regnault et al. 2011). The consequences of this effect with regard to later risk for metabolic diseases however remain to be clarified. It has been reported that girls are intrinsically more insulin-resistant compared with boys already at birth (Shields et al. 2007) and also later in childhood by the age of 5 (Murphy et al. 2004). Furthermore, type 2 diabetes in the young population is more common in girls (Murphy et al. 2004, Wilkin and Murphy 2006) and observational studies have related slower early growth patterns with insulin resistance or diabetes in later life (Bhargava et al. 2004, Barker et al. 2005).

The inverse association with infant weight gain might reflect the central effects of insulin as an adiposity signal synergistically to leptin by activating neurocircuits in the hypothalamus

involved in suppressing food intake and enhancing energy expenditure (Schwartz et al. 2000, Benoit et al. 2004). However, as the effect was only apparent in girls, this seems questionable. Alternatively, higher insulin levels in cord blood reflecting increased fetal insulin resistance may confer protection against excessive weight gain or even adipose tissue growth, at least in girls. Yet, this interpretation has to be taken cautiously as we could not detect significant associations between cord blood insulin and measures of body fat at 2 years of age, which is compatible to findings of the HAPO-study showing no association of cord blood C-peptide with rates of overweight or obesity and the sum of skinfolds $\geq 90^{th}$ percentile in the offspring of non-diabetic mothers at the age of 2 years (Pettitt et al. 2010).

To conclude, cord blood insulin was strongly correlated with birth weight and newborn adiposity, and significantly inversely associated with weight gain over the first 2 years of life in girls. This might suggest a role for fetal insulin in programming energy homeostasis in early life, which takes effect differently in both sexes.

6.9.4 Maternal triglycerides in relation to infant clinical outcomes

Maternal triglyceride levels were found to be mostly unrelated to the infant growth and body composition variables from birth up to 2 years of life in the present analysis. Increasing triglyceride concentrations over the course of pregnancy are a characteristic feature of normal pregnancy (Desoye et al. 1987). Higher triglyceride levels in the maternal circulation may enhance the concentration gradient across the placenta, resulting in accelerated transport and deposition of lipids in fetal tissues (Shafrir and Khassis 1982, Heerwagen et al. 2010). Against this theory, we could not find any significant associations of maternal triglycerides with neonatal body composition, apart from a weak positive relationship between the change of maternal serum triglyceride levels from 15^{th} until the 32^{nd} week of gestation and subsequent Ponderal Index at 4 months. However, this slight effect seems clinically irrelevant or even might have occurred by chance with regard to the numerous correlations explored. Thus, we could not confirm previous findings of a small pilot study showing the increase in triglycerides from early to late pregnancy to be highly predictive for neonatal adiposity (Barbour et al. 2009). Other studies reported associations between absolute maternal triglycerides levels and birth weight or large-for-gestational-age infants (Di Cianni et al. 2005, Misra et al. 2011, Vrijkotte et al. 2012). Different sampling time-points, weight and health status of the women might explain the discrepancy to our findings.

6.9.5 Maternal HMW adiponectin in relation to infant clinical outcomes

In the present study, we could not find any associations between maternal HMW adiponectin concentrations in the last trimester of pregnancy and anthropometric outcomes of the infants at birth or within the first two years of life, despite a positive relationship of maternal adiponectin with neonatal lean body mass at birth, and a positive association with infant weight at 2 years.

Previous studies reporting on maternal adiponectin in relation to infant anthropometric variables focused almost exclusively on growth outcomes at birth, predominantly birth weight, and largely did not provide data on longer-term associations with infant growth or body composition measured at later follow-up. In view of the negative relationship of circulating adiponectin with adiposity in adults, an inverse association with neonatal anthropometry might be expected. However, available data with respect to the relationship of maternal adiponectin and birth weight are inconsistent, ranging from an inverse relationship (Lopez-Bermejo et al. 2004, Ong et al. 2007) to no correlation at all (Chan et al. 2004) to even a positive association (Cseh et al. 2004).

When comparing the results of recent larger studies investigating the role of various maternal anthropometric and metabolic factors for neonatal body size, data remain conflicting: In a subanalysis of the HAPO study comprising almost 1500 pregnant women, maternal adiponectin levels were found to be negatively associated with birth weight, the sum of skinfolds, and percent body fat before and after adjustment for potential confounders including maternal BMI, fasting glucose and C-peptide. Exclusion of women with GDM did not change the results (Lowe et al. 2010). Likewise, in a recent study by Retnakaran et al. including almost 500 healthy pregnant women, maternal adiponectin emerged as a significant negative predictor of birth weight, even after controlling for several covariates, such as pre-pregnancy BMI and gestational weight gain (Retnakaran et al. 2012).

By contrast, the findings of other large trials were less clear. Weyermann et al. reported no relationship of maternal adiponectin at delivery with birth weight or birth weight according to gestational age in a German population-based cohort of more than 700 mothers and their newborns, although lower adiponectin concentrations in maternal serum were associated with higher Ponderal Index at birth (Weyermann et al. 2006). Moreover, in a cohort of 631 pregnant women maternal adiponectin was found to be negatively correlated with birth weight in the univariate analysis, but this association was lost after adjusting for potential

confounding factors including maternal body size, and metabolic markers like glucose and insulin (Verhaeghe et al. 2006). Thus, the authors concluded that the potential of adipokines to improve the prediction of birth weight based on clinical parameters such as maternal body weight or weight gain is rather limited.

All these previous studies have merely focused on total adiponectin concentrations and it has been hypothesized that differences in adiponectin isoform distribution might partly explain the conflicting findings in the existing literature. In this context, Ong et al. found the ratio of HMW to total adiponectin to be independently and inversely associated with infant birth weight suggesting that the proportion of maternal adiponectin in the HMW form rather than total adiponectin concentration may be a correlate of fetal growth (Ong et al. 2007).

With respect to longer-term associations of maternal adiponectin with infant anthropometric variables beyond birth, Woo et al. reported maternal serum adiponectin measured within the first 3 weeks postpartum to be associated with lower infant weight-for-age z-scores at birth, 3 and 6 months postpartum. However, later on, maternal adiponectin was not significantly associated with either mean weight-for-length or weight-for-age z-scores or growth trajectories during the second year of life (Woo et al. 2012). This is in contrast to our finding showing a significant positive association of maternal HMW adiponectin with infant body weight at 2 years of life, which remained even significant in the adjusted model correcting for several potential influencing variables. Overall, the paucity of existing data concerning associations between maternal adiponectin and infant anthropometric outcomes at later age does not allow a clear conclusion at the present time. A putative link between maternal plasma adiponectin and subsequent infant growth might be provided by adiponectin present in breast milk, which will be discussed in the following section.

6.9.6 Breast milk adipokines in relation to infant clinical outcomes

Besides various biomarkers in maternal and cord blood, we explored the relationship of adipokines present in breast milk with infant weight development and body composition up to 2 years of life. Whereas milk leptin was largely unrelated to the infants` anthropometric data, milk adiponectin was found to be significantly associated with a number of infant clinical outcomes up to 2 years pp. Milk adiponectin tended to be inversely related to infant anthropometry in the early postnatal period (up to 4 months pp), but afterwards was positively associated with infant weight gain and fat mass up to 2 years of life.

To our knowledge this is the first prospective study investigating long-term associations of milk adipokines with measures of infant body composition that go beyond the rather general growth parameters like weight or common growth indices in providing also data on infant fat mass and regional fat distribution assessed by skinfold thickness measurements. Only one previous study, which was cross-sectional, applied a more complex technique by exploring associations of various appetite-regulating hormones, growth factors and inflammatory factors in human breast milk with infant body composition by DEXA at 1 month of age (Fields and Demerath 2012).

Leptin in breast milk

Several previous studies reported associations between breast milk leptin levels and infant weight or BMI, suggesting a role for milk-borne leptin in the early regulation of body weight. Miralles et al. provided the first evidence for such an effect by showing an inverse relationship between milk leptin levels at 1 month of lactation and infant BMI at 1 and 2 years of age (Miralles et al. 2006). Likewise, three other studies reported negative associations between milk leptin and BMI development or infant weight gain within the first months of life (Doneray et al. 2009, Schuster et al. 2011, Fields and Demerath 2012), further arguing for a contribution of milk leptin in the control of appetite and food intake in early infancy. In view of the strong positive correlation between breast milk leptin levels and maternal fat mass, exposure to high leptin levels might protect infants nursed by overweight or obese mothers from excessive early weight gain as a result of the central actions of leptin providing a signal to the brain to increase satiety and enhance energy expenditure (Miralles et al. 2006, Schuster et al. 2011).

In contrast, we could not demonstrate associations of milk leptin concentrations with infant weight gain or adiposity, despite a transient negative relationship with infant weight, BMI and lean body mass at 4 months pp. This is in agreement with other human studies (Ucar et al. 2000, Uysal et al. 2002), including one large observational study of more than 670 mother-infant pairs, showing no clear relationship between milk leptin content at 6 weeks pp and the odds of overweight up to 2 years in infants breastfed for at least 6 months (Weyermann et al. 2007). The considerable disparity between the available studies concerning the time-points of milk sampling and infant anthropometric assessment might explain this discrepancy to some

extent. It could thus be possible, that effects conveyed by milk leptin at earlier stages after delivery (i.e. before 6 weeks pp) remained undetected in the present analysis.

Regardless of the somewhat inconsistent findings from human studies, animal studies provided some evidence for a direct cause-effect of orally supplied leptin in regulating food intake and weight gain (Sanchez et al. 2005, Pico et al. 2007, Palou and Pico 2009). Rats treated with physiological doses of leptin during the suckling period showed reduced caloric intake, lower body weight and fat mass compared to controls, suggesting that early leptin supply might confer protection against later fat accumulation up to adulthood (Pico et al. 2007). These and other observations indicate a role for exogenous leptin supply in the short- and long-term regulation of food intake and further metabolic adaptations, resulting in decreased fat mass and markers of the metabolic syndrome (Pico et al. 2007, Sanchez et al. 2008, Priego et al. 2010). Such a function of oral leptin could also be conceivable in breastfed infants, especially in very early life, when the appetite regulatory system is still immature (Sanchez et al. 2005, Schuster et al. 2011). However, our data do not support a sustained effect in the longer term up to 2 years of life.

Adiponectin in breast milk

The relationship of breast milk adiponectin with infant anthropometry has only been addressed by few previous studies (Weyermann et al. 2007, Woo et al. 2009, Woo et al. 2012). In a large cohort study including more than 670 mother-infant pairs, Weyermann et al. reported higher milk adiponectin to be related with greater odds of overweight at the age of 2 years (Weyermann et al. 2007). In contrast, in another study combining two independent cohorts from Mexico and USA, milk adiponectin concentrations were associated with lower infant weight and weight-for-length z-scores over the first 6 months of life (Woo et al. 2009). Interestingly, follow-up of the infants up to 2 years of life revealed that infants exposed to high milk total adiponectin experienced increasing weight-for-age and weight-for-length z-scores within the 2nd year of life whereas those exposed to lower milk adiponectin levels showed almost no change. These differences in growth trajectory remained significant even after adjustment for growth in the first 6 months and other covariates, indicating a reversal of the effect seen in early infancy.

In our study, milk adiponectin tended to be inversely related to infant anthropometric outcomes up to 4 months pp, but was significantly positively related to infant weight gain and

fat mass up to 2 years of life. Thus, our results are in agreement with the findings by Woo et al., suggesting that breast milk adiponectin may exert differential effects on weight gain during the period of active breastfeeding compared to later periods in infancy (Woo et al. 2012). In particular, the association of high human milk adiponectin with accelerated growth trajectories over the 2nd year of life was suggested to reflect catch-up growth after less pronounced weight gain in the early postpartum period (Woo et al. 2012). Our results extend previous findings in demonstrating that higher adiponectin in breast milk was not only associated with more pronounced weight gain, but also with higher fat mass assessed as the sum of skinfolds and percentage body fat in the 2nd year of life.

Considering the biological effects of adiponectin, an association with higher infant fat mass might appear counter-intuitive. However, although not supported by our data, milk adiponectin, in contrast to serum levels, has been shown to be positively associated with maternal pre-pregnancy and postpartum weight or BMI (Bronsky et al. 2006, Martin et al. 2006). Thus, higher breast milk adiponectin might reflect higher maternal BMI, which is an accepted risk factor for offspring overweight (Desai et al. 2013). Nonetheless, in the present study, the association of breast milk adiponectin with higher infant fat mass persisted even after adjustment for several confounders including maternal BMI, which might suggest other mechanisms to account for the observed relationship. Alternatively, a potential mechanistic link for our findings might be provided by the central effects of adiponectin, as it was shown to stimulate food intake and decrease energy expenditure in the hypothalamus (Kubota et al. 2007).

Bioavailability of breast milk adipokines

Generally, the potential of breast milk adipokines to affect infant growth and metabolism depends on their bioavailability. However, whether milk derived adipokines are gastrointestinally absorbed and reach the infants´ circulation is questionable. Due to their molecular size, absorption of both leptin and adiponectin might be nil or negligibly low (Gillman and Mantzoros 2007, Thompson 2012). In rats, orally ingested leptin was shown to be absorbed by the immature stomach of the suckling pups and transferred to the infant circulation (Casabiell et al. 1997, Sanchez et al. 2005), but this has not yet been proven in humans. For adiponectin, the transport across the intestinal mucosa into the serum was

recently demonstrated in mice after administering adiponectin through oro-gastric intubation (Newburg et al. 2010).

In human studies, both breast milk leptin and adiponectin levels have been shown to correlate with their respective plasma concentrations in the infant (Ucar et al. 2000, Newburg et al. 2010, Savino et al. 2012, Woo et al. 2012). Moreover, higher leptin levels were found in breast-fed vs. formula fed infants in some (Savino et al. 2002, Savino et al. 2004, Savino et al. 2005), but not all (Lonnerdal and Havel 2000, Petridou et al. 2005) studies, favoring the possibility that leptin from breast milk might reach the infant blood stream. Another major issue is whether these milk proteins, when absorbed, undergo degradation in the infant digestive tract or can be transferred to the target organs in its biologically active form (Newburg et al. 2010). It has been hypothesized that leptin might be protected against degradation by the infant digestive tract due to its association with milk fat globules (Sanchez et al. 2005). Furthermore, the reduced acidity of the infant stomach and limited gastric proteolysis might prevent degradation of milk proteins in the stomach (Martin et al. 2006, Newburg et al. 2010). Since adiponectin in human milk was shown to be predominantly present in a highly glycosylated form, this might support resistance against proteolytic degradation, as suggested for other proteins in human milk (Woo et al. 2009, Newburg et al. 2010).

In conclusion, milk leptin did not show a clear relationship with infant growth and body composition up to 2 years, whereas adiponectin was positively associated with infant fat mass and weight gain up to 2 years of life. Although the latter finding is compatible with previous studies (Weyermann et al. 2007, Woo et al. 2012), the underlying mechanisms for such an effect need to be clarified. Further larger longitudinal studies covering all stages of lactation are desirable to provide more conclusive evidence about various bioactive factors in breast milk, their interplay as well as their relevance for infant body composition.

6.10 Strengths and limitations

Strengths of the study are its longitudinal nature including the extensive assessment of infant growth and body composition over multiple time-points from birth through the first two years of life and the concurrent collection of maternal (plasma and breast milk) and fetal (cord blood) biological samples. This enabled us to precisely define the time window when associations between maternal or cord blood parameters and infant anthropometric variables become apparent, or change their direction. Furthermore, the analyses are based on a well-characterized study population allowing us to consider a range of potential maternal and child confounding factors in the multivariate analysis.

Nevertheless some limitations should be noted: It has to be acknowledged that skinfold thickness measurements do not represent a direct method for the assessment of total body fat and have limitations (Lingwood et al. 2012). The significance of the presented findings might further be limited by the relatively small sample size as well as the short observation period up to now. However, despite considerable loss to follow-up, selection bias seems unlikely, as the participants lost to follow-up did not significantly differ from those who continued the study regarding the major socio-demographic and clinical characteristics.

With regard to maternal markers of glucose metabolism, another limitation is that the study protocol did not include a standardized glucose tolerance test to assess maternal insulin resistance under a glucose challenge, so that we had to rely on measures of insulin resistance (HOMA-IR) in the fasting state. Thus, potential associations with insulin resistance or sensitivity markers based on a glucose challenge might have remained undetected.

Due to the limited available sample volume we were not able to measure other parameters related to fetal insulin secretion, such as C-peptide or proinsulin, or lipid parameters in umbilical cord blood which might also come into consideration as potential determinants of fetal growth (Schaefer-Graf et al. 2008).

Concerning our data on milk adipokines it should be considered, that both milk samples collected within the study reflect mature milk. Therefore, we could have missed the influence of adipokines in earlier stages of lactation mediated by colostrum or transitional milk.

7 Conclusion and perspectives

This study provides comprehensive data on the effect of lowering the dietary n-6/n-3 LCPUFA-ratio during pregnancy and lactation on various maternal and cord blood variables (adipokines, markers of glucose and lipid metabolism) and, based on an observational approach, their relationship with infant body composition and weight gain from birth up to 2 years of life.

In conclusion, the dietary intervention to reduce the n-6/n-3 fatty acids ratio in maternal nutrition did not have a major impact on maternal plasma adipokines (leptin and HMW adiponectin), fasting insulin and HOMA-IR over the course of pregnancy and lactation, but resulted in a less pronounced increase in triglycerides during pregnancy compared to the control group. Likewise, the respective parameters analyzed in umbilical cord blood (leptin axis and insulin) and in breast milk (leptin and total adiponectin) remained unaffected by the intervention. Thus, the results suggest that an enhanced supply of n-3 LCPUFAs is unlikely to have a clinically relevant effect on either maternal or cord blood adipokines and insulin metabolism. In contrast, the marked effect on maternal serum triglyceride levels over the course of pregnancy is comparable to the triglyceride-lowering potential of n-3 LCPUFAs in non-pregnant subjects (Harris 1997) and reflects the high compliance of the women in implementing the dietary intervention as previously evidenced by the analysis of the fatty acid composition in various compartments (Much et al. 2013).

Besides, the observational analyses based on the study population as a whole revealed several significant and striking results: Both, maternal and cord blood leptin, were significantly associated with infant growth and body composition outcomes at birth and up to 2 years of age. Overall, the contribution of cord leptin to the infant outcomes was stronger compared with maternal plasma leptin, suggesting that direct fetal exposures might have a greater impact on subsequent weight gain and body composition compared to the maternal metabolic background. The negative relationship of cord blood leptin with infant weight gain up to 2 years of life, through gain in both, fat and lean mass, suggests that fetal leptin could have long-term effects on regulating energy balance in early infancy and affects both, adipose and lean tissues.

Whilst maternal insulin, insulin resistance, HMW adiponectin and triglyceride levels were largely unrelated to infant growth and body composition, cord blood insulin was found to be

strongly positively associated with birth weight and neonatal body fat, and inversely with infant weight gain up to 2 years. This relationship with weight gain was stronger and significant only in girls suggesting that fetal insulin exposure might differentially impact on subsequent weight gain in both sexes as previously hypothesized (Regnault et al. 2011).

The observation that both cord blood leptin and insulin were inversely associated with infant weight gain might suggest a certain interaction of both signals. This would be in agreement with the concept that both hormones are part of a dual hormonal feedback loop, the so called adipoinsular axis, regulating the maintenance of nutrient balance and energy homeostasis (Kieffer and Habener 2000). A better understanding of the interplay of these hormones or other factors involved in the control of energy metabolism as well as potential gender-specific differences should be the focus of future research.

With regard to breast milk adipokines, milk leptin did not show a consistent relationship with infant growth and body composition up to 2 years, whereas adiponectin was positively associated with infant fat mass and weight gain up to 2 years of life. Although the latter finding is in agreement with previous studies (Weyermann et al. 2007, Woo et al. 2012), the underlying mechanisms for such an effect remain to be elucidated. Further larger longitudinal studies covering all stages of lactation are desirable to get a more detailed picture of the various bioactive factors present in breast milk as well as their relevance for the infant appetite regulatory system and later body composition. Moreover, additional work is needed to gain more insight into the uptake of proteins from breast milk into the infant circulation.

Taken together, our results support the notion of the perinatal period being a time window of high developmental plasticity in which certain environmental exposures could have implications for subsequent weight gain and body composition in early childhood. Whether the observed effects persist until preschool age will be further pursued in ongoing follow-up investigations up to the age of 5 years. However, the long-term clinical consequences, such as the risk for certain metabolic diseases, deserve additional study. Further prospective studies with sufficient power and applying more sophisticated techniques to measure body composition are warranted to evaluate not only potential effects of early exposures on weight gain and subcutaneous fat mass, but also on the development of other fat depots (e.g. intra-abdominal fat), which might be a better indicator of metabolic risk.

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10 Publications and other contributions

Publications

Original articles (in chronological order):

1. Hauner H, Much D, Vollhardt C, **Brunner S**, Schmid D, Sedlmeier EM, Heimberg E, Schuster T, Zimmermann A, Schneider KTM, Bader BL, Amann-Gassner U. Effect of reducing the n-6/n-3 long-chain polyunsaturated fatty acid (LCPUFA) ratio during pregnancy and lactation on infant adipose tissue growth within the first year of life (INFAT-study): an open-label, randomized, controlled trial. Am J Clin Nutr (2012) 95 (2): 383–94

2. **Brunner S**, Holub I, Theis S, Gostner A, Melcher R, Wolf P, Amann-Gassner U, Scheppach W, Hauner H. Metabolic effects of replacing sucrose by isomaltulose in subjects with type 2 diabetes – a randomized double-blind trial. Diabetes Care (2012) 35 (6): 1249−51 [not associated with the present work]

3. Much D, **Brunner S**, Vollhardt C, Schmid D, Sedlmeier EM, Brüderl M, Heimberg E, Bartke N, Boehm G, Bader BL, Amann-Gassner U, Hauner H. Effect of dietary intervention to reduce the n-6/n-3 fatty acid ratio on maternal and fetal fatty acid profile and its relation to offspring growth and body composition at 1 year of age. Eur J Clin Nutr (2013) 67 (3): 282−8

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6. **Brunner S**, Schmid D, Hüttinger K, Much D, Heimberg E, Sedlmeier EM, Brüderl M, Kratzsch J, Bader BL, Amann-Gassner U, Hauner H. Maternal metabolic variables and cord blood insulin in relation to offspring body composition and weight gain up to 2 years. Diabet Med. (2013) 30 (12):1500−7

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Reviews

1. **Brunner S**, Much D, Amann-Gassner U., Hauner H. Fischöl in der Schwangerschaft? – Neue Erkenntnisse. Adipositas (2011) 5 (4): 188−94

2. Hauner H, **Brunner S**, Amann-Gassner U: The role of dietary fatty acids for early human adipose tissue growth. Am J Clin Nutr. (2013) 98 (2):549S−55S

Congress contributions

Talks

1. 10th Congress of the International Society for the Study of Fatty Acids and Lipids (ISSFAL), 26−30. Mai 2012, Vancouver, BC, Kanada: "Breast milk fatty acid profile in relation to infant fat mass during the first year of life – Results from the INFAT-study"

2. 28. Jahrestagung der Deutschen Adipositasgesellschaft e.V., 4.−6. Okt. 2012, Stuttgart: „Effect of a dietary intervention to reduce the n-6/n-3 fatty acid ratio on the maternal and cord blood leptin axis and relation of leptin to body composition in the offspring – Results of the INFAT study"

Posters

1. 27. Jahrestagung der Deutschen Adipositas Gesellschaft e.V., 6.−8. Nov. 2011, Bochum: "The INFAT-study: Establishment of a follow-up programme. PEPO-Consortium of the Competence Network Obesity"

2. 50. Wissenschaftlicher Kongress der Deutschen Gesellschaft für Ernährung e. V., 20.−22. März 2013, Bonn: „Effect of a dietary intervention to reduce the n-6/n-3 fatty acid ratio on maternal parameters of glucose and lipid metabolism and cord blood insulin in relation to body composition and weight gain in the offspring up to 2 years of life"

3. Hot Topics Conference "Obesity and Pregnancy" of The International Association for the Study of Obesity (IASO) and The Obesity Society (TOS), 15.−17. Mai 2013, Boston, MA, USA: "Maternal metabolic variables and cord blood insulin in relation to offspring body composition and weight gain up to 2 years"

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12 Appendix

Table A- 1 Median values of the parameters in maternal plasma/serum over the course of pregnancy and lactation

		15th week gest.	**32nd week gest.**	**6 weeks pp**	**4 months pp**	$p_{over\ time}$ [1]	$p_{15th\ wk}$ [2]	$p_{32nd\ wk}$ [2]	p_{6wks} [2]	p_{4mo} [2]	p_{LMM} [3]	$p_{LMM\ adj}$ [4]
Leptin [ng/ml]	CG	13.32 [12.11] (92)	18.98 [17.48] (91)	10.98 [8.67] (41)	9.38 [9.00] (41)	**<0.001**	>0.99	>0.99	0.064	0.646	**0.027**	0.144[5]
	IG	13.60 [9.50] (91)	18.70 [14.52] (91)	7.19 [6.41] (41)	6.73 [8.58] (41)	**<0.001**						
sOB-R [ng/ml]	CG	31.73 [8.66] (41)	33.10 [10.84] (41)	26.88 [7.36] (41)	26.10 [6.97] (41)	**<0.001**	>0.99	0.534	>0.99	>0.99	0.692	0.820
	IG	31.02 [8.58] (41)	34.33 [7.61] (41)	27.28 [7.10] (41)	26.28 [7.56] (41)	**<0.001**						
FLI	CG	0.46 [0.45] (41)	0.73 [0.64] (41)	0.38 [0.41] (41)	0.35 [0.37] (41)	**<0.001**	0.165	0.084	0.217	>0.99	0.065	0.126
	IG	0.36 [0.26] (41)	0.49 [0.48] (41)	0.29 [0.28] (41)	0.25 [0.41] (41)	**<0.001**						
Insulin [pmol/l]	CG	37.20 [32.10] (92)	70.90 [49.65] (91)	30.70 [16.40] (41)	36.60 [40.60] (41)	**<0.001**	0.978	0.396	0.426	>0.99	**0.034**	0.097[6]
	IG	34.50 [21.50] (91)	64.70 [43.75] (91)	26.00 [18.00] (41)	33.40 [22.10] (41)	**<0.001**						
Glucose [mg/dl]	CG	79.00 [16.00] (101)	77.00 [16.50] (91)	77.00 [10.58] (75)	79.00 [11.00] (59)	0.095	>0.99	>0.99	>0.99	>0.99	0.652	0.763[6]
	IG	78.00 [14.50] (87)	78.50 [12.75] (90)	79.00 [13.00] (77)	80.50 [9.50] (64)	0.984						
HOMA-IR	CG	1.05 [1.07] (91)	1.92 [1.59] (87)	0.87 [0.61] (41)	1.13 [1.20] (40)	**<0.001**	>0.99	>0.99	0.276	0.919	0.123	0.465[6]
	IG	0.98 [0.65] (77)	1.89 [1.26] (88)	0.74 [0.48] (41)	0.91 [0.73] (41)	**<0.001**						
TG [mg/dl]	CG	100.50 [57] (101)	207.00 [106] (93)	78.50 [43] (75)	57.00 [34] (63)	**<0.001**	0.724	**<0.001**	**0.004**	0.088	**0.017**	**0.025**
	IG	96.00 [34] (102)	165.50 [60] (94)	57.50 [26] (72)	52.00 [23] (60)	**<0.001**						
HMW-AdipoQ [µg/ml]	CG	3.36 [2.09] (91)	2.33 [1.84] (92)	2.16 [1.67] (41)	1.80 [1.59] (41)	**<0.001**	0.132	>0.99	>0.99	0.668	0.10	0.09
	IG	2.85 [1.56] (91)	2.18 [1.25] (91)	1.97 [0.77] (41)	2.13 [1.03] (41)	**<0.001**						

AdipoQ: Adiponectin; CG: control group; FLI: free leptin index; gest.: gestation; IG: intervention group; pp: postpartum;TG: triglycerides

Data are presented as median [interquartile range] (N); p-values <0.05 are in bold face

[1] p-value for change over time within groups (Friedman-Test)

[2] p-values obtained from group comparisons by Mann-Whitney-Tests (CG vs. IG) at the different time-points, Bonferroni-corrected for multiple testing (original p-value *4)

[3] p-value obtained from linear mixed models including N=41 women per group in the longitudinal analysis over the course of pregnancy and lactation

[4] adjusted p-value from the linear mixed model (corrected for maternal pre-pregnancy BMI and age)

[5] additionally corrected for baseline levels

[6] additionally corrected for glucose tolerance status

Table A- 2 Change over time in maternal plasma/serum parameters by visit interval

		Level 1 vs. 2	Level 2 vs. 3	Level 3 vs. 4	Total period
Leptin	CG	<0.001	<0.001	n.s.	<0.001
	IG	<0.001	<0.001	n.s.	<0.001
sOB-R	CG	n.s.	<0.001	0.039	<0.001
	IG	0.003	<0.001	0.009	<0.001
FLI	CG	<0.001	<0.001	n.s.	<0.001
	IG	<0.001	<0.001	n.s.	<0.001
Insulin	CG	<0.001	<0.001	n.s.	<0.001
	IG	<0.001	<0.001	n.s.	<0.001
Glucose	CG	n.s.	n.s.	n.s.	n.s.
	IG	n.s.	n.s.	n.s.	n.s.
HOMA-IR	CG	<0.001	<0.001	n.s.	<0.001
	IG	<0.001	<0.001	n.s.	<0.001
Triglycerides	CG	<0.001	<0.001	0.003	<0.001
	IG	<0.001	<0.001	0.024	<0.001
HMW-Adiponectin	CG	<0.001	n.s.	n.s.	<0.001
	IG	<0.001	n.s.	0.006	<0.001

Level 1: 15th week of gestation, Level 2: 32nd week of gestation, Level 3: 6 weeks pp, Level 4: 4 months pp
CG: control group; IG: intervention group; FLI: free leptin index; n.s.: non significant (>0.05)
Data are presented as p-values from Wilcoxon or Friedman-Tests, respectively.
Results of Wilcoxon-Tests were Bonferroni-corrected for multiple testing (original p-value *3).

Table A- 3 Cord plasma leptin [ng/ml] in relation to infant growth and body composition outcomes up to 2 years (complete dataset)

		Unadjusted analysis		**Adjusted analysis**[1]	
Outcome variable	N	Beta [95 % CI]	p	Beta [95 % CI]	p
Birth					
Birth weight [g]	135	18.35 [11.29; 25.41]	**<0.001**	16.69 [8.81; 24.56]	**<0.001**
Placental weight [g]	126	2.71 [0,98; 4.44]	**0.002**	3.10 [1.15; 5.04]	**0.002**
PI [kg/m^3]	135	0.04 [0; 0.08]	0.066	0.05 [0; 0.09]	0.068
BMI [kg/m^2]	135	0.04 [0.02; 0.06]	**<0.001**	0.04 [0.01; 0.06]	**0.002**
Sum 4 SFT [mm]	128	0.09 [0.05; 0.13]	**<0.001**	0.11 [0.06; 0.16]	**<0.001**
Body fat [%]	128	0.10 [0.05; 0.14]	**<0.001**	0.12 [0.07; 0.17]	**<0.001**
Fat mass [g]	128	5.93 [3.72; 8.14]	**<0.001**	6.63 [4.06; 9.19]	**<0.001**
LBM [g]	128	12.38 [6.71; 18.05]	**<0.001**	9.97 [3.82; 16.12]	**0.002**
6 weeks pp					
Weight [g]	133	9.32 [-0.55; 19.19]	0.066	5.88 [-4.82; 16.58]	0.284
PI [kg/m^3]	133	0.05 [0.01; 0.1]	**0.019**	0.04 [-0.01; 0.10]	0.103
BMI [kg/m^2]	133	0.02 [0; 0.04]	0.062	0.01 [-0.01; 0.03]	0.437
Sum 4 SFT [mm]	133	0.02 [-0.05; 0.08]	0.621	0.01 [-0.06; 0.09]	0.752
Body fat [%]	133	0.02 [-0.03; 0.07]	0.538	0.01 [-0.05; 0.07]	0.672
Fat mass [g]	133	2.31 [-1.37; 6.00]	0.221	1.51[-2.80; 5.83]	0.492
LBM [g]	133	7.01 [-0.03; 14.05]	0.053	4.36 [-3.03; 11.76]	0.249
4 months pp					
Weight [g]	130	-1.75 [-13,89; 10.39]	0.778	-1.94 [-15.04; 11.16]	0.772
PI [kg/m^3]	130	0.01 [-0.03; 0.05]	0.565	0.02 [-0.03; 0.07]	0.417
BMI [kg/m^2]	130	0.00 [-0.02; 0.03]	0.837	0.01 [-0.02; 0.03]	0.713
Sum 4 SFT [mm]	129	0.00 [-0.06; 0.07]	0.920	0.01 [-0.06; 0.09]	0.731
Body fat [%]	129	0.00 [-0.04; 0.05]	0.893	0.01 [-0.04; 0.06]	0.691
Fat mass [g]	129	-0.29 [-4.81; 4.23]	0.900	0.13 [-5.17; 5.43]	0.962
LBM [g]	129	-2.07 [-11.1; 6.95]	0.653	-2.72 [-12.01; 6.56]	0.567
Weight gain [g] (birth – 4 months pp)	132	-20.04 [-31.07; -9.01]	**0.001**	-14.01 [-25.89; -2.12]	**0.021**
1 year pp					
Weight [g]	127	-9.58 [-28.1; 8.94]	0.312	-10.32[-30.26; 9.62]	0.312
PI [kg/m^3]	127	-0.01 [-0.04; 0.03]	0.722	-0.01 [-0.05; 0.03]	0.719
BMI [kg/m^2]	128	-0.01 [-0.03; 0.02]	0.517	-0.01 [-0.04; 0.02]	0.516
Sum 4 SFT [mm]	123	0.01 [-0.07; 0.08]	0.856	0.02 [-0.07; 0.11]	0.631
Body fat [%]	123	0.00 [-0.05; 0.06]	0.871	0.01 [-0.05; 0.07]	0.648
Fat mass [g]	123	-1.72 [-9.53; 6.08]	0.666	-0.79[-9.75; 8.17]	0.863
LBM [g]	123	-8.28 [-20.91; 4.36]	0.202	-9.90 [-23.13; 3.33]	0.145
Weight gain [g] (birth – 1 year pp)	128	-27.87 [-44.56; -11.19]	**0.001**	-23.44 [-41.06; -5.82]	**0.010**
2 years pp					
Weight [g]	93	-43.70 [-79.13; -8.26]	**0.016**	-49.72 [-88.27; -11.17]	**0.012**
PI [kg/m^3]	93	-0.03 [-0.07; 0.01]	0.116	-0.04 [-0.08; 0]	0.063
BMI [kg/m^2]	93	-0.04 [-0.07; 0]	**0.032**	-0.05 [-0.08; -0.01]	**0.017**
Sum 4 SFT [mm]	90	-0.05 [-0.14; 0.04]	0.305	-0.06 [-0.15; 0.04]	0.255
Body fat [%]	90	-0.03 [-0.09; 0.03]	0.293	-0.04 [-0.11; 0.03]	0.228
Fat mass [g]	90	-12.40 [-25.53; 0.735]	0.064	-14.86 [-29.49; -0.23]	**0.047**
LBM [g]	90	-32.27 [-57.40; -7.15]	**0.012**	-37.22 [-64.06; -10.37]	**0.007**
Weight gain [g] (birth – 2 years)	93	-65.24 [-97.26; -33.22]	**<0.001**	-66.50 [-101.86; -31.14]	**<0.001**
Gain fat mass [g] (birth – 2 years)	87	-20.26 [-33.37; -7.15]	**0.003**	-22.60 [-37.24; -7.96]	**0.003**
Gain LBM [g] (birth -2 years)	87	-47.94 [-70.08; -25.80]	**<0.001**	-47.82[-72.38; -23.25]	**<0.001**

Legend for **Table A- 3**:

LBM: lean body mass; PI: Ponderal Index; pp: postpartum; SFT: skinfold thicknesses
Data are presented as the unadjusted or adjusted regression coefficient beta (b) along with the 95 % confidence interval; $p \leq 0.05$ are in bold face
[1] Results were adjusted for maternal pre-pregnancy BMI, gestational weight gain, pregnancy duration, group and sex for the data at birth. Beyond birth, results were additionally adjusted for Ponderal Index at birth and breastfeeding status (exclusively/partially breastfed or formula) at 6 weeks or 4 months, respectively.

Table A- 4 Maternal insulin [pmol/l] and HOMA-IR at 32nd week of gestation in relation to infant growth and body composition outcomes up to 2 years pp (fully adjusted data)

	Maternal insulin			Maternal HOMA-IR		
Outcome variable	N	Beta [95 % CI][1]	p[1]	N	Beta [95 % CI][1]	p[1]
Birth						
Birth weight [g]	179	-1.19 [-2.84; 0.46]	0.159	172	-55.52 [-112.25; 1.20]	0.057
PI [kg/m^3]	179	-0.01 [-0.02; 0]	0.153	172	-0.23 [-0.56; 0.1]	0.175
BMI [kg/m^2]	179	-0.00 [-0.01; 0]	0.089	172	-0.15[-0.31; 0.01]	0.060
Sum 4 SFT [mm]	164	-0.00 [-0.01; 0.01]	0.676	157	-0.09 [-0.47; 0.29]	0.651
Body fat [%]	164	-0.00 [-0.01; 0.01]	0.754	157	-0.10 [-0.49; 0.3]	0.631
Fat mass [g]	164	-0.30 [-0.88; 0.28]	0.310	157	-12.61 [-32.63; 7.41]	0.219
LBM [g]	164	-1.11 [-2.40; 0.18]	0.094	157	-54.94 [-99.23; -10.64]	**0.016**
6 weeks pp						
Weight [g]	174	0.141 [-2.07; 2.35]	0.902	167	-17.16 [-98.29; 61.97]	0.671
PI [kg/m^3]	174	0.00 [-0.01; 0.01]	0.947	167	-0.16 [-0.53; 0.22]	0.416
BMI [kg/m^2]	174	0.00 [0; 0.01]	0.783	167	-0.07 [-0.25; 0.11]	0.430
Sum 4 SFT [mm]	174	0.01 [-0.01; 0.02]	0.396	167	0.34 [-0.22; 0.89]	0.235
Body fat [%]	174	0.00 [-0.01; 0.02]	0.531	167	0.22 [-0.23; 0.66]	0.339
Fat mass [g]	174	0.23 [-0.66; 1.12]	0.613	167	7.20 [-25.36; 39.75]	0.665
LBM [g]	174	-0.09 [-1.60; 1.42]	0.907	167	-24.36 [-77.53; 28.81]	0.371
4 months pp						
Weight [g]	171	-0.09 [-2.75; 2.57]	0.948	166	-4.57 [-108.23; 99.09]	0.931
PI [kg/m^3]	171	0.00 [-0.01; 0.01]	0.575	166	0.170 [-0.2; 0.54]	0.372
BMI [kg/m^2]	171	0.00 [0; 0.01]	0.692	166	0.060 [-0.15; 0.27]	0.577
Sum 4 SFT [mm]	171	0.01 [-0.01; 0.03]	0.273	166	0.561 [-0.09; 1.21]	0.095
Body fat [%]	171	0.01 [-0.01; 0.02]	0.315	166	0.350 [-0.08; 0.78]	0.114
Fat mass [g]	170	0.28 [-0.87; 1.43]	0.637	165	20.083 [-24.26; 64.43]	0.376
LBM [g]	170	-0.69 [-2.56; 1.17]	0.468	165	-34.487 [-105.59; 36.62]	0.343
1 year pp						
Weight [g]	168	-2.95 [-6.90; 1.00]	0.146	162	-65.676 [-218.76; 87.41]	0.402
PI [kg/m^3]	168	-0.01 [-0.02; 0]	0.100	162	-0.270 [-0.58;0.04]	0.091
BMI [kg/m^2]	168	-0.01 [-0.01; 0]	0.060	157	-0.177 [-0.39; 0.03]	0.101
Sum 4 SFT [mm]	163	-0.01 [-0.02; 0.01]	0.474	157	0.100 [-0.58; 0.78]	0.773
Body fat [%]	163	-0.01 [-0.02; 0.01]	0.442	157	0.045 [-0.42;0.51]	0.847
Fat mass [g]	163	-1.05 [-2.80; 0.70]	0.242	157	-6.343 [-74.86; 62.17]	0.856
LBM [g]	163	-1.67 [-4.36; 1.02]	0.225	157	-40.538 [-144.14; 63.07]	0.444
Weight gain [g] (birth – 1 year pp)	169	-2.74 [-6.51; 1.03]	0.153	162	-51.70 [-197.70; 94.30]	0.485
2 years pp						
Weight [g]	118	-3.56 [-10.32; 3.21]	0.300	115	-104.03 [-376.38; 168.3]	0.451
PI [kg/m^3]	118	-0.01 [-0.01; 0.00]	0.250	115	-0.11 [-0.46; 0.23]	0.518
BMI [kg/m^2]	118	-0.01 [-0.01; 0.0]	0.200	115	-0.12 [-0.40; 0.16]	0.413
Sum 4 SFT [mm]	110	-0.00 [-0.02; 0.02]	0.832	107	0.19 [-0.49; 0.87]	0.586
Body fat [%]	110	-0.00 [-0.01; 0.02]	0.793	107	0.13 [-0.34; 0.60]	0.580
Fat mass [g]	110	-0.96 [-3.45; 1.54]	0.449	107	-7.37 [-109.25; 94.51]	0.886
LBM [g]	110	-2.73 [-7.35; 1.89]	0.244	107	-95.96 [-283.98; 92.05]	0.314
Weight gain [g] (birth – 2 years pp)	118	-4.01 [-10.47; 2.28]	0.206	115	-72.44 [-326.58; 181.70]	0.573

LBM: lean body mass; PI: Ponderal Index; pp: postpartum; SFT: skinfold thicknesses
Data are presented as the adjusted regression coefficient beta (b) along with the 95 % confidence interval; p≤0.05 are in bold face

[1] Results were adjusted for maternal pre-pregnancy BMI, gestational weight gain, glucose tolerance status, pregnancy duration, group and sex for the data at birth. Beyond birth, results were additionally adjusted for Ponderal Index at birth and breastfeeding status (exclusively/partially breastfed or formula) at 6 weeks or 4 months, respectively.

Table A- 5 Cord plasma insulin [pmol/l] in relation to infant growth and body composition outcomes up to 2 years (complete dataset)

		Unadjusted analysis		**Adjusted analysis**[1]	
Outcome variable	N	Beta [95 % CI]	p	Beta [95 % CI]	p
Birth					
Birth weight [g]	137	1.68 [0.03; 3.33]	**0.048**	1.87 [0.27;3.47]	**0.023**
PI [kg/m^3]	137	-0.00 [-0.01; 0.01]	0.875	-0.00 [-0.01;0.01]	0.803
BMI [kg/m^2]	137	0.00 [0; 0.01]	0.434	0.00 [-0.00; 0.01]	0.423
Sum 4 SFT [mm]	130	0.02 [0.01; 0.02]	**0.001**	0.02 [0.01; 0.03]	**0.003**
Body fat [%]	130	0.02 [0.01; 0.03]	**0.001**	0.02 [0.01; 0.03]	**0.002**
Fat mass [g]	130	0.81 [0.31; 1.31]	**0.002**	0.80 [0.28; 1.32]	**0.003**
LBM [g]	130	0.89 [-0.39; 2.17]	0.176	1.01 [-0.21; 2.24]	0.105
6 weeks pp					
Weight [g]	133	0.87 [-1.32; 3.07]	0.437	1.39 [-0.68; 3.46]	0.186
PI [kg/m^3]	133	0.00 [-0.01; 0.01]	0.480	0.00 [-0.01; 0.01]	0.438
BMI [kg/m^2]	133	0.00 [0; 0.01]	0.930	0.00 [-0.00; 0.01]	0.767
Sum 4 SFT [mm]	133	0.01 [0; 0.02]	0.109	0.01 [-0.00; 0.03]	0.069
Body fat [%]	133	0.01 [0; 0.02]	0.143	0.01 [-0.00; 0.02]	0.102
Fat mass [g]	133	0.50 [-0.3; 1.31]	0.224	0.67 [-0.15; 1.49]	0.108
LBM [g]	133	0.37 [-1.2; 1.94]	0.646	0.72 [-0.72; 2.15]	0.325
4 months pp					
Weight [g]	131	-1.03 [-3.71; 1.64]	0.450	-0.31 [-2.85; 2.23]	0.809
PI [kg/m^3]	131	0.00 [-0.01; 0.01]	0.927	0.00 [-0.01; 0.01]	0.817
BMI [kg/m^2]	131	-0.00 [-0.01; 0]	0.578	0.00 [-0.01; 0.01]	0.904
Sum 4 SFT [mm]	130	0.01 [-0.01; 0.02]	0.503	0.01 [-0.01; 0.02]	0.529
Body fat [%]	130	0.00 [-0.01; 0.01]	0.603	0.00 [-0.01; 0.01]	0.624
Fat mass [g]	130	-0.04 [-1.04; 0.95]	0.933	0.12 [-0.91; 1.14]	0.821
LBM [g]	130	-0.91 [-2.89; 1.07]	0.368	-0.29 [-2.10; 1.52]	0.753
Weight gain [g] (birth – 4 months pp)	131	-2.710 [-5.22; -0.2]	**0.036**	-2.11[-4.56; 0.33]	0.092
1 year pp					
Weight [g]	127	-3.00 [-7.06; 1.06]	0.150	-1.58 [-5.48; 2.32]	0.424
PI [kg/m^3]	127	-0.01 [-0.02; 0]	0.066	-0.01 [-0.02; 0.00]	0.097
BMI [kg/m^2]		-0.01 [-0.01; 0]	0.053	-0.00 [-0.01; 0.00]	0.121
Sum 4 SFT [mm]	127	-0.00 [-0.02; 0.02]	0.930	-0.00 [-0.02; 0.02]	0.901
Body fat [%]	123	-0.00 [-0.01; 0.01]	0.733	-0.00 [-0.01; 0.01]	0.765
Fat mass [g]	123	-0.73 [-2.44; 0.99]	0.408	-0.43 [-2.18; 1.33]	0.630
LBM [g]	123	-2.39 [-5.15; 0.37]	0.092	-1.17 [-3.78; 1.44]	0.375
Weight gain [g] (birth – 1 year pp)	127	-4.65 [-8.39; -0.92]	**0.016**	-3.36 [-7.02; 0.28]	0.071
2 years pp					
Weight [g]	93	-7.60 [-13.79; -1.41]	**0.017**	-5.50 [-12.12; 1.13]	0.103
PI [kg/m^3]	93	-0.01 [-0.01; 0.00]	0.064	-0.01 [-0.01; 0.00]	0.211
BMI [kg/m^2]	93	-0.01 [-0.01; -0.00]	**0.021**	-0.01 [-0.01; 0.00]	0.115
Sum 4 SFT [mm]	90	-0.01 [-0.01; 0.00]	0.064	-0.01 [-0.03; 0.01]	0.226
Body fat [%]	90	-0.01 [-0.02; 0.01]	0.269	-0.01 [-0.02; 0.01]	0.249
Fat mass [g]	90	-2.36 [-4.65; -0.07]	**0.043**	-2.02 [-4.48; 0.43]	0.105
LBM [g]	90	-5.60 [-10.00; -1.21]	**0.013**	-3.80 [-8.45; 0.85]	0.108
Weight gain [g] (birth – 2 years pp)	93	-8.66 [-14.46; -2.85]	**0.004**	-6.90 [-13.13; -0.67]	**0.030**

LBM: lean body mass; PI: Ponderal Index; pp: postpartum; SFT: skinfold thicknesses
Data are presented as the adjusted regression coefficient beta (b) along with the 95 % confidence interval.
p≤0.05 are in bold face
[1] Results were adjusted for maternal pre-pregnancy BMI, gestational weight gain, glucose tolerance status, pregnancy duration, group and sex for the data at birth. Beyond birth, results were additionally adjusted for Ponderal Index at birth and breastfeeding status (exclusively/partially breastfed or formula) at 6 weeks or 4 months, respectively.

Table A- 6 Maternal triglycerides (TG) [mg/dl] at 32nd week of gestation and change in TG (ΔTG) [mg/dl] from 15th week until 32nd week of gestation in relation to infant growth and body composition outcomes up to 2 years pp (fully adjusted data)

	Maternal TG			Maternal ΔTG		
Outcome variable	N	Beta [95 % CI][1]	p[1]	N	Beta [95 % CI] [1]	p[1]
Birth						
Birth weight [g]	186	-0.54 [-1.56; 0.49]	0.308	184	0.12 [-1.02; 1.26]	0.842
PI [kg/m^3]	186	-0.00 [-0.01; 0]	0.539	184	-0.00 [-0.01; 0.01]	0.991
BMI [kg/m^2]	186	-0.00 [0; 0]	0.449	184	0.00[0; 0]	0.865
Sum 4 SFT [mm]	167	0.00 [0; 0.01]	0.524	165	0.00 [0; 0.01]	0.253
Body fat [%]	167	0.00 [0; 0.01]	0.441	165	0.01 [0; 0.01]	0.179
Fat mass [g]	167	-0.00 [-0.37; 0.36]	0.983	165	0.18 [-0.21; 0.57]	0.358
LBM [g]	167	-0.52 [-1.33; 0.29]	0.211	165	0.03 [-0.86; 0.91]	0.956
6 weeks pp						
Weight [g]	178	-0.97 [-2.33; 0.4]	0.167	176	-0.34 [-1.87; 1.19]	0.663
PI [kg/m^3]	178	-0.00 [-0.01; 0]	0.693	176	0.00 [-0.01; 0.01]	0.661
BMI [kg/m^2]	178	-0.00 [0; 0]	0.397	176	-0.00 [0; 0]	0.766
Sum 4 SFT [mm]	178	-0.00 [-0.01; 0.01]	0.489	176	-0.00 [-0.01; 0.01]	0.681
Body fat [%]	178	-0.00 [-0.01; 0]	0.461	176	-0.00 [-0.01; 0.01]	0.688
Fat mass [g]	178	-0.355 [-0.9; 0.19]	0.205	176	-0.22 [-0.83; 0.4]	0.489
LBM [g]	178	-0.611 [-1.54; 0.32]	0.200	176	-0.12 [-1.17; 0.92]	0.816
4 months pp						
Weight [g]	172	-0.62 [-2.27; 1.03]	0.463	170	-0.23 [-2.1; 1.65]	0.814
PI [kg/m^3]	172	0.01 [0; 0.01]	0.085	170	0.01 [0; 0.01]	**0.025**
BMI [kg/m^2]	172	0.00 [0; 0]	0.347	170	0.00 [0; 0.01]	0.211
Sum 4 SFT [mm]	172	0.01 [0; 0.02]	0.260	170	0.00 [-0.01; 0.01]	0.729
Body fat [%]	172	0.00 [0; 0.01]	0.320	170	0.00 [-0.01; 0.01]	0.935
Fat mass [g]	171	0.06 [-0.64; 0.76]	0.864	169	-0.06 [-0.85; 0.73]	0.879
LBM [g]	171	-0.72 [-1.86; 0.41]	0.214	169	-0.18 [-1.47; 1.12]	0.787
1 year pp						
Weight [g]	168	-1.46 [-3.83; 0.92]	0.231	166	-0.74 [-3.42; 1.94]	0.587
PI [kg/m^3]	168	-0.00 [-0.01; 0]	0.725	166	0.00 [0; 0.01]	0.697
BMI [kg/m^2]	168	-0.00 [0; 0]	0.427	166	0.00 [0; 0]	0.991
Sum 4 SFT [mm]	163	0.00 [-0.01; 0.01]	0.515	161	0.01 [-0.01; 0.02]	0.366
Body fat [%]	163	0.00 [0; 0.01]	0.535	161	0.00 [0; 0.01]	0.426
Fat mass [g]	163	-0.12 [-1.18; 0.94]	0.821	161	0.13 [-1.06; 1.33]	0.826
LBM [g]	163	-1.46 [-3.06; 0.15]	0.078	161	-1.01 [-2.82; 0.8]	0.276
Weight gain [g] (birth – 1 year pp)	168	-0.98 [-3.29; 1.34]	0.411	166	-0.82 [-3.46; 1.81]	0.541
2 years pp						
Weight [g]	118	-2.32 [-6.31; 1.67]	0.257	116	-0.61 [-5.21; 4]	0.798
PI [kg/m^3]	118	0.00 [0; 0.01]	0.651	116	0.01 [0; 0.01]	0.071
BMI [kg/m^2]	118	-0.00 [0; 0]	0.876	116	0.00 [0; 0.01]	0.238
Sum 4 SFT [mm]	110	-0.01 [-0.02; 0]	0.208	109	-0.00 [-0.01; 0.01]	0.829
Body fat [%]	110	-0.00 [-0.01; 0]	0.198	109	-0.00 [-0.01; 0.01]	0.794
Fat mass [g]	110	-1.12 [-2.58; 0.33]	0.133	109	-0.48 [-2.18; 1.22]	0.581
LBM [g]	110	-1.98 [-4.71; 0.74]	0.157	109	-1.34 [-4.54; 1.86]	0.415
Weight gain [g] (birth – 2 year pp)	118	-1.85 [-5.63; 1.93]	0.339	116	-0.77[-5.16; 3.62]	0.730

LBM: lean body mass; PI: Ponderal Index; pp: postpartum; SFT: skinfold thicknesses
Data are presented as the adjusted regression coefficient beta (b) along with the 95 % confidence interval.
p≤0.05 are in bold face
[1] Results were adjusted for maternal pre-pregnancy BMI, gestational weight gain, glucose tolerance status, pregnancy duration, group and sex for the data at birth. Beyond birth, results were additionally adjusted for Ponderal Index at birth and breastfeeding status (exclusively/partially breastfed or formula) at 6 weeks or 4 months, respectively.

Table A- 7 Maternal plasma HMW adiponectin [µg/ml] at the 32nd week of gestation in relation to infant growth and body composition outcomes up to 2 years pp (complete dataset)

		Unadjusted analysis		**Adjusted analysis**[1]	
Outcome variable	N	Beta [95 % CI]	p	Beta [95 % CI]	p
birth					
Weight [g]	183	55.51 [-3.74; 114.77]	0.07	45.36 [-10.86; 101.58]	0.12
PI [kg/m^3]	183	0.12 [-0.18; 0.42]	0.44	0.11 [-0.21; 0.42]	0.51
BMI [kg/m^2]	183	0.11 [-0.05; 0.26]	0.17	0.09 [-0.06; 0.24]	0.23
Sum 4 SFT [mm]	168	-0.02 [-0.37; 0.33]	0.91	-0.01 [-0.38; 0.35]	0.94
Body fat [%]	168	-0.06 [0.43; 0.30]	0.73	-0.06 [-0.44; 0.31]	0.75
Fat mass [g]	168	7.15 [-11.80; 26.18]	0.46	6.92 [-12.63; 26.47]	0.49
LBM [g]	167	49.48 [2.62; 96.34]	**0.04**	44.92 [1.74; 88.10]	**0.04**
6 weeks pp					
Weight [g]	178	49.92 [-29.01; 128.85]	0.22	45.43 [-31.18; 122.04]	0.25
PI [kg/m^3]	178	0.14 [-0.20; 0.47]	0.42	0.10 [-0.26; 0.45]	0.60
BMI [kg/m^2]	178	0.10 [-0.07; 0.27]	0.25	0.08 [-0.09; 0.25]	0.35
Sum 4 SFT [mm]	178	0.04 [-0.45; 0.53]	0.87	0.16 [-0.37; 0.69]	0.55
Body fat [%]	178	0.02 [-0.37; 0.41]	0.91	0.12 [-0.30; 0.54]	0.58
Fat mass [g]	178	11.86 [-17.69; 41.40]	0.43	15.10 [-15.79; 45.98]	0.34
LBM [g]	178	38.07 [-17.56; 93.69]	0.18	30.33 [-21.88; 82.55]	0.26
4 months pp					
Weight [g]	173	7.72 [-85.3; 100.75]	0.87	9.09 [-81.08; 99.25]	0.84
PI [kg/m^3]	173	0.04 [-0.27; 0.35]	0.81	0.07 [-0.26; 0.40]	0.68
BMI [kg/m^2]	173	0.03 [-0.15; 0.20]	0.78	0.04 [-0.15; 0.22]	0.68
Sum 4 SFT [mm]	173	-0.29 [-0.83; 0.26]	0.31	-0.20 [-0.77; 0.37]	0.50
Body fat [%]	173	-0.17 [-0.53; 0.19]	0.35	-0.12 [-0.50; 0.25]	0.52
Fat mass [g]	172	-8.80 [-44.94; 27.33]	0.63	-5.11 [-43.18; 32.95]	0.79
LBM [g]	172	17.72 [-49.98; 85.42]	0.61	16.42 [-45.8; 78.65]	0.61
Weight gain (birth ─ 4 mo pp)	173	-53.41 [-140.29; 22.48]	0.23	-37.76 [-124.97; 49.45]	0.40
1 year pp					
Weight [g]	169	106.07 [-30.84; 242.98]	0.13	110.99 [-19.41; 241.39]	0.10
PI [kg/m^3]	169	0.07 [-0.21; 0.34]	0.63	0.07 [-0.21; 0.34]	0.63
BMI [kg/m^2]	169	0.10 [-0.09, 0.29]	0.30	0.10 [-0.08; 0.28]	0.27
Sum 4 SFT [mm]	164	0.21 [-0.35; 0.77]	0.47	0.34 [-0.25; 0.92]	0.26
Body fat [%]	164	0.16 [-0.22; 0.55]	0.41	0.24 [-0.16; 0.64]	0.23
Fat mass [g]	164	39.05 [-18.09; 96.19]	0.18	46.49 [-11.66; 104.64]	0.12
LBM [g]	164	74.40 [-20.54; 169.33]	0.13	68.33 [-20.59; 157.24]	0.13
Weight gain (birth ─ 1 year pp)	169	45.03 [-82.98; 173.04]	0.49	82.20 [-44.55; 208.95]	0.21
2 years pp[2]					
weight [g]	169	189.61 [10.56; 368.66]	**0.04**	198.34 [16.49; 380.19]	**0.03**
PI [kg/m^3]	169	0.10 [-0.13; 0.33]	0.40	0.08 [0.17; 0.32]	0.53
BMI [kg/m^2]	169	0.14 [-0.04; 0.33]	0.13	0.14 [-0.06; 0.33]	0.17
Sum 4 SFT [mm]	110	0.58 [0.03; 1.13]	**0.04**	0.54 [-0.05; 10.14]	0.08
Body fat [%]	110	0.42 [0.05; 0.80]	**0.03**	0.39 [-0.02; 0.80]	0.06
Fat mass [g]	110	88.16 [7.43; 168.89	**0.04**	85.07 [-3.52; 173.66]	0.06
LBM [g]	110	125.02 [-32.94; 282.97]	0.12	133.12 [-31.59; 297.84]	0.12
Weight gain (birth ─ 2 years pp)	169	120.21 [-48.42; 288.83]	0.16	149.15 [-25.36; 323.67]	0.10

LBM: lean body mass; PI: Ponderal Index; pp: postpartum; SFT: skinfold thicknesses
Data are presented as the adjusted regression coefficient beta (b) along with the 95 % confidence interval.
p≤0.05 are in bold face[1] Results were adjusted for maternal pre-pregnancy BMI, gestational weight gain, pregnancy duration, group and sex for the data at birth. Beyond birth, results were additionally adjusted for Ponderal Index at birth and breastfeeding status (exclusively/partially breastfed or formula) at 6 weeks or 4 months, respectively.
[2] Infants, for whom only data from the well-child visit at 2 years were available, were also included.

Table A- 8 Breast milk leptin [ng/ml] at 6 weeks pp in relation to infant growth and body composition outcomes up to 2 years pp (complete dataset)

		Unadjusted analysis		**Adjusted analysis**[1]	
Outcome variable	N	Beta [95 % CI]	p	Beta [95 % CI]	p
6 weeks pp					
Weight [g]	152	-148.90 [-542.09; 244.29]	0.46	-39.15 [-436.87; 358.58]	0.85
PI [kg/m^3]	152	-0.60 [2.26; 1.06]	0.48	-0.62 [-2.47; 1.22]	0.51
BMI [kg/m^2]	152	-0.29 [-1.13; 0.54]	0.49	-0.14 [-1.04; 0.76]	0.76
Sum 4 SFT [mm]	152	-0.60 [-3.04; 1.83]	0.63	-0.26 [-3.02; 2.5]	0.85
Body fat [%]	152	-0.34 [-2.25; 1.57]	0.73	-0.13 [-2.3; 2.04]	0.91
Fat mass [g]	152	-48.42 [-195.77; 98.93]	0.52	-17.86 [-180.17; 144.45]	0.83
LBM [g]	152	-100.46 [-376.11; 175.19]	0.48	-22.26 [-289.7; 247.18]	0.88
4 months pp					
Weight [g]	148	75.24 [-385.43; 535.9]	0.75	135.16 [-357.41; 627.74]	0.59
PI [kg/m^3]	148	0.18 [-1.3; 1.67]	0.81	0.39 [-1.38; 2.15]	0.67
BMI [kg/m^2]	148	0.27 [-0.6; 1.14]	0.55	0.31 [-0.71; 1.31]	0.55
Sum 4 SFT [mm]	148	0.99 [-1.76; 3.74]	0.48	0.61 [-2.59; 3.8]	0.71
Body fat [%]	148	0.66 [-1.14; 2.46]	0.47	0.50 [-1.6; 2.59]	0.64
Fat mass [g]	147	58.78 [-122.26; 239.83]	0.53	65.89 [-146.51; 277.3]	0.54
LBM [g]	147	23.09 [309.9; 356.07]	0.89	66.53 [-270.15; 403.2]	0.70
Weight gain (6 wks – 4 mo pp)	148	227.59 [-64.73; 519.92]	0.13	146.33 [-165.06; 457.71]	0.36
1 year pp					
Weight [g]	146	326.17 [-345.44; 997.78]	0.34	305.84 [-411.98; 1023.66]	0.40
PI [kg/m^3]	146	-0.09 [-1.4; 1.23]	0.90	0.10 [-1.37; 1.57]	0.90
BMI [kg/m^2]	146	0.16 [0.75; 1.07]	0.73	0.25 [-0.73; 1.24]	0.61
Sum 4 SFT [mm]	141	0.71 [-2; 3.42]	0.61	0.29 [-2.86; 3.44]	0.85
Body fat [%]	141	0.57 [-1.26; 2.4]	0.54	0.36 [-1.76; 2.49]	0.74
Fat mass [g]	141	110.65 [-166.43; 387.74]	0.43	93.11 [-224.1; 410.31]	0.57
LBM [g]	141	174.47 [-291.05; 639.98]	0.46	202.41 [-281.08; 685.89]	0.41
Weight gain (6 wks – 1 year pp)	146	478.54 [-105.07; 1062.14]	0.11	425.65 [-204.85; 1056.16]	0.19
2 years pp[2]					
Weight [g]	145	86.85 [-801.22; 974.92]	0.85	2.00 [-980.19; 984.19]	1.00
PI [kg/m^3]	145	-0.08 [-1.21; 1.04]	0.89	-0.10 [-1.53; 1.11]	0.75
BMI [kg/m^2]	145	0.00 [-0.91; 0.91]	1.00	-0.21 [-1.13; 0.94]	0.85
Sum 4 SFT [mm]	97	0.34 [-2.01; 2.7]	0.78	-0.18 [-3.05; 2.68]	0.90
Body fat [%]	97	0.31 [-1.3; 1.92]	0.71	-0.06 [-2.02; 1.9]	0.95
Fat mass [g]	97	36.11 [-314.38; 386.6]	0.84	-85.53 [-513.75; 342.69]	0.70
LBM [g]	97	11.94 [-654.05; 677.93]	0.97	-242.34 [-1003.14; 518.46]	0.53
Weight gain (6 wks – 2 years pp)	145	205.08 [-598.74; 1008.9]	0.62	-72.73 [-951.14; 805.68]	0.87

LBM: lean body mass; mo: month; PI: Ponderal Index; pp: postpartum; SFT: skinfold thicknesses; wk: week
Data are presented as the adjusted regression coefficient beta (b) along with the 95 % confidence interval.
p≤0.05 are in bold face

[1] Results were adjusted for maternal pre-pregnancy BMI, gestational weight gain, pregnancy duration, group, sex, Ponderal Index at birth and breastfeeding status (exclusively/partially breastfed or formula) at 6 weeks or 4 months, respectively.

[2] Infants, for whom only data from the well-child visit at 2 years were available, were also included.

Table A- 9 Breast milk leptin [ng/ml] at 4 months pp in relation to infant growth and body composition outcomes up to 2 years pp (complete dataset)

		Unadjusted analysis		**Adjusted analysis**[1]	
Outcome variable	N	Beta [95 % CI]	p	Beta [95% CI]	p
4 months pp					
Weight [g]	119	-188.85 [-743.83; 366.13]	0.51	-635.54 [-1194.86; -76.22]	**0.03**
PI [kg/m^3]	119	-1.16 [-2.91; 0.59]	0.20	-1.58 [-3.64; 0.49]	0.14
BMI [kg/m^2]	119	-0.63 [-1.69; 0.42]	0.24	-1.20 [-2.37; 0.02]	**0.05**
Sum 4 SFT [mm]	120	-2.02 [-5.25; 1.2]	0.22	-1.70 [-5.42; 2.02]	0.37
Body fat [%]	120	-1.31 [-3.44; 0.81]	0.23	-1.03 [-3.48; 1.42]	0.41
Fat mass [g]	119	-130.76 [-349.84; 88.32]	0.24	-209.63 [-459.63; 40.38]	0.10
LBM [g]	119	-58.05 [-454.63; 338.53]	0.78	-425.90 [-797.1; 54.69]	**0.03**
1 year pp					
Weight [g]	117	129.19 [-648.26; 906.64]	0.74	-417.59 [-1228.71; 393.53]	0.32
PI [kg/m^3]	117	-0.81 [-2.29; 0.67]	0.29	-1.10 [-2.72; 0.51]	0.18
BMI [kg/m^2]	117	-0.29 [-1.35; 0.76]	0.59	-0.76 [-1.87; 0.35]	0.18
Sum 4 SFT [mm]	112	-0.66 [-3.94; 2.62]	0.69	-1.32 [-5.02; 2.37]	0.48
Body fat [%]	112	-0.37 [-2.57; 1.84]	0.74	-0.61 [-3.1; 1.87]	0.63
Fat mass [g]	112	-22.66 [-352.63; 307.31]	0.89	-150.66 [-518.85; 217.53]	0.42
LBM [g]	112	108.58 [-424.39; 641.55]	0.69	-239.78 [-779.03; 299.48]	0.39
Weight gain (4 mo ─ 1 year pp)	116	280.93 [-265.2; 827.06]	0.32	196.78 [-398.43; 792]	0.52
2 years pp[2]					
Weight [g]	118	-116.91 [-1097.95; 864.13]	0.82	-539.33 [-1612.85; 534.18]	0.33
PI [kg/m^3]	118	-0.98 [-2.29; 0.33]	0.14	-1.00 [-2.52; 0.53]	0.20
BMI [kg/m^2]	118	-0.61 [-1.65; 0.44]	0.26	-0.80 [-1.99; 0.38]	0.19
Sum 4 SF [mm]	78	0.27 [-2.49; 3.02]	0.85	0.63 [-2.49; 3.76]	0.69
Body fat [%]	78	0.12 [-1.81; 2.04]	0.91	0.39 [-1.79; 2.56]	0.73
Fat mass [g]	78	12.80 [-385.23; 410.84]	0.95	-64.73 [-524.51; 395.06]	0.78
LBM [g]	78	-18.97 [-732.67; 694.74]	0.96	-498.08 [-1271.77; 275.62]	0.21
Weight gain (4 mo ─ 2 years pp)	117	53.83 [-737.49; 845.15]	0.89	113.24 [-797.44; 1023.92]	0.81

LBM: lean body mass; mo: month; PI: Ponderal Index; pp: postpartum; SFT: skinfold thicknesses
Data are presented as the adjusted regression coefficient beta (b) along with the 95 % confidence interval.
p≤0.05 are in bold face
[1] Results were adjusted for maternal pre-pregnancy BMI, gestational weight gain, pregnancy duration, group, sex, Ponderal Index at birth and breastfeeding status (exclusively/partially breastfed or formula) at 4 months.
[2] Infants, for whom only data from the well-child visit at 2 years were available, were also included.

Table A- 10 Breast milk total adiponectin [ng/ml] at 4 months pp in relation to infant growth and body composition outcomes up to 2 years pp (complete dataset)

		Unadjusted analysis		**Adjusted analysis**[1]	
Outcome variable	N	Beta [95% CI]	p	Beta [95 % CI]	p
4 months pp					
Weight [g]	119	-12.45 [-30.06; 5.16]	0.17	-9.68 [-26.53; 7.16]	0.26
PI [kg/m^3]	119	-0.02 [-0.08; 0.04]	0.51	-0.01 [-0.07; 0.05]	0.66
BMI [kg/m^2]	119	-0.02 [-0.05; 0.01]	0.25	-0.01 [-0.05; 0.02]	0.40
Sum 4 SFT [mm]	120	-0.06 [-0.17; 0.04]	0.22	-0.04 [-0.15; 0.07]	0.46
Body fat [%]	120	-0.05 [-0.11; 0.02]	0.18	-0.03 [0.1; 0.04]	0.39
Fat mass [g]	119	-5.76 [-12.72; 1.19]	0.11	-4.25 [-11.63; 3.13]	0.26
LBM [g]	119	-6.68 [-19.29; 5.93]	0.30	-5.43 [-16.7; 5.84]	0.35
1 year pp					
Weight [g]	117	3.79 [-21.05; 28.62]	0.77	-2.25 [-26.41; 21.91]	0.85
PI [kg/m^3]	117	-0.01 [-0.06; 0.04]	0.70	-0.02 [-0.07; 0.03]	0.46
BMI [kg/m^2]	117	-0.00 [-0.04; 0.03]	0.88	-0.01 [-0.05; 0.02]	0.52
Sum 4 SFT [mm]	112	0.04 [-0.07; 0.15]	0.48	0.05 [-0.06; 0.16]	0.33
Body fat [%]	112	0.03 [-0.04; 0.1]	0.40	0.04 [-0.04; 0.12]	0.25
Fat mass [g]	112	5.30 [-5.51; 16.12]	0.34	4.71 [-6.62; 16.03]	0.39
LBM [g]	112	5.28 [-11.34; 24.07]	0.48	-0.46 [-17.11; 16.18]	0.94
Weight gain (4 mo ─ 1 year pp)	116	14.88 [-2.42; 32.19]	0.10	5.45 [-12.25; 23.14]	0.55
2 years pp[2]					
Weight [g]	118	25.32 [-6.13; 56.77]	0.12	19.20 [-12.45; 50.85]	0.24
PI [kg/m^3]	118	0.01 [-0.03; 0.05]	0.66	0.01 [-0.04; 0.05]	0.82
BMI [kg/m^2]	118	0.02 [-0.02; 0.05]	0.35	0.01 [-0.02; 0.05]	0.54
Sum 4 SFT [mm]	78	0.16 [0.05; 0.27]	**<0.001**	0.14 [0.02; 0.25]	**0.02**
Body fat [%]	78	0.11 [0.04; 0.19]	**<0.001**	0.10 [0.02; 0.18]	**0.02**
Fat mass [g]	78	20.96 [5.07; 36.85]	**0.01**	17.36 [0.37; 34.36]	**0.05**
LBM [g]	78	15.86 [-13.64; 45.36]	0.29	11.45 [-17.77; 40.68]	0.44
Weight gain (4 mo ─ 2 years pp)	117	38.08 [13.41; 62.75]	**<0.001**	29.38 [2.66; 55.44]	**0.03**

LBM: lean body mass; mo: month; PI: Ponderal Index; pp: postpartum; SFT: skinfold thicknesses
Data are presented as the adjusted regression coefficient beta (b) along with the 95 % confidence interval.
p≤0.05 are in bold face
[1] Results were adjusted for maternal pre-pregnancy BMI, gestational weight gain, pregnancy duration, group, sex, Ponderal Index at birth and breastfeeding status (exclusively/partially breastfed or formula) at 4 months.
[2] Infants, for whom only data from the well-child visit at 2 years were available, were also included.

Curriculum Vitae

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Studium

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10/2003–07/2006	Studium der Ernährungswissenschaft (Bachelor of Science) an der Technischen Universität München, Freising-Weihenstephan Bachelorarbeit: „Einfluss von Apfelsaftkonsum auf Diabetes- und Adipositasmarker bei gesunden Probanden" am Lehrstuhl für Biofunktionalität der Lebensmittel

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