TUM School of Life Sciences

The value of maternal and fetal biomarkers for predicting early childhood obesity: results from the INFAT cohort

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If life were measured by accomplishments, most of us would die in infancy.

Adolph P. Gouthey

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Abstract

Childhood obesity is increasing globally, prompting an urgent need for novel prevention strategies that will reverse current trends. Strong evidence demonstrates that adverse events in early life can influence a child's risk of developing obesity. However, the biological mechanisms driving early adipose tissue expansion is poorly understood, and longitudinal studies examining the long-term effects of intrauterine exposures are lacking. Identifying biomarkers that reflect the underlying metabolic changes involved in obesity development could have a tremendous public health impact, allowing clinicians to predict whether children are at increased risk for chronic diseases later on.

This work investigated the influence of perinatal metabolic and fatty acid parameters on adipose tissue growth in children at 2, 3, 4 and 5 years old (n= 169, 162, 159 & 152, respectively). Pooled cohort data were derived from the INFAT study (The **i**mpact of **n**utritional **f**atty acids during pregnancy and lactation on early human **a**dipose **t**issue development), a prospective intervention trial that examined the effect of reducing the maternal dietary ratio of n-6/n-3 long-chain polyunsaturated fatty acids (LCPUFAs) during pregnancy and lactation on offspring adipose tissue growth.

Multiple linear regression models were fitted to explore associations between 1) insulin, leptin, HOMA-IR, triglycerides, and n-6 and n-3 LCPUFAs in maternal blood at 32 weeks gestation, 2) insulin, leptin and n-6 and n-3 LCPUFAs in cord blood, and 3) breast milk n-6 and n-3 LCPUFAs at 6 weeks and 16 weeks postpartum on child growth and body composition. The interaction between child sex and cord blood insulin was also examined with multiple regression. Models were adjusted for several maternal and child confounders, including pre-pregnancy BMI, gestational weight gain, gestational duration, ponderal index at birth, study group, sex, and mode of infant feeding. Due to the exploratory nature of the analysis, no corrections were made for multiple testing.

Overall, the investigated metabolic and nutritional markers in maternal blood were largely unrelated to body composition in children ages 2 to 5 years old. Cord blood insulin and leptin demonstrated some transient inverse associations with child clinical outcomes, but most of these relationships had small effects and disappeared by age 5. Breast milk LCPUFAs (at 6 weeks postpartum) were related to some adiposity measurements at 2 and 4 years of age. However, these relationships were not significant across all age groups, and almost all associations were no longer present at 5 years.

In summary, we could not identify maternal or fetal biomarkers that were consistently associated with adipose tissue development in early childhood. This doctoral work forwards our understanding of the mechanisms that link the maternal nutritional and hormonal milieu to growth and adipose tissue accretion in young children. The long-term follow-up provided rare and important insights into the nature of many of the relationships between perinatal metabolic and fatty acid parameters and offspring body composition over time. Results from this thesis emphasize the need to generate longitudinal studies that examine the impact of perinatal nutritional and metabolic changes on child health outcomes.

Zusammenfassung

Die Fettleibigkeit bei Kindern nimmt weltweit zu, so dass ein dringender Bedarf an neuen Präventionsstrategien besteht, die den aktuellen Trend umkehren. Es gibt deutliche Hinweise, dass ungünstige Ereignisse im Mutterleib bzw. in der frühen Kindheit das Risiko einer Adipositas im Kindesalter erhöhen können. Da Langzeitstudien über die langfristigen Auswirkungen der intrauterinen Exposition fehlen, sind die biologischen Mechanismen, die eine frühe Ausdehnung des Fettgewebes vorantreiben, noch unzureichend verstanden. Die Identifikation von Biomarkern, welche die zugrundeliegenden metabolischen Veränderungen bei der Entwicklung von Adipositas widerspiegeln, könnte einen enormen Einfluss auf das Gesundheitswesen haben, da gefährdete Kinder erkannt und gezielte Ernährungs- und Präventionsstrategien ermöglicht werden. Diese Arbeit untersuchte den Einfluss von perinatalen metabolischen und Fettsäuren Variablen auf die Zunahme des Fettgewebes bei Kindern im Alter von 2, 3, 4 und 5 Jahren (n= 169, 162, 159 & 152). Die gepoolten Kohortendaten stammen aus der INFAT Studie (The impact of nutritional fatty acids during pregnancy and lactation on early human adipose tissue development), einer prospektiven Interventionsstudie, die den Effekt einer Reduzierung des mütterlichen Verhältnisses von Omega-6/Omega-3-Fettsäuren in der Ernährung während der Schwangerschaft und Stillzeit auf das Wachstum des Fettgewebes der Kinder untersuchte.

Multiple lineare Regressionsmodelle wurden angewendet um die Assoziationen zwischen 1) Insulin, Leptin, HOMA-IR, Triglyceride und Omega-6 und Omega-3 langkettigen, mehrfach ungesättigten Fettsäuren (LCPUFAs) im mütterlichen Blut in der 32. Schwangerschaftswoche, 2) Insulin, Leptin und und Omega-6 und Omega-3 LCPUFAs in Nabelschnurblut, und 3) Omega-3 und Omega-6 LCPUFAs in der Muttermilch 6 und 16 Wochen nach der Geburt, und dem Wachstum des Kindes sowie der Körperzusammensetzung zu untersuchen.

Die Interaktion zwischen dem Geschlecht des Kindes und dem Insulingehalt des Nabelschnurbluts wurde ebenfalls mithilfe von multiplen Regressionen untersucht. Die Modelle wurden auf verschiedene mütterliche und kindliche Störgrößen, inklusive dem BMI vor der Schwangerschaft, der Gewichtszunahme während der Schwangerschaft, dem Ponderal Index bei der Geburt, der Gruppenzugehörigkeit, dem Geschlecht und der Art der Säuglingsernährung adjustiert. Aufgrund des explorativen Ansatzes der Auswertungen, wurden keine Korrekturen für multiples Testen angewendet. Im Wesentlichen bestand bei

den untersuchten metabolischen und ernährungsbezogenen Biomarkern im mütterlichen Blut kein Zusammenhang mit der kindlichen Körperzusammensetzung im Alter von 2 bis 5 Jahren. Die Biomarker Insulin und Leptin im Nabelschnurblut zeigten einige vorübergehende inverse Assoziationen mit den klinischen Ergebnissen der Kinder. Die meisten dieser Beziehungen hatten jedoch nur kleine Effekte und verschwanden im Alter von 5 Jahren. LCPUFAs in der Muttermilch (6 Wochen postpartum) waren mit einigen Adipositas Indikatoren im Alter von 2 und 4 Jahren assoziiert. Diese Beziehungen waren aber nicht über alle Altersgruppen hinweg signifikant, fast alle Assoziationen waren im Alter von 5 Jahren nicht mehr vorhanden.

Insgesamt konnte das Follow-up vom 2. bis 5. Lebensjahr keine mütterlichen oder fetalen Biomarker identifizieren, die durchgehend mit der Entwicklung des Fettgewebes in der frühen Kindheit verbunden waren.

Diese Doktorarbeit verbessert das bestehende Verständnis der Mechanismen, die das mütterliche Ernährungs- und Hormonmilieu mit dem Wachstum und der Fettgewebsakkumulation bei Kleinkindern verbinden. Die Langzeitbeobachtung lieferte seltene und wichtige Einblicke in die Beschaffenheit vieler Zusammenhänge zwischen perinatalen Biomarkern und der Körperzusammensetzung der Kinder im Laufe der Zeit. Die Ergebnisse dieser Arbeit unterstreichen die Notwendigkeit von Langzeitstudien, die den Einfluss von perinatalen Ernährungs- und Stoffwechselveränderungen auf die gesundheitliche Entwicklung von Kindern untersuchen.

Abbreviations

AA	Arachidonic acid (C20:4n-6)
AgRP	Agouti-related protein
BAT	Brown adipose tissue
BMI	Body mass index
BMI SDS	Standardized BMI
DHA	Docosahexaenoic acid (C22:6n-3)
DOHaD	Developmental Origins of Health and Disease
DXA	Dual-energy x-ray absorptiometry
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
EPA	Eicosapentaenoic acid (C20:5n-3)
FA	Fatty acid
FAME	Fatty acid methyl esters
FLI	Free leptin index
GDM	Gestational diabetes mellitus
GWG	Gestational weight gain
HOMA-IR	Homeostatic model assessment of insulin resistance
IGF	Insulin-like growth factor
INFAT	Impact of Nutritional Fatty acids during pregnancy and lactation on early human Adipose Tissue development
IOM	Institute of Medicine
IQR	Interquartile range
LCPUFA	Long chain polyunsaturated fatty acid

LGA	Large for gestational age
MRI	Magnetic resonance imaging
NAT	Nonadipose tissue
NPY	Neuropeptide Y
Ob/ob	Leptin deficiency induced obesity
ObRb	Leptin receptor, long-form
POMC	Pro-opiomelanocortin
PUFA	Polyunsaturated fatty acid
RBC	Red blood cell
RCT	Randomized controlled trial
SAT	Subcutaneous adipose tissue
SD	Standard deviation
SFT	Skinfold thickness
sObR	Soluble leptin receptor
VAT	Visceral adipose tissue
VLDL	Very low density lipoprotein
WAT	White adipose tissue

1 Introduction

1.1 Childhood obesity - current challenges and opportunities

Childhood obesity is a growing public health concern. Its prevalence is rising in every country and contributes to an increasing proportion of the global non-communicable disease burden (World Health Organization (WHO) 2016). Children with obesity are at a higher risk of developing chronic diseases, such as type 2 diabetes mellitus and cardiovascular disease, even before they reach adulthood (Bhave et al. 2004; World Health Organization (WHO) 2016). Children with excess weight are also more likely to become adults with obesity (Ebbeling et al. 2002; Simmonds et al. 2016), and adiposity-related complications are exacerbated if obesity begins early in life (Styne 2001). These trends have led the World Health Organization to prioritize the goal of halting the rise in child obesity by 2025 (World Health Organization (WHO) 2016)(www.who.int/beat-ncds).

Obesity is a complex, largely preventable disease with a multifactorial etiology. Treating childhood obesity has proven difficult and success is limited (Ells et al. 2018; Foster et al. 2015). Rather than focusing on obesity treatment, prioritizing obesity prevention necessitates an expansion in research goals to identify factors contributing to early adipose tissue accretion. While mechanisms underlying obesity development are not fully understood, a relatively new scientific and public health model embraces a life-course perspective by linking environmental exposures in early life to later disease risk. This obesity origins framework, known as the Developmental Origins of Health and Disease (DOHaD) (Gluckman et al. 2008), maintains that obesity begins within the first 1000 days post-conception. Exposure to a range of nutritional and environmental conditions programs offspring with metabolic characteristics that increase the likelihood of obesity later on (Godfrey et al. 2013; Hanson and Gluckman 2014; Sutton et al. 2016).

The first 1000 days – a unique opportunity for obesity prevention

The first 1000 days of life, defined as the period from conception to age 2, are characterized by dynamic growth and development. This period corresponds to a time of heightened vulnerability but can also be viewed as a unique window of opportunity when interventions

to optimize health and nutrition could have a tremendous impact on later disease risk. The DOHaD model gave rise to research that advanced our understanding of the continuity between early development and long-term health outcomes by providing insights into how perinatal health and nutrition shape offspring outcomes (van Dijk et al. 2015).

The ability to screen in pregnancy for childhood obesity would be a major advancement in antenatal care and clinical surveillance. In clinical practice, pregnancies are routinely categorized as “low” or “high” risk based on the perceived likelihood of suffering adverse obstetric outcomes. However, this classification does not fully capture the spectrum of risk that exists for the mother and her offspring, particularly in relation to long-term outcomes. Several hormones and nutritional substrates affect fetal growth, which suggests that they could serve as surrogate markers for later obesity risk. The National Institutes of Health Definitions Working Group defines a biomarker as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” (Biomarkers Definitions Working Group 2001). Identifying biomarkers that can predict child obesity outcomes would be a valuable tool for early intervention approaches.

1.2 Perinatal determinants associated with child health outcomes

The hormone- and nutrient-rich intrauterine environment has a profound influence on fetal development and growth. Evidence shows that unfavorable maternal and placental conditions can have lasting health consequences (Gluckman et al. 2008). Among prenatal risk factors, maternal prepregnancy body mass index (BMI) and gestational weight gain (GWG) appear to have the greatest impact on short- and long-term offspring health, such as pre-term birth, macrosomia, and obesity (Institute of Medicine and National Research Council 2007; Oken et al. 2008; Oken 2009; Viswanathan et al. 2008; Woo Baidal et al. 2016). This is concerning when one considers that increasing numbers of European women begin their pregnancies with overweight or obesity (Devlieger et al. 2016). For instance, in Germany, it is estimated that around a third of women of childbearing age present with a BMI ≥ 25 kg/m^2 (Mensink et al. 2013). Mothers with obesity are at an elevated risk for several metabolic complications compared to their normal-weight counterparts, including insulin resistance, hypertension, glucose intolerance, and hyperlipidemia (Catalano 2010; Gaillard et al. 2016). Metabolic

consequences extend to the next generation, with children born to obese mothers being more likely to suffer from obesity themselves (Institute of Medicine and National Research Council 2007; Yu et al. 2013).

The most widely-used guidelines for weight gain in pregnancy were developed by the U.S. Institute of Medicine (IOM). Optimal weight gain ranges are outlined according to maternal prepregnancy BMI (Institute of Medicine 2009). Parallel to the rising prevalence of high preconception BMI is the increasing proportion of women whose GWG exceeds IOM recommendations (Goldstein et al. 2017). Robust evidence links excess GWG with offspring adiposity (Goldstein et al. 2017; Lau et al. 2014; Mamun et al.; Oken et al. 2008). Thus, the proportion of children exposed to an obesigenic intrauterine environment is increasing, which promotes the transmission of intergenerational obesity (Institute of Medicine and National Research Council 2007). However, the underlying biological mechanisms that explain the connection between excess maternal fat and offspring health outcomes are less understood. Adipose tissue functions as an endocrine organ, secreting various cytokines and adipokines that play a role in energy regulation and appetite control (Bouret 2012; Zeltser 2015; Zorena et al. 2020). There is a growing appreciation that elucidating these mechanisms will forward our understanding of early adipose tissue expansion.

Among postnatal determinants, breastfeeding seems to confer some protection against childhood adiposity (Horta and Victora 2013; Weng et al. 2012). Breastfeeding facilitates the self-regulation of infant milk intake, thus promoting better appetite control (Li et al. 2010). There is also a multitude of bioactive components present in breast milk that regulate energy metabolism and appetite (Fields et al. 2016). Additionally, some evidence supports a relationship between increased protein intake and higher adiposity in infancy (Koletzko et al. 2009). The protein intake of breastfed babies is generally lower than formula-fed babies, which may have important implications for early growth and fat development (Arenz et al. 2004; Weng et al. 2012). Thus, the protective effects of breastfeeding are most likely due to a combination of behavioral, non-nutritive, and nutritive factors.

1.3 Metabolic adaptations in pregnancy

Fetal growth is mainly determined by a mother's ability to provide nourishment, which underscores the importance of the maternal diet in fetal development. The quality of substrate provision depends on not only the components of the maternal diet but also the placenta's ability to deliver oxygen and provide nutrients, including glucose, amino acids, and fatty acids (Zhang et al. 2015). Several metabolic changes take place in the course of pregnancy to ensure a continued supply of nutrients. The overall health status of the mother is an important determinant of offspring health, and obesity may be programmed in the womb from exposure to maternal metabolic dysregulation. The following chapters will outline normal and maladaptive metabolic changes that occur during pregnancy and their impact on offspring development.

Changes in carbohydrate metabolism

Glucose accounts for the major source of energy used by the fetus and is largely responsible for fetal growth and metabolism (Hay 2006). In early pregnancy, glucose tolerance and hepatic glucose production are normal, while insulin sensitivity may increase or decrease (Catalano et al. 1993). As pregnancy advances, pancreatic beta cells undergo hyperplasia which increases both basal and postprandial insulin secretion (Butte 2000). The hyperinsulinemic-euglycemic clamp technique shows a progressive decrease in insulin sensitivity by 50–70% (Catalano et al. 1993; Catalano et al. 1999; Catalano 2014). This is thought to be partly mediated by hormonal changes, such as increased growth hormone, cortisol, prolactin, progesterone, and human placental lactogen (Catalano 2014; Ryan and Enns 1988; Sonagra et al. 2014). During pregnancy, maternal postprandial glucose levels are elevated with longer peaks (Cousins et al. 1980) while hepatic glucose production is augmented by as much as 16 – 30% (Assel et al. 1993; Catalano et al. 1992; Kalhan et al. 1979). The physiological state of gestational insulin resistance results in marked changes in postprandial concentrations of key nutrients that are shunted to the growing fetus, such as glucose, lipids, and amino acids.

Elevated maternal glucose concentrations beyond the physiologically expected range are strongly associated with increased fetal adiposity (HAPO Cooperative Research Group 2009). Long-term effects appear to extend into childhood, whereby children born to mothers with gestational diabetes mellitus (GDM) are more likely to develop obesity (Gillman et al. 2003;

Kim et al. 2012). Importantly, the combined effect of GDM and maternal obesity seems to amplify risk to offspring (Kim et al. 2012), a relevant finding considering the increasing trends in prepregnancy overweight.

Lipid changes in pregnancy

Hormonal and metabolic adaptations that take place in a normal pregnancy lead to changes in the maternal lipid profile. Altered lipid metabolism has been described as occurring in two phases (Blackburn 2015). Initially, an anabolic phase occurs in the first two trimesters. Maternal hyperphagia and increased insulin sensitivity facilitate *de novo* lipogenesis and fat deposition. The switch to a net catabolic phase in the third trimester is characterized by decreased insulin sensitivity and increased lipolysis of stored triglycerides. The breakdown of fat depots precipitates maternal hyperlipidemia, with a marked increase in plasma triglyceride levels (Herrera and Ortega-Senovilla 2010). By late pregnancy, triglyceride levels are doubled while cholesterol levels are around 50% higher than those seen in prepregnancy (Grimes and Wild 2000). The pronounced rise in maternal triglycerides results from both the enhanced production of very-low-density lipoprotein (VLDL) in the liver and decreased clearance of lipoprotein triglycerides (Alvarez et al. 1996). **Figure 1** presents the key changes in lipid metabolism that occur during the two phases of pregnancy.

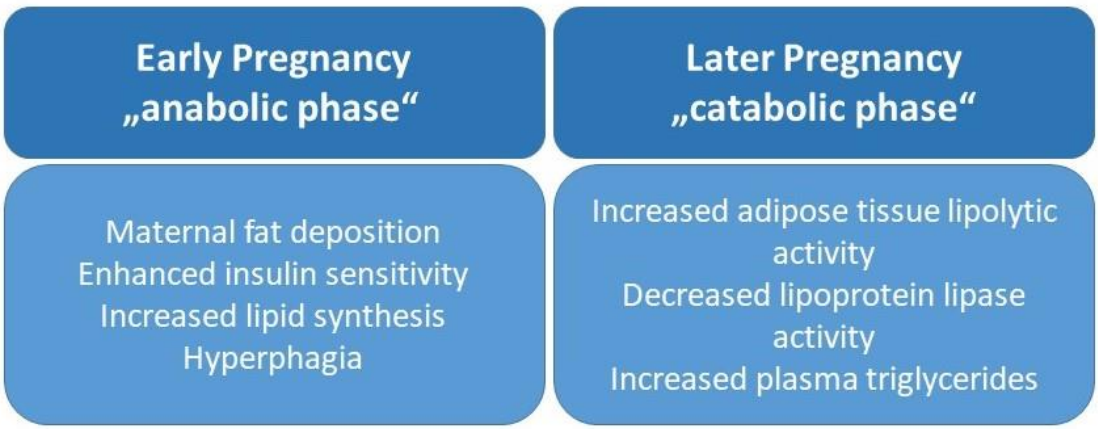

Figure 1 Lipid metabolism in early and late pregnancy

Early pregnancy corresponds to approximately the first and second trimesters.
Later pregnancy takes place in the third trimester. The switch from an anabolic to a catabolic state is partly due to a progressive decrease in insulin sensitivity.

Data source: Herrera and Ortega-Senovilla 2010

The physiological adaptations in the maternal lipid profile serve an important role in pregnancy by providing additional energy to facilitate fetal growth and possibly contributing to fat storage (Schaefer-Graf et al. 2008). Cholesterol is important in normal fetal development, contributing to the formation of cell membranes and regulating the expression of proteins involved in metabolic pathways (Woollett 2008). It is also the precursor to several hormones, including steroids and vitamin D. Elevated triglycerides help meet the mother's metabolic needs while sparing glucose for the fetus. It is important, however, to distinguish between physiological and pathological gestational hyperlipidemia and the implications on maternal and offspring health. Abnormally high levels of triglycerides have been linked to an increased risk of obstetric complications, including preeclampsia and GDM (Wiznitzer et al. 2009). Adverse neonatal outcomes have also been reported, such as an increased incidence of babies born large for gestational age (LGA) or with macrosomia (Jin et al. 2016; Schaefer-Graf et al. 2008).

1.4 Hormones that modulate energy homeostasis in pregnancy

The hypothalamus plays an important role in regulating body weight by controlling the activation of pathways that stimulate (orexigenic) or suppress (anorexic) appetite. These pathways are activated by circulating hormones that act as messenger molecules, communicating energy stores to the brain (Myers Jr and Olson 2012). Leptin, a hormone secreted by adipocytes, functions as a key regulator in this negative feedback loop.

Leptin

Leptin is a protein encoded by the obese (ob) gene and primarily secreted by white adipocytes (Zhang et al. 1994). Since its identification over two decades ago, research has aimed to elucidate its role in regulating energy homeostasis, neuroendocrine functioning, and metabolism. Circulating leptin concentrations correlate closely with fat mass (Considine et al. 1996) and preclinical animal studies show that ob/ob mice who lack endogenous leptin become massively obese (Zhang et al. 1994).

The arcuate nucleus in the hypothalamus is the primary site of both leptin receptor expression and an abundance of neurons that directly respond to leptin (Schwartz et al. 2000). Leptin functions as a signal of the body's nutritional status on two groups of neurons that exert

differential effects on food intake and energy expenditure. Neuropeptide Y (NPY) and agouti-related peptide (AgRP) are orexigenic, meaning that their expression increases appetite, while pro-opiomelanocortin (POMC) expression exerts the opposite, anorectic effect by reducing food intake **(Figure 2)** (Sahu 2003; Schwartz et al. 2000). Circulating leptin crosses the blood-brain barrier and binds to leptin (ObRb) receptors on NPY/AGRP and POMC neurons which results in decreased food intake and increased energy expenditure (Ladyman and Grattan 2013).

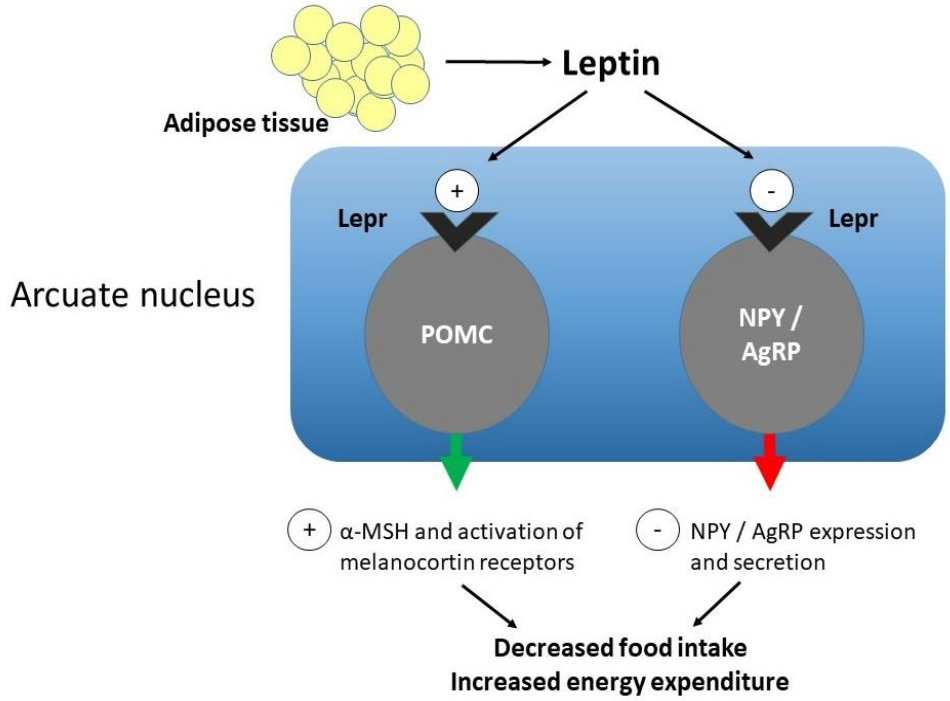

Figure 2 Schematic presentation of leptin action on signals governing food intake in the hypothalamus

Increased fat cell mass results in an increase in leptin expression. Leptin binds to ObRb (Lepr) receptors on a subset of hypothalamic neurons that are involved in energy regulation. In the arcuate nucleus, leptin inhibits orexigenic NPY/AgRP neurons and activates neurons that produce POMC. This activity results in decreased food intake and increased energy expenditure.

AgRP, agouti-related peptide; α-MSH, α-melanocyte-stimulating hormone; Lepr, leptin receptor; POMC, pro-opiomelanocortin; NPY, neuropeptide Y

Data source: Schwartz, et al. 2000

Leptin resistance in pregnancy

Pregnancy is characterized by considerable changes in leptin expression and concentration. Maternal leptin concentrations rise early in pregnancy before any notable increase in body weight (Teppa et al. 2000). Levels remain elevated throughout gestation, with peak concentrations observed in the second trimester (Hardie et al. 1997; Teppa et al. 2000). Although primarily expressed in adipose tissue in non-pregnant women, placental production of leptin is partly responsible for an increase in circulating gestational levels (Masuzaki et al. 1997). In contrast to leptin's usual effect on satiety, pregnancy is characterized by hyperphagia despite an increase in circulating leptin, and direct injection of leptin into the brains of pregnant rats has no effect on reducing food intake (Ladyman and Grattan 2004). These findings support the theory that pregnancy induces a state of central leptin resistance, possibly mediated by a decrease in ObRb leptin receptor expression in the hypothalamus (Grattan et al. 2007; Szczepankiewicz et al. 2006). The ventromedial nucleus appears to be a key site in the hypothalamus involved in pregnancy-associated leptin resistance (Ladyman and Grattan 2005). Temporal leptin resistance seen in pregnancy serves an important function by increasing energy and nutrients to the growing fetus.

The role of leptin in early brain development

Besides its involvement in regulating energy balance in adults, leptin also plays a key role in early brain development, particularly in the hypothalamus. Important preclinical research by Bouret and colleagues identified a link between postnatal leptin and the development of a subpopulation of hypothalamic neurons involved in metabolic regulation (Bouret and Simerly 2006). Leptin deficient (ob/ob) mice pups were found to have permanent defects in neural circuitry responsible for appetite control and energy regulation in adulthood, such as NPY and AgRP co-expressing neurons and POMC-containing neurons. These changes resulted in reduced leptin sensitivity, reduced neural cell numbers, and altered neural projections. Interestingly, when the newborn pups were infused with leptin many axonal connection defects were corrected. However, these improvements were not observed when a leptin infusion was delivered to ob/ob mice in adulthood (Bouret and Simerly 2006; Bouret 2012). These experiments suggest that leptin plays a neurotrophic role in hypothalamic development, and low postnatal leptin exposure may result in the brain being "hard-wired"

for obesity in adulthood. Although the findings are compelling, their relevance for humans is unclear given the stark differences in the time-frame of brain maturation between humans and rodents. Notably, hypothalamic development in humans occurs late in the second trimester and not postnatally as seen in mice (Koutcherov et al. 2003).

Leptin and adipose tissue accretion in early life

Maternal leptin may have an important role in the regulation of offspring development, although more studies are needed to explore long-term effects. Some evidence has shown that higher maternal leptin levels are associated with newborn adiposity (Josefson et al. 2014), though others could not confirm this association (Castro et al. 2017).

Fetal leptin is detectable around 18 weeks gestation and rises dramatically by the end of the third trimester (Jaquet et al. 1998). Cord blood leptin correlates with both placenta- and birth weight, and babies born small for gestational age present with lower levels of cord leptin than those born appropriate for gestational age. (Karakosta et al.; Kiess et al. 1998; Koistinen et al. 1997; Martos-Moreno et al. 2009; Matsuda et al. 1997; Sivan et al. 1997; Stefaniak et al. 2019; Tamura et al. 1998; Tsai et al. 2015). These findings suggest that fetal leptin levels mirror adipose tissue expansion and may modulate metabolic regulation in early life. Limited research has examined outcomes beyond birth and shown modest inverse relationships between perinatal leptin and adiposity at 3 and 4 years (Boeke et al. 2013; Karakosta et al. 2016; Mantzoros et al. 2009). However, there is not enough data to conclude whether programming effects persist in childhood.

Crosstalk between leptin and insulin

In addition to leptin, insulin also functions as a signaling molecule to regulate food intake and energy homeostasis through a negative feedback loop. Although these two hormones are unrelated and act on different receptors, both leptin and insulin enter the brain from the peripheral circulation and exert potent anorexigenic effects by activating the same signaling pathways in the arcuate nucleus (Woods and Seeley 2000). Consistent with their function as adiposity signals, both leptin and insulin concentrations are correlated with fat mass (Niswender and Schwartz 2003; Schwartz et al. 2000) and direct injection of either hormone in the brain decreases food intake (Niimi et al. 1999; Woods et al. 1979). Moreover, a

disruption in neuronal signaling of either molecule can result in hyperphagia and altered glucose homeostasis (Brüning et al. 2000; Niswender and Schwartz 2003).

Research suggests that insulin and leptin interact synergistically at the level of the arcuate nucleus to potentiate effects on energy balance and food intake (Carvalheira et al. 2005; Niswender and Schwartz 2003).

1.5 Long-chain PUFAs in the prenatal and early postnatal period

The origins of adipose tissue growth

Adipose tissue was historically considered an inert organ whose function was to store excess energy. An abundance of research has established that adipose tissue functions as a metabolically active organ, responsible for synthesizing and secreting hormones involved in energy regulation. The two types of adipose tissue found in humans have markedly different characteristics and perform different functions. White adipose tissue (WAT) plays an important role in energy storage (Large et al. 2004) and is also recognized as an active endocrine organ, while brown adipose tissue (BAT) is active in the dissipation of energy, mainly through the production of heat, ensuring effective adaptation to the extrauterine environment after birth (Himms-Hagen 1990). Fat lobules appear in human fetuses around the 14^{th} week of gestation, with a progressive increase in number as gestational age advances (Poissonnet et al. 1984). Adipose tissue development takes place during critical developmental periods. Peak activity in the proliferation and differentiation of adipocytes is observed primarily in early infancy, and to a lesser extent, in the preadolescent years (Baum et al. 1986; Hauner et al. 1989; Salans et al. 1973). The total number of adipocytes is believed to be established early in life and is mostly stable in adulthood (Spalding et al. 2008).

Numerous animal studies support the concept that maternal dietary fat plays a role in the development of obesity (Taylor and Poston 2007). Dams fed with high-fat diets are more likely to give birth to pups with an increased likelihood of obesity later on (Johnson et al. 2017). It is also known that selected fatty acids modulate several genes and transcriptional factors involved in energy metabolism (Hammad and Jones 2017). Against this background, studies have explored associations beyond the overall quantity of maternal fats by investigating the composition of maternal fats in relation to offspring adiposity outcomes.

LCPUFAs are critical for the proper growth and development of the fetus. The availability of LCPUFAs for placental transfer to the fetus is largely dependant on maternal dietary intake (Koletzko et al. 2007). Given their importance in early life development and metabolic functioning, it is particularly concerning to observe the dramatic increase in the dietary intake of n-6 fatty acids and a concomitant decline in n-3 fatty acid intake over the past few decades. This has resulted in an estimated dietary ratio of n-6/n-3 fatty acids of ~15:1 in the European diet (Simopoulos 2009; Simopoulos 2016), which contrasts sharply with the ideal ratio of around 2:1 (Simopoulos 2009; Simopoulos 2016). There are some indications that early exposure to LCPUFAs can modulate metabolic pathways involved in cell differentiation and gene expression (Bordoni et al. 2006; Jump 2008; Uauy et al. 2000). In-vitro and animal studies also provide evidence that arachidonic acid (AA) of the n-6 fatty acid family (AA,20:4n-6) enhances fat cell differentiation at the preadipocyte stage, mainly mediated by AA-derived prostaglandin (Massiera et al. 2003; Muhlhausler and Ailhaud 2013). In contrast, fatty acids from the n-3 family, namely eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), appear to inhibit fat cell differentiation (Simopoulos 2016). Importantly, evidence of these relationships comes largely from experimental studies, and data in humans are limited.

1.6 Predicting obesity – estimating body fat in children

The literature addressing associations between perinatal determinants and offspring obesity outcomes is characterized by wide variations in the methods used to measure adiposity. Frequently, BMI, waist circumference, and skinfold thickness measurements are used in clinical research. Direct methods of body composition in young children include air displacement plethysmography and dual-energy x-ray absorptiometry (DXA), the latter of which has also been shown to accurately estimate regional fat depots (Micklesfield et al. 2012; Wells and Fewtrell 2006). This heterogeneity of assessment methods complicates the interpretation of the data. Importantly, measurements used as proxies for adiposity, such as body weight and BMI, are estimates of growth rather than body fat. This is an important consideration given the inherent inaccuracy of these measurements for estimating true obesity in children. Body fatness, as opposed to body size, is a key predictor of adverse health consequences related to obesity, underscoring the importance of choosing accurate methods.

Besides measuring total body fat, the distribution of fat is also of interest, as associations between central obesity and diseases such as coronary heart disease and type 2 diabetes mellitus have been observed (Cook and Kavey 2011; Daniels et al. 1999). Magnetic resonance imaging (MRI) and computer tomography (CT) are known as the gold standards for measuring visceral fat but are often impractical due to cost and time constraints, which limit their clinical application (Wells and Fewtrell 2006). Recently, the INFAT study demonstrated that abdominal ultrasound could be used in young children to accurately estimate abdominal subcutaneous and preperitoneal fat mass (Brei et al. 2015; Brei et al. 2018), the latter recognized as a proxy for visceral fat. Ultrasound has a considerable advantage over other methods due to its low cost and portability.

Furthermore, few studies have examined how perinatal metabolic and nutritional exposures influence fat development and distribution throughout early childhood. To successfully address this knowledge gap, longitudinal maternal/child cohort studies that employ accurate methods for measuring body composition are needed.

1.7 Aim of the thesis

Greater insights into the biological mechanisms driving early childhood adipose tissue expansion are needed. Placental transport of nutrients is critical to fetal growth, and a wide range of maternal hormones and cytokines, including insulin and leptin, have been shown to regulate transplacental nutrient supply to the fetus (Jansson et al. 2003; Roos et al. 2009). Placental responses to changes in the maternal environment during pregnancy can affect fetal growth (Rosario et al. 2015). Experimental and epidemiological studies suggest that the dysregulation of appetite-regulating hormones and other substrates in utero are associated with adiposity outcomes in childhood. Identifying biomarkers that reflect the underlying metabolic changes putatively involved in obesity development could have a tremendous public health impact. Early screening can identify vulnerable offspring for dietary and other obesity prevention strategies. Against this background, this work investigated several perinatal metabolic and hormonal variables to explore their association with adipose tissue accretion in early childhood.

The three aims presented in this thesis are the following:

1. Publication 1 – To explore whether leptin in maternal blood at 32 weeks of gestation and cord blood were associated with body composition in children at 3, 4, and 5 years of age.
2. Publication 2 – To investigate whether maternal metabolic variables of glucose and lipid metabolism at 32 weeks of gestation and cord blood insulin were related to child growth and adipose tissue development at 3, 4, and 5 years of age.
3. Publication 3 – To observe whether perinatal exposure to n-6 and n-3 LCPUFAs influences offspring growth and fat accretion. Maternal biosamples (red blood cells at 32 weeks' gestation and breast milk at 6 and 16 weeks postpartum) and cord blood were analyzed.

2 Methods

Data for this thesis were derived from the "Impact of Nutritional Fatty acids during pregnancy and lactation on early human Adipose Tissue development" (INFAT) study. The primary outcome of the study was to determine whether reducing the ratio of n-6/n-3 LCPUFAs in pregnant and breastfeeding women helped limit offspring adipose tissue expansion in early childhood up to the age of 5 years (Brei et al. 2016; Hauner et al. 2012). The study was established in 2006, with the last examination taking place in 2014. Healthy non-obese pregnant women were supplemented with n-3 LCPUFAs from 15 weeks gestation until 4 months postpartum and reduced their intake of foods high in AA. Offspring were followed from birth until 5 years of age. Findings from the initial and follow-up studies did not support the premise that reducing the maternal n-6/n-3 fatty acid ratio influenced adipose tissue development in early life, arguing against this method as a strategy for childhood obesity prevention (Brei et al. 2016; Hauner et al. 2012). These results are in agreement with systematic reviews and meta-analyses (Stratakis et al. 2014; Delgado-Noguera et al. 2015; Vahdaninia et al. 2019). This thesis describes several secondary cohort analyses that were carried out to elucidate relationships between selected perinatal exposures on offspring growth and adipose tissue development.

2.1 Design of the INFAT study

INFAT is an open-label, monocenter, prospective randomized controlled intervention trial. Recruitment took place in Munich and the surrounding area between July 2006 and May 2009 by research assistants at the Institute of Nutritional Medicine, University Hospital Klinikum rechts der Isar, Munich. The study was advertised in newspapers and relevant maternity websites, as well as in a monthly maternity journal. Screening for eligibility was performed by telephone or at the outpatient clinic using a structured checklist. **Table 1** lists the study inclusion and exclusion criteria.

Table 1 Study inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Pregnant women aged 18 - 43 years ≤ 15th week of gestation Prepregnancy BMI 18 – 30 kg/m^2 Willingness to adhere to dietary recommendations Sufficient German language skills Written informed consent	High-risk pregnancy (e.g. multiple pregnancies, rhesus incompatibility, hepatitis B, or parity >4) Hypertension Gastrointestinal disorders accompanied by maldigestion, malabsorption or elevated energy and nutritional requirements Chronic diseases (e.g. diabetes) Psychiatric illness Hyperemesis gravidarum n-3 LCPUFA supplementation prior to randomization Alcohol abuse Known metabolic birth defects (e.g. phenylketonuria)

Participants who met the eligibility criteria were randomly assigned to the intervention or control group using a computer-generated block randomization method (Institute for Medical Statistics and Epidemiology, Klinikum rechts der Isar). The intervention group (n = 104) was supplemented with fish oil capsules containing 1,200 mg n-3 long-chain PUFAs (1,020 mg DHA, 180 mg EPA plus 9 mg Vitamin E as an antioxidant) daily from 15 weeks gestation until 4 months postpartum. Women in the intervention group were also given detailed dietary advice to reduce their intake of meat and eggs to achieve an AA level within the recommended range of 50 – 90 mg per day. Women in the control group (n = 104) received general dietary recommendations according to the German Nutrition Society guidelines (Deutsche Gesellschaft für Ernährung, DGE) and were asked to refrain from taking fish oil or DHA supplements for the duration of the intervention.

The study protocol adhered to the rules of the International Conference of Harmonization Good Clinical Practice Guidelines (valid from January 17, 1997), the declaration of Helsinki (October 2000, Edinburgh, UK), and followed local regulatory requirements and laws. The

protocol was approved by the Technical University of Munich Ethics Committee (1479/06/2006/2/21) and is registered at clinicaltrials.gov, number NCT00362089.

Women who participated in the study had a mean age of 32 years and a mean prepregnancy BMI of 22 kg/m^2. There were no significant differences in baseline maternal lifestyle or socio-demographic characteristics between women in the intervention and control groups.

Maternal adherence to the intervention was confirmed by fatty acid analysis of several biosamples, including plasma phospholipids, erythrocyte membranes, placenta, umbilical cord blood, and breast milk. There was also a reduced intake of AA from meat products and other foods at 32 weeks gestation in the intervention group (Hauner et al. 2012). Infants were assessed at four time points during the initial study (birth, 6 weeks, 4 months, and 1 year).

The primary endpoint was adipose tissue expansion in infancy, measured by skinfold thicknesses (SFT). No evidence of significant differences in SFT, either individually or the sum of 4 SFT, was detected between the two groups. Results from the initial study were previously reported (Hauner et al. 2012). A planned follow-up study took place between February 2008 and November 2014 to investigate the intervention effects in offspring between 2 and 5 years of age. Written informed consent was obtained from both parents for the follow-up study. The study protocol was approved by the Technical University of Munich Ethics Commission (1479/06/2009/10/26).

Children were examined at the study center or during home visits biannually until 2 years, and then annually until their fifth birthday. Additional secondary endpoints included assessing the children's diet, activity level, and neurodevelopment at selected time points. An overview of the INFAT study can be seen in **Figure 3**.

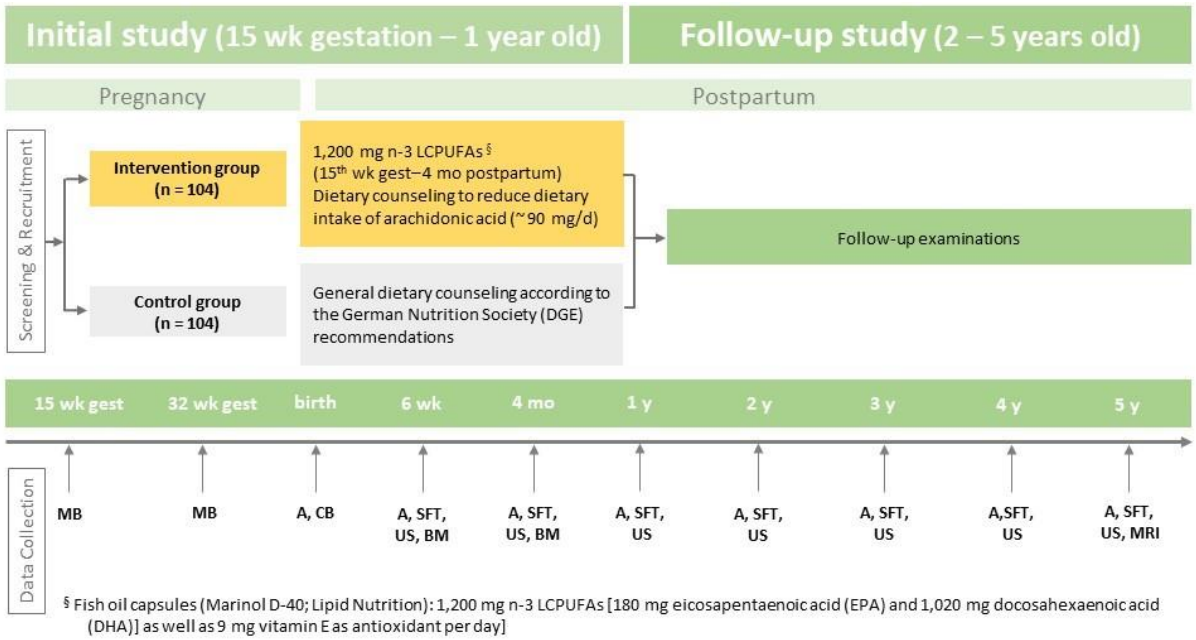

Figure 3 Design of the INFAT study

A, anthropometry; BM, breast milk; CB, umbilical cord blood; gest, gestation; LCPUFAs, long-chain polyunsaturated fatty acids; MB, maternal blood; mo, months; MRI, abdominal magnetic resonance imaging (subgroup of 44 children); SFT, skinfold thickness measurements; US, abdominal ultrasound; wk, weeks; y, years

Adapted from Meyer et al. 2020

2.2 Maternal characteristics and measurements

Prepregnancy weight was self-reported and height was recorded from maternity records (*Mutterpass*). BMI was calculated by INFAT researchers. Glucose tolerance screening took place at gynecological practices. Pregnant women were diagnosed with GDM following a 75 g oral glucose tolerance test, except for one participant, who was diagnosed with GDM based on a random elevated glucose level. The proportion of women with GDM did not statistically differ between the intervention (n = 7) and control (n = 10) groups (Hauner et al. 2012). Two women in each group were treated with insulin according to maternity records.

2.3 Child body composition measurements

2.3.1 Growth and anthropometric measurements

Growth measurements were assessed in children aged 2 to 5 years by trained researchers in a standardized manner. A standard flat scale (Seca Clara 803; seca) was used to measure child weight to the nearest 100 g. Height was measured to the nearest 0.5 cm with a stadiometer (Stadiometer seca 214; seca). BMI percentiles were calculated from a German reference group (Kromeyer-Hauschild et al. 2001). Skinfold thickness was measured at the left body axis at four sites (biceps, triceps, subscapular and suprailiac) in triplicate with a Holtain caliper (Holtain Ltd.). The mean from each site was calculated. Means from all four sites were added to obtain the sum of four SFT to estimate subcutaneous fat (Hauner et al. 2012). Two ratio indices served as proxies for fat patterning: the subscapular-to-triceps SFT ratio, described by Haffner et al. (Haffner et al. 1987) approximated central to peripheral fat distribution, and the trunk-to-total SFT ratio according to Weststrate and Deurenberg (Weststrate and Deurenberg 1989) estimated central-to-total fat patterning. Body fat percentage was estimated with predictive SFT equations as previously described (Weststrate and Deurenberg 1989). Lean body mass (kg), percentage of lean body mass, and total body fat were calculated using body fat percentage and body weight.

2.3.2 Measurement of abdominal subcutaneous and preperitoneal fat with ultrasound

Ultrasound was performed at the study center by trained staff using a high–resolution ultrasonographic system (Siemens Acuson X150 Premium, Siemens) with a 10 MHz linear probe (VFX 13-5, Siemens Medical Solutions, Erlangen, Germany) in b-picture mode. Ultrasound methods are reported in detail in INFAT publications (Brei et al. 2015; Brei et al. 2018), following minor adaptations from previous methods (Holzhauer et al. 2009). Ultrasonography was performed on children lying supine with their arms at their sides. Subcutaneous and preperitoneal fat were estimated by measuring two pre-defined abdominal regions and all measurements were taken at the end of normal expiration. The evaluation process took place at an off-line working station with the OsiriX software (http://osirix-viewer.com; Geneva, Switzerland). The xiphoid process was set as a starting point for measurements on the sagittal plane, and the linea alba on the axial plane. Fat areas were

evaluated as layers 1 cm in length. The areas of preperitoneal fat and subcutaneous fat in the sagittal plane and subcutaneous fat in the axial plane were calculated from the means of ultrasound measurement distances.

2.3.3 Abdominal subcutaneous and visceral fat volumes by MRI

Abdominal MRI was performed in a sub-group of 44 children at 5 years of age to estimate subcutaneous (SAT) and visceral adipose tissue (VAT) volumes (Brei et al. 2016). The children underwent abdominal MRIs at the University Hospital Klinikum rechts der Isar on a whole-body scanner with no sedation. Children were placed in the scanner, feet first, in a supine position with their arms at their sides. Imaging took approximately 10 minutes to complete. Quantitative scans under normal-breathing conditions were followed by a 4-point Dixon technique (echo time: 2.38, 4.76, 7.15 and 9.53) (Glover 1991), with children holding their breath (3.9 s) to obtain water- and fat-separated images.

MRI images were exported to a remote workstation for data analysis using a customized MATLAB program (R2014b; MathWorks) (Cordes et al. 2015). Water and fat images were calculated and used to estimate subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) volumes (Brei et al. 2016). Non adipose tissue volumes (NAT) (composed mostly of water) and ratios of SAT, VAT and NAT to total volumes were also computed.

2.4 Collection of biosamples

2.4.1 Blood

Maternal blood was collected after an overnight fast at 32 weeks gestation. Blood was collected in EDTA-treated tubes from the umbilical vein at delivery. All blood samples were prepared by centrifugation for 10 minutes at 2000 x g at 4°C to separate plasma and erythrocytes. The erythrocytes were washed three times with 0.9% NaCl. Plasma and erythrocytes were aliquoted and stored at −86 °C until analysis.

2.4.2 Breast milk

Lactating mothers expressed fasting breast milk samples from an electric breast pump (Medela Symphony; Eching, Germany) at 6 weeks and 4 months postpartum. The samples

were aliquoted and stored at −80 °C until analysis. Skim breast milk was extracted from whole breast milk to prepare samples for analysis with the following steps: Three milk samples, each containing 1 ml of breast milk, were thawed to room temperature and vortexed. The milk was then sonicated with an ultrasound stick three times at 5-second bursts (ultrasonic processor UP100 H, 100 W, 30 kHz, 100 % amplitude; Hielscher Ultrasonics GmbH, Teltow, Germany). The breast milk was centrifuged at 14,000 rpm for 30 minutes (Eppendorf Centrifuge 5415C/D, Eppendorf AG, Hamburg, Germany) to separate milk fat from skim milk.

Biochemical samples were measured at the Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University Hospital Leipzig, Leipzig, Germany, unless otherwise indicated.

2.5 Laboratory analysis

2.5.1 Insulin in maternal and cord blood

Chemiluminescence immunoassay technology was used to measure maternal and cord blood insulin in EDTA plasma samples, performed on the fully automated LIAISON analyzer (DiaSorin, Saluggia, Italy). The inter- and intra-assay coefficients of variability were both 4%.

Fasting glucose from maternal plasma in sodium fluoride-containing tubes as well as maternal serum triglycerides were measured by an external laboratory (Synlab Labordienstleistungen, Munich, Germany). The degree of insulin resistance was approximated by the homeostasis model assessment for insulin resistance (HOMA-IR), as previously described (Matthews et al. 1985).

2.5.2 Leptin in maternal and cord blood

Leptin and leptin receptor (sObR) in maternal plasma were measured using the ELISA method (Mediagnost, Reutlingen, Germany) with an automated instrument (DYNEX Technologies, Chantilly, VA, USA) set to a sensitivity of 0.2 ng/ml. Inter- and intra-assay coefficients of variability were both below 5.2 %. sObR was estimated in a subset of maternal samples with an in-house ligand-immunofunctional assay (Lammert et al. 2001; Kratzsch et al. 2002). The free leptin index (FLI) was calculated as the ratio of leptin to sObR.

2.5.3 Fatty acids in maternal blood, cord blood, and breast milk

Fatty acid analysis from erythrocytes and breast milk was performed in the Laboratory of Lipid Research, Danone Research – Centre for Specialised Nutrition, Friedrichsdorf, Germany.

Formation and analysis of fatty acid methyl esters (FAME).

Frozen erythrocytes and breast milk were thawed at room temperature. Erythrocytes and breast milk samples were then dissolved in 2 ml methanol/hexane (4:1, v/v) in the presence of 0.5 % pyrogallol. The solution was mixed with 200 µl acetyl chloride and methylated by heating to 100 °C for 60 minutes according to published methods (Lepage and Roy 1984). Five ml of 6 % potassium carbonate was added and the sample was centrifuged at 4 °C for 10 minutes at 3200 rpm. The upper phase, containing the fatty acid methyl esters (FAMEs), was reserved for analysis. The individual FAMEs were quantified by capillary gas chromatography using a cold-on-column injector technique. All analyses were performed in duplicate. FAMEs were identified using GLC-85 as an external standard (GLC 85 standard mix, NuChekPrep, Inc. Elysian, Minnesota, USA). The instrumental configuration and experimental conditions are summarized in **Table 2**.

Table 2 Instrumental configuration and experimental conditions for FAME analysis

Instrumentation	
Chromatographic system	Agilent 6890N (Agilent Technologies, Waldbronn, Germany)
Detector	Flame ionization detector
Column	60 m X 0.25 mm ID, 0.25 µm, DB-23 (J&W Scientific, Agilent Technologies, USA)
Experimental conditions	
Carrier gas	Hydrogen, 1,8 ml/min
Oven temperature	60 °C for 0.1 min; 60 °C to 160 °C at 40 °C/min; 160 °C for 2 min; 160 °C to 190 °C at 3 °C/min; 190 °C to 220 °C at 4,5 °C/min, 220 °C for 5 min; 220 °C to 240 °C at 5 °C/min; 240 °C for 25 min
Detector temperature	280 °C

FAMEs were used to estimate fatty acid values (% FAs/total FAs). **Table 3** shows the composition of fatty acids used for analysis.

Table 3 Fatty acid groups analyzed in maternal blood, cord blood, and breast milk

Fatty acid group	Composition of fatty acids
Total n-3 fatty acids	C20:3n-3; C20:4n-3; C20:5n-3; C21:5n-3; C22:3n-3; C22:5n-3; C22:6n-3
Total n-6 fatty acids	C20:2n-6; C20:3n-6; C20:4n-6; C22:2n-6; C22:4n-6; C22:5n-6
Ratio n-6 / n-3 fatty acids	C20:2n-6; C20:3n-6; C20:4n-6; C22:2n-6; C22:4n-6; C22:5n-6 / C20:3n-3; C20:4n-3; C20:5n-3; C21:5n-3; C22:3n-3; C22:5n-3; C22:6n-3

2.6 Timeline and collection measurements for the INFAT secondary analyses

This doctoral work investigated secondary endpoints from INFAT, the first RCT to explore whether modifying the n-6/n-3 LCPUFA ratio in the maternal diet through dietary n-3 LCPUFA supplementation in pregnancy and lactation influenced adipose tissue expansion in offspring. No significant evidence of an intervention effect on fat mass or distribution was observed in offspring from birth to 5 years of age (Brei et al. 2017; Hauner et al. 2012). Therefore, data from the control and intervention groups were pooled for analyses.

Metabolic and fatty acid variables in maternal blood at 32 weeks gestation, cord blood, and breast milk were analyzed. Offspring body composition data from 2 to 5 years old were considered for outcome variables. **Table 4** provides a list of the biomarker candidates investigated in this work.

Table 4. Maternal and fetal biomarker candidates and offspring follow-up time-period.

Biomarker candidates	Offspring follow-up ages
n-6 & n-3 LCPUFAs Maternal blood at 32 weeks gestation, Cord blood & Breast milk at 6 and 16 weeks postpartum	2, 3, 4 & 5 years
Leptin Maternal blood at 32 weeks gestation & Cord blood	3, 4 & 5 years
Insulin Maternal blood at 32 weeks gestation & Cord blood	3, 4 & 5 years
Triglycerides Maternal blood at 32 weeks gestation	3, 4 & 5 years
HOMA-IR Maternal blood at 32 weeks gestation	3, 4 & 5 years

Figure 4 provides an overview of the INFAT cohort maternal and offspring biomarker candidates and body composition measurements.

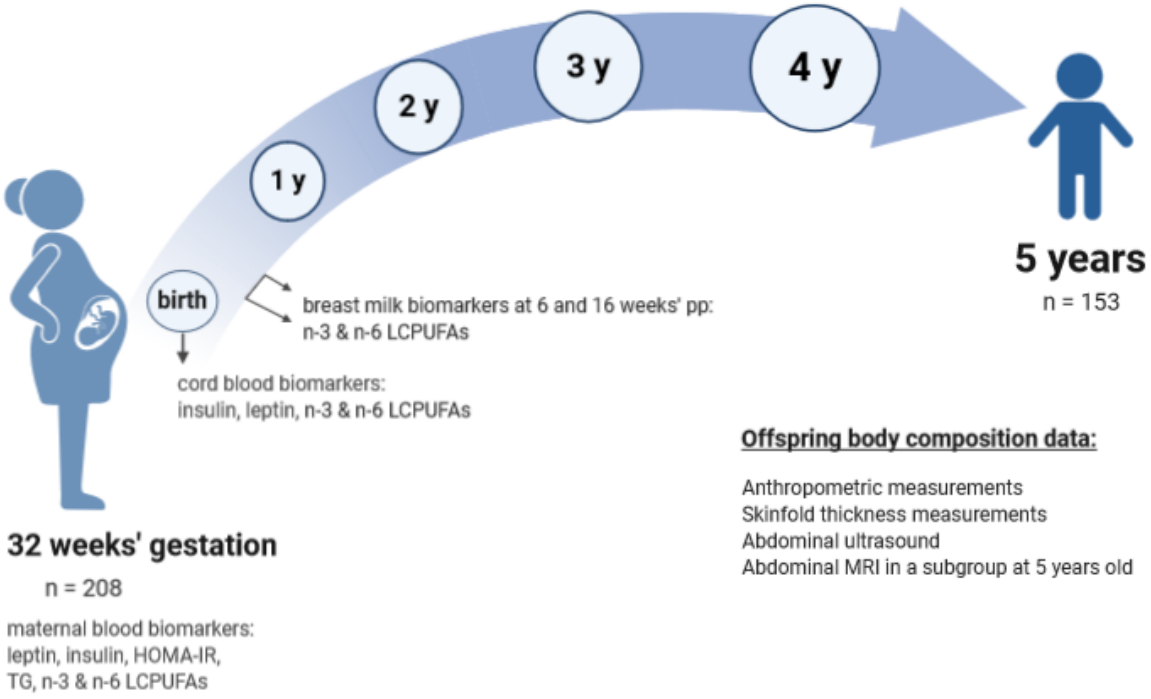

Figure 4 Timeline of data collection for the INFAT cohort biomarker analysis.

Body composition measurements for this doctoral work were analyzed from 2 – 5 years old.
Abdominal MRI was performed in a subgroup of 44 children at 5 years old.

HOMA-IR, homeostatic model assessment of insulin resistance; LCPUFAS, long-chain polyunsaturated fatty acids; MRI, magnetic resonance imaging; n, number; pp, postpartum; TG, triglycerides; wk, weeks; y, years

Figure created with BioRender.com

2.7 Statistical analyses

All analyses were performed with the software package IBM SPSS Statistics for Windows, version 23 (IBM Corp., Armonk, N.Y., USA). The Kolmogorov-Smirnov test was used to assess the normality of distribution for variables. Most child body composition parameters were normally distributed, while all maternal and fetal metabolic and fatty acid variables were non-normally distributed. Maternal and child characteristics are presented as percentages or

means and standard deviation (SD). Cord blood insulin is given as median and interquartile range (IQR). Mann-Whitney U tests were performed to determine differences in cord blood insulin between sexes.

Relationships between candidate biomarkers and child body composition were examined with linear regression models. Child outcome parameters, maternal and fetal metabolic variables, and perinatal fatty acid variables were continuous. Confounders were chosen in consultation with a statistician who is also a co-author of the manuscripts included in this doctoral work. Due to a sizable attrition rate, the number of observations for outcomes of interest was rather small, particularly for parameters estimating adipose tissue mass and distribution. Moreover, these analyses were a follow-up from similar analyses performed in the infant cohort. To maintain continuity and avoid over-fitting the models with too many predictor variables, no additional variables were considered for adjustment that were not included in previous INFAT analyses.

Neonatal growth outcomes in the intervention group were strongly associated with pregnancy duration, which was on average ~5 days longer in this group (Hauner et al. 2012). Therefore, multivariate models that included maternal and fetal metabolic variables were adjusted for ponderal index at birth (kg/m^3) and pregnancy duration. Associations between leptin in maternal plasma (at 32 weeks gestation) and cord blood on offspring clinical parameters were adjusted for maternal pre-pregnancy BMI, gestational weight gain, pregnancy duration, study group, sex (except for analyses with BMI percentiles), ponderal index at birth (kg/m^3) and mode of infant feeding (exclusively breastfed, partially breastfed or formula fed at 4 months postpartum). Relationships with sObR and FLI on offspring outcomes were examined by multiple regression in a subset of 72 maternal blood samples (collected at 32 weeks gestation) and 52 cord blood samples. Multiple regression analyses that examined associations between insulin, HOMA-IR, and triglycerides in maternal blood and cord blood insulin on child body composition included the same confounding variables as above, with the additional adjustment for maternal glucose tolerance (i.e. GDM diagnosis, yes/no).

Multiple linear regression models were fitted to examine associations between LCPUFAs in 1) maternal and cord blood LCPUFAs in RBCs, and 2) breast milk LCPUFAs at 6 weeks and 16 weeks postpartum on child outcomes. Due to the low number of observations for several of

the outcomes of interest, these models only adjusted for pregnancy duration, sex, and mode of infant feeding (exclusively breastfed or a combination of partially breastfed/formula). Our interest was examining LCPUFA status in pregnancy/lactation on adipose tissue growth. Therefore, these models were not adjusted for group allocation in order to avoid obscuring the estimates for fatty acid levels. A 2-sided P value of ≤ 0.05 was considered significant. No corrections were made for multiple comparisons.

All analyses were performed by the author of this doctoral work. Statistical advice and assistance with interpreting some results were provided by a statistician.

3 Results

This work investigated whether perinatal metabolic and fatty acid parameters were associated with growth and adipose tissue development in young children aged 2 – 5 years. This cumulative thesis is based on three original articles that have been published in peer-reviewed journals. Longitudinal data allowed investigating the relationships between candidate perinatal biomarkers and offspring adipose tissue over time. Results and manuscripts from secondary analyses of the INFAT study will be briefly summarized below.

3.1 Publication 1 – Associations between leptin in maternal and cord blood and offspring adipose tissue expansion

Title: "Leptin in Maternal Plasma and Cord Blood as a Predictor of Offspring Adiposity at 5 Years: A Follow-up Study"

Authors: **Dorothy Meyer**, Christina Brei, Lynne Stecher, Daniela Much, Stefanie Brunner, and Hans Hauner

Obesity (Silver Spring) 26(2): 279-283, 2018

Personal contribution: Dorothy Meyer reviewed the literature and developed the framework for the paper. She performed all of the statistical analyses, prepared tables, and wrote and revised the manuscript. All co-authors contributed to the manuscript by discussing the work progress, giving scientific advice, and editing.

Summary of findings:

This secondary analysis aimed to explore relationships between perinatal leptin concentrations and child body composition. This analysis is a follow-up of previous work by INFAT researchers that investigated the same associations in INFAT offspring from birth to 2 years old (Brunner et al. 2014). Leptin concentrations were assessed in maternal blood, measured at the 32nd week of gestation, and cord blood. Body composition parameters, including anthropometrics and predictive and direct adipose tissue measurements, were collected in offspring at 3, 4, and 5 years of age. Maternal plasma leptin was negatively

associated with child growth parameters, such as weight and BMI percentiles, but not with adipose tissue mass or distribution. Additional analysis that investigated maternal and cord blood FLI and sObR in a subset of 72 mother/child pairs found no associations with child body composition at 5 years. Cord leptin was inversely associated with weight, weight gain, and lean body mass at 3 and 4 years. However, most relationships were attenuated toward the null at 5 years except for inverse relationships with higher fat mass and body fat percentage. Importantly, effect sizes for most associations were very small and many associations were not consistently present at all investigated time-points. Notably, no associations between maternal or cord leptin and abdominal adipose tissue, measured by ultrasound and MRI, were found. In summary, several weak negative associations between perinatal leptin concentrations and child body composition were observed. However, many relationships were transient and did not persist up to 5 years old. Moreover, no evidence of associations between maternal or fetal leptin and abdominal fat mass or distribution was identified.

3.2 Publication 2 – Insulin and indices of glucose and lipid metabolism as determinants of offspring adiposity

Title: "Maternal insulin resistance, triglycerides, and cord blood insulin are not determinants of offspring growth and adiposity up to 5 years: a follow-up study"

Authors: **Dorothy Meyer**, Christina Brei, Lynne Stecher, Stefanie Brunner, and Hans Hauner

Diabetic Medicine: 35 (10): 1399-1403, 2018.

Personal contribution: Dorothy Meyer reviewed all relevant literature and developed the structure of the paper. She performed all of the statistical analyses, prepared tables, and wrote and revised the manuscript. All co-authors contributed to the manuscript by discussing the work progress and giving scientific and editing advice.

Summary of findings:

This cohort analysis examined the role of maternal insulin, insulin resistance, and triglycerides on offspring growth and fat development. Associations with cord blood insulin on child clinical outcomes were also investigated. This analysis was a follow-up of work performed by Brunner and colleagues who found that cord blood insulin was inversely related to weight gain in infant girls at 2 years, while maternal metabolic variables were largely unrelated to child body composition (Brunner et al. 2013). Maternal triglycerides, insulin, and insulin resistance (estimated by HOMA-IR) were measured at 32 weeks gestation. Cord blood insulin was collected at delivery. Child anthropometric measurements and abdominal ultrasound were assessed annually from 3 to 5 years. Direct measurements of fat patterning and distribution were assessed by abdominal MRI in a subgroup of 44 children. Associations between the aforementioned maternal and fetal parameters and body composition in children at 3, 4, and 5 years old were explored by multiple regression with adjustments for putative confounders. Maternal metabolic variables were largely unrelated to child clinical outcomes, which suggests that minor changes in glucose metabolism and insulin sensitivity in healthy non-obese women do not have adverse effects on growth or fat development in young children. Some negative temporal relationships between cord insulin and child fat mass were observed but did not persist until 5 years. Similar to observations in 2-year-old INFAT children, inverse associations

between cord blood insulin and weight gain from birth to 5 years were observed in girls only. There were no observed relationships between any of the investigated metabolic or fetal variables and direct measurements (by MRI or ultrasound) of abdominal adipose tissue in children. In conclusion, these findings did not provide sufficient evidence that markers of maternal insulin resistance or triglycerides are related to adipose tissue accretion in early childhood. Cord blood insulin was associated with weight gain in girls, albeit with small effect sizes.

3.3 Publication 3 – Maternal and fetal long-chain PUFAs in relation to offspring body composition

Title: "Associations between long-chain PUFAs in maternal blood, cord blood, and breast milk and offspring body composition up to 5 years: follow-up from the INFAT study".

Authors: **Dorothy Meyer**, Christina Brei, Lynne Stecher, Daniela Much, Stefanie Brunner, and Hans Hauner

European Journal of Clinical Nutrition: 73(3): 458-464, 2019

Personal contribution: Dorothy Meyer reviewed the literature and developed the conceptual framework for the paper. She performed all of the statistical analyses, prepared tables, and wrote and revised the manuscript. All co-authors contributed to the manuscript by discussing the work progress and providing editing and scientific advice.

Summary of findings:

This secondary analysis investigated the influence of perinatal LCPUFAS on early childhood growth and fat development. This work builds upon previous INFAT findings, which indicated that both maternal n-6 and n-3 LCPUFAs promote gestational growth, whereas n-6 LCPUFAs play a larger role in postnatal development (Much et al. 2013b). Moreover, higher concentrations of n-3 LCPUFAs in breast milk were associated with greater fat depots in infants up to 1 year old (Much et al. 2013a). This follow-up longitudinal analysis investigates these relationships in INFAT children from 2 to 5 years of age. Maternal blood at 32 weeks gestation, cord blood, and breast milk at 6 weeks and 4 months postpartum were collected. N-6 and n-3 fatty acid profiles were assessed from the maternal and fetal biosamples. LCPUFAs in maternal RBCs, cord RBCs, and breast milk at 4 months postpartum were largely unrelated to child clinical outcomes. The key finding was that LCPUFAs in breast milk measured at 6 weeks postpartum appeared to have the most profound effects on child body composition. N-3 LCPUFAs and DHA in breast milk at 6 weeks postpartum were associated with higher weight and adiposity measures at 2 years, and with higher BMI percentiles and increased lean body mass at 4 years. Moreover, the ratio of breast milk n-6/n-3 LCPUFAs at 6 weeks postpartum was inversely related to weight and BMI percentiles at 2 years, and lean body

mass at 4 and 5 years. Notably, most associations were transient and disappeared by 5 years of age. In conclusion, this follow-up analysis of INFAT offspring did not find evidence that maternal and cord blood LCPUFAs were consistently associated with adiposity outcomes in young children. While some temporal relationships between breast milk LCPUFAs at 6 weeks postpartum and child body development existed, most were not present at 5 years old.

4 Discussion

Using data from the INFAT cohort, this project sought to identify maternal and fetal metabolic and fatty acid parameters that were associated with adipose tissue expansion in early childhood. The long-term follow-up provided rare and important insights into the nature of many of these relationships over time. Altogether, this work sheds new light on the relationships between perinatal blood and breast milk parameters and child body composition. The following discussion highlights the uniqueness of the INFAT longitudinal study design and considers the scope and limitations in biomarker research for risk profiling of early obesity.

4.1 Insulin, triglycerides, and biomarkers of insulin resistance

The insulin/IGF pathway plays an essential role in regulating fetal growth and fat accretion. Alterations in maternal insulin levels have been proposed as a plausible biological mechanism underlying obesity in offspring (Hiden et al. 2009). Although the link between GDM and offspring adiposity is well-established (Catalano 2010), surprisingly few studies have examined whether the degree of maternal insulin resistance, independent of GDM, is associated with child outcomes. Findings thus far have focused on newborns and are largely inconsistent (Bomba-Opon et al. 2009; Voldner et al. 2010; Yamashita et al. 2014). Yamashita and colleagues observed that HOMA-IR in healthy, non-diabetic women was positively associated with birth weight and an independent risk factor for babies born LGA (Yamashita et al. 2014). Yet, other research groups were unable to confirm these findings (Bomba-Opon et al. 2009; Voldner et al. 2010). INFAT is one of the few groups to examine relationships beyond neonatal outcomes. Similar to observations in the Rhea pregnancy cohort (Daraki et al. 2015) we saw no evidence that maternal insulin or markers of insulin resistance (HOMA-IR) were related to obesity outcomes in preschool-aged children. Drawing conclusions from our findings and others, it appears that small changes in maternal insulin resistance in healthy pregnancies do not have an impact on offspring growth.

Gender differences in insulin concentrations have been documented from birth onwards. Girls are born lighter and with intrinsically higher insulin levels, suggesting that newborn girls are more insulin resistant than boys (Murphy et al. 2004; Shields et al. 2007). The observed gender

difference in insulin resistance persists throughout childhood, with adolescent girls more likely to develop type 2 diabetes mellitus than boys (Feltbower et al. 2003; McMahon et al. 2004; Wabitsch 2000). Consistent with earlier findings in INFAT babies (Brunner et al. 2013), this follow-up analysis revealed that lower cord blood insulin was associated with weight gain from birth to 5 years old in girls only, suggesting that changes in the hormonal milieu in utero may have different phenotypic effects on male and female offspring. A plausible biological explanation for the observed sex differences is that gender-specific genes mediate insulin sensitivity. Insulin exposure during fetal development could influence early weight gain, whereby a sex-differential response results in girls demonstrating higher susceptibility to changes in concentrations (Wilkin and Murphy 2006). It is unclear if these sex-related differences have consequences for increased risk of obesity later on. The increase in weight gain seen in INFAT 5-year-old girls was rather small, which raises questions about whether findings are clinically relevant. Importantly, there was no evidence that cord insulin was associated with adipose tissue mass in preschool children, confirming findings from others (Thaware et al. 2015).

Analogous to observations in our infant cohort (Brunner et al. 2013), this work found no evidence that triglyceride levels in the third trimester of pregnancy were associated with child body composition. Further research is needed to elucidate relationships between maternal triglycerides and offspring growth, and studies investigating these associations are scarce. It has been theorized that triglycerides may be used as an additional energy source for the fetus (Kulkarni et al. 2013). Hence, higher maternal triglyceride concentrations lead to enhanced placental transfer of lipids, subsequently resulting in increased fat deposition in fetal tissues (Heerwagen et al. 2010). While some evidence shows a link between maternal triglycerides and birthweight (Geraghty et al. 2016; Kulkarni et al. 2013), the relationship disappears in late infancy (Geraghty et al. 2016).

In summary, this work did not identify maternal or fetal parameters of insulin resistance, insulin, or triglycerides that were related to growth or body composition in early childhood.

4.2 Leptin

Evidence of perinatal leptin's role in regulating early offspring growth and development in humans is limited. Consistent with earlier INFAT findings (Brunner et al. 2014), we could not confirm that maternal leptin was associated with fat development in preschool children, and observed a stronger contribution of fetal leptin to early childhood growth. Research investigating the association between maternal leptin levels and early offspring growth has reported inconsistent findings (Hinkle et al. 2019; Josefson et al. 2014; Telschow et al. 2019). Hinkle et al. demonstrated that the direction of the relationship between maternal leptin and neonatal adiposity differed according to the maternal BMI category (Hinkle et al. 2019). For instance, free leptin was positively associated with birth length and skinfold thickness in offspring of women with obesity, but negative associations were observed in women with normal- and overweight. Conversely, Telschow et al. found that high maternal leptin levels corresponded to a more pronounced BMI-SDS increase in the first year of life irrespective of maternal BMI (Telschow et al. 2019). Others have demonstrated a link between higher concentrations of maternal leptin and lower BMI z-score and waist circumference at 3 and 7 years old, though no associations between maternal leptin and SFTs were noted (Boeke et al. 2013). Some inconsistencies may be explained due to differences in the timing of maternal leptin measurements or child body composition measurement methods. Taken together, it appears that maternal leptin may influence offspring body composition differently depending on the gestational period and the existence of other maternal determinants, such as obesity, but more studies are needed before drawing any meaningful conclusions.

An important deficit in research conducted so far is the lack of accurate methods to estimate offspring fat depots. The studies presented above rely largely on BMI or other predictive measurements. One of the few studies that looked at direct fat measurements could find no evidence that maternal leptin was related to fat mass by DXA at 7 years (Boeke et al. 2013). These results are in line with INFAT follow-up observations, presented herein, that found no associations between leptin and abdominal fat at 5 years measured by abdominal ultrasound and MRI.

The relationship between cord blood leptin and growth has been demonstrated at birth (Chaoimh et al. 2016; Clapp and Kiess 1998; Donnelly et al. 2015; Ong et al. 1999; Valūniene

et al. 2007), but less is known about later time-points. Project Viva, an American maternal/child cohort, reported that lower cord leptin led to an increased rate of weight gain in the first year of life (Mantzoros et al. 2009), an observation also noted by others (Kaar et al. 2014). Project Viva also reported that higher cord leptin levels were associated with slower weight gain in the first 6 months among babies of diabetic mothers (Parker et al. 2011). These studies support the role of fetal leptin as a key regulator of energy metabolism and food intake in early infancy. However, data in childhood is rare. Follow-up observations from Project Viva report that inverse relationships between cord leptin and adiposity persist at 3 years (estimated by BMI and SFT), but are largely absent by 7 years of age (Boeke et al. 2013). This observation in early childhood was confirmed by the Rhea study, which noted similar BMI patterns in children from birth to 4 years old (Karakosta et al. 2016). This INFAT follow-up analysis observed some transient relationships with cord blood leptin and body composition at 3 and 4 years, but not all associations were consistently significant and effect sizes were small. Importantly, no relationships between cord blood leptin and measures of abdominal adipose tissue by ultrasound or MRI were identified. In summary, it appears that cord leptin may be a modulator of childhood obesity, and therefore hold promise as a potential perinatal biomarker. More studies are needed with larger and diverse cohorts to further explore these relationships. Importantly, these studies should be designed with methods that accurately measure body composition and fat patterning.

4.3 Perinatal fatty acid biomarkers

LCPUFAs in maternal and fetal blood

Limited human data suggests that offspring body composition is influenced by perinatal LCPUFA status. Earlier work by INFAT colleagues demonstrated that exposure to n-6 and n-3 fatty acids in utero influences growth and fat development in the first year of life (Much et al. 2013b). This follow-up analysis was not able to confirm that these findings persisted in INFAT children from 2 to 5 years old. In contrast to our results, Project Viva found that enhanced levels of prenatal and cord blood n-3 LCPUFAs were associated with lower adiposity outcomes at 3 years (estimated by SFTs) (Donahue et al. 2011). When considering the n-3 LCPUFA status, it is important to examine the relative intake of n-6 LCPUFAs due to their pro-adipogenic activity and putative role in offspring growth (Muhlhausler and Ailhaud 2013). Research

suggests that maternal n-6 LCPUFAs are strongly associated with offspring adiposity outcomes (Vries et al. 2014). A large maternal/child cohort, the Southampton Women's Survey (SWS), assessed these associations by measuring fat patterning with DXA (Moon et al. 2013). While they could not confirm inverse relationships between n-3 LCPUFAs and child fat mass seen in Project Viva, they did report that higher concentrations of prenatal n-6 LCPUFAs were associated with a small increase in fat mass measurements (~2%) at 4 and 6 years of age, an observation confirmed by others (Vries et al. 2014). Other work has explored whether the n-6/n-3 ratio in maternal plasma and cord blood influences childhood obesity risk and report inconsistent relationships with offspring adiposity outcomes (Moon et al. 2013; Vidakovic et al. 2016; Vries et al. 2014). However, most studies to date lack the longitudinal design to examine if these effects are persistent and clinically meaningful, suggesting a need for further research in this area.

LCPUFAs in breast milk

The role of breast milk LCPUFAs on offspring adipose tissue development is also unclear and data from human studies are limited. Previous INFAT analysis found consistent positive associations between n-3 LCPUFAs in breast milk at 6 weeks postpartum and fat mass at 1 year of age (Much et al. 2013a). In this follow-up analysis, higher concentrations of DHA and total n-3 LCPUFAs in breast milk at 6 weeks postpartum were positively associated with several measurements of growth and adiposity at 2 and 4 years old. These findings argue against the hypothesis that exposure to enhanced n-3 LCPUFAs in early postpartum protects against obesity development in early childhood. The INFAT study measured breast milk fatty acids at both 6 weeks and 16 weeks postpartum, allowing us to observe if relationships between breast milk LCPUFAs and child body composition differed depending on the measurement time-point. It is interesting to note that LCPUFAs measured in later postpartum were largely unassociated with offspring clinical outcomes. This suggests that exposure to breast milk fatty acids earlier in the postpartum period may have a more pronounced effect on adipose tissue development in early childhood. These findings contrast results from another study that found that higher breast milk n-3 LCPUFAs at 4 months postpartum was associated with increased BMI at 2.5 years old (Lauritzen et al. 2005), though associations were attenuated toward the null at the 7-year follow-up assessment (Asserhøj et al. 2009).

In summary, analysis from the INFAT cohort could not demonstrate a clear relationship between perinatal LCPUFA status and offspring obesity risk. A Cochrane review of eight RCTs evaluating the effects of n-3 LCPUFA supplementation in pregnancy and lactation on offspring obesity outcomes confirms these results (Delgado-Noguera et al. 2015). When discussing inconsistent findings across studies, it is important to consider the wide variations in study design and methodological approaches. Mixed results may be partly due to differences in body composition assessment methods, variation in maternal fatty acid intake, and measurement time-points of fatty acids and body composition. It should also be mentioned that the risks and benefits of modifying the maternal dietary fat intake in pregnancy and lactation are not clear, and the safety of high intake of LCPUFAs in pregnancy is not established (Eritsland 2000; Hamosh 1998; Lauritzen et al. 2001). Given these considerations, recommendations to increase LCPUFA intake in pregnancy beyond current recommendations are not advised.

4.4 Biomarkers and a clinical approach to obesity prevention

Physicians use biomarkers for screening, diagnosis, prognosis, and treatment. Considering their importance in clinical practice, it is not surprising that the interest in identifying predictive biomarkers for early obesity prevention has grown. Prenatal markers could provide a source of biological information about the intrauterine environment and metabolic processes that may contribute to excessive fetal growth. This knowledge can be used in combination with other clinical methods to generate risk profiles for early overweight/obesity. Our ability to predict child outcomes during fetal development is limited and centers mainly around maternal characteristics, such as prepregnancy BMI, GWG, and lifestyle factors (Liao et al. 2019; Monasta et al. 2010). As outlined in this work, the field of fetal programming is just beginning to investigate candidate biomarkers that could guide prevention-directed actions.

What makes a good biomarker?

A comprehensive systematic review of prenatal markers found that most circulating markers, including adipokines, lipids, and markers of glucose tolerance, were either unrelated to neonatal fat mass or effect sizes were small (Roelants et al. 2016). When considering potential

biomarkers of obesity, assessing clinical relevance is paramount. Roelants et al. (2016) concede that while some prenatal biomarker candidates were statistically significant in large cohorts they had no clinical value for individual patients. Research described in this work highlight the dearth of large, heterogenous maternal/child cohorts that would allow researchers to determine the clinical validity of the investigated perinatal parameters. A good biomarker is generalizable and consistent across populations and various individual characteristics (Steyerberg 2009). Sensitivity and specificity are also important. Specificity refers to the ability to correctly rule out offspring who are not at increased risk, while sensitivity refers to the ability to correctly predict those who are (Steyerberg 2009). Another consideration is the cost-effectiveness and convenience of biomarker collection and processing. All biomarkers investigated in this work were derived from blood and breast milk, which increases the feasibility of wide-scale application since collection and analysis are cheap and minimally invasive. High-throughput technologies are also emerging as novel methods to identify biomarkers in the nutrigenomics, epigenomics, and metabolomics fields, as summarized in a recent review (Wu et al. 2020).

Important limitations in perinatal biomarker research cannot be ignored when evaluating the evidence to date. First, most studies, including INFAT, examine candidate biomarker relationships as secondary endpoints. INFAT was not powered for secondary outcomes which must be taken into account when interpreting results. Second, for myriad reasons, many maternal/child cohorts are small and may not represent the general population. Thus, relationships observed between perinatal markers and offspring outcomes may not be present in other cohorts with different characteristics. Third, the timing of measurements needs to be addressed. Many studies measured prenatal candidate biomarkers in the third trimester (Roelants et al. 2016), yet the DOHaD model emphasizes the importance of exposures that occur throughout the first 1,000 days (Barker 2001). This doctoral work took a comprehensive approach by investigating potential biomarkers from maternal and fetal samples that were collected at four time-points in pregnancy and lactation: 32 weeks gestation, birth, and twice in postpartum. Moreover, many effects of perinatal programming can only be ascertained years after exposure. Most studies investigated adiposity outcomes at birth or in infancy. Repeated body composition measurements need to be collected throughout childhood and even into adolescence. Finally, many studies investigate single

biological pathways, which overlook complex interactions between multiple mechanisms and their combined contribution towards obesity-related outcomes.

Concomitant with improving risk profiling is the need to reassess current standards for measuring obesity in children. Anthropometric measurements, such as BMI and body weight, have been predominantly used to estimate obesity in both research and clinical settings. However, measuring fat patterning and distribution provides a better picture of metabolic risk and should be consistently applied in obesity research. INFAT has provided a valuable contribution to the research community by validating abdominal ultrasound as a reliable and reproducible method in preschoolers for measuring subcutaneous and preperitoneal fat.

The findings presented herein also emphasize the need to generate longitudinal studies that examine the impact of perinatal nutritional and metabolic changes on child health outcomes. INFAT stands out as one of the few studies that has followed-up children throughout the entire period of early childhood. Many of the relationships between candidate perinatal biomarkers and body composition in the INFAT cohort were only found to be temporal and not consistently present at 5 years of age. This is important to consider when planning future studies that investigate biomarkers in early childhood, as around 5 years is thought to be the age of adiposity rebound that can predict the risk of obesity later on (Rolland-Cachera et al. 2006). A recent German study confirms this hypothesis by observing that accelerated weight gain in the preschool age is predictive of overweight and obesity in adolescence (Geserick et al. 2018).

Along with developing a predictive screening approach, evidence-based interventions that are proven to mitigate risk must also be available. Unfortunately, few interventions that target early obesity prevention exist, and most focus on individual- and family-level changes with only moderate success rates (Blake-Lamb et al. 2016). Maternal dietary interventions are also largely ineffective, as seen in the 5-year follow-up from the INFAT trial that demonstrated that n-3 LCPUFA supplementation did not influence offspring adipose tissue development (Brei et al. 2017; Hauner et al. 2012), a finding confirmed in a recent meta-analysis (Vahdaninia et al. 2019). A meaningful prevention strategy must include individual, community, and population changes that address not only diet and physical activity but also policy and legislation (Huang et al. 2007). To summarize, concomitant research in early childhood obesity prevention must

be intensified, so that clinicians will have access to evidence-based strategies to effectively intervene at an early stage of life.

Limitations

This doctoral work has several limitations to consider. All analyses presented in this work were exploratory and sought to identify associations between candidate perinatal markers and offspring outcomes. It is worth mentioning that the INFAT study was powered to detect differences in adipose tissue mass (assessed by SFT measurements) between intervention and control group infants at 4 months postpartum (Hauner et al. 2009; Hauner et al. 2012). The power calculation is based on a total of 204 women (104 in each group) and INFAT was not powered for secondary analyses. Hence, we cannot conclude from our non-significant findings that there are no effects between predictor and outcome variables. Our sample size was small which further limits the ability to interpret effect sizes. Moreover, with the exception of examining the combined effect of child sex and cord blood insulin on body composition, we did not examine whether interactions between perinatal markers may have had additive or synergistic effects on child outcomes.

In consultation with the statistician and agreement from the principal investigator, predictors were chosen a priori that were also considered in earlier INFAT analyses and were within the sample size limits to avoid overfitting regression models. However, residual confounding due to other factors cannot be excluded. For instance, lifestyle factors are known to play a role in childhood obesity development. Data on maternal education level, child physical activity, and child dietary habits were collected but were not included as confounding variables. Further, we adjusted for the mode of infant feeding (i.e. exclusively breastfed, partially breastfed or formula-fed) but did not distinguish between exclusive and predominant breastfeeding, the latter of which includes water or water-based drinks (World Health Organization (WHO) 2008) and has been shown to influence breastfeeding duration and other outcomes (Giovannini et al. 2005).

Child body composition variables were largely normally distributed and extreme outliers were removed prior to fitting regression models. Some outcome parameters violated the normality assumption, however, linear regression is somewhat robust to some departures from normality (Ghasemi and Zahediasl 2012). Due to the exploratory nature of these analyses,

there were no corrections made for multiple testing. Therefore, significant findings and effect sizes should be interpreted with caution.

With respect to metabolic and fatty acid parameters, we measured insulin but not C-peptide, which may be a better indicator of insulin secretion (O'Rahilly et al. 1987) and has been associated with offspring growth and metabolic outcomes (Regnault et al. 2011; HAPO Cooperative Research Group 2009). Moreover, breast milk was measured at 6 and 16 weeks postpartum. Importantly, both of these samples are mature breast milk, and the lack of sample collection at earlier time points precluded the ability to assess the influence of LCPUFAs in colostrum or transitional milk on offspring body composition.

The women in our cohort were relatively well educated, with 70% (n= 119) reporting to have completed >12 years of school. Moreover, our participants were generally healthy and non-obese, with a mean BMI of 22 kg/m^2, and only four women (2.4%) reported to have smoked during pregnancy. There also was a considerable loss to follow-up throughout the longitudinal observation period which may have introduced selection bias, although mothers lost to follow-up did not differ significantly in sociodemographic or clinical characteristics from those who completed the study. We cannot assume that our findings can be generalized to groups from other socioeconomic backgrounds or in women with obesity. In summary, our cohort is not representative of the general population and further research is warranted to confirm our findings. Importantly, validation studies investigating associations between perinatal biomarker candidates and child obesity should use accurate methods for measuring offspring adipose tissue as the primary outcome. Moreover, when deciding on adequate sample size, meaningful effect sizes should be considered at the onset, as well as factors that may reduce the sample size, such as drop-out rates and missing data (Velentgas et al. 2013). Larger and more diverse sample sizes are needed, including women from all BMI classes and varied socioeconomic backgrounds.

4.5 Conclusion and Outlook

Robust evidence clearly indicates that early life events can have an impact on long-term health. This window can also be viewed as a unique opportunity for interventions that can potentially improve health over the entire life course. To our knowledge, INFAT is the first research group that not only measured maternal and fetal parameters repeatedly in the perinatal period but also investigated their associations with body composition across the entire span of infancy and early childhood. The INFAT cohort contributes valuable data to the emerging field of biomarker research as a strategy to prevent or mitigate childhood obesity.

Identifying biomarkers of adipose tissue expansion that confer clinical utility could be a valuable tool in the battle to reverse current obesity trends, as early prediction will allow for targeted perinatal interventions. Unfortunately, results from the INFAT cohort could not identify maternal or fetal metabolic and LCPUFA variables that were consistently associated with adipose tissue development in infancy and early childhood. A review of the literature also demonstrates that research into clinically relevant biomarkers to identify children at risk of obesity is lacking (Roelants et al. 2016; Wu et al. 2020), particularly studies in early childhood (age 2-5 years) despite it being an important developmental period.

Complex interactions between biological and environmental exposures overlap to contribute to early obesity. Thus, a clinically useful predictive model will most likely be a multi-parametric panel, comprised of biomarkers, offspring-, and maternal factors to gain a clearer picture of disease etiology and identify risk profiles.

To accelerate perinatal biomarker research, collaborative efforts and data-sharing on a large scale would need to be implemented. Data pooling would allow for meta-analyses and the generation of data-driven hypotheses. Pooled, diverse cohorts could also provide insights into the heterogeneous effects of perinatal factors in different subgroups. This may generate tailored obesity prevention strategies, particularly for vulnerable populations.

Going forward, the importance of research that focuses on the early prevention of obesity cannot be overemphasized as a global public health goal. This doctoral work forwards our understanding of the mechanisms that link the maternal nutritional and hormonal milieu with growth and adipose tissue accretion in early childhood.

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7 Appendix

Appendix 1: Maternal free leptin index at 32 weeks gestation in relation to child body composition at 5 years of age

Body composition variables	n	Unadjusted Analysis		Adjusted Analysis*	
		beta (95% CI)	*P*	beta (95% CI)	*P*
5 years					
Weight (kg)	72	0.04 (−1.15; 1.22)	0.950	−1.38 (−3.17; 0.41)	0.127
BMI Percentiles	71	−2.65 (−16.22; 10.92)	0.698	−25.34 (−43.89; −6.79)	0.008
Sum 4 SFT (mm)	56	−0.68 (−3.28; 1.91)	0.600	−1.96 (−5.86; 1.95)	0.318
Fat mass (kg)	58	−0.26 (−0.81; 0.29)	0.346	−0.54 (−1.34; 0.25)	0.177
Body fat (%)	56	−0.56 (−2.59; 1.48)	0.596	−1.28 (−3.98; 1.42)	0.345
Lean Body Mass (kg)	56	−0.63 (−1.61; 0.35)	0.201	−1.18 (−2.54; 0.19)	0.089
Weight gain (birth – 5 y)	72	−0.11 (−1.20; 0.98)	0.944	−1.61 (−3.30; 0.08)	0.061
Area sag pp, (mm^2)	50	−3.22 (−10.84; 4.40)	0.399	−4.54 (−16.32; 7.23)	0.440
Area sag sc, (mm^2)	51	−4.11 (−12.24; 4.02)	0.315	−3.83 (−15.18; 7.51)	0.499
Area ax sc, (mm^2)	52	−4.43 (−16.18; 7.33)	0.453	−7.23 (−23.93; 9.50)	0.387
VAT volume, (cm^3)	23	2.06 (−28.21; 32.33)	0.889	−32.80 (−102.04; 41.45)	0.359
SAT volume, (cm^3)	23	6.24 (−113.70; 126.19)	0.915	−110.09 (−423.66; 203.49)	0.464

Data are presented as the regression coefficient beta (95% CI) from linear regression analyses.

*Adjusted for maternal pre-pregnancy BMI, gestational weight gain, pregnancy duration, sex (except for BMI percentiles), ponderal index at birth, group, and mode of infant feeding (partially or exclusively breastfed or formula) at 4 months postpartum.

Abbreviations: Area ax sc, area of subcutaneous fat in the axial plane; area sag pp, area of preperitoneal fat in the sagittal plane; area sag sc, area of subcutaneous fat in the sagittal plane; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue

Appendix

Appendix 2: Maternal plasma sObR at 32 weeks gestation in relation to child body composition at 5 years of age

Body composition variables	n	Unadjusted Analysis		Adjusted Analysis*	
		Beta (95% CI)	*P*	Beta (95% CI)	*P*
5 years					
Weight (kg)	72	0.03 (−0.03; 0.09)	0.316	0.08 (0.02; 0.14)	0.011
BMI Percentiles	71	0.35 (−0.29; 0.99)	0.283	0.88 (0.22; 1.55)	0.010
Sum 4 SFT (mm)	56	0.03 (−0.08; 0.15)	0.572	0.07 (−0.07; 0.20)	0.319
Fat mass (kg)	56	0.01 (−0.01; 0.04)	0.327	0.02 (−0.01; 0.05)	0.128
Body fat (%)	56	0.02 (−0.07; 0.11)	0.609	0.05 (−0.05; 0.14)	0.313
Lean Body Mass (kg)	56	0.03 (−0.02; 0.07)	0.244	0.04 (−0.01; 0.09)	0.085
Weight gain (birth – 5 y)	72	0.04 (−0.02; 0.09)	0.161	0.08 (0.03; 0.14)	0.006
Area sag pp, (mm^2)	50	0.06 (−0.28; 0.40)	0.732	0.04 (−0.40; 0.48)	0.856
Area sag sc, (mm^2)	51	−0.03(−0.39; 0.34)	0.889	0.06 (−0.36; 0.48)	0.778
Area ax sc, (mm^2)	52	−0.18 (−0.70; 0.34)	0.484	−0.06 (−0.66; 0.55)	0.856
VAT volume, (cm^3)	23	−2.61 (−6.61; 2.29)	0.324	0.09 (−7.45; 7.62)	0.980
SAT volume, (cm^3)	23	−0.55 (−1.67; 0.58)	0.322	−0.17 (−1.99; 1.64)	0.847

Data are presented as the regression coefficient beta (95% CI) from linear regression analyses.

*Adjusted for maternal pre-pregnancy BMI, gestational weight gain, pregnancy duration, sex (except for BMI percentiles), ponderal index at birth, group, and mode of infant feeding (partially or exclusively breastfed or formula) at 4 months postpartum.

Abbreviations: Area ax sc, area of subcutaneous fat in the axial plane; area sag pp, area of preperitoneal fat in the sagittal plane; area sag sc, area of subcutaneous fat in the sagittal plane; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue

Appendix

Appendix 3: Cord blood free leptin index in relation to child body composition at 5 years of age

Body composition variables	n	Unadjusted Analysis		Adjusted Analysis*	
		beta (95% CI)	*P*	beta (95% CI)	*P*
5 years					
Weight (kg)	52	0.20 (−0.82; 1.23)	0.691	−0.78 (−1.93; 0.37)	0.179
BMI Percentiles	51	3.77 (−7.83; 15.37)	0.517	−0.78 (−12.09; 10.53)	0.890
Sum 4 SFT (mm)	41	1.58 (−0.77; 3.83)	0.183	1.00 (−1.94; 3.95)	0.492
Fat mass (kg)	41	0.33 (−0.19; 0.85)	0.209	0.00(−0.61; 0.62)	0.994
Body fat (%)	41	1.62 (−0.20; 3.45)	0.080	0.71 (−1.31; 2.73)	0.479
Lean Body Mass (kg)	41	−0.08 (−0.90; 0.74)	0.841	−0.49 (−1.45; 0.47)	0.307
Weight gain (birth – 5 y)	52	−0.11 (−1.06; 0.85)	0.822	−0.95 (−2.06; 0.15)	0.089
Area sag pp, (mm^2)	36	3.16 (−3.34; 9.66)	0.330	5.34 (−2.79; 13.47)	0.189
Area sag sc, (mm^2)	37	4.46 (−2.51; 11.43)	0.202	1.73 (−6.69; 10.14)	0.677
Area ax sc, (mm^2)	38	9.01 (−0.51; 19.52)	0.063	3.51 (−8.59; 15.62)	0.557
VAT volume, (cm^3)	19	14.56 (−8.81; 37.92)	0.206	31.50 (1.74; 61.26)	0.040
SAT volume, (cm^3)	19	94.04 (21.01; 167.07)	0.015	121.05 (1.07; 241.02)	0.048

Data are presented as the regression coefficient beta (95% CI) from linear regression analyses.

*Adjusted for maternal pre-pregnancy BMI, gestational weight gain, pregnancy duration, sex (except for BMI percentiles), ponderal index at birth, group, and mode of infant feeding (partially or exclusively breastfed or formula) at 4 months postpartum.

Abbreviations: Area ax sc, area of subcutaneous fat in the axial plane; area sag pp, area of preperitoneal fat in the sagittal plane; area sag sc, area of subcutaneous fat in the sagittal plane; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue

Appendix

Appendix 4: Cord blood sObR in relation to child body composition at 5 years of age

Body composition variables	n	Unadjusted Analysis		Adjusted Analysis*	
		beta (95% CI)	*P*	beta (95% CI)	*P*
5 years					
Weight (kg)	52	−0.02 (−0.17; 0.14)	0.823	−0.06 (0.22; 0.11)	0.509
BMI Percentiles	51	−0.40 (−2.17; 1.37)	0.650	−0.92 (−2.73; 0.90)	0.315
Sum 4 SFT (mm)	41	−0.31 (−0.63; 0.02)	0.063	−0.48 (−0.86; −0.10)	0.016
Fat mass (kg)	41	0.05 (−0.13; 0.02)	0.147	− 0.35(−0.61; −0.10)	0.009
Body fat (%)	41	−0.25 (−0.51; 0.01)	0.056	−0.09 (−0.17; − 0.01)	0.037
Lean Body Mass (kg)	41	−0.02 (−0.13; 0.10)	0.772	−0.07 (−0.21; 0.07)	0.297
Weight gain (birth – 5 y)	52	0.00 (−0.14; 0.14)	0.996	−0.03 (−0.19; 0.14)	0.718
Area sag pp, (mm^2)	36	−0.97 (−1.93; −0.01)	0.047	−0.78 (−2.08; 0.52)	0.228
Area sag sc, (mm^2)	37	−0.75(−1.82; 0.32)	0.164	−1.07 (−2.35; 0.21)	0.097
Area ax sc, (mm^2)	38	−1.37 (−2.85; 0.11)	0.068	−1.70 (−3.54; 0.13)	0.069
SAT volume, (cm^3)	19	−10.98 (−24.11; 2.16)	0.098	−20.06 (−37.23; 2.90)	0.026
VAT volume, (cm^3)	19	−1.43 (−5.37; 2.51)	0.454	−2.90 (−8.12; 2.33)	0.245

Data are presented as the regression coefficient beta (95% CI) from linear regression analyses.

*Adjusted for maternal pre-pregnancy BMI, gestational weight gain, pregnancy duration, sex (except for BMI percentiles), ponderal index at birth, group, and mode of infant feeding (partially or exclusively breastfed or formula) at 4 months postpartum.

Abbreviations: Area ax sc, area of subcutaneous fat in the axial plane; area sag pp, area of preperitoneal fat in the sagittal plane; area sag sc, area of subcutaneous fat in the sagittal plane; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue

Appendix

Appendix 5: Maternal insulin (pmol/l) at 32 weeks gestation in relation to adipose tissue measurements (by ultrasound and MRI) at 3, 4, and 5 years of age.

Body composition variables	n	Unadjusted Analysis		Adjusted Analysis*	
		b (95% CI)	*P*	b (95% CI)	*P*
3 years					
Area sag pp, mm^2	102	0 (−0.05; 0.05)	0.909	0.03 (−0.03; 0.10)	0.289
Area sag sc, mm^2	103	−0.03 (−0.08; 0.03)	0.354	−0.03 (−0.09; 0.04)	0.412
Area ax sc, mm^2	102	−0.02 (−0.11; 0.07)	0.616	−0.03 (−0.12; 0.07)	0.591
4 years					
Area sag pp, mm^2	94	0 (−0.06; 0.07)	0.900	0.03 (−0.05; 0.11)	0.509
Area sag sc, mm^2	93	−0.04 (−0.09; 0.02)	0.178	−0.06 (−0.12; 0.01)	0.096
Area ax sc, mm^2	95	−0.02 (−0.11; 0.07)	0.680	−0.05 (−0.16; 0.06)	0.367
5 years					
Area sag pp, mm^2	96	0 (−0.06; 0.07)	0.919	0.01 (−0.07; 0.09)	0.794
Area sag sc, mm^2	97	−0.02 (−0.08; 0.04)	0.507	0.03 (−0.11; 0.04)	0.376
Area ax sc, mm^2	98	0 (−0.09; 0.09)	0.994	−0.02 (−0.14; 0.09)	0.700
SAT volume, cm^3	44	0.99 (−0.72; 2.70)	0.248	−0.17 (−2.62; 2.29)	0.891
VAT volume, cm^3	44	0.28 (−0.09; 0.64)	0.132	0.12 (−0.44; 0.67)	0.671

Data are presented as the regression coefficient b (95% CI) from linear regression analyses.

*Adjusted for maternal pre-pregnancy BMI, gestational weight gain, pregnancy duration in days, maternal glucose status, sex (except for BMI percentiles), ponderal index at birth, group, and mode of infant feeding (exclusively or partially breastfed or formula) at 4 months postpartum.

Abbreviations; Area ax sc, area of subcutaneous fat in the axial plane; area sag pp, area of preperitoneal fat in sagittal plane; area sag sc, area of subcutaneous fat in the sagittal plane; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

Appendix

Appendix 6: Maternal plasma insulin pmol/l at 32 weeks gestation in relation to child body composition at 3, 4, and 5 years of age.

Body composition variables	n	Unadjusted Analysis		Adjusted Analysis*	
		b (95% CI)	*P*	b (95% CI)	*P*
3 years					
Weight (kg)	162	0 (−0.01; 0)	0.311	−0.01 (−0.01; 0)	0.161
BMI percentiles	162	−0.05 (−0.14; 0.05)	0.335	−0.10 (−0.21; 0.01)	0.073
Sum 4 SFT (mm)	113	−0.01 (−0.02; 0.01)	0.496	0 (−0.02; 0.02)	0.693
Fat mass (kg)	113	0 (0; 0)	0.321	0 (0; 0)	0.493
Body fat (%)	113	0 (−0.02; 0.01)	0.433	0 (−0.02; 0.01)	0.663
Lean body mass (kg)	113	0 (−0.01; 0)	0.530	0 (−0.01; 0)	0.521
4 years					
Weight (kg)	159	0 (−0.01; 0)	0.451	−0.01 (−0.01; 0)	0.178
BMI percentiles	159	−0.05 (−0.15; 0.05)	0.354	−0.10 (−0.21; 0.01)	0.073
Sum 4 SFT (mm)	102	0 (−0.02; 0.02)	0.899	−0.01 (−0.03; 0.02)	0.635
Fat mass (kg)	102	0 (0; 0)	0.414	0 (−0.01; 0)	0.422
Body fat (%)	102	0 (−0.02; 0.01)	0.725	0 (−0.02; 0.01)	0.552
Lean body mass (kg)	102	0 (−0.01; 0)	0.328	0 (−0.01; 0.01)	0.604
5 years					
Weight (kg)	153	0 (−0.01; 0.01)	0.775	0 (−0.01; 0.01)	0.754
BMI percentiles	153	0.01 (−0.10; 0.10)	0.928	−0.07 (−0.19; 0.04)	0.212
Sum 4 SFT (mm)	112	0.01 (−0.02; 0.03)	0.595	0 (−0.03; 0.02)	0.780
Fat mass (kg)	112	0 (0; 0.01)	0.870	0 (−0.01; 0.01)	0.787
Body fat (%)	112	0 (−0.01; 0.02)	0.741	0 (−0.02; 0.01)	0.780
Lean body mass (kg)	112	0 (−0.01; 0.01)	0.863	0 (−0.01; 0.01)	0.928
Weight gain (kg) (birth – 5y)	153	0 (−0.0; 0.01)	0.781	0 (−0.0; 0.01)	0.743

Data are presented as the regression coefficient b (95% CI) from linear regression analyses.
*Adjusted for maternal pre-pregnancy BMI, gestational weight gain, pregnancy duration in days, maternal glucose status, sex (except for BMI percentiles), ponderal index at birth, group, and mode of infant feeding (exclusively or partially breastfed, or formula) at 4 months postpartum.

Abbreviations; BMI, body mass index; LBM, lean body mass; SFT, skinfold thickness.

Appendix

Appendix 7: Maternal plasma HOMA-IR at 32 weeks gestation in relation to child body composition at 3, 4, and 5 years of age.

Body composition variables	n	Unadjusted Analysis		Adjusted Analysis*	
		b (95% CI)	*P*	b (95% CI)	*P*
3 years					
Weight (kg)	156	−0.04 (−0.27; 0.19)	0.749	−0.10 (−0.36; 0.17)	0.474
BMI percentiles	156	−0.74 (−4.38; 2.90)	0.689	−2.19 (−6.32; 1.94)	0.297
Sum 4 SFT (mm)	109	−0.06 (−0.68; 0.56)	0.848	0 (−0.75; 0.76)	0.996
Fat mass (kg)	109	−0.03 (−0.13; 0.07)	0.564	−0.03 (−0.16; 0.10)	0.647
Body fat (%)	109	−0.07 (−0.51; 0.38)	0.767	−0.01 (−0.53; 0.50)	0.956
Lean body mass (kg)	109	−0.06 (−0.27; 0.15)	0.561	0.09 (−0.35; 0.16)	0.468
4 years					
Weight (kg)	152	−0.01 (−0.28; 0.26)	0.957	−0.11 (−0.42; 0.21)	0.498
BMI percentiles	152	−0.67 (−4.47; 3.14)	0.730	−2.53 (−6.86; 1.81)	0.251
Sum 4 SFT (mm)	99	0.22 (−0.44; 0.89)	0.505	−0.05 (−0.80; 0.91)	0.902
Fat mass (kg)	99	−0.01 (−0.13; 0.11)	0.866	−0.03 (−0.19; 0.12)	0.655
Body fat (%)	99	0.11 (−0.39; 0.61)	0.660	0.01 (−0.58; 0.60)	0.978
Lean body mass (kg)	99	−0.16 (−0.44; 0.12)	0.250	−0.16 (−0.50; 0.18)	0.344
5 years					
Weight (kg)	146	0.14 (−0.23; 0.50)	0.458	0.01 (−0.43; 0.45)	0.958
BMI percentiles	146	0.20 (−3.72; 4.12)	0.919	−3.28 (−7.89; 1.33)	0.162
Sum 4 SFT (mm)	108	−0.02 (−0.87; 0.84)	0.972	−0.73 (−1.78; 0.32)	0.172
Fat mass (kg)	108	−0.02 (−0.19; 0.15)	0.819	−0.14 (−0.35; 0.08)	0.203
Body fat (%)	108	−0.06 (−0.68; 0.56)	0.853	−0.50 (−1.18; 0.18)	0.146
Lean body mass (kg)	108	−0.05 (−0.38; 0.27)	0.746	−0.10 (−0.49; 0.29)	0.622
Weight gain (kg) (birth – 5y)	146	0.13 (−0.21; 0.48)	0.447	0.02 (−0.40; 0.44)	0.925

Data are presented as the regression coefficient b (95% CI) from linear regression analyses.
*Adjusted for maternal pre-pregnancy BMI, gestational weight gain, pregnancy duration in days, maternal glucose status, sex (except for BMI percentiles), ponderal index at birth (kg/m^3), group, and mode of infant feeding (exclusively or partially breastfed, or formula) at 4 months postpartum.

Abbreviations; BMI, body mass index; LBM, lean body mass; SFT, skinfold thickness.

Appendix

Appedix 8: Maternal HOMA-IR at 32 weeks gestation in relation to adipose tissue measurements (by ultrasound and MRI) at 3, 4, and 5 years of age.

Body composition variables	n	Unadjusted Analysis		Adjusted Analysis*	
		b (95% CI)	*P*	b (95% CI)	*P*
3 years					
Area sag pp, mm^2	98	0.18 (−1.87; 2.23)	0.860	1.29 (−1.29; 3.86)	0.324
Area sag sc, mm^2	99	−0.72 (−2.91; 1.47)	0.518	−1.16 (−3.81; 1.49)	0.387
Area ax sc, mm^2	98	−0.63 (−3.91; 2.64)	0.701	−1.11 (−5.11; 2.88)	0.581
4 years					
Area sag pp, mm^2	90	0.55 (−2.13; 3.23)	0.685	1.31 (−2.07; 4.69)	0.442
Area sag sc, mm^2	89	−0.80 (−3.14; 1.55)	0.501	−1.72 (−4.65; 1.20)	0.245
Area ax sc, mm^2	91	0.18 (−3.57; 3.93)	0.925	−1.15 (−5.85; 3.56)	0.630
5 years					
Area sag pp, mm^2	92	0.48 (−3.19; 2.24)	0.729	−1.04 (−4.52; 2.44)	0.553
Area sag sc, mm^2	93	−1.10 (−3.52; 1.32)	0.369	−2.23 (−5.31; 0.84)	0.152
Area ax sc, mm^2	94	−0.36 (−4.13; 3.40)	0.849	−2.11 (−6.93; 2.72)	0.388
SAT volume, cm^3	44	30.77 (−19.02; 80.55)	0.219	6.13 (−69.14; 81.40)	0.870
VAT volume, cm^3	44	9.00 (−1.64; 19.64)	0.095	5.87 (−11.04; 22.707)	0.485

Data are presented as the regression coefficient b (95% CI) from linear regression analyses.

*Adjusted for maternal pre-pregnancy BMI, gestational weight gain, pregnancy duration in days, maternal glucose status, sex (except for BMI percentiles), ponderal index at birth, group, and mode of infant feeding (exclusively or partially breastfed or formula) at 4 months postpartum.

Abbreviations; Area ax sc, area of subcutaneous fat in the axial plane; area sag pp, area of preperitoneal fat in the sagittal plane; area sag sc, area of subcutaneous fat in the sagittal plane; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

Appendix

Appendix 9: Associations between maternal triglycerides (mg/dl) at 32 weeks gestation and child body composition at 3, 4, and 5 years.

Body composition variables	n	Unadjusted Analysis		Adjusted Analysis*	
		b (95% CI)	*P*	b (95% CI)	*P*
3 years					
Weight (kg)	161	0 (−0.01; 0)	0.100	0 (−0.01; 0)	0.071
BMI percentiles	161	−0.04 (−0.10; 0.02)	0.239	−0.05 (−0.11; 0.02)	0.151
Sum 4 SFT (mm)	113	0 (−0.01; 0.01)	0.680	0 (−0.01; 0.01)	0.761
Fat mass (kg)	113	0 (0; 0)	0.173	0 (0; 0)	0.265
Body fat (%)	113	0 (−0.01; 0.01)	0.671	0 (−0.01; 0.01)	0.747
Lean body mass (kg)	113	0 (−0.01; 0)	0.029	0 (−0.01; 0)	0.042
4 years					
Weight (kg)	158	0 (−0.01; 0)	0.362	0 (−0.01; 0)	0.285
BMI percentiles	158	0 (−0.06; 0.06)	0.994	0 (−0.07; 0.07)	0.981
Sum 4 SFT (mm)	102	0.01 (−0.01; 0.02)	0.373	0 (−0.01; 0.02)	0.457
Fat mass (kg)	102	0 (0; 0)	0.892	0 (0; 0)	0.976
Body fat (%)	102	0 (−0.01; 0.01)	0.417	0 (−0.01; 0.01)	0.456
Lean body mass (kg)	102	0 (−0.01; 0)	0.109	0 (−0.01; 0)	0.265
5 years					
Weight (kg)	152	0 (−0.01; 0)	0.381	0 (−0.01; 0)	0.249
BMI percentiles	152	−0.02 (−0.08; 0.04)	0.545	0.04 (−0.10; 0.03)	0.268
Sum 4 SFT (mm)	112	0.01 (−0.01; 0.02)	0.492	0 (−0.01; 0.02)	0.785
Fat mass (kg)	112	0 (0; 0)	0.842	0 (0; 0)	0.723
Body fat (%)	112	0 (−0.01; 0.01)	0.610	0 (−0.01; 0.01)	0.856
Lean body mass (kg)	112	0 (−0.01; 0)	0.175	0 (−0.01; 0)	0.213
Weight gain (kg) (birth – 5y)	152	0 (−0.01; 0)	0.404	0 (−0.01; 0)	0.265

Data are presented as the regression coefficient b (95% CI) from linear regression analyses.
*Adjusted for maternal pre-pregnancy BMI, gestational weight gain, pregnancy duration in days, maternal glucose status, sex (except for BMI percentiles), ponderal index at birth, group, and mode of infant feeding (exclusively or partially breastfed, or formula) at 4 months postpartum.

Abbreviations; BMI, body mass index; LBM, lean body mass; SFT, skinfold thickness.

Appendix

Appendix 10: Associations between maternal triglycerides (mg/dl) at 32 weeks gestation and adipose tissue measurements (by ultrasound and MRI).

Body composition variables	n	Unadjusted Analysis		Adjusted Analysis*	
		b (95% CI)	*P*	b (95% CI)	*P*
3 years					
Area sag pp, mm^2	102	−0.01 (−0.04; 0.02)	0.606	0 (−0.04; 0.04)	0.883
Area sag sc, mm^2	103	0 (−0.03; 0.04)	0.929	0 (−0.04; 0.04)	0.924
Area ax sc, mm^2	102	0.02 (−0.04; 0.07)	0.518	0.01 (−0.04; 0.07)	0.653
4 years					
Area sag pp, mm^2	94	0.01 (−0.03; 0.05)	0.728	0.01 (−0.03; 0.06)	0.602
Area sag sc, mm^2	93	0.03 (−0.01; 0.06)	0.170	0.06 (−0.03; 0.07)	0.181
Area ax sc, mm^2	95	0.05 (0; 0.11)	0.066	0.05 (−0.02; 0.11)	0.157
5 years					
Area sag pp, mm^2	96	0.01 (−0.04; 0.05)	0.796	0.01 (−0.04; 0.05)	0.822
Area sag sc, mm^2	97	0.01 (−0.03; 0.05)	0.528	0 (−0.04; 0.05)	0.853
Area ax sc, mm^2	98	0.05 (−0.01; 0.11)	0.127	0.04 (−0.03; 0.10)	0.305
SAT volume, cm^3	44	0.47 (−0.30; 1.24)	0.224	0.25 (−0.76; 1.26)	0.617
VAT volume, cm^3	44	0.06 (−0.11; 0.23)	0.464	0.04 (−0.19; 0.27)	0.742

Data are presented as the regression coefficient b (95% CI) from linear regression analyses.

*Adjusted for maternal pre-pregnancy BMI, gestational weight gain, pregnancy duration in days, maternal glucose status, sex (except for BMI percentiles), ponderal index at birth, group, and mode of infant feeding (exclusively or partially breastfed or formula) at 4 months postpartum.

Abbreviations; Area ax sc, area of subcutaneous fat in the axial plane; area sag pp, area of preperitoneal fat in the sagittal plane; area sag sc, area of subcutaneous fat in the sagittal plane; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

Appendix

Appendix 11: Supplemental table from Meyer, et al. 2018. Maternal insulin resistance, triglycerides, and cord blood insulin are not determinants of offspring growth and adiposity up to 5 years: a follow-up study. Diabetic Medicine: 35 (10).

Cord blood insulin pmol/l in relation to child body composition in boys and girls at 5 years of age.

Body composition variables	n	Boys, unadj.		Boys, adj.		n	Girls, unadj.		Girls, adj.	
		b (95% CI)	*P*	b (95% CI)	*P*		b (95% CI)	*P*	*b (95% CI)*	*P*
5 years										
Weight (kg)	62	0.01 (−0.01; 0.02)	0.373	0.01 (−0.01; 0.02)	0.601	55	−0.01 (−0.03; 0)	0.066	−0.01 (−0.03; 0)	0.092
BMI percentiles	62	−0.05 (−0.12; 0.22)	0.576	0 (−0.18; 0.19)	0.965	55	−0.08 (−0.22; 0.06)	0.236	−0.08 (−0.21; 0.06)	0.283
Sum 4 SFT (mm)	49	−0.02 (−0.05; 0.02)	0.358	−0.02 (−0.06; 0.02)	0.348	39	−0.01 (−0.06; 0.03)	0.555	−0.01 (−0.06; 0.05)	0.807
Fat mass (kg)	49	0 (−0.01; 01)	0.429	0 (−0.01; 01)	0.511	39	−0.01 (−0.01; 0)	0.272	0 (−0.02; 0.01)	0.403
Body fat (%)	49	−0.01 (−0.03; 0.01)	0.394	−0.01 (−0.04; 0.02)	0.394	39	−0.01 (−0.04; 0.02)	0.561	0 (−0.04; 0.03)	0.801
Lean body mass (kg)	49	0 (−0.02; 0.01)	0.752	0 (−0.02; 0.02)	0.960	39	−0.01 (−0.02; 0.01)	0.181	−0.01 (−0.03; 0.01)	0.187
Weight gain (kg) (birth – 5y)	62	0.01 (−0.01; 0.02)	0.539	0 (−0.01; 0.02)	0.777	55	−0.01 (−0.03; 0)	**0.037**	−0.01 (−0.03; 0)	**0.050**
Area sag pp, mm^2	38	−0.04 (−0.18; 0.09)	0.527	0 (−0.16; 0.17)	0.963	36	−0.04 (−0.12; 0.05)	0.381	−0.01 (−0.10; 0.08)	0.802
Area sag sc, mm^2	39	−0.06 (−0.16; 0.04)	0.225	−0.05 (−0.18; 0.08)	0.421	36	−0.04 (−0.13; 0.04)	0.307	−0.05 (−0.15; 0.05)	0.302
Area ax sc, mm^2	39	−0.08 (−0.23; 0.09)	0.351	−0.05 (−0.25; 0.15)	0.604	37	−0.03 (−0.15; 0.09)	0.620	−0.05 (−0.19; 0.09)	0.466
SAT volume, cm^3	14	−0.80 (−2.29; 0.69)	0.264	−0.15 (−2.61; 2.30)	0.879	19	−1.39 (−4.01; 1.24)	0.280	0.89 (−2.23; 4.00)	0.540
VAT volume, cm^3	14	−0.13 (−0.50; 0.24)	0.460	−0.04 (−0.79; 0.70)	0.893	19	−0.32 (−0.87; 0.24)	0.243	−0.11 (−0.85; 0.63)	0.749

Data are presented as the regression coefficient b (95% CI) from linear regression analyses.
*Adjusted for maternal pre-pregnancy BMI, gestational weight gain, pregnancy duration, maternal glucose status, sex (except for BMI percentiles), ponderal index at birth, group, and mode of infant feeding (exclusively or partially breastfed, or formula) at 4 months postpartum.
Abbreviations; Area ax sc, area of subcutaneous fat in the axial plane; area sag pp, area of preperitoneal fat in the sagittal plane; area sag sc, area of subcutaneous fat in the sagittal plane; BMI, body mass index; SAT, subcutaneous adipose tissue; SFT, skinfold thickness; VAT, visceral adipose tissue

Appendix 12: Associations between cord blood insulin (pmol/l) and adipose tissue measurements (by ultrasound and MRI) at 3, 4, and 5 years of age.

Body composition variables	n	Unadjusted Analysis		Adjusted Analysis*	
		b (95% CI)	*P*	b (95% CI)	*P*
3 years					
Area sag pp, mm^2	79	−0.03 (−0.09; 0.03)	0.286	−0.03 (−0.09; 0.03)	0.286
Area sag sc, mm^2	79	−0.03 (−0.09; 0.03)	0.356	−0.04 (−0.10; 0.02)	0.178
Area ax sc, mm^2	78	−0.02 (−0.11; 0.07)	0.616	−0.04 (−0.13; 0.05)	0.360
4 years					
Area sag pp, mm^2	75	−0.01 (−0.08; 0.06)	0.811	0 (−0.08; 0.07)	0.901
Area sag sc, mm^2	74	−0.01 (−0.07; 0.05)	0.715	−0.03 (−0.09; 0.03)	0.351
Area ax sc, mm^2	75	0 (−0.08; 0.09)	0.941	−0.02 (−0.11; 0.08)	0.720
5 years					
Area sag pp, mm^2	74	−0.03 (−0.10; 0.04)	0.428	−0.02 (−0.10; 0.05)	0.539
Area sag sc, mm^2	75	−0.03 (−0.09; 0.03)	0.321	−0.05 (−0.11; 0.02)	0.176
Area ax sc, mm^2	75	−0.02 (−0.11; 0.07)	0.650	−0.04 (−0.14; 0.06)	0.436
SAT volume, cm^3	33	−0.93 (−2.41; 0.55)	0.209	−1.14 (−2.87; 0.60)	0.188
VAT volume, cm^3	33	−0.20 (−0.51; 0.12)	0.216	−0.27 (−0.64; 0.10)	0.145

Data are presented as the regression coefficient b (95% CI) from linear regression analyses.

*Adjusted for maternal pre-pregnancy BMI, gestational weight gain, pregnancy duration, maternal glucose status, sex (except for BMI percentiles), ponderal index at birth, group, and mode of infant feeding (exclusively or partially breastfed or formula) at 4 months postpartum.

Abbreviations; Area ax sc, area of subcutaneous fat in the axial plane; area sag pp, area of preperitoneal fat in the sagittal plane; area sag sc, area of subcutaneous fat in the sagittal plane; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

Appendix

Appendix 13: Associations between body composition at 5 years and the interaction between cord blood insulin (pmol/l) and child sex

		CB insulin (pmol/l)		sex		Interaction between CB insulin & sex	
	n	beta (CI)	*P*	beta (CI)	*P*	beta (CI)	*P*
Body weight (kg)	117						
Unadjusted		0.01 (−0.01; 0.02)	0.381	0.49 (−0.83; 1.82)	0.462	−0.02 (−0.04; 0)	0.064
Adjusted*		0.01 (−0−.01; 0.02)	0.495	0.54 (−0.82; 1.89)	0.434	−0.02 (−0.04; 0.01)	0.137
Weight gain, birth to 5 years (kg)	117						
Unadjusted		0.01 (−0.01; 0.02)	0.548	0.43 (−0.73; 1.78)	0.409	−0.02 (−0.04; 0)	0.071
Adjusted*		0 (−0.01; 0.02)	0.677	0.55 (−0.74; 1.84)	0.400	−0.02 (−0.04; 0.01)	0.145
Sum 4 SFT (mm)	88						
Unadjusted		−0.02 (−0.06; 0.03)	0.458	3.20 (0.03; 6.37)	0.048	0 (−0.05; 0.06)	0.934
Adjusted*		−0.02 (−0.06; 0.03)	0.453	3.07 (−0.22; 6.36)	0.067	0.01 (−0.05; 0.07)	0.786
Body fat (%)	88						
Unadjusted		−0.01 (−0.04; 0.02)	0.463	3.81 (1.79; 5.84)	0.000	0 (−0.03; 0.04)	0.919
Adjusted*		−0.01 (−0.04; 0.02)	0.479	3.75 (1.65; 5.85)	0.001	0.01 (−0.03; 0.04)	0.797
Lean body mass (kg)	88						
Unadjusted		0 (−0.02; −0.01)	0.744	−0.74 (−1.95; −0.47)	0.227	−0.01 (0.03; 0.02)	0.537
Adjusted*		0 (−0.02; −0.02)	0.927	−0.66 (−1.90; −0.58)	0.291	−0.01 (0.03; 0.01)	0.471

Appendix

Area sag pp (mm^2)	74						
Unadjusted		−0.04 (−0.18; −0.09)	0.530	4.91 (−4.54; 14.36)	0.303	0.01 (−0.15; 0.16)	0.943
Adjusted*		0 (−0.15; 15)	0.994	5.42 (−4.12; 14.96)	0.260	−0.03 (−0.20; 0.14)	0.733
Area sag sc (mm^2)	75						
Unadjusted		−0.06 (−0.18; 0.06)	0.297	6.92 (−1.33; 15.18)	0.099	0.02 (−0.12; 0.16)	0.776
Adjusted*		−0.06 (−0.19; 0.08)	0.404	7.08 (−1.73; 15.89)	0.113	0.02 (−0.14; 0.17)	0.851
Area ax sc (mm^2)	76						
Unadjusted		−0.08 (−0.25; 0.10)	0.398	7.90 (−4.25; 20.05)	0.199	0.05 (−0.15; 0.25)	0.659
Adjusted*		−0.07 (−0.27; 0.13)	0.486	8.26 (−4.66; 21.18)	0.206	0.04 (−0.19; 0.27)	0.719
SAT (cm^3)	33						
Unadjusted		−0.80(−2.81; 1.21)	0.422	123.343 (−68.93; −315.62)	0.200	−0.59 (−3.55; 2.37)	0.688
Adjusted*		−1.77(−4.15; 0.61)	0.138	−9.77 (−270.61; −251.08)	0.939	1.63 (−2.53; 5.79)	0.426
VAT (cm^3)	33						
Unadjusted		−0.13 (−0.57; −0.31	0.555	22.61 (−19.51; 64.73)	0.281	−0.19 (−0.84;0.46)	0.555
Adjusted*		−0.28 (−0.80; −0.23	0.267	9.26 (−47.35; 65.88)	0.738	0.04 (−0.87;0.94)	0.937

Data are presented as the regression coefficient beta (95% CI) from linear regression analyses.

*Adjusted for maternal pre−pregnancy BMI, gestational weight gain, pregnancy duration, maternal glucose status, sex (except for BMI percentiles), ponderal index at birth, group, and mode of infant feeding (exclusively or partially breastfed or formula) at 4 months postpartum.

Abbreviations; Area ax sc, area of subcutaneous fat in the axial plane; area sag pp, area of preperitoneal fat in the sagittal plane; area sag sc, area of subcutaneous fat in the sagittal plane; BMI, body mass index; CB, cord blood; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

Appendix

Appendix 14: Maternal RBC arachidonic acid at 32 weeks gestation in relation to offspring body composition at 2−5 years of age

Body composition variables	n	Unadjusted Analysis		Adjusted Analysis*	
		beta (95% CI)	*P*	beta (95% CI)	*P*
2 years					
Weight (kg)	169	−0.06 (−0.12; 0)	0.052	−0.05 (−0.11; 0.01)	0.117
BMI percentiles	169	−1.03 (−2.27; 0.21)	0.103	−0.93 (−2.18; 0.32)	0.142
Sum 4 SFT (mm)	110	0.02 (−0.16; 0.20)	0.846	0 (−0.18; 0.18)	0.999
Body fat (%)	110	0.01 (−0.11; 0.1	0.842	0 (−0.12; 0.13	0.985
Lean body mass (kg)	110	−0.03 (−0.08; 0.02)	0.244	−0.02 (−0.07; 0.03)	0.339
Area sag pp (mm^2)	111	0.21 (−0.18; 0.60)	0.294	0.19 (−0.21; 0.59)	0.348
Area sag sc (mm^2)	111	0.22 (−0.35; 0.79)	0.450	0.16 (−0.41; 0.74)	0.574
Area ax sc (mm^2)	111	0.18 (−0.47; 0.83)	0.584	0.10 (−0.53; 0.73)	0.754
3 years					
Weight (kg)	162	−0.47 (−0.13; 0.03)	0.229	−0.03 (−0.11; 0.04)	0.389
BMI percentiles	162	−0.83 (−2.04; 0.37)	0.174	−0.74 (−1.94; 0.47)	0.227
Sum 4 SFT (mm)	113	0.05 (−0.15; 0.25)	0.629	0 (−0.19; 0.19)	0.995
Body fat (%)	113	0.05 (−0.10; 0.19)	0.522	0 (−0.12; 0.13)	0.951
Lean body mass (kg)	113	−0.03 (−0.10; 0.03)	0.331	−0.02 (−0.09; 0.04)	0.519
Area sag pp (mm^2)	102	0.03 (−0.62; 0.69)	0.922	−0.02 (−0.69; 0.64)	0.944
Area sag sc (mm^2)	103	0.09 (−0.61; 0.79)	0.797	−0.04 (−0.72; 0.64)	0.901
Area ax sc (mm^2)	102	0.28 (−0.78; 1.34)	0.603	0.09 (−0.93; 1.11)	0.864
4 years					
Weight (kg)	159	−0.03 (−0.12; 0.06)	0.535	−0.01 (−0.10; 0.07)	0.750
BMI percentiles	159	−0.83 (−2.04; 0.38)	0.177	−0.70 (−1.91; 0.50)	0.251
Sum 4 SFT (mm)	102	0.04 (−0.17; 0.25)	0.702	0.01 (−0.19; 0.22)	0.896
Body fat (%)	102	0.06 (−0.10; 0.22)	0.445	0.02 (−0.13; 0.16)	0.826
Lean body mass (kg)	102	−0.06 (−0.16; 0.03)	0.168	−0.04 (−0.13; 0.05)	0.406
Area sag pp (mm^2)	94	−0.21 (−1.05; 0.64)	0.628	−0.31 (−1.15; 0.53)	0.466
Area sag sc (mm^2)	93	0.04 (−0.70; 0.78)	0.907	−0.12 (−0.83; 0.59)	0.737
Area ax sc (mm^2)	95	0.19 (−0.96; 1.34)	0.749	0 (−1.13; 1.13)	0.999
5 years					
Weight (kg)	153	−0.02 (−0.13; 0.10)	0.787	0 (−0.11; 0.12)	0.968
BMI percentiles	153	−0.63 (−1.82; 0.56)	0.299	−0.52 (−1.71; 0.68)	0.394
Sum 4 SFT (mm)	112	0.06 (−0.21; 0.32)	0.673	−0.01 (−0.26; 0.24)	0.943
Body fat (%)	112	0.07 (−0.12; 0.26)	0.439	0 (−0.16; 0.16)	0.983
Lean body mass (kg)	112	−0.02 (−0.12; 0.08)	0.691	0.01 (−0.09; 0.11)	0.857
Weight gain (kg) (birth – 5y)	153	−0.04 (−0.15; 0.07)	0.466	−0.03 (−0.14; 0.08)	0.612
Area sag pp (mm^2)	96	0.07 (−0.79; 0.92)	0.874	−0.03 (−0.88; 0.82)	0.940
Area sag sc (mm^2)	97	0.14 (−0.64; 0.91)	0.724	0.06 (−0.71; 0.82)	0.884
Area ax sc (mm^2)	98	−0.09 (−1.29; 1.11)	0.881	−0.26 (−1.45; 0.93)	0.664
SAT volume (cm^2)	44	5.63 (−10.79; 22.05)	0.493	4.76 (−11.61; 21.12)	0.560
VAT volume (cm^2)	44	0.51 (−3.07; 4.09)	0.774	−0.02 (−3.73; 3.69)	0.990

*Adjusted for pregnancy duration in days, sex (except for BMI percentiles), and mode of infant feeding (exclusively breastfed or combination of partially breastfed//formula) at 4 months postpartum. Abbreviations; area ax sc, area of subcutaneous fat in the axial plane; area sag pp, area of preperitoneal fat in the sagittal plane; area sag sc, area of subcutaneous fat in the sagittal plane; BMI, body mass index; kg, kilograms; mm, millimeters; n, number of children; SAT, subcutaneous adipose tissue; SFT, skinfold thickness; VAT, visceral adipose tissue. Data are presented as the regression coefficient beta (95% CI) from linear regression analyses.

Appendix 15: Maternal RBC docosahexaenoic acid in relation to offspring body composition at 2−5 years of age

Body composition variables	n	Unadjusted Analysis		Adjusted Analysis*	
		beta (95% CI)	*P*	beta (95% CI)	*P*
2 years					
Weight (kg)	169	0.01 (−0.07; 0.08)	0.866	0.02 (−0.05; 0.09)	0.534
BMI percentiles	169	−0.04 (−1.53; 1.45)	0.955	0.20 (−1.30; 1.70)	0.795
Sum 4 SFT (mm)	110	0 (−0.22; 0.22)	0.995	0.01 (−0.20; 0.23)	0.907
Body fat (%)	110	0.01 (−0.14; 0.16)	0.932	0.02 (−0.13; 0.17)	0.831
Lean body mass (kg)	110	0 (−0.06; 0.06)	0.966	0 (−0.06; 0.06)	0.966
Area sag pp (mm^2)	111	0.15 (−0.33; 0.63)	0.539	0.14 (−0.35; 0.63)	0.573
Area sag sc (mm^2)	111	−0.21 (−0.91; 0.49)	0.554	−0.25 (−0.95; 0.45)	0.479
Area ax sc (mm^2)	111	−0.51 (−1.30; 0.27)	0.195	−0.56 (−1.33; 0.21)	0.149
3 years					
Weight (kg)	162	0.01 (−0.08; 0.11)	0.763	0.03 (−0.06; 0.12)	0.470
BMI percentiles	162	−0.16 (−1.59; 1.27)	0.822	0.07 (−1.36; 1.50)	0.919
Sum 4 SFT (mm)	113	0.06 (−0.17; 0.29)	0.624	0.03 (−0.19; 0.25)	0.768
Body fat (%)	113	0.05 (−0.11; 0.22)	0.520	0.03 (−0.13; 0.18)	0.736
Lean body mass (kg)	113	0.02 (−0.06; 0.10)	0.662	0.04 (−0.04; 0.11)	0.344
Area sag pp (mm^2)	102	0.28 (−0.48; 1.04)	0.464	0.26 (−0.52; 1.04)	0.504
Area sag sc (mm^2)	103	−0.11 (−0.93; 0.70)	0.782	−0.22 (−1.01; 0.58)	0.592
Area ax sc (mm^2)	102	−0.22 (−1.44; 1.00)	0.718	−0.37 (−1.57; 0.83)	0.541
4 years					
Weight (kg)	159	0.02 (−0.08; 0.12)	0.703	0.04 (−0.07; 0.14)	0.490
BMI percentiles	159	−0.15 (−1.59; 1.30)	0.844	−0.09 (−1.54; 1.37)	0.907
Sum 4 SFT (mm)	102	0.03 (−0.21; 0.28)	0.788	0.01 (−0.23; 0.26)	0.923
Body fat (%)	102	0.07 (−0.12; 0.25)	0.477	0.02 (−0.15; 0.19)	0.855
Lean body mass (kg)	102	0.03 (−0.08; 0.14)	0.577	0.07 (−0.04; 0.18)	0.190
Area sag pp (mm^2)	94	0.07 (−0.89; 1.03)	0.882	−0.02 (−1.00; 0.96)	0.968
Area sag sc (mm^2)	93	−0.03 (−0.88; 0.81)	0.943	−0.26 (−1.08; 0.57)	0.541
Area ax sc (mm^2)	95	−0.31 (−1.62; 1.01)	0.645	−0.60 (−1.91; 0.71)	0.368
5 years					
Weight (kg)	153	0.04 (−0.09; 0.18)	0.528	0.07 (−0.07; 0.21)	0.320
BMI percentiles	153	−0.17 (−1.58; 1.25)	0.814	−0.08 (−1.51; 1.34)	0.908
Sum 4 SFT (mm)	112	−0.24 (−0.67; 0.19)	0.277	−0.07 (−0.37; 0.22)	0.625
Body fat (%)	112	−0.05 (−0.36; 0.27)	0.774	−0.05 (−0.24; 0.15)	0.644
Lean body mass (kg)	112	0.07 (−0.05; 0.19)	0.234	0.11 (0; 0.23)	0.058
Weight gain (kg) (birth – 5y)	153	0.01 (−0.12; 0.14)	0.889	0.04 (−0.09; 0.17)	0.560
Area sag pp (mm^2)	96	0.30 (−0.66; 1.27)	0.536	0.26 (−0.70; 1.23)	0.589
Area sag sc (mm^2)	97	−0.01 (−0.89; 0.86)	0.977	−0.15 (−1.02; 0.72)	0.731
Area ax sc (mm^2)	98	−0.47 (−1.83; 0.88)	0.490	−0.75 (−2.10; 0.59)	0.269
SAT volume (cm^2)	44	−0.36 (−18.56; 17.86)	0.969	0.38 (−17.20; 17.97)	0.965
VAT volume (cm^2)	44	−0.79 (−4.74; 3.16)	0.688	−0.70 (−4.66; 3.27)	0.724

* Adjusted for pregnancy duration in days, sex (except for BMI percentiles), and mode of infant feeding (exclusively breastfed or combination of partially breastfed/formula) at 4 months postpartum. Abbreviations; area ax sc, area of subcutaneous fat in the axial plane; area sag pp, area of preperitoneal fat in the sagittal plane; area sag sc, area of subcutaneous fat in the sagittal plane; BMI, body mass index; kg, kilograms; mm, millimeters; n, number of children; SAT, subcutaneous adipose tissue; SFT, skinfold thickness; VAT, visceral adipose tissue. Data shown is the regression coefficient beta (95% CI) from linear regression analyses.

Appendix

Appendix 16: Associations between maternal RBC n-3 LCPUFAs and offspring body composition at 2-5 years of age.

Body composition variables	n	Unadjusted Analysis		Adjusted Analysis*	
		beta (95% CI)	*P*	beta (95% CI)	*P*
2 years					
Weight (kg)	169	0.01 (−0.05; 0.06)	0.856	0.01 (−0.04; 0.07)	0.653
BMI percentiles	169	0.01 (−1.14; 1.16)	0.985	0.16 (−1.00; 1.32)	0.784
Sum 4 SFT (mm)	110	−0.01 (−0.18; 0.16)	0.909	0 (−0.17; 0.16)	0.960
Body fat (%)	110	0 (−0.12; 0.11)	0.944	0 (−0.12; 0.12)	0.996
Lean body mass (kg)	110	0 (−0.05; 0.04)	0.866	0 (−0.05; 0.05)	0.997
Area sag pp (mm^2)	111	0.15 (−0.22; 0.52)	0.427	0.11 (−0.27; 0.49)	0.570
Area sag sc (mm^2)	111	−0.11 (−0.64; 0.43)	0.700	−0.15 (−0.69; 0.40)	0.595
Area ax sc (mm^2)	111	−0.32 (−0.92; 0.29)	0.300	−0.39 (−0.98; 0.20)	0.197
3 years					
Weight (kg)	162	0.01 (−0.06; 0.09)	0.696	0.03 (−0.05; 0.10)	0.486
BMI percentiles	162	−0.02 (−1.13; 1.09)	0.975	0.15 (−0.95; 1.26)	0.784
Sum 4 SFT (mm)	113	0.05 (−0.13; 0.23)	0.609	0.01 (−0.16; 0.19)	0.879
Body fat (%)	113	0.04 (−0.09; 0.17)	0.526	0.01 (−0.11; 0.13)	0.869
Lean body mass (kg)	113	0.01 (−0.05; 0.08)	0.689	0.03 (−0.04; 0.09)	0.399
Area sag pp (mm^2)	102	0.21 (−0.39; 0.81)	0.486	0.15 (−0.45; 0.75)	0.624
Area sag sc (mm^2)	103	−0.04 (−0.68; 0.60)	0.898	−0.14 (−0.75; 0.48)	0.659
Area ax sc (mm^2)	102	−0.08 (−1.04; 0.87)	0.863	−0.24 (−1.16; 0.67)	0.599
4 years					
Weight (kg)	159	0.01 (−0.07; 0.09)	0.741	0.02 (−0.06; 0.10)	0.577
BMI percentiles	159	−0.16 (−1.28; 0.96)	0.777	−0.06 (−1.17; 1.06)	0.922
Sum 4 SFT (mm)	102	0 (−0.19; 0.20)	0.964	−0.03 (−0.22; 0.16)	0.730
Body fat (%)	102	0.04 (−0.11; 0.18)	0.634	−0.02 (−0.15; 0.11)	0.787
Lean body mass (kg)	102	0.02 (−0.07; 0.10)	0.678	0.04 (−0.05; 0.12)	0.366
Area sag pp (mm^2)	94	0.07 (−0.68; 0.82)	0.860	−0.10 (−0.86; 0.66)	0.798
Area sag sc (mm^2)	93	−0.03 (−0.69; 0.62)	0.917	−0.22 (−0.86; 0.42)	0.495
Area ax sc (mm^2)	95	−0.26 (−1.29; 0.76)	0.609	−0.47 (−1.48; 0.54)	0.355
5 years					
Weight (kg)	153	0.04 (−0.07; 0.14)	0.479	0.05 (−0.06; 0.16)	0.370
BMI percentiles	153	−0.06 (−1.16; 1.03)	0.913	0.04 (−1.06; 1.13)	0.947
Sum 4 SFT (mm)	112	0 (−0.24; 0.24)	0.995	−0.05 (−0.28; 0.18)	0.676
Body fat (%)	112	0.03 (−0.14; 0.20)	0.729	−0.03 (−0.18; 0.12)	0.712
Lean body mass (kg)	112	0.05 (−0.04; 0.15)	0.278	0.07 (−0.02; 0.16)	0.119
Weight gain (kg) (birth – 5y)	153	0.01 (−0.09; 0.11)	0.848	0.02 (−0.08; 0.12)	0.655
Area sag pp (mm^2)	96	0.23 (−0.52; 0.98)	0.543	0.11 (−0.65; 0.86)	0.775
Area sag sc (mm^2)	97	0 (−0.68; 0.68)	0.994	−0.14 (−0.81; 0.54)	0.689
Area ax sc (mm^2)	98	−0.39 (−1.44; 0.66)	0.463	−0.64 (−1.68; 0.39)	0.221
SAT volume (cm^2)	44	(0.43 (−13.73; 14.59)	0.951	−0.38 (−14.57; 13.81)	0.957
VAT volume (cm^2)	44	−0.53 (−3.60; 2.54)	0.729	−0.50 (−3.70; 2.70)	0.755

* Adjusted for pregnancy duration in days, sex (except for BMI percentiles), and mode of infant feeding (exclusively breastfed or combination of partially breastfed/formula) at 4 months postpartum. Abbreviations; area ax sc, area of subcutaneous fat in the axial plane; area sag pp, area of preperitoneal fat in the sagittal plane; area sag sc, area of subcutaneous fat in the sagittal plane; BMI, body mass index; kg, kilograms; LCPUFAs, long-chain polyunsaturated fatty acids; mm, millimeters; n, number of children; SAT, subcutaneous adipose tissue; SFT, skinfold thickness; VAT, visceral adipose tissue. Values for fatty acids are expressed as a percentage of the weight of total fatty acids (% FA of total FA). PUFA: sum of all cis-FAs with two or more double bonds: n3 LCPUFAs: C20:3n-3; C20:4n-3; 20:5n-3; C21:5n-3; C22:3n-3; 22:5n-3; 22:6n-3. Data shown is the regression coefficient (95% CI) from linear regression analysis.

Appendix 17: Associations between maternal RBC n-6 LCPUFAs at 32 weeks gestation and offspring body composition at 2−5 years of age

Body composition variables	n	Unadjusted Analysis		Adjusted Analysis*	
		beta (95% CI)	*P*	beta (95% CI)	*P*
2 years					
Weight (kg)	167	−0.04 (−0.08; 0)	0.050	−0.03 (−0.07; 0.01)	0.122
BMI percentiles	167	−0.78 (−1.63; 0.08)	0.075	−0.71 (−1.58; 0.15)	0.104
Sum 4 SFT (mm)	109	0.01 (−0.12; 0.13)	0.901	−0.01 (−0.13; 0.12)	0.927
Body fat (%)	109	0.13 (−0.08; 0.09)	0.893	0 (−0.09; 0.08)	0.946
Lean body mass (kg)	109	−0.02 (−0.06; 0.01)	0.206	−0.02 (−0.05; 0.02)	0.292
Area sag pp (mm^2)	111	0.16 (−0.11; 0.44)	0.241	0.15 (−0.13; 0.43)	0.290
Area sag sc (mm^2)	111	0.20 (−0.20; 0.60)	0.326	0.16 (−0.24; 0.56)	0.439
Area ax sc (mm^2)	111	0.17 (−0.28; 0.62)	0.454	0.11 (−0.33; 0.55)	0.622
3 years					
Weight (kg)	160	−0.03 (−0.09; 0.02)	0.260	−0.02 (−0.07; 0.03)	0.449
BMI percentiles	160	−0.63 (−1.46; 0.20)	0.134	−0.57 (−1.40; 0.26)	0.179
Sum 4 SFT (mm)	113	0.04 (−0.10; 0.18)	0.581	0 (−0.13; 0.13)	0.971
Body fat (%)	113	0.04 (−0.06; 0.14)	0.470	0.01 (−0.09; 0.10)	0.910
Lean body mass (kg)	113	−0.03 (−0.07; 0.02)	0.264	−0.02 (−0.06; 0.03)	0.435
Area sag pp (mm^2)	102	0.02 (−0.44; 0.48)	0.935	−0.03 (−0.49; 0.44)	0.915
Area sag sc (mm^2)	103	0.09 (−0.40; 0.58)	0.721	−0.01 (−0.49; 0.46)	0.959
Area ax sc (mm^2)	102	0.21 (−0.53; 0.95)	0.567	0.07 (−0.64; 0.79)	0.838
4 years					
Weight (kg)	157	−0.01 (−0.07; 0.05)	0.668	0 (−0.06; 0.06)	0.918
BMI percentiles	157	−0.54 (−1.38; 0.30)	0.202	−0.46 (−1.29; 0.38)	0.281
Sum 4 SFT (mm)	102	0.04 (−0.10; 0.19)	0.557	0.02 (−0.12; 0.17)	0.740
Body fat (%)	102	0.05 (−0.06; 0.16)	0.343	0.02 (−0.08; 0.12)	0.670
Lean body mass (kg)	102	−0.05 (−0.11; 0.02)	0.141	−0.03 (−0.09; 0.03)	0.337
Area sag pp (mm^2)	94	−0.17 (−0.76; 0.42)	0.568	−0.25 (−0.84; 0.34)	0.397
Area sag sc (mm^2)	93	0.07 (−0.45; 0.59)	0.778	−0.04 (−0.54; 0.46)	0.875
Area ax sc (mm^2)	95	0.19 (−0.62; 0.99)	0.646	0.05 (−0.74; 0.84)	0.895
5 years					
Weight (kg)	152	0 (−0.09; 0.08)	0.924	0.01 (−0.07; 0.09)	0.820
BMI percentiles	152	−0.40 (−1.23; 0.43)	0.343	−0.32 (−1.15; 0.51)	0.442
Sum 4 SFT (mm)	112	0.05 (−0.13; 0.24)	0.572	0.01 (−0.17; 0.18)	0.933
Body fat (%)	112	0.06 (−0.07; 0.19)	0.361	0.01 (−0.10; 0.12)	0.872
Lean body mass (kg)	112	−0.02 (−0.09; 0.06)	0.666	0 (−0.07; 0.07)	0.913
Weight gain (kg) (birth – 5y)	152	−0.02 (−0.10; 0.06)	0.591	−0.01 (−0.09; 0.06)	0.758
Area sag pp (mm^2)	96	0.06 (−0.54; 0.66)	0.838	−0.01 (−0.60; 0.58)	0.972
Area sag sc (mm^2)	97	0.13 (−0.41; 0.68)	0.626	0.07 (−0.46; 0.61)	0.783
Area ax sc (mm^2)	98	−0.01 (−0.85; 0.84)	0.989	−0.12 (−0.96; 0.71)	0.768
SAT volume (cm^2)	44	3.80 (−7.62; 15.22)	0.505	3.35 (−8.04; 14.74)	0.555
VAT volume (cm^2)	44	0.37 (−2.12; 2.86)	0.766	0.02 (−2.56; 2.60)	0.988

Data are presented as the regression coefficient beta (95% CI) from linear regression analyses.
* Adjusted for pregnancy duration in days, sex (except for BMI percentiles), and mode of infant feeding (exclusively breastfed or combination of partially breastfed/formula) at 4 months postpartum. Abbreviations; area ax sc, area of subcutaneous fat in the axial plane; area sag pp, area of preperitoneal fat in the sagittal plane; area sag sc, area of subcutaneous fat in the sagittal plane; BMI, body mass index; kg, kilograms; LCPUFAs, long-chain polyunsaturated fatty acids; mm, millimeters; n, number of children; SAT, subcutaneous adipose tissue; SFT, skinfold thickness; VAT, visceral adipose tissue. Values for fatty acids are expressed as a percentage of the weight of total fatty acids (% FA of total FA). PUFA: sum of all cis-FAs with two or more double bonds, n6 LCPUFAs: C20:2n-6; 20:3n-6; 20:4n-6; C22:2n-6; C22:4n6; C22:5n-6

Appendix

Appendix 18: Associations between the maternal RBC ratio of n-6/n-3 of LCPUFAs at 32 weeks gestation and offspring body composition at 2−5 years of age

Body composition variables	n	Unadjusted Analysis		Adjusted Analysis*	
		beta (95% CI)	*P*	beta (95% CI)	*P*
2 years					
Weight (kg)	167	−0.06 (−0.25; 0.13)	0.542	−0.07 (−0.26; 0.11)	0.450
BMI percentiles	167	−1.53 (−5.39; 2.34)	0.437	−2.04 (−5.92; 1.85)	0.302
Sum 4 SFT (mm)	109	−0.15 (−0.81; 0.52)	0.664	−0.23 (−0.89; 0.44)	0.499
Body fat (%)	109	−0.10 (−0.56; 0.35)	0.653	−0.16 (−0.61; 0.30)	0.491
Lean body mass (kg)	109	−0.01 (−0.20; 0.18)	0.900	−0.02 (−0.20; 0.16)	0.801
Area sag pp (mm^2)	111	−0.32 (−1.67; 1.02)	0.633	−0.37 (−1.75; 1.02)	0.601
Area sag sc (mm^2)	111	1.20 (−0.74; 3.14)	0.222	1.10 (−0.86; 3.06)	0.270
Area ax sc (mm^2)	111	2.02 (−0.15; 4.20)	0.067	1.79 (−0.35; 3.93)	0.101
3 years					
Weight (kg)	160	−0.05 (−0.29; 0.19)	0.672	−0.07 (−0.31; 0.17)	0.573
BMI percentiles	160	−0.73 (−4.44; 2.98)	0.699	−1.17 (−4.88; 2.54)	0.534
Sum 4 SFT (mm)	113	−0.07 (−0.80; 0.66)	0.858	−0.08 (−0.77; 0.61)	0.817
Body fat (%)	113	−0.07 (−0.59; 0.46)	0.793	−0.06 (−0.54; 0.41)	0.795
Lean body mass (kg)	113	−0.08 (−0.34; 0.17)	0.509	−0.11 (−0.35; 0.13)	0.349
Area sag pp (mm^2)	102	−0.83 (−3.20; 1.54)	0.489	−0.92 (−3.35; 1.52)	0.456
Area sag sc (mm^2)	103	1.23 (−1.31; 3.76)	0.339	1.24 (−1.23; 3.72)	0.322
Area ax sc (mm^2)	102	1.50 (−2.27; 5.27)	0.431	1.53 (−2.16; 5.23)	0.412
4 years					
Weight (kg)	157	−0.08 (−0.36; 0.19)	0.547	−0.10 (−0.37; 0.17)	0.469
BMI percentiles	157	−1.14 (−4.94; 2.67)	0.556	−1.29 (−5.09; 2.51)	0.503
Sum 4 SFT (mm)	102	−0.01 (−0.75; 0.74)	0.987	0 (−0.73; 0.73)	0.993
Body fat (%)	102	−0.09 (−0.66; 0.47)	0.750	−0.03 (−0.53; 0.48)	0.912
Lean body mass (kg)	102	−0.14 (−0.47; 0.18)	0.385	−0.21 (−0.53; 0.11)	0.197
Area sag pp (mm^2)	94	−0.92 (−3.83; 2.00)	0.534	−0.89 (−3.80; 2.02)	0.545
Area sag sc (mm^2)	93	1.19 (−1.36; 3.74)	0.356	1.51 (−0.93; 3.96)	0.221
Area ax sc (mm^2)	95	2.67 (−1.34; 6.67)	0.189	3.15 (−0.78; 7.09)	0.115
5 years					
Weight (kg)	152	−0.07 (−0.43; 0.29)	0.690	−0.10 (−0.46; 0.26)	0.575
BMI percentiles	152	−1.32 (−5.00; 2.37)	0.482	−1.53 (−5.23; 2.16)	0.414
Sum 4 SFT (mm)	112	−0.03 (−0.99; 0.93)	0.950	−0.04 (−0.97; 0.88)	0.925
Body fat (%)	112	−0.10 (−0.80; 0.59)	0.773	−0.05 (−0.64; 0.55)	0.881
Lean body mass (kg)	112	−0.17 (−0.55; 0.20)	0.365	−0.24 (−0.59; 0.12)	0.193
Weight gain (kg) (birth – 5y)	152	0 (−0.34; 0.34)	0.998	−0.05 (−0.38; 0.29)	0.787
Area sag pp (mm^2)	96	−0.77 (−3.80; 2.25)	0.612	−0.90 (−3.89; 2.09)	0.551
Area sag sc (mm^2)	97	1.51 (−1.21; 4.24)	0.272	1.71 (−0.96; 4.38)	0.206
Area ax sc (mm^2)	98	3.02 (−1.19; 7.23)	0.157	3.47 (−0.66; 7.60)	0.098
SAT volume (cm^2)	44	−4.11 (−52.97; 44.75)	0.866	−3.73 (−51.32; 43.87)	0.875
VAT volume (cm^2)	44	2.90 (−7.67; 13.47)	0.583	2.84 (−7.86; 13.55)	0.594

Data are presented as the regression coefficient beta (95% CI) from linear regression analyses.
* Adjusted for pregnancy duration in days, sex (except for BMI percentiles), and mode of infant feeding (exclusively breastfed or combination of partially breastfed/formula) at 4 months postpartum. Abbreviations; area ax sc, area of subcutaneous fat in the axial plane; area sag pp, area of preperitoneal fat in the sagittal plane; area sag sc, area of subcutaneous fat in the sagittal plane; BMI, body mass index; kg, kilograms; LCPUFAs, long-chain polyunsaturated fatty acids; mm, millimeters; n, number of children; SAT, subcutaneous adipose tissue; SFT, skinfold thickness; VAT, visceral adipose tissue. Values for fatty acids are expressed as a percentage of the weight of total fatty acids (% FA of total FA). PUFA: sum of all cis-FAs with two or more double bonds, n6 LCPUFAs: C20:2n-6; 20:3n-6; 20:4n-6; C22:2n-6; C22:4n6; C22:5n-6, n3 LCPUFAs: C20:3n-3; C20:4n-3; 20:5n-3; C21:5n-3; C22:3n-3; 22:5n-3; 22:6n-3.

Appendix 19: Associations between cord blood arachidonic acid and body composition at 2−5 years of age

Body composition variables	n	Unadjusted Analysis		Adjusted Analysis*	
		beta (95% CI)	*P*	beta (95% CI)	*P*
2 years					
Weight (kg)	122	−0.01 (−0.06; 0.05)	0.810	−0.03 (−0.08; 0.03)	0.373
BMI percentiles	122	−0.43 (−1.57; 0.72)	0.466	−0.60 (−1.76; 0.56)	0.307
Sum 4 SFT (mm)	86	0.08 (−0.08; 0.25)	0.316	0.09 (−0.07; 0.26)	0.255
Body fat (%)	86	0.05 (−0.07; 0.16	0.406	0.05 (−0.06; 0.17	0.348
Lean body mass (kg)	86	0.01 (−0.03; 0.06)	0.536	0 (−0.04; 0.05)	0.959
Area sag pp (mm^2)	85	0.35 (−0.01; 0.72)	0.058	0.40 (0.03; 0.77)	0.037
Area sag sc (mm^2)	85	−0.07 (−0.58; 0.44)	0.789	−0.02 (−0.54; 0.50)	0.932
Area ax sc (mm^2)	85	0.12 (−0.48; 0.72)	0.696	0.19 (−0.40; 0.79)	0.517
3 years					
Weight (kg)	116	0 (−0.08; 0.07)	0.900	−0.03 (−0.10; 0.04)	0.452
BMI percentiles	116	−0.47 (−1.58; 0.63)	0.397	−0.70 (−1.81; 0.42)	0.217
Sum 4 SFT (mm)	86	0.02 (−0.16; 0.19)	0.839	0.05 (−0.12; 0.22)	0.527
Body fat (%)	86	0 (−0.13; 0.13)	0.987	0.04 (−0.08; 0.15)	0.546
Lean body mass (kg)	86	0.02 (−0.05; 0.08)	0.623	−0.01 (−0.07; 0.05)	0.743
Area sag pp (mm^2)	76	0.42 (−0.16; 1.00)	0.151	0.56 (−0.03; 1.14)	0.061
Area sag sc (mm^2)	76	−0.10 (−0.72; 0.52)	0.751	0.03 (−0.58; 0.65)	0.915
Area ax sc (mm^2)	75	−0.32 (−1.27; 0.64)	0.514	−0.09 (−1.04; 0.86)	0.855
4 years					
Weight (kg)	116	0.01 (−0.08; 0.09)	0.890	−0.02 (−0.11; 0.06)	0.593
BMI percentiles	116	−0.49 (−1.62; 0.65)	0.398	−0.80 (−1.96; 0.37)	0.177
Sum 4 SFT (mm)	76	0 (−0.18; 0.18)	0.997	0 (−0.18; 0.19)	0.967
Body fat (%)	76	−0.02 (−0.16; 0.12)	0.782	0.01 (−0.12; 0.14)	0.887
Lean body mass (kg)	76	0.03 (−0.06; 0.11)	0.493	0 (−0.09; 0.09)	0.988
Area sag pp (mm^2)	71	0.13 (−0.62; 0.88)	0.727	0.26 (−0.52;(1.03)	0.512
Area sag sc (mm^2)	70	−0.30 (−0.96; 0.35)	0.360	−0.14 (−0.80; 0.53)	0.681
Area ax sc (mm^2)	71	−0.17 (−1.14; 0.81)	0.731	0.15 (−0.84; 1.15)	0.758
5 years					
Weight (kg)	114	−0.01 (−0.12; 0.10)	0.897	−0.04 (−0.15; 0.07)	0.477
BMI percentiles	114	−0.32 (−1.46; 0.82)	0.578	−0.55 (−1.72; 0.61)	0.348
Sum 4 SFT (mm)	85	−0.15 (−0.40; 0.10)	0.246	−0.09 (−0.34; 0.16)	0.467
Body fat (%)	85	−0.14 (−0.32; 0.04)	0.127	−0.07 (−0.22; 0.09)	0.412
Lean body mass (kg)	85	0.06 (−0.04; 0.15)	0.249	0.02 (−0.08; 0.11)	0.740
Weight gain (kg) (birth – 5y)	114	−0.02 (−0.12; 0.09)	0.731	−0.04 (−0.15; 0.06)	0.425
Area sag pp (mm^2)	70	−0.16 (−0.89; 0.56)	0.654	0.11 (−0.63; 0.84)	0.767
Area sag sc (mm^2)	71	−0.46 (−1.17; 0.25)	0.203	−0.33 (−1.07; 0.41)	0.381
Area ax sc (mm^2)	72	−0.35 (−1.37; 0.67)	0.495	−0.06 (−1.11; 1.00)	0.917
SAT volume (cm^2)	31	0.76 (−13.97; 15.49)	0.917	5.14; (−10.85; 21.13)	0.515
VAT volume (cm^2)	31	0.43 (−2.64; 3.49)	0.779	1.28 (−2.16; 4.73)	0.451

Data are presented as the regression coefficient beta (95% CI) from linear regression analyses.
* Adjusted for pregnancy duration in days, sex (except for BMI percentiles), and mode of infant feeding (exclusively breastfed or a combination of partially breastfed/formula) at 4 months postpartum. Abbreviations; area ax sc, area of subcutaneous fat in the axial plane; area sag pp, area of preperitoneal fat in the sagittal plane; area sag sc, area of subcutaneous fat in the sagittal plane; BMI, body mass index; kg, kilograms; mm, millimeters; n, number of children; SAT, subcutaneous adipose tissue; SFT, skinfold thickness; VAT, visceral adipose tissue.

Appendix 20: Cord blood docosahexaenoic acid in relation to body composition at 2−5 years of age

Body composition variables	n	Unadjusted Analysis		Adjusted Analysis*	
		beta (95% CI)	*P*	beta (95% CI)	*P*
2 years					
Weight (kg)	122	0.02 (−0.08; 0.12)	0.679	−0.03 (−0.13; 0.07)	0.611
BMI percentiles	122	−0.11 (−2.12; 1.91)	0.917	−0.56 (−2.67; 1.55)	0.602
Sum 4 SFT (mm)	86	0.11 (−0.18; 0.39)	0.452	0.17 (−0.12; 0.47)	0.251
Body fat (%)	86	0.07 (−0.13; 0.26)	0.508	0.10 (−0.10; 0.31)	0.312
Lean body mass (kg)	86	0.05 (−0.03; 0.13)	0.196	0.02 (−0.06; 0.10)	0.649
Area sag pp (mm^2)	85	0.34 (−0.30; 0.98)	0.298	0.48 (−0.19; 1.15)	0.157
Area sag sc (mm^2)	85	−0.32 (−1.20; 0.57)	0.481	−0.18 (−1.10; 0.75)	0.703
Area ax sc (mm^2)	85	−0.17 (−1.21; 0.87)	0.749	0.07 (−0.99; 1.13)	0.895
3 years					
Weight (kg)	116	0.03 (−0.09; 0.16)	0.621	−0.03 (−0.16; 0.10)	0.670
BMI percentiles	116	−0.02 (−1.97; 1.94)	0.987	−0.60 (−2.67; 1.47)	0.569
Sum 4 SFT (mm)	86	0.04 (−0.27; 0.34)	0.804	0.16 (−0.15; 0.47)	0.306
Body fat (%)	86	0.01 (−0.21; 0.23)	0.947	0.11 (−0.10; 0.33)	0.303
Lean body mass (kg)	86	0.07 (−0.04; 0.17)	0.196	0.01 (−0.10; 0.12)	0.859
Area sag pp (mm^2)	76	0.53 (−0.50; 1.55)	0.311	0.93 (−0.14; 2.01)	0.087
Area sag sc (mm^2)	76	−0.29 (−1.39; 0.82)	0.607	0.07 (−1.06; 1.21)	0.896
Area ax sc (mm^2)	75	−0.82 (−2.51; 0.86)	0.333	−0.19 (−1.93; 1.55)	0.829
4 years					
Weight (kg)	116	0.06 (−0.09; 0.20)	0.439	−0.01 (−0.16; 0.15)	0.926
BMI percentiles	116	0.23 (−1.73; 2.20)	0.814	−0.44 (−2.56; 1.67)	0.678
Sum 4 SFT (mm)	76	0.05 (−0.27; 0.38)	0.754	0.05 (−0.30; 0.39)	0.791
Body fat (%)	76	0 (−0.25; 0.25)	0.976	0.05 (−0.20; 0.29)	0.717
Lean body mass (kg)	76	0.12 (−0.03; 0.27)	0.116	0.06 (−0.10; 0.22)	0.430
Area sag pp (mm^2)	71	0.09 (−1.18; 1.36)	0.889	0.37 (−0.99; 1.73)	0.589
Area sag sc (mm^2)	70	−0.61 (−1.73; 0.51)	0.282	−0.30 (−1.47; 0.87)	0.614
Area ax sc (mm^2)	71	−0.46 (−2.11; 1.20)	0.585	0.19 (−1.56; 1.93)	0.830
5 years					
Weight (kg)	114	0.03 (−0.16; 0.22)	0.735	−0.05 (−0.26; 0.15)	0.620
BMI percentiles	114	0.02 (−1.93; 1.97)	0.981	−0.57 (−2.66; 1.52)	0.591
Sum 4 SFT (mm)	85	−0.24 (−0.67; 0.19)	0.277	−0.13 (−0.58; 0.31)	0.549
Body fat (%)	85	−0.23 (−0.53; 0.08)	0.151	−0.09 (−0.38; 0.19)	0.523
Lean body mass (kg)	85	0.15 (−0.02; 0.31)	0.077	0.07 (−0.11; 0.24)	0.439
Weight gain (kg) (birth – 5y)	114	0.02 (−0.17; 0.20)	0.868	−0.05 (−0.24; 0.15)	0.647
Area sag pp (mm^2)	70	−0.55 (−1.81; 0.71)	0.384	0.05 (−1.28; 1.38)	0.943
Area sag sc (mm^2)	71	−0.73 (−1.96; 0.50)	0.240	−0.50 (−1.83; 0.83)	0.455
Area ax sc (mm^2)	72	−0.46 (−2.23; 1.30)	0.603	0.17 (−1.72; 2.05)	0.861
SAT volume (cm^2)	31	0.55 (−28.19; 29.30)	0.969	11.72 (−19.45; 42.89)	0.447
VAT volume (cm^2)	31	−0.38 (−6.37; 5.62)	0.899	1.63 (−5.15; 8.41)	0.625

Data are presented as the regression coefficient beta (95% CI) from linear regression analyses.
* Adjusted for pregnancy duration in days, sex (except for BMI percentiles), and mode of infant feeding (exclusively breastfed or combination of partially breastfed/formula) at 4 months postpartum. Abbreviations; area ax sc, area of subcutaneous fat in the axial plane; area sag pp, area of preperitoneal fat in the sagittal plane; area sag sc, area of subcutaneous fat in the sagittal plane; BMI, body mass index; kg, kilograms; mm, millimeters; n, number of children; SAT, subcutaneous adipose tissue; SFT, skinfold thickness; VAT, visceral adipose tissue.

Appendix

Appendix 21: Cord blood n-3 LCPUFAs in relation to body composition at 2−5 years of age

Body composition variables	n	Unadjusted Analysis		Adjusted Analysis*	
		beta (95% CI)	*P*	beta (95% CI)	*P*
2 years					
Weight (kg)	122	0.02 (−0.06; 0.11)	0.602	−0.02 (−0.11; 0.07)	0.692
BMI percentiles	122	0.04 (−1.72; 1.80)	0.965	−0.34 (−2.19; 1.51)	0.717
Sum 4 SFT (mm)	86	0.09 (−0.16; 0.34)	0.456	0.15 (−0.11; 0.41)	0.241
Body fat (%)	86	0.06 (−0.11; 0.23)	0.508	0.09 (−0.08; 0.27)	0.298
Lean body mass (kg)	86	0.05 (−0.02; 0.12)	0.161	0.02 (−0.05; 0.09)	0.576
Area sag pp (mm^2)	85	0.29 (−0.27; 0.86)	0.301	0.43 (−0.17; 1.02)	0.155
Area sag sc (mm^2)	85	−0.30 (−1.08; 0.47)	0.438	−0.18 (−0.99; 0.63)	0.663
Area ax sc (mm^2)	85	−0.18 (−1.09; 0.74)	0.704	0.05 (−0.89; 0.98)	0.923
3 years					
Weight (kg)	116	0.03 (−0.08; 0.14)	0.550	−0.02 (−0.13; 0.10)	0.753
BMI percentiles	116	0.06 (−1.64; 1.76)	0.942	−0.43 (−2.24; 1.38)	0.638
Sum 4 SFT (mm)	86	0.04 (−0.22; 0.31)	0.740	0.16 (−0.12; 0.43)	0.257
Body fat (%)	86	0.02 (−0.18; 0.21)	0.872	0.11 (−0.08; 0.30)	0.251
Lean body mass (kg)	86	0.06 (−0.03; 0.15)	0.171	0.01 (−0.08; 0.11)	0.782
Area sag pp (mm^2)	76	0.46 (−0.43; 1.34)	0.310	0.81 (−0.12; 1.74)	0.088
Area sag sc (mm^2)	76	−0.22 (−1.17; 0.74)	0.651	0.09 (−0.90; 1.07)	0.859
Area ax sc (mm^2)	75	−0.69 (−2.15; 0.77)	0.349	−0.15 (−1.66; 1.36)	0.845
4 years					
Weight (kg)	116	0.06 (−0.07; 0.18)	0.383	0 (−0.13; 0.14)	0.988
BMI percentiles	116	0.32 (−1.39; 2.02)	0.715	−0.26 (−2.10; 1.59)	0.783
Sum 4 SFT (mm)	76	0.05 (−0.23; 0.33)	0.723	0.05 (−0.25; 0.35)	0.749
Body fat (%)	76	0 (−0.22; 0.22)	0.991	0.05 (−0.17; 0.26)	0.672
Lean body mass (kg)	76	0.11 (−0.02; 0.24)	0.092	0.07 (−0.08; 0.21)	0.360
Area sag pp (mm^2)	71	0.08 (−1.02; 1.19)	0.885	0.33 (−0.85; 1.51)	0.581
Area sag sc (mm^2)	70	−0.51 (−1.48; 0.46)	0.297	−0.24 (−1.26; 0.77)	0.633
Area ax sc (mm^2)	71	−0.39 (−1.82; 1.05)	0.594	0.17 (−1.35; 1.69)	0.825
5 years					
Weight (kg)	114	0.03 (−0.13; 0.20)	0.684	−0.04 (−0.22; 014)	0.669
BMI percentiles	114	0.08 (−1.61; 1.77)	0.925	−0.43 (−2.25; 1.39)	0.642
Sum 4 SFT (mm)	85	−0.20 (−0.57; 0.18)	0.295	−0.11 (−0.49; 0.28)	0.594
Body fat (%)	85	−0.19 (−0.46; 0.08)	0.163	−0.07 (−0.32; 0.18)	0.572
Lean body mass (kg)	85	0.13 (−0.01; 0.27)	0.063	0.07 (−0.08; 0.22)	0.382
Weight gain (kg) (birth – 5y)	114	0.02 (−0.14; 0.18)	0.802	−0.03 (−0.20; 0.14)	0.717
Area sag pp (mm^2)	70	−0.47 (−1.56; 0.62)	0.393	0.07 (−1.09; 1.24)	0.899
Area sag sc (mm^2)	71	−0.62 (−1.69; 0.45)	0.250	−0.42 (−1.58; 0.74)	0.472
Area ax sc (mm^2)	72	−0.39 (−1.92; 1.14)	0.613	0.16 (−1.48; 1.80)	0.846
SAT volume (cm^2)	31	−0.64 (−26.29; 25.01)	0.960	9.87 (−18.43; 38.16)	0.480
VAT volume (cm^2)	31	−0.47 (−5.82; 4.87)	0.858	1.42 (−4.73; 7.57)	0.638

Data are presented as the regression coefficient beta (95% CI) from linear regression analyses.
* Adjusted for pregnancy duration in days, sex (except for BMI percentiles), and mode of infant feeding (exclusively breastfed or combination of partially breastfed/formula) at 4 months postpartum. Abbreviations; area ax sc, area of subcutaneous fat in the axial plane; area sag pp, area of preperitoneal fat in the sagittal plane; area sag sc, area of subcutaneous fat in the sagittal plane; BMI, body mass index; kg, kilograms; LCPUFAs, long-chain polyunsaturated fatty acids; mm, millimeters; n, number of children; SAT, subcutaneous adipose tissue; SFT, skinfold thickness; VAT, visceral adipose tissue. Values for fatty acids are expressed as a percentage of the weight of total fatty acids (% FA of total FA). PUFA: sum of all cis-FAs with two or more double bonds: n3 LCPUFAs: C20:3n-3; C20:4n-3; 20:5n-3; C21:5n-3; C22:3n-3; 22:5n-3; 22:6n-3.

Appendix 22: Cord blood n-6 LCPUFAs in relation to body composition at 2−5 years of age

Body composition variables	n	Unadjusted Analysis		Adjusted Analysis*	
		beta (95% CI)	*P*	beta (95% CI)	*P*
2 years					
Weight (kg)	122	0 (−0.04; 0.04)	0.930	−0.01 (−0.05; 0.02)	0.468
BMI percentiles	122	−0.26 (−1.01; 0.50)	0.502	−0.36 (−1.12; 0.39)	0.343
Sum 4 SFT (mm)	86	0.06 (−0.05; 0.17)	0.274	0.07 (−0.04; 0.18)	0.202
Body fat (%)	86	0.03 (−0.04; 0.11)	0.357	0.04 (−0.03; 0.11)	0.282
Lean body mass (kg)	86	0.01 (−0.02; 0.04)	0.425	0 (−0.03; 0.03)	0.840
Area sag pp (mm^2)	85	0.25 (0.01; 0.49)	0.042	0.28 (0.04; 0.52)	0.025
Area sag sc (mm^2)	85	−0.03 (−0.37; 0.30)	0.846	0 (−0.34; 0.35)	0.980
Area ax sc (mm^2)	85	0.08 (−0.32; 0.47)	0.699	0.14 (−0.25; 0.53)	0.486
3 years					
Weight (kg)	116	0 (−0.04; 0.05)	0.907	−0.01 (−0.06; 0.04)	0.629
BMI percentiles	116	−0.27 (−0.99; 0.46)	0.466	−0.40 (−1.13; 0.32)	0.274
Sum 4 SFT (mm)	86	0.02 (−0.10; 0.13)	0.790	0.04 (−0.07; 0.15)	0.436
Body fat (%)	86	0 (−0.08; 0.09)	0.950	0.03 (−0.05; 0.11)	0.450
Lean body mass (kg)	86	0.02 (−0.02; 0.05)	0.457	0 (−0.04; 0.04)	0.934
Area sag pp (mm^2)	76	0.29 (−0.08; 0.67)	0.126	0.39 (0.01; 0.77)	0.044
Area sag sc (mm^2)	76	−0.05 (−0.46; 0.36)	0.808	0.05 (−0.35; 0.45)	0.805
Area ax sc (mm^2)	75	−0.20 (−0.83; 0.42)	0.517	−0.04 (−0.66; 0.58)	0.902
4 years					
Weight (kg)	116	0.01 (−0.04; 0.07)	0.679	−0.01 (−0.06; 0.05)	0.817
BMI percentiles	116	−0.25 (−1.00; 0.49)	0.505	−0.44 (−1.21; 0.32)	0.252
Sum 4 SFT (mm)	76	0.01 (−0.11; 0.12)	0.925	0.01 (−0.11; 0.13)	0.859
Body fat (%)	76	−0.01 (−0.10; 0.08)	0.827	0.01 (−0.07; 0.10)	0.780
Lean body mass (kg)	76	0.03 (−0.03; 0.08)	0.343	0.01 (−0.05; 0.06)	0.806
Area sag pp (mm^2)	71	0.09 (−0.40; 0.58)	0.713	0.19 (−0.32; 0.69)	0.463
Area sag sc (mm^2)	70	−0.17 (−0.61; 0.25)	0.408	−0.06 (−0.49; 0.38)	0.798
Area ax sc (mm^2)	71	−0.08 (−0.72; 0.55)	0.795	0.14 (−0.50; 0.79)	0.659
5 years					
Weight (kg)	114	0.01 (−0.07; 0.08)	0.891	−0.02 (−0.09; 0.06)	0.682
BMI percentiles	114	−0.15 (−0.90; 0.60)	0.690	−0.29 (−1.05; 0.47)	0.451
Sum 4 SFT (mm)	85	−0.09 (−0.25; 0.08)	0.296	−0.04 (−0.21; 0.12)	0.597
Body fat (%)	85	−0.09 (−0.21; 0.03)	0.148	−0.03 (−0.14; 0.07)	0.540
Lean body mass (kg)	85	0.05 (−0.02; 0.11)	0.156	0.02 (−0.04; 0.08)	0.550
Weight gain (kg) (birth – 5y)	114	0 (−0.07; 0.07)	0.918	−0.02 (−0.09; 0.05)	0.603
Area sag pp (mm^2)	70	−0.11 (−0.58; 0.37)	0.662	0.09 (−0.39; 0.57)	0.716
Area sag sc (mm^2)	71	−0.26 (−0.73; 0.20)	0.265	−0.16 (−0.65; 0.32)	0.509
Area ax sc (mm^2)	72	−0.18 (−0.84; 0.49)	0.602	0.04 (−0.65; 0.73)	0.910
SAT volume (cm^2)	31	0.79 (−8.73; 10.31)	0.866	3.65 (−6.66; 13.96)	0.473
VAT volume (cm^2)	31	0.29 (−1.69; 2.27)	0.766	0.84 (−1.39; 3.06)	0.448

Data are presented as the regression coefficient beta (95% CI) from linear regression analyses.
* Adjusted for pregnancy duration in days, sex (except for BMI percentiles), and mode of infant feeding (exclusively breastfed or combination of partially breastfed/formula) at 4 months postpartum.
Abbreviations; area ax sc, area of subcutaneous fat in the axial plane; area sag pp, area of preperitoneal fat in the sagittal plane; area sag sc, area of subcutaneous fat in the sagittal plane; BMI, body mass index; kg, kilograms; LCPUFAs, long-chain polyunsaturated fatty acids; mm, millimeters; n, number of children; SAT, subcutaneous adipose tissue; SFT, skinfold thickness; VAT, visceral adipose tissue. Values for fatty acids are expressed as a percentage of the weight of total fatty acids (% FA of total FA). PUFA: sum of all cis-FAs with two or more double bonds, n6 LCPUFAs: C20:2n-6; 20:3n-6; 20:4n-6; C22:2n-6; C22:4n6; C22:5n-6

Appendix 23: Cord blood ratio of n-6/ n-3 LCPUFAs in relation to body composition at 2–5 years of age

Body composition variables	n	Unadjusted Analysis		Adjusted Analysis*	
		beta (95% CI)	*P*	beta (95% CI)	*P*
2 years					
Weight (kg)	122	−0.06 (−0.20; 0.08)	0.398	−0.03 (−0.17; 0.12)	0.709
BMI percentiles	122	−0.84 (−3.79; 2.12)	0.575	−0.70 (−3.73; 2.33)	0.647
Sum 4 SFT (mm)	86	−0.10 (−0.54; 0.34)	0.664	−0.20 (−0.65; 0.25)	0.373
Body fat (%)	86	−0.06 (−0.36; 0.24)	0.705	−0.13 (−0.43; 0.18)	0.419
Lean body mass (kg)	86	−0.04 (−0.16; 0.09)	0.567	0 (−0.13; 0.12)	0.973
Area sag pp (mm^2)	85	−0.06 (−1.08; 0.97)	0.910	−0.21 (−1.27; 0.86)	0.702
Area sag sc (mm^2)	85	1.17 (−0.22; 2.55)	0.098	0.94 (−0.49; 2.37)	0.195
Area ax sc (mm^2)	85	1.07 (−0.56; 2.71)	0.195	0.66 (−1.00; 2.32)	0.430
3 years					
Weight (kg)	116	−0.06 (−0.25; 0.13)	0.537	0 (−0.20; 0.19)	0.970
BMI percentiles	116	0.16 (−2.82; 3.13)	0.918	0.64 (−2.44; 3.72)	0.683
Sum 4 SFT (mm)	86	−0.16 (−0.70; 0.38)	0.562	−0.27 (−0.81; 0.27)	0.316
Body fat (%)	86	−0.10 (−0.49; 0.29)	0.615	−0.19 (−0.56; 0.19)	0.326
Lean body mass (kg)	86	−0.14 (−0.32; 0.05)	0.140	−0.07 (−0.26; 0.11)	0.435
Area sag pp (mm^2)	76	−0.84 (−2.58; 0.89)	0.335	−1.17 (−2.93; 0.60)	0.193
Area sag sc (mm^2)	76	0.27 (−1.59; 2.13)	0.773	0.04 (−1.81; 1.89)	0.969
Area ax sc (mm^2)	75	1.40 (−1.45; 4.24)	0.331	0.91 (−1.93; 3.75)	0.525
4 years					
Weight (kg)	116	−0.04 (−0.27; 0.18)	0.722	0.02 (−0.21; 0.26)	0.842
BMI percentiles	116	0.35 (−2.70; 3.39)	0.822	1.06 (−2.12; 4.24)	0.509
Sum 4 SFT (mm)	76	−0.26 (−0.79; 0.26)	0.317	−0.19 (−0.73; 0.36)	0.497
Body fat (%)	76	−0.18 (−0.58; 0.22)	0.378	−0.13 (−0.51; 0.26)	0.508
Lean body mass (kg)	76	−0.18 (−0.42; 0.06)	0.147	−0.15 (−0.40; 0.10)	0.248
Area sag pp (mm^2)	71	−1.15 (−3.25; 0.94)	0.276	−1.33 (−3.48; 0.81)	0.218
Area sag sc (mm^2)	70	0.27 (−1.60; 2.14)	0.774	0.11 (−1.76; 1.97)	0.909
Area ax sc (mm^2)	71	0.26 (−2.50; 3.01)	0.853	−0.17 (−2.95; 2.61)	0.902
5 years					
Weight (kg)	114	−0.06 (−0.35; 0.24)	0.701	0.02 (−0.28; 0.33)	0.879
BMI percentiles	114	−0.11 (−3.13; 2.92)	0.944	0.46 (−2.71; 3.63)	0.774
Sum 4 SFT (mm)	85	0.09 (−0.64; 0.82)	0.809	0.03 (−0.70; 0.76)	0.927
Body fat (%)	85	0.10 (−0.43; 0.62)	0.721	0.04 (−0.43; 0.50)	0.874
Lean body mass (kg)	85	−0.21 (−0.49; 0.06)	0.127	−0.15 (−0.43; 0.14)	0.304
Weight gain (kg) (birth – 5y)	114	−0.05 (−0.33; 0.23)	0748	0 (−0.29; 0.29)	0.997
Area sag pp (mm^2)	70	−0.72 (−2.83; 1.39)	0.498	−1.47 (−3.59; 0.65)	0.171
Area sag sc (mm^2)	71	0.07 (−2.01; 2.15)	0.946	0 (−2.16; 2.16)	0.997
Area ax sc (mm^2)	72	−0.33 (−3.25; 2.59)	0.820	−0.73 (−3.72; 2.26)	0.628
SAT volume (cm^2)	31	9.91 (−61.89; 81.71)	0.780	−16.86 (−100.52; 66.81)	0.682
VAT volume (cm^2)	31	4.49 (−10.40; 19.39)	0.542	−0.17 (−18.32; 17.97)	0.984

Data are presented as the regression coefficient beta (95% CI) from linear regression analyses.
* Adjusted for pregnancy duration in days, sex (except for BMI percentiles), and mode of infant feeding (exclusively breastfed or combination of partially breastfed/formula) at 4 months postpartum. Abbreviations; area ax sc, area of subcutaneous fat in the axial plane; area sag pp, area of preperitoneal fat in the sagittal plane; area sag sc, area of subcutaneous fat in the sagittal plane; BMI, body mass index; kg, kilograms; LCPUFAs, long-chain polyunsaturated fatty acids; mm, millimeters; n, number of children; SAT, subcutaneous adipose tissue; SFT, skinfold thickness; VAT, visceral adipose tissue. Values for fatty acids are expressed as a percentage of the weight of total fatty acids (% FA of total FA). PUFA: sum of all cis-FAs with two or more double bonds, n6 LCPUFAs: C20:2n-6; 20:3n-6; 20:4n-6; C22:2n-6; C22:4n6; C22:5n-6, n3 LCPUFAs: C20:3n-3; C20:4n-3; 20:5n-3; C21:5n-3; C22:3n-3; 22:5n-3; 22:6n-3.

Appendix 24: Associations between LCPUFAs in breast milk at 6 weeks postpartum and offspring body composition from 2−5 years of age (unadjusted analysis)

Body composition variables	n	AA		DHA		n-3 LCPUFAs		n-6 LCPUFAs		Ratio n-6 LCPUFAs/ n-3 LCPUFAs	
		b (95% CI)	*P*	b (95% CI)	*P*	b (95% CI)	*P*	b (95% CI)	*P*	b (95% CI)	*P*
2 years											
Weight (kg)	145	−1.64 (−4.47; 1.19)	0.254	0.33 (0.01; 0.64)	0.041	0.24 (0.02; 0.47)	0.031	−0.03 (−1.14; 1.07)	0.953	−0.32 (−0.60; 0.03)	0.032
BMI percentiles	145	−31.13 (−88.24; 25.97)	0.283	6.44 (0.17; 12.72)	0.044	5.02 (0.48; 9.55)	0.030	−4.92 (−27.18; 17.34)	0.663	−6.20 (−12.02; 0.38)	0.037
Sum 4 SFT (mm)	97	−2.18 (−11.37; 7.02)	0.639	1.01 (−0.11; 2.14)	0.077	0.78 (−0.10; 1.65)	0.081	−0.21 (−3.92; 3.50)	0.912	−0.57 (−1.47; 0.33)	0.209
Body fat (%)	97	−1.46 (−7.76; 4.83	0.645	0.71 (−0.06; 1.48)	0.071	0.55 (−0.05; 1.14)	0.072	−0.08 (−2.62; 2.46)	0.952	−0.40 (−1.01; 0.22)	0.204
Lean body mass	97	−0.36 (−2.96; 2.25)	0.785	0.17 (−0.16; 0.49)	0.306	0.15 (−0.10; 0.40)	0.223	0.26 (−0.79; 1.31)	0.625	−0.20 (−0.45; 0.06)	0.127
Area sag pp, mm^2	98	−3.31 (−22.83; 16.21)	0.737	−0.03 (−2.42; 2.36)	0.980	0.27 (−1.59; 2.14)	0.773	2.70 (−5.43; 10.83)	0.511	0.59 (−1.33; 2.51)	0.540
Area sag sc, mm^2	98	−19.72 (−49.17; 9.73)	0.187	−2.31 (−5.91; 1.30)	0.207	−1.77 (−4.58; 1.04)	0.215	−2.52 (−14.92; 9.87)	0.687	2.15 (−0.74; 5.05)	0.143
Area ax sc, mm^2	98	−22.01 (−54.89; 10.88)	0.187	−3.20 (−7.21; 0.81)	0.116	−2.46 (−5.59; 0.67)	0.122	−4.15 (−17.98; 9.67)	0.552	2.10 (−1.15; 5.34)	0.202
3 years											
Weight (kg)	139	−1.30 (−4.84; 2.25)	0.470	0.33 (−0.06; 0.73)	0.097	0.24 (−0.04; 0.53)	0.096	−0.28 (−1.69; 1.14)	0.698	−0.27 (−0.64; 0.10)	0.152
BMI percentiles	139	−22.45 (−76.71; 31.81)	0.415	5.18 (−0.87; 11.22)	0.093	3.77 (−0.58; 8.13)	0.089	−8.90 (−30.52; 12.71)	0.417	−3.96 (−9.66; 1.74)	0.172
Sum 4 SFT (mm)	99	0.62 (−9.20; 10.45)	0.900	0.44 (−0.71; 1.60)	0.451	0.42 (−0.48; 1.31)	0.359	0.98 (−2.93; 4.86)	0.620	−0.32 (−1.24; 0.60)	0.492
Body fat (%)	99	1.00 (−5.98; 7.98)	0.777	0.30 (−0.52; 1.12)	0.467	0.28 (−0.36; 0.91)	0.394	1.00 (−1.77; 3.77)	0.476	−0.20 (−0.86; 0.46)	0.545
Lean body mass	99	−2.04 (−5.30; 1.46)	0.250	0.29 (−0.12; 0.70)	0.168	0.23 (−0.09; 0.55)	0.156	−0.65 (−2.05; 0.74)	0.356	−0.29 (−0.62; 0.03)	0.077
Area sag pp, mm^2	89	−5.91 (−38.34; 26.53)	0.718	−0.33 (−4.01; 3.36)	0.861	−0.01 (−2.86; 2.84)	0.994	4.27 (−8.64; 17.18)	0.513	0.41 (−2.57; 3.39)	0.784
Area sag sc, mm^2	90	−24.30 (−59.01; 10.41)	0.168	−1.95 (−5.89; 1.99)	0.328	−1.51 (−4.56; 1.53)	0.326	−1.28 (−15.27; 12.72)	0.857	1.29(−1.91; 4.50)	0.424
Area ax sc, mm^2	89	−30.67 (−84.49; 23.15)	0.260	−1.87 (−8.03; 4.30)	0.549	−1.48 (−6.24; 3.29)	0.539	−3.95 (−25.66; 17.76)	0.718	0.15 (−4.83; 5.13)	0.952

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4 years											
Weight (kg)	137	−0.70 (−4.81; 3.42)	0.739	0.42 (−0.03; 0.87)	0.065	0.30 (−0.02; 0.63)	0.064	0.28 (−1.33; 1.88)	0.736	−0.30 (−0.72; 0.12)	0.157
BMI percentiles	137	−14.51 (−70.35; 41.33)	0.608	6.67 (0.63; 12.71)	0.031	4.85 (−0.49; 9.21)	0.030	−6.11 (−27.91; 15.70)	0.581	−5.78 (−11.46; −0.11)	0.046
Sum 4 SFT (mm)	91	−0.19 (−10.71; 10.33)	0.972	0.06 (−1.22; 1.34)	0.921	0.06 (−0.97; 1.09)	0.916	1.08 (−3.24; 5.39)	0.621	−0.13 (−1.09; 0.83)	0.790
Body fat (%)	91	−0.82 (−7.11; 8.75)	0.837	0.16 (−0.81; 1.12)	0.749	0.11 (−0.67; 0.89)	0.782	1.54 (−1.70; 4.79)	0.347	−0.10 (−0.83; 0.62)	0.778
Lean body mass	91	−2.39 (−7.16; 2.38)	0.321	0.67 (0.10; 1.24)	0.021	0.55 (0.10; 1.01)	0.018	−1.04 (−3.00; 0.92)	0.294	−0.55 (−0.98; −0.13)	0.010
Area sag pp, mm^2	85	−5.42 (−46.18; 35.35)	0.792	2.19 (−2.24; 6.62)	0.329	1.86 (−1.56; 5.28)	0.282	5.43 (−10.66; 21.53)	0.504	−1.12 (−4.76; 2.52)	0.543
Area sag sc, mm^2	84	−24.10 (−62.88; 14.67)	0.220	−1.88 (−6.08; 2.33)	0.378	−1.57 (−4.82; 1.68)	0.340	−0.97 (−16.45; 14.50)	0.901	1.81 (−1.74; 5.36)	0.314
Area ax sc, mm^2	86	−30.06 (−91.48; 31.37)	0.333	−3.50 (−10.20; 3.20)	0.302	−2.81 (−7.98; 2.36)	0.283	−4.28 (−28.79; 20.23)	0.729	2.63 (−2.84; 8.10)	0.342
5 years											
Weight (kg)	132	−1.34 (−6.84; 4.17)	0.632	0.45 (−0.16; 1.05)	0.148	0.35 (−0.09; 0.78)	0.115	−0.16 (−2.31; 2.00)	0.887	−0.28 (−0.86; 0.30)	0.342
BMI percentiles	132	−27.61 (−83.00; 27.78)	0.326	4.53 (−1.57; 10.63)	0.144	3.57 (−0.82; 7.96)	0.110	−14.03 (−35.63; 7.57)	0.201	−4.56 (−10.41; 1.29)	0.126
Sum 4 SFT (mm)	99	−0.77 (−13.55; 12.01)	0.906	−0.66 (−2.25; 0.93)	0.412	−0.44 (−1.68; 0.80)	0.484	0.02 (−5.15; 5.19)	0.995	−0.04 (−1.31; 1.24)	0.956
Body fat (%)	99	−0.24 (−9.42; 8.95)	0.959	−0.45 (−1.60; 0.69)	0.434	−0.34 (−1.23; 0.56)	0.455	0.52 (−3.19; 4.24)	0.780	0.05 (−0.87; 0.96)	0.921
Lean body mass	99	−2.78 (−7.78; 2.22)	0.272	0.56 (−0.06; 1.18)	0.077	0.46 (−0.03; 0.94)	0.064	−1.21 (−3.22; 0.82)	0.239	−0.54 (−1.03; −0.05)	0.030
Weight gain (kg) (birth – 5y)	132	0.03 (−5.13; 5.19)	0.991	0.44 (−0.12; 1.01)	0.125	0.34 (−0.06; 0.75)	0.098	0.19 (−1.82; 2.21)	0.849	−0.22 (−0.77; 0.32)	0422
Area sag pp, mm^2	87	−1.04 (−43.45; 41.37)	0.961	2.17 (−2.56; 6.90)	0.365	2.00 (−1.64; 5.64)	0.277	11.82 (−4.96; 28.59)	0.165	−0.06 (−3.84; 3.72)	0.975
Area sag sc, mm^2	88	−19.20 (−58.08; 19.68)	0.329	−1.00 (−5.37; 3.37)	0.650	−0.89 (−4.26; 2.48)	0.601	−3.20 (−18.78; 12.38)	0.684	0.62 (−2.85; 4.09)	0.722
Area ax sc, mm^2	89	−15.34 (−77.09; 46.42)	0.623	−2.38 (−9.24; 4.48)	0.492	−2.07 (−7.38; 3.24)	0.440	3.44 (−21.03; 27.91)	0.781	1.55 (−3.91; 7.01)	0.574
SAT volume, cm^3	42	−316.86 (−931.25; 297.54)	0.304	−12.31 (−92.78; 68.16)	0.759	−12.33 (−72.01; 47.35)	0.679	−168.90 (−438.87; 101.08)	0.213	−11.56 (−82.40; 59.29)	0.743
VAT volume, cm^3	42	1.48 (−131.71; 134.68)	0.982	−6.08 (−23.20; 11.04)	0.477	−4.12 (−16.85; 8.61)	0.517	2.58 (−56.31; 61.47)	0.930	5.03 (−10.06; 20.12)	0.504

Data are presented as the regression coefficient b (95% CI) from linear regression analyses.Values for fatty acids are expressed as a percentage of the weight of total fatty acids (% FA of total FA). PUFA: sum of all cis-FAs with two or more double bonds, n6 LCPUFAs: C20:2n-6; 20:3n-6; 20:4n-6; C22:2n-6; C22:4n6; C22:5n-6, n3 LCPUFAs: C20:3n-3; C20:4n-3; 20:5n-3; C21:5n-3; C22:3n-3; 22:5n-3; 22:6n-3. Abbreviations; AA, arachidonic acid, area ax sc, area of subcutaneous fat in the axial plane; area sag pp, area of preperitoneal fat in the sagittal plane; area sag sc, area of subcutaneous fat in the sagittal plane; BMI, body mass index; DHA, docosahexaenoic acid; kg, kilograms; LCPUFAs, long-chain polyunsaturated fatty acids; mm, millimeters; n, number of children; SAT, subcutaneous adipose tissue; SFT, skinfold thickness; VAT, visceral adipose tissue.

Appendix 25: Associations between LCPUFAs in breast milk at 4 months postpartum and offspring body composition from 2−5 years of age (unadjusted analysis)

Body composition variables	n	AA		DHA		n-3 LCPUFAs		n-6 LCPUFAs		Ratio n-6 LCPUFAs/ n-3 LCPUFAs	
		b (95% CI)	*P*	b (95% CI)	*P*	b (95% CI)	*P*	b (95% CI)	*P*	b (95% CI)	*P*
2 years											
Weight (kg)	118	−0.94 (−3.96; 2.07)	0.536	0.30 (−0.14; 0.74)	0.178	0.22 (−0.15; 0.58)	0.241	0.24 (−0.95; 1.42)	0.691	−0.23 (−0.56; 1.00)	0.166
BMI percentiles	118	−35.58 (−100.47; 29.32)	0.280	6.27 (−3.17; 15.72)	0.191	4.88 (−2.98; 12.73)	0.221	−1.61 (−27.19; 23.96)	0.901	−3.16 (−10.33; 4.02)	0.385
Sum 4 SFT (mm)	78	1.81 (−8.00; 11.62)	0.714	1.12 (−0.30; 2.54)	0.121	0.74 (−0.45; 1.92)	0.219	2.53 (−1.34; 6.40)	0.196	−0.69 (−1.88; .0.49)	0.248
Body fat (%)	78	1.07 (−5.77; 7.91	0.756	0.74 (−0.25; 1.73)	0.142	0.48 (−0.35; 1.30)	0.253	1.69 (−1.01; 4.39)	0.217	−0.46 (−1.28; 0.37)	0.274
Lean body mass	78	0.45 (−2.09; 2.99)	0.724	0.15 (−0.22; 0.52)	0.432	0.10 (−0.21; 0.41)	0.514	0.44 (−0.57; 1.44)	0.393	−0.16 (−0.47; 0.14)	0.290
Area sag pp, mm^2	78	−6.06 (−26.82; 14.70)	0.563	−1.74 (−4.70; 1.21)	0.243	−1.74 (−4.18; 0.70)	0.160	1.16 (−7.12; 9.44)	0.781	1.61 (−0.89; 4.10)	0.205
Area sag sc, mm^2	78	−13.66 (−41.49; 14.18)	0.332	−3.19 (−7.14; 0.75)	0.111	−3.05 (−6.31; 0.20)	0.066	0.18 (−10.97; 11.33)	0.974	2.21 (−1.15; 5.57)	0.194
Area ax sc, mm^2	78	−16.52 (−47.64; 14.61)	0.294	−2.82 (−7.27; 1.62)	0.210	−2.66 (−6.33; 1.02)	0.154	−0.13 (−12.62; 12.35)	0.983	1.71 (−2.08; 5.49)	0.372
3 years											
Weight (kg)	113	−0.49 (−4.21; 3.23)	0.794	0.20 (−0.34; 0.74)	0.465	0.11 (−0.34; 0.56)	0.622	0.03 (−1.47; 1.52)	0.972	−0.18 (−0.60; 0.24)	0.394
BMI percentiles	113	−3.27 (−66.12; 59.57)	0.918	6.36 (−2.71; 15.43)	0.168	5.07 (−2.45; 12.60)	0.184	3.38 (−21.84; 28.60)	0.791	−2.85 (−9.88; 4.18)	0.424
Sum 4 SFT (mm)	81	4.64 (−5.36; 14.64)	0.358	0.22 (−1.22; 1.66)	0.765	0.08 (−1.11; 1.27)	0.893	2.92 (−1.16; 6.99)	0.158	0.02 (−1.17; 1.21)	0.975
Body fat (%)	81	3.86 (−3.34; 11.06)	0.289	0.24 (−0.79; 1.28)	0.640	0.13 (−0.73; 0.99)	0.765	2.31 (−0.62; 5.24)	0.121	−0.03 (−0.89; 0.82)	0.941
Lean body mass	81	0.54 (−2.62; 3.69)	0.736	0.28 (−0.17; 0.72)	0.221	0.20 (−0.17; 0.57)	0.291	0.36 (−0.94; 1.65)	0.586	−0.25 (−0.61; 0.12)	0.189
Area sag pp, mm^2	72	−1.72 (−33.48; 30.05)	0.914	0 (−4.64; 4.63)	0.999	0.08 (−3.74; 3.91)	0.965	4.74 (−8.38; 17.85)	0.474	0.51 (−3.36; 4.38)	0.794
Area sag sc, mm^2	73	0.93 (−32.14; 33.99)	0.956	−0.55 (−5.37; 4.28)	0.822	−0.27 (−4.25; 3.71)	0.892	3.67 (−10.01; 17.34)	0.595	1.27 (−2.75; 5.29)	0.532
Area ax sc, mm^2	72	0.73 (−50.66; 52.13)	0.977	−1.29 (−8.89; 6.32)	0.737	−0.77 (−7.04; 5.49)	0.807	5.82 (−16.07; 27.71)	0.598	1.22 (−5.06; 7.50	0.699

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4 years											
Weight (kg)	112	0.27 (−4.07; 4.61)	0.902	0.31 (−0.32; 0.93)	0.334	0.16 (−0.36; 0.68)	0.538	0.87 (−0.85; 2.58)	0.318	−0.12 (−0.60; 0.36)	0.626
BMI percentiles	112	−4.63 (−68.78; 59.53)	0.887	7.69 (−1.46; 16.85)	0.099	5.38 (−2.24; 13.01)	0.165	6.81 (−18.61; 32.23)	0.597	−3.77 (−10.84; 3.29)	0.292
Sum 4 SFT (mm)	75	−6.87 (−17.45; 3.70)	0.199	0.05 (−1.51; 1.60)	0.950	−0.18 (−1.46; 1.11)	0.785	−0.81 (−5.18; 3.56)	0.714	−0.18 (−1.47; 1.11)	0.780
Body fat (%)	75	−4.58 (−12.64; 3.47	0.260	0.18 (−1.00; 1.36)	0.766	−0.01 (−1.00; 1.00)	0.982	−0.23 (−3.55; 3.10)	0.893	−0.24 (−1.22; 0.74)	0.623
Lean body mass	75	0.52 (−3.72; 4.76)	0.809	0.52 (−0.09; 1.12)	0.093	0.35 (−0.15; 0.86)	0.168	0.49 (−1.24; 2.22)	0.577	−0.42 (−0.92; 0.08)	0.101
Area sag pp, mm^2	69	−0.67 (−38.16; 36.81)	0.971	1.39 (−4.02; 6.80)	0.610	1.04 (−3.44; 5.52)	0.644	9.41 (−5.69; 24.51)	0.218	−0.24 (−4.91; 4.43)	0.919
Area sag sc, mm^2	69	−14.52 (−49.36; 20.31)	0.408	−0.07 (−5.13; 4.99)	0.978	0.01 (−4.18; 4.20)	0.997	0.72 (−13.54; 14.99)	0.920	0.46 (−3.90; 4.82)	0.833
Area ax sc, mm^2	70	−25.87 (−75.64; 23.91)	0.303	−1.49 (−8.75; 5.76)	0.682	−0.92 (−6.92; 5.09)	0.761	−2.51 (−22.97; 17.94)	0.807	0.10 (−6.14; 6.34)	0.975
5 years											
Weight (kg)	108	0.43 (−5.31; 6.16)	0.883	0.09 (−0.76; 0.94)	0.841	−0.05 (−0.75; 0.66)	0.895	0.92 (−1.35; 3.20)	0.423	0.03 (−0.62; 0.68)	0.929
BMI percentiles	108	−4.70 (−67.69; 58.30)	0.883	4.37 (−4.92; 13.66)	0.354	2.71 (−5.00; 10.42)	0.487	6.39 (−18.62; 31.40)	0.613	−1.77 (−8.90; 5.36)	0.624
Sum 4 SFT (mm)	85	4.31 (−8.67; 17.29)	0.510	−0.64 (−2.53; 1.26)	0.505	−0.69 (−2.25; 0.87)	0.380	2.74 (−2.49; 7.97)	0.300	0.42 (−1.11; 1.95)	0.587
Body fat (%)	85	3.28 (−6.20; 12.76)	0.494	−0.25 (−1.64; 1.13)	0.717	−0.32 (−1.46; 0.82)	0.579	2.25 (−1.56; 6.07)	0.244	0.16 (−0.96; 1.28)	0.778
Lean body mass	85	−0.24 (−4.85; 4.37)	0.918	0.30 (−0.37; 0.97)	0.376	0.15 (−0.40; 0.71)	0.589	0.31 (−1.56; 2.18)	0.743	−0.22 (−0.76; 0.33)	0.428
Weight gain (kg) (birth – 5y)	108	1.00 (−4.29; 6.26)	0.711	0.09 (−0.69; 0.88)	0.814	−0.03 (−0.67; 0.62)	0.939	0.92 (−1.17; 3.01)	0.384	0 (−0.60; 0.60)	0993
Area sag pp, mm^2	73	6.54 (−32.72; 45.80)	0.741	−0.34 (−5.99; 5.31)	0.905	−0.42 (−5.09; 4.25)	0.857	14.60 (−1.09; 30.29)	0.068	0.25 (−4.38; 4.88)	0.915
Area sag sc, mm^2	74	10.44 (−27.76; 48.63)	0.588	−0.70 (−6.18; 4.78)	0.800	−0.55 (−5.09; 3.99)	0.810	10.78 (−4.66; 26.22)	0.168	1.06 (−3.42; 5.54)	0.640
Area ax sc, mm^2	75	18.12 (−35.91; 72.15)	0.506	−0.72 (−8.52; 7.09)	0.855	−0.10 (−6.56; 6.36)	0.975	16.06 (−5.68; 37.80)	0.145	0.39 (−6.02; 6.79)	0.905
SAT volume, cm^3	38	322.99 (−286.92; 932.90)	0.290	19.16 (−77.98;116.30)	0.691	(9.44 (−67.61; 86.50)	0.805	222.84 (−31.95; 481.63)	0.084	−23.19 (−94.11; 47.74)	0.512
VAT volume, cm^3	38	64.24 (−85.41; 213.88)	0.390	−7.62 (−31.24; 16.00)	0.517	(−8.09 (−26.71; 10.53)	0.384	40.21 (−23.72; 104.14)	0.210	4.04 (−13.32; 21.40)	0.640

Data are presented as the regression coefficient b (95% CI) from linear regression analyses. Values for fatty acids are expressed as a percentage of the weight of total fatty acids (% FA of total FA). PUFA: sum of all cis-FAs with two or more double bonds, n6 LCPUFAs: C20:2n-6; 20:3n-6; 20:4n-6; C22:2n-6; C22:4n6; C22:5n-6, n3 LCPUFAs: C20:3n-3; C20:4n-3; 20:5n-3; C21:5n-3; C22:3n-3; 22:5n-3; 22:6n-3. Abbreviations; AA, arachidonic acid, area ax sc, area of subcutaneous fat in the axial plane; area sag pp, area of preperitoneal fat in the sagittal plane; area sag sc, area of subcutaneous fat in the sagittal plane; BMI, body mass index; DHA, docosahexaenoic acid; kg, kilograms; LCPUFAs, long-chain polyunsaturated fatty acids; mm, millimeters; n, number of children; SAT, subcutaneous adipose tissue; SFT, skinfold thickness; VAT, visceral adipose tissue

Appendix

Appendix 26: LCPUFAs at in breast milk at 6 weeks postpartum and offspring adipose tissue measurements at 2−5 years of age (adjusted analysis)

Body composition variables	n	AA		DHA		n-3 LCPUFAs		n-6 LCPUFAs		Ratio n-6 LCPUFAs/ n-3 LCPUFAs	
		b (95% CI)	*P*	b (95% CI)	*P*	b (95% CI)	*P*	b (95% CI)	*P*	b (95% CI)	*P*
2 years											
Area sag pp, mm^2	98	−2.25 (−22.20; 17.71)	0.824	0.01 (−2.41; 2.43)	0.992	0.30 (−1.59; 2.19)	0.752	3.26 (−5.11; 11.63)	0.441	0.58 (−1.38; 2.54)	0.558
Area sag sc, mm^2	98	−20.63 (−49.60; 8.33)	0.161	−2.17 (−5.69; 1.35)	0.224	−1.54 (−4.29; 1.21)	0.270	−5.02 (−17.29; 7.25)	0.418	1.72 (−1.14; 4.57)	0.236
Area ax sc, mm^2	98	−22.33 (−59.98; 9.31)	0.164	−2.99 (−6.82; 0.84)	0.124	−2.13 (−5.13; 0.87)	0.161	−7.26 (−20.62; 6.11)	0.284	1.47 (−1.66; 4.60)	0.354
3 years											
Area sag pp, mm^2	89	−8.15 (−40.58; 24.28)	0.619	−0.09 (−3.77; 3.59)	0.960	0.22 (−2.63; 3.07)	0.880	3.41 (−9.74; 16.56)	0.607	0.12 (−2.90; 3.13)	0.936
Area sag sc, mm^2	90	−30.42 (−63.37; 2.53)	0.070	−1.56 (−5.32; 2.20)	0.412	−1.05 (−3.97; 1.87)	0.478	−5.57 (−19.15; 8.01)	0.417	0.59 (−2.50; 3.68)	0.705
Area ax sc, mm^2	89	−40.79 (−92.19; 10.62)	0.118	−1.53 (−7.42; 4.37)	0.608	−0.99 (−5.57; 3.58)	0.667	−10.57 (−31.72; 10.57)	0.323	−0.76 (−5.56; 4.05)	0.755
4 years											
Area sag pp, mm^2	85	−8.15 (−48.66; 32.36)	0.690	2.25 (−2.12; 6.62)	0.308	2.07 (−1.30; 5.44)	0.225	4.28 (−11.93; 20.50)	0.601	−1.32 (−4.91; 2.28)	0.468
Area sag sc, mm^2	84	−30.21 (−66.13; 5.72)	0.098	−1.96 (−5.86; 1.95)	0.322	−1.37 (−4.40; 1.65)	0.369	−4.97 (−19.56; 9.62)	0.500	1.59 (−1.72; 4.90)	0.342
Area ax sc, mm^2	86	−38.30 (−97.45; 20.84)	0.201	−3.32 (−9.77; 3.12)	0.308	−2.33 (−7.32; 2.66)	0.356	−10.44 (−34.37; 13.49)	0.388	1.95 (−3.34; 7.24)	0.466
5 years											
Area sag pp, mm^2	87	−2.80 (−44.96; 33.35)	0.895	2.12 (−2.57; 6.82)	0.371	2.11 (−1.51; 5.72)	0.250	10.86 (−6.09; 27.81)	0.206	−0.21 (−3.98; 3.55)	0.910
Area sag sc, mm^2	88	−20.94 (−58.21; 16.34)	0.267	−0.98 (−5.18; 3.21)	0.642	−0.65 (−3.89; 2.60)	0.693	−4.12 (−19.29; 11.06)	0.591	0.46 (−2.88; 3.80)	0.783
Area ax sc, mm^2	89	−18.03 (−77.73; 41.67)	0.550	−2.20 (−8.83; 4.43)	0.512	−1.60 (−6.74; 3.55)	0.539	1.36 (−22.63; 25.35)	0.910	1.18 (−4.12; 6.47)	0.659
SAT volume, cm^3	42	−318.92 (−963.65; 325.81)	0.323	−20.24 (−99.54; 59.07)	0.608	(−15.64 (−74.47; 43.18)	0.593	−228.10 (−504.09; 47.89)	0.102	−4.39 (−76.30; 67.53)	0.902
VAT volume, cm^3	42	8.84 (−137.59; 155.26)	0.903	−6.90 (−24.59; 10.79)	0.434	−4.59 (−17.74; 8.56)	0.484	−1.62 (−65.78; 62.54)	0.959	5.60 (−10.42; 21.61)	0.483

Data are presented as the regression coefficient b (95% CI) from linear regression analyses.Values for fatty acids are expressed as a percentage of the weight of total fatty acids (% FA of total FA). PUFA: sum of all cis-FAs with two or more double bonds, n6 LCPUFAs: C20:2n-6; 20:3n-6; 20:4n-6; C22:2n-6; C22:4n6; C22:5n-6, n3 LCPUFAs: C20:3n-3; C20:4n-3; 20:5n-3; C21:5n-3; C22:3n-3; 22:5n-3; 22:6n-3. *Adjusted for pregnancy duration in days, sex (except for BMI percentiles), and mode of infant feeding (exclusively breastfed or partially breastfed) at 6 weeks' postpartum. Abbreviations; AA, arachidonic acid, area ax sc, area of subcutaneous fat in the axial plane; area sag pp, area of preperitoneal fat in the sagittal plane; area sag sc, area of subcutaneous fat in the sagittal plane; BMI, body mass index; DHA, docosahexaenoic acid; kg, kilograms; LCPUFAs, long-chain polyunsaturated fatty acids; mm, millimeters; n, number of children; SAT, subcutaneous adipose tissue; SFT, skinfold thickness; VAT, visceral adipose tissue.

Appendix

Appendix 27: Associations between breast milk LCPUFAs at 4 months postpartum and offspring adipose tissue measurements at 2−5 years of age (adjusted analysis)

Body composition variables	n	AA		DHA		n-3 LCPUFAs		n-6 LCPUFAs		Ratio n-6 LCPUFAs/ n-3 LCPUFAs	
		b (95% CI)	*P*	b (95% CI)	*P*	b (95% CI)	*P*	b (95% CI)	*P*	b (95% CI)	*P*
2 years											
Area sag pp, mm^2	78	−5.52 (−26.65; 15.60)	0.604	−1.76 (−4.81; 1.29)	0.254	−1.77 (−4.29; 0.75)	0.166	1.35 (−7.10; 9.81)	0.750	1.61 (−0.98; 4.20)	0.218
Area sag sc, mm^2	78	−14.68 (−41.73; 12.37)	0.283	−3.62 (−7.50; 0.25)	0.066	−3.44 (−6.63; 0.25)	0.035	−0.55 (−11.45; 10.35)	0.920	2.64 (−0.67; 5.96)	0.116
Area ax sc, mm^2	78	−18.20 (−48.11; 11.72)	0.229	−3.27 (−7.60; 1.06)	0.136	−3.06 (−6.63; 0.51)	0.092	−1.38 (−13.45; 10.69)	0.821	2.19 (−1.51; 5.89)	0.241
3 years											
Area sag pp, mm^2	72	−5.34 (−37.42; 26.75)	0.741	−0.16 (−4.87; 4.54)	0.945	−0.11 (−3.98; 3.77)	0.956	4.14 (−9.19; 17.47)	0.537	0.53 (−3.38; 4.44)	0.788
Area sag sc, mm^2	73	−5.47 (−36.53; 25.58)	0.726	−1.22 (−5.77; 3.34)	0.596	−0.91 (−4.66; 2.84)	0.629	1.92 (−11.03; 14.87)	0.768	1.63 (−2.14; 5.40)	0.391
Area ax sc, mm^2	72	−7.91 (−57.89; 42.07)	0.753	−2.28 (−9.69; 5.13)	0.542	−1.71 (−7.81; 4.40)	0.579	2.89 (−18.76; 24.54)	0.791	1.67 (−4.43; 7.77)	0.587
4 years											
Area sag pp, mm^2	69	−2.23 (−39.05; 34.60)	0.904	0.56 (−4.75; 5.86)	0.835	0.33 (−4.07; 4.72)	0.883	9.84 (−5.12; 24.79)	0.194	0.31 (−4.26; 4.87)	0.893
Area sag sc, mm^2	69	−18.95 (−50.35; 12.45)	0.232	−1.14 (−5.70; 3.43)	0.621	−0.90 (−4.68; 2.89)	0.637	−0.17 (−13.24; 12.89)	0.979	1.11 (−2.81; 5.04)	0.573
Area ax sc, mm^2	70	−30.29 (−77.41; 16.83)	0.204	−2.76 (−9.62; 4.11)	0.425	−2.00 (−7.69; 3.69)	0.485	−3.29 (−22.95; 16.37)	0.740	0.85 (−5.06; 6.76)	0.774
5 years											
Area sag pp, mm^2	73	1.00 (−38.30; 40.31)	0.960	−0.66 (−6.34; 5.01)	0.817	−0.75 (−5.43; 3.93)	0.750	12.11 (−3.95; 28.16)	0.137	0.57 (−4.04; 5.17)	0.806
Area sag sc, mm^2	74	3.85 (−32.56; 40.26)	0.834	−1.85 (−7.05; 3.36)	0.481	−1.52 (−5.82; 2.79)	0.485	8.72 (−6.24; 23.69)	0.249	1.69 (−2.53; 5.91)	0.427
Area ax sc, mm^2	75	11.34 (−39.81; 62.48)	0.660	−2.16 (−9.51; 5.18)	0.559	−1.29 (−7.37; 4.79)	0.673	14.64 (−6.26; 35.54)	0.167	1.13 (−4.87; 7.13)	0.709
SAT volume, cm^3	38	109.43 (−507.74; 726.59)	0.721	6.48 (−87.24; 100.20)	0.889	(−1.69 (−75.95; 72.57)	0.963	136.81 (−130.44; 404.06)	0.305	−9.15 (−78.49; 60.19)	0.790
VAT volume, cm^3	38	44.32 (−112.34; 200.98)	0.569	−9.10 (−32.75; 14.55)	0.439	(−8.93 (−27.57; 9.71)	0.337	31.80 (−36.43; 100.03)	0.350	6.65 (−10.87; 24.16)	0.446

Data are presented as the regression coefficient b (95% CI) from linear regression analyses. Values for fatty acids are expressed as a percentage of the weight of total fatty acids (% FA of total FA). PUFA: sum of all cis-FAs with two or more double bonds, n6 LCPUFAs: C20:2n-6; 20:3n-6; 20:4n-6; C22:2n-6; C22:4n6; C22:5n-6, n3 LCPUFAs: C20:3n-3; C20:4n-3; 20:5n-3; C21:5n-3; C22:3n-3; 22:5n-3; 22:6n-3. Adjusted for pregnancy duration in days, sex (except for BMI percentiles), and mode of infant feeding (exclusively breastfed orpartially breastfed) at 4 months postpartum. Abbreviations; AA, arachidonic acid, area ax sc, area of subcutaneous fat in the axial plane; area sag pp, area of preperitoneal fat in the sagittal plane; area sag sc, area of subcutaneous fat in the sagittal plane; BMI, body mass index; DHA, docosahexaenoic acid; kg, kilograms; LCPUFAs, long-chain polyunsaturated fatty acids; mm, millimeters; n, number of children; SAT, subcutaneous adipose tissue; SFT, skinfold thickness; VAT, visceral adipose tissue

Appendix 28: Approval for a publication-based dissertation

Einverständniserklärung zur publikationsbasierten Promotion[1]

Anlage 6 (für § 6 Abs. 2)

Hiermit erkläre ich mein Einverständnis, dass die Dissertation von

Frau/Herrn Dorothy Meyer

als publikationsbasierte Dissertation eingereicht wird. Sie erfüllt die nachfolgenden Kriterien:

1. Einleitungs- und Methodenteil (20 Seiten). Ein themenübergreifender Diskussionsteil mit Reflexion zur bestehenden Literatur.
2. Kumulative Einbindung von mindestens zwei akzeptierten Erstautorenveröffentlichungen (full paper in einem englischsprachigen, international verbreiteten Publikationsorgan, peer reviewed)
3. Die eingebundenen Veröffentlichungen müssen federführend vom Doktoranden abgefasst sein.
4. Eingebunden muss sein: je eine einseitige Zusammenfassung der jeweiligen Veröffentlichungen unter Hervorhebung der individuellen Leistungsbeiträge des Kandidaten.
5. Einbindung von ausgewählten Originalveröffentlichungen nur mit einem separaten schriftlichen „Erlaubnisschreiben des jeweiligen Verlags". Alle anderen Originalveröffentlichungen werden unter Nennung der bibliografischen Angaben aufgelistet. In den Exemplaren für die Mitglieder der Prüfungskommission sind alle Originalveröffentlichungen separat dazu abzugeben.

01.02.2021
Datum

Unterschrift Betreuer/in.
Prof. Dr. med. H. Hauner
Institut für Ernährungsmedizin
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Georg-Brauchle-Ring 62, 80992 München

[1] Zur Vorlage bei der Einreichung der Dissertation.

Appendix 29: Declaration of Authorship

Anhang I

Eidesstattliche Erklärung

Ich erkläre an Eides statt, dass ich die bei der promotionsführenden Einrichtung
Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Um-welt

der TUM zur Promotionsprüfung vorgelegte Arbeit mit dem Titel:
The value of maternal and fetal biomarkers for predicting early childhood obesity: results from the INFAT cohort

in Lehrstuhl für Klinische Ernährungsmedizin, Technische Universität München
Fakultät, Institut, Lehrstuhl, Klinik, Krankenhaus, Abteilung

unter der Anleitung und Betreuung durch: Univ. Prof. med. Dr. J.J. Hauner ohne sonstige Hilfe erstellt und bei der Abfassung nur die gemäß § 6 Ab. 6 und 7 Satz 2 angebotenen Hilfsmittel benutzt habe.

☒ Ich habe keine Organisation eingeschaltet, die gegen Entgelt Betreuerinnen und Betreuer für die Anfertigung von Dissertationen sucht, oder die mir obliegenden Pflichten hinsichtlich der Prüfungsleistungen für mich ganz oder teilweise erledigt.

☒ Ich habe die Dissertation in dieser oder ähnlicher Form in keinem anderen Prüfungsverfahren als Prüfungsleistung vorgelegt.

☐ Die vollständige Dissertation wurde in _____ veröffentlicht. Die promotionsführende Einrichtung

_____

hat der Veröffentlichung zugestimmt.

☒ Ich habe den angestrebten Doktorgrad noch nicht erworben und bin nicht in einem früheren Promotionsverfahren für den angestrebten Doktorgrad endgültig gescheitert.

☐ Ich habe bereits am _____ bei der Fakultät für _____

der Hochschule _____

unter Vorlage einer Dissertation mit dem Thema _____

die Zulassung zur Promotion beantragt mit dem Ergebnis: _____

Die öffentlich zugängliche Promotionsordnung der TUM ist mir bekannt, insbesondere habe ich die Bedeutung von § 28 (Nichtigkeit der Promotion) und § 29 (Entzug des Doktorgrades) zur Kenntnis genommen. Ich bin mir der Konsequenzen einer falschen Eidesstattlichen Erklärung bewusst.

Mit der Aufnahme meiner personenbezogenen Daten in die Alumni-Datei bei der TUM bin ich

☒ einverstanden, ☐ nicht einverstanden.

München, den 09.02.2021 Dorothy Nyga

Ort, Datum, Unterschrift

Appendix

Appendix 30: List of Publications

Meyer DM[1], Brei C[1], Stecher L, Much D, Brunner S, Hauner H. 2017. Cord blood and Child Plasma Adiponectin Levels in Relation to Childhood Obesity Risk and Fat Distribution up to 5 years. *Pediatr Res*. 81(5):745-751([1] The first two authors contributed equally to this work).

Meyer DM, Brei C, Stecher L, Much D, Brunner S, Hauner H. 2017. The relationship between breast milk leptin and adiponectin with child body composition from 3 to 5 years: a follow-up study. *Pedatric Ob.* 12: 125-129. Supplement: 1

Brei C, Stecher L, **Meyer DM**, Young V, Much D, Brunner S, Hauner H. 2018. Impact of Dietary Macronutrient Intake during Early and Late Gestation on Offspring Body Composition at Birth, 1, 3, and 5 Years of Age. *Nutrients*. 8;10(5).

Meyer DM, Brei C, Stecher L, Brunner S, Hauner H. 2018. Maternal insulin resistance, triglycerides and cord blood insulin are not determinants of offspring growth and adiposity up to 5 years: a follow-up study. *Diabet Med*. 35(10):1399-1403.

Meyer DM, Brei C, Stecher L, Much D, Brunner S, Hauner H. 2018. Leptin in Maternal Plasma and Cord Blood as a Predictor of Offspring Adiposity at 5 Years: A Follow-up Study. *Obesity* (Silver Spring). 26(2):279-283.

Meyer DM, Brei C, Stecher L, Much D, Brunner S, Hauner H. 2019. Associations between long chain PUFAs in maternal plasma, cord blood, and breast milk and offspring body composition up to 5 years: follow-up from the INFAT study. *Eur J Clin Nutr* 73:458-464.

Hoffmann J, Günther J, Stecher L, Spies M**, Meyer D,** Kunath J, Raab R, Rauh K, Hauner H. 2019. Effects of a Lifestyle Intervention in Routine Care on Short- and Long-Term Maternal Weight Retention and Breastfeeding Behavior—12 Months Follow-up of the Cluster-Randomized GeliS Trial. *J Clin Med*. 8(6), 876.

Günther J, Hoffmann J, Kunath J, Spies M, **Meyer D**, Stecher L, Rosenfeld E, Kick L, Rauh K, Hauner H. 2019. Effects of a Lifestyle Intervention in Routine Care on Prenatal Dietary Behavior—Findings from the Cluster-Randomized GeliS Trial. *J Clin Med*. 8(7), 960.

Günther J, Hoffmann J, Spies M, **Meyer D**, Kunath J, Stecher L, Rosenfeld E, Kick L, Rauh K, Hauner H. 2019. Associations between the Prenatal Diet and Neonatal Outcomes—A Secondary Analysis of the Cluster-Randomised GeliS Trial. *Nutrients*. 11(8), 1889.

Hoffmann J, Günther J, Geyer K, Stecher L, Kunath J, **Meyer D,** Spies M, Rosenfeld E, Kick L, Rauh K, Hauner H. 2019. Associations between Prenatal Physical Activity and Neonatal and Obstetric Outcomes – A Secondary Analysis of the Cluster-Randomized GeliS Trial. *J Clin Med* 8(10), 1735.

Hoffmann J, Günther J, Geyer, K, Stecher L, Rauh K, Kunath J, **Meyer D,** Sitzberger C, Spies M, Rosenfeld E, Kick L, Oberhoffer R, Hauner H. 2019. Effects of a lifestyle intervention in routine care on prenatal physical activity – findings from the cluster-randomised GeliS trial. *BMC Pregnancy Childbirth*. (19)1.

Meyer DM, Brei C, Bader BL, Hauner H. 2020. Evaluation of maternal dietary n-3 LCPUFA supplementation as a primary strategy to reduce offspring obesity: Lessons from the INFAT trial and implications for future research. *Frontiers in Nutrition.*7:156.

Meyer DM, Stecher L, Brei C, Hauner H. 2020. Mid-pregnancy weight gain is associated with offspring adiposity outcomes in early childhood. *Ped Res* 90(2):390-396.

Hoffmann J, Günther J, Stecher L, Spies M, Geyer K, Raab R, **Meyer D**, Rauh K, Hauner H. 2021. Infant growth during the first year of life following a pregnancy lifestyle intervention in routine care – findings from the cluster-randomised GeliS trial. *Pediatric Ob*. 16(2).

Sedlmeier E, **Meyer DM**, Stecher L, Sailer M; Daniel H, Hauner H, Bader BL. 2021. Fetal sex modulates placental microRNA expression, potential microRNA-mRNA interactions, and levels of amino acid transporter expression and substrates: n-3 LCPUFA intervention during pregnancy and associations with offspring body composition. *BMC Molecular and Cell Biology* 3;22(1):15.

Geyer K, Spies M, Günther J, Hoffmann J, Raab R, **Meyer D**, Rauh K, Hauner H. 2021. Effects of a Prenatal Lifestyle Intervention in Routine Care on Maternal Health Behaviour in the First Year Postpartum—Secondary Findings of the Cluster-Randomised GeliS Trial. *Nutrients* 13(4):1310

Günther J, Hoffmann J, Stecher L, Spies M, Geyer K, Raab R, **Meyer D**, Rauh K, Hauner H. 2021. How does antenatal lifestyle affect the risk for gestational diabetes mellitus? A secondary cohort analysis from the GeliS trial. *Eur Clin Nutr*. Epub ahead of print.