Turun yliopisto
University of Turku

GENETIC, PRENATAL AND POSTNATAL DETERMINANTS OF WEIGHT GAIN AND OBESITY IN YOUNG CHILDREN – The STEPS Study

Johanna Mäkelä

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
Sarja - ser. D osa - tom. 1187 | Medica - Odontologica | Turku 2015

University of Turku

Faculty of Medicine
Department of Paediatrics
University of Turku Doctoral Program of Clinical Investigation (CLIPD)
Turku Institute for Child and Youth Research

Supervised by

Adjunct professor Hanna Lagström, PhD
Turku Institute for Child and Youth Research
University of Turku
Turku, Finland

Professor Harri Niinikoski, MD, PhD
Department of Paediatrics and
Department of Physiology
University of Turku
Turku, Finland

Reviewed by

Adjunct professor Maijaliisa Erkkola, PhD
Department of Food and
Environmental Sciences
University of Helsinki
Helsinki, Finland

Adjunct professor Ulla Sankilampi, MD, PhD
Department of Paediatrics
Kuopio University Hospital
Kuopio, Finland

Opponent

Professor Inga Thorsdottir, PhD
School of Health Sciences
University of Iceland
Reykjavik, Iceland

ISBN 978-951-29-6234-1 (PRINT)
ISBN 978-951-29-6235-8 (PDF)
ISSN 0355-9483
Painosalama Oy - Turku, Finland 2015

ABSTRACT

Johanna Mäkelä

Genetic, Prenatal and Postnatal Determinants of Weight Gain and Obesity in Young Children – The STEPS Study

University of Turku, Faculty of Medicine, Department of Paediatrics, University of Turku Doctoral Program of Clinical Investigation (CLIPD), Turku Institute for Child and Youth Research.

Conditions of being overweight and obese in childhood are common health problems with long-lasting effects into adulthood. Currently 22% of Finnish boys and 12% of Finnish girls are overweight and 4% of Finnish boys and 2% of Finnish girls are obese. The foundation for later health is formed early, even before birth, and the importance of prenatal growth on later health outcomes is widely acknowledged. When the mother is overweight, had high gestational weight gain and disturbances in glucose metabolism during pregnancy, an increased risk of obesity in children is present. On the other hand, breastfeeding and later introduction of complementary foods are associated with a decreased obesity risk. In addition to these, many genetic and environmental factors have an effect on obesity risk, but the clustering of these factors is not extensively studied.

The main objective of this thesis was to provide comprehensive information on prenatal and early postnatal factors associated with weight gain and obesity in infancy up to two years of age. The study was part of the STEPS Study (Steps to Healthy Development), which is a follow-up study consisting of 1797 families. This thesis focused on children up to 24 months of age.

Altogether 26% of boys and 17% of girls were overweight and 5% of boys and 4% of girls were obese at 24 months of age according to New Finnish Growth references for Children BMI-for-age criteria. Compared to children who remained normal weight, the children who became overweight or obese showed different growth trajectories already at 13 months of age. The mother being overweight had an impact on children's birth weight and early growth from birth to 24 months of age. The mean duration of breastfeeding was almost 2 months shorter in overweight women in comparison to normal weight women. A longer duration of breastfeeding was protective against excessive weight gain, high BMI, high body weight and high weight-for-length SDS during the first 24 months of life. Breast milk fatty acid composition differed between overweight and normal weight mothers, and overweight women had more saturated fatty acids and less n-3 fatty acids in breast milk. Overweight women also introduced complementary foods to their infants earlier than normal weight mothers. Genetic risk score calculated from 83 obesogenic- and adiposity-related single nucleotide polymorphisms (SNPs) showed that infants with a high genetic risk for being overweight and obese were heavier at 13 months and 24 months of age than infants with a low genetic risk, thus possibly predisposing to later obesity in obesogenic environment. Obesity Risk Score showed that children with highest number of risk factors had almost 6-fold risk of being overweight and obese at 24 months compared to children with lowest number of risk factors. The accuracy of the Obesity Risk Score in predicting overweight and obesity at 24 months was 82%.

This study showed that many of the obesogenic risk factors tend to cluster within children and families and that children who later became overweight or obese show different growth trajectories already at a young age. These results highlight the importance of early detection of children with higher obesity risk as well as the importance of prevention measures focused on parents**.**

Keywords: Breastfeeding, Child, Complementary Feeding, Genes, Glucose metabolism, Growth, Infant Nutrition Physiology, Nutrition, Obesity, Overweight, Programming

TIIVISTELMÄ

Johanna Mäkelä

Painonnousun ja ylipainon geneettiset, prenataaliset ja postnataaliset tekijät pienillä lapsilla Hyvän kasvun avaimet –tutkimuksessa
Turun yliopisto, Lääketieteellinen tiedekunta, Lastentautioppi, Turun yliopiston kliininen tohtoriohjelma (TKT), Turun lapsi- ja nuorisotutkimuskeskus.

Lapsuusiän ylipaino ja lihavuus ovat yleisiä terveysongelmia, joilla on pitkäkantoisia vaikutuksia terveyteen. Tällä hetkellä suomalaisista pojista 22 % ja tytöistä 12 % on ylipainoisia ja 4 % pojista 2 % tytöistä lihavia. Pohja myöhemmälle terveydelle luodaan jo varhain, jopa ennen syntymää ja sikiöaikaisen kasvun vaikutukset myöhempään terveyteen ovat tunnistettuja monissa tutkimuksissa. Äidin ylipaino, glukoosiaineenvaihdunnan häiriöt ja liiallinen painonnousu raskausaikana ovat yhteydessä lapsen suurempaan ylipainoriskiin. Toisaalta, imetyksen ja myöhäisemmän lisäruokien aloituksen on havaittu olevan yhteydessä pienentyneeseen ylipainoriskiin. Näiden tekijöiden lisäksi erilaiset geneettiset tekijät sekä muut ympäristötekijät ovat yhteydessä varhaiseen painonkehitykseen ja ylipainoriskiin, mutta näiden tekijöiden kasaantumista on toistaiseksi tutkittu vain vähän.

Tutkimuksen päätavoitteena oli tuottaa aiempaa kokonaisvaltaisempi kuva ylipainoriskiin ja varhaiseen kasvuun liittyvistä ympäristö- ja perimätekijöistä. Tämä tutkimus oli osa Hyvän kasvun avaimet –seurantatutkimusta (the STEPS Study), jossa seurataan monitieteisesti 1797 perhettä. Tämä väitöstutkimus keskittyi lasten kahteen ensimmäiseen ikävuoteen.

Väitöstutkimuksen tulokset osoittivat, että monet eri tekijät ovat yhteydessä varhaiseen kasvuun ja ylipainoriskiin. Hyvän kasvun avaimet –tutkimuksessa 26 % pojista ja 17 % tytöistä oli ylipainoisia ja 5 % pojista ja 4 % tytöistä lihavia kahden vuoden iässä. Ylipainoisten ja lihavien lasten kasvu erosi normaalipainoisten lasten kasvusta jo 13 kuukauden iässä. Äidin ylipaino vaikutti lasten syntymäpainoon ja varhaiseen kasvuun syntymästä kahden vuoden ikään. Ylipainoisilla äideillä imetyksen kesto oli keskimäärin 1,7 kk lyhyempi kuin normaalipainoisilla äideillä. Imetyksen pidempi kesto suojasi liialliselta painonnousulta, korkealta painoindeksiltä, korkealta painolta ja korkealta pituus-painon keskihajonnalta ensimmäisten kahden vuoden aikana. Ylipainoisilla äideillä oli rintamaidossaan enemmän tyydyttyneitä rasvahappoja ja vähemmän n-3 rasvahappoja kuin normaalipainoisilla äideillä. Lisäksi ylipainoiset äidit aloittivat kiinteät ruoat lapsilleen normaalipainoisia äitejä aiemmin. Ylipainoon liittyvistä 83:sta yhden emäksen geenipolymorfioista laskettu geneettinen riskipisteytys osoitti, että lapset, joilla oli suurempi geneettinen riski ylipainoon painoivat enemmän 13 kuukauden ja 2 vuoden iässä kuin lapset, joilla oli pienempi geneettinen riski ylipainoon. Ylipainon geneettisistä, prenataalisista ja postnataalisista riskitekijöistä laskettu ylipainoriskin summamuuttuja osoitti, että lapsilla, joilla oli korkein määrä yksittäisiä riskitekijöitä oli lähes 6-kertainen riski ylipainoon ja lihavuuteen 2 vuoden iässä verrattuna lapsiin, joilla oli vähän tai ei lainkaan riskitekijöitä. Summamuuttujan tarkkuus 2 vuoden ylipainoa ja lihavuutta ennustettaessa oli 82 %.

Tutkimus osoitti että monet ylipainon varhaisista riskitekijöistä kasaantuivat samoille lapsille ja perheille. Lapset, joista myöhemmin tuli ylipainoisia erosivat kasvultaan normaalipainoisista lapsista jo varhaisessa vaiheessa. Nämä tulokset korostavat lasten ylipainon varhaisen tunnistamisen ja erityisesti vanhempiin kohdistuvien ennaltaehkäisevien toimenpiteiden tärkeyttä.

Avainsanat: Imetys, Imeväisen ravitsemus, Kasvu, Lapset, Lihavuus, Ohjelmoituminen, Perintötekijät, Ravitsemus, Sokeriaineenvaihdunta Ylipaino, Äidinmaito

TABLE OF CONTENTS

ABBREVIATIONS

AGA	Appropriate for gestational age
ANOVA	Analysis of variance
BMI	Body mass index (kg/m^2)
BF	Breastfeeding
CF	Complementary feeding (solid foods)
CI	Confidence interval
DOHaD	Developmental Origins of Health and Disease
EBF	Exclusive breastfeeding
ESPHGAN	European Society for Paediatric Gastroenterology, Hepatology and Nutrition
FA	Fatty acid
FTO	Fat mass and obesity associated gene
GRS	Genetic risk score
GWAS	Genome-wide association study
HAPO	The Hyperglycemia and Adverse Pregnancy Outcome Study
HDL	High-density lipoprotein
HOMA	Homeostasis model assessment
LCPUFA	Long-chain polyunsaturated fatty acids
LDL	Low-density lipoprotein
LGA	Large for gestational age
IDQ	Index of diet quality
IOTF	International Obesity Task Force
Matsuda ISI	Matsuda insulin sensitivity index
MUFA	Monounsaturated fatty acids
OBERISK	Early risk factors of childhood obesity study
OGTT	Oral glucose tolerance test
PBF	Partial breastfeeding
PI	Ponderal index (kg/m^3)
PUFA	Polyunsaturated fatty acids
QUICKI	Quantitative insulin sensitivity check index
SD	Standard deviation
SDS	Standard deviation score
SFA	Saturated fatty acids
SGA	Small for gestational age
SNP	Single nucleotide polymorphism
STAKES	National Research and Development Centre for Welfare and Health
STEPS	Steps to healthy development study
WHO	World Health Organization
WeightGRS	Weight genetic risk score

LIST OF ORIGINAL PUBLICATIONS

I Mäkelä J, Lagström H, Kaljonen A, Simell O, Niinikoski H. Hyperglycemia, increased infant birth size and risk of prematurity in pregnant overweight women –STEPS Study. Early Human Development 2013;89:439-444.

II Mäkelä J, Linderborg K, Niinikoski H, Yang B, Lagström H. Breast milk fatty acid composition differs between overweight and normal weight women. European Journal of Nutrition 2013;52:727-735.

III Mäkelä J, Vaarno J, Kaljonen A, Niinikoski H, Lagström H. Maternal overweight impacts infant feeding patterns—the STEPS Study. European Journal of Clinical Nutrition 2014;68:43-49.

IV Mäkelä J, Lagström H, Pitkänen N, Kuulasmaa T, Kaljonen A, Laakso M, Niinikoski H. Genetic risk clustering increases children's body weight at 24 months of age – the STEPS Study (Submitted)

1. INTRODUCTION

Being overweight and obese in childhood are common health problems in developed countries, including Finland. Currently in Finland, 22% of boys and 12% of girls are overweight and 4% of boys and 2% of girls are obese (Saari et al. 2011; Working group appointed by the Finnish Paediatric Society, 2013). Despite some stabilizing trends in the prevalence of overweight children and childhood obesity worldwide, the rates are still high and increasing in many countries.

The basis for later health is formed early, even before birth. The importance of prenatal growth on later health outcomes, first proposed by Barker and colleagues (Barker & Osmond 1986), is widely acknowledged. According to this theory, events occurring early in life may have long-term effects on health later in life. The associations were first observed with low birth weight, but studies since then have shown that also high birth weight increases the disease risk later in life. This suggests that the relationship between birth weight and later disease risk is U-shaped (Dabelea et al. 1999; Harder et al. 2007).

Many environmental as well as genetic factors are associated with weight gain and obesity risk. Breastfeeding is known to protect against excessive weight gain and obesity (Hörnell et al. 2013). Also, later introduction of complementary foods may be associated with a lower risk of obesity (Schack-Nielsen et al. 2010). On the other hand, parents being overweight is a strong risk factor for childhood obesity (Danielzik et al. 2002). Also other environmental factors such as a chronic lack of sleep in children (Taveras et al. 2014), maternal smoking (Oken et al. 2008), high maternal gestational weight gain (Wrotniak et al. 2008) and low socio-economic class (Kleiser et al. 2009) have been associated with increased obesity risk later in life. Genetic factors, such as obesogenic and adiposity-related single-nucleotide polymorphisms (SNPs), are known to affect adult weight and BMI, may also associate with early growth and obesity risk in childhood (Warrington et al. 2013).

The main aim of this study was to provide comprehensive and new information on genetic, prenatal and postnatal risk factors associated with weight gain and obesity. Modulation of environmental obesity risk factors by maternal overweight was also evaluated.

2. REVIEW OF THE LITERATURE

2.1 Epidemiology of being overweight and obese

2.1.1 Definition and prevalence in adults and in children

Being overweight and obese are consequences of a continuously larger energy intake than expenditure. This results in excessive accumulation of adipose tissue. The most widely used methods for defining being overweight and obese in children are body mass index (BMI) and waist circumference. BMI and waist circumference are inexpensive, easy to perform and well-producible. In addition to BMI, ponderal index (PI) and weight-for-length/height are used to describe growth and size in children. In epidemiological studies involving children, population and gender specific standard deviation scores (SDS or z-scores) for BMI, weight and weight-for-length/height are also used. Methods directly measuring adipose tissue include underwater weighing, dual-energy X-ray absorptiometry, air displacement plethysmography and isotope dilution. These provide accurate measurements of adipose tissue but are not feasible to be conducted in large epidemiological studies in children. Methods that provide estimates of adiposity but require equations for calculation of body fat percentage include bioelectrical impedance and skin fold thickness.

For adults, there are internationally defined BMI cut-off points for being underweight (<18.5 kg/m^2), normal weight (18.5-24.9 kg/m^2), overweight (25-29.9 kg/m^2) and obesity (≥30 kg/m^2). BMI is also used as an estimate of adiposity in children, because it correlates well with more direct measures of obesity (Pietrobelli et al. 1998). However, for children, there is currently no consensus on what definitions of obesity should be used. National growth charts have been developed, including the New Finnish Growth References for Children (Saari et al. 2011), which determine the age- and sex-specific BMI cut-off points for being overweight and obese from 2 years onwards. In addition to BMI, weight-for-length/height is an estimate used to determine growth in young children and is widely used in Finland. In addition to Finland, the weight-for-length/height is also used in Norway (Juliusson et al. 2013). In children under 7 years, being overweight is determined as +10 to +20% and obesity as >+20% weight-for-length (Working group appointed by the Finnish Paediatric Society 2013). In order to facilitate international comparison, the International Obesity Task Force (IOTF) has developed age- and sex-specific BMI cut-off points for being underweight, normal weight, overweight and obese in children (Cole et al. 2000). The IOTF definitions are based on international reference data and provide cutoff points for being overweight and correspond to an adult BMI of 25 and for obesity, corresponding to an adult BMI of 30 (Cole et al. 2000). The New Finnish Growth References for Children are based on a similar calculation method as the IOTF criteria. In both criteria BMI-for-age percentiles passing through various adult BMI cutoffs were used to define various grade of thinness, being overweight and obese

but instead of international reference data, the national growth reference data comprises from measurements of 73,659 Finnish children (Saari et al. 2011). In addition, the WHO has determined a child as being overweight by having a BMI-for-age ≥85th percentile and obesity as BMI-for-age ≥97th percentile (WHO, 2006).

The prevalence of being overweight and obese has increased throughout the world and it is estimated that by year 2030 as many as 1.12 billion adults will be obese and more than 2.2 billion overweight (Kelly et al. 2008). A recent study in United States showed that the levels of obesity in children are levelling off and no significant increase in obesity prevalence between 2003 to 2004 and 2011 to 2012 were found (Ogden et al. 2014). However, even with the possible stabilization, the prevalence of obesity is high: 31.8% of children are overweight and 16.9% of children are obese in the United States. A similar stabilization has been reported in France in 2009 (Peneau et al. 2009; Salanave et al. 2009). However, this trend may be partly explained by a selection bias. Moreover, the awareness and stigmatization of being overweight and obese may have resulted in non-participation of overweight children in studies (Schmidt Morgen et al. 2013). It is also possible that the trend is different in subgroups and while being overweight and obese prevalence may have decreased in higher socio-economic families, it may have increased among other groups. In Finland the rates of being overweight and obese are still increasing (Vuorela et al. 2011a).

In 2006 in Finland, the prevalence of being overweight and obese in 5-year-old boys was 9.8% and 2.5% and in girls 17.7% and 4.3% according to IOTF criteria (Vuorela et al. 2009). In the same study the prevalence of being overweight and obese in 12 year-old boys was 23.6% and 4.7% and in 12-year-old girls, 19.1% and 3.2%. In 16-year-old children, the reported prevalence of being overweight or obese was 17.1% in boys and 13.8% in girls according to the IOTF criteria (Pirkola et al. 2008). According to New Finnish Growth References, the BMI-criteria showed 12% of girls and 22% of boys were overweight and 2% and 4% obese (Saari et al. 2011; Working group appointed by the Finnish Paediatric Society 2013).

Between 1974 and 2001, being overweight and obese have become more prevalent in Finnish young adolescents, especially in boys (Vuorela et al. 2011a). However, at the same time 2-year old children have become slimmer (Vuorela et al. 2011b). This slimming of toddlers is probably due to changes in infant feeding patterns since the duration of breastfeeding has increased during the past 30 years and at the same time the introduction of complementary foods has been postponed to later months (Erkkola et al. 2005). Interestingly, the changes in infant nutrition have not impacted infant's BMI at 6 months of age (Vuorela et al. 2011b).

2.1.2 Consequences of childhood obesity

Obese children have excess adipose tissue that acts as energy storage as well as an active endocrine organ. The metabolic alteration most commonly seen in overweight

or obese children is insulin resistance, which is also involved in many other metabolic complications (Weiss & Kaufman 2008). Children who are overweight and obese have high rates of impaired glucose tolerance and some may even develop type 2 diabetes in childhood. The prevalence of impaired glucose tolerance is 25% in obese children and further 4% have undiagnosed type 2 diabetes (Sinha et al. 2002). Overweight or obese children have a higher risk for elevated triglycerides, total cholesterol and LDL-cholesterol as well as low HDL-cholesterol. From 546 obese children in Germany, 45.8% had an abnormal lipid profile (Korsten-Reck et al. 2008). Obese children also have an increased risk of high systolic and diastolic blood pressure. The cardiovascular risk factor clustering is evident in overweight and obese children (Raitakari et al. 1995).

The clustering of cardiometabolic risk factors, including impaired insulin sensitivity, impaired glucose metabolism dyslipidemia and elevated blood pressure, is referred to as the metabolic syndrome (Alberti et al. 2005). The first signs of metabolic syndrome start developing already in childhood and frequently track into adulthood (Mattsson et al. 2008). Being overweight is a risk factor for metabolic syndrome. A higher metabolic risk in childhood leads to a higher risk of metabolic syndrome (Mattsson et al. 2008), type 2 diabetes (Nguyen et al. 2010) and premature death (Franks et al. 2010) in adulthood. For early intervention and prevention of later complications, the identification of children with increased cardiometabolic risk is critical.

Obese children have a higher risk of becoming obese adults (Singh et al. 2008), especially if their parents are obese (Whitaker et al. 1997). Being overweight at a young age is associated with increased all-cause mortality in men and the association is independent of weight status in adulthood (Must et al. 1992). Moreover, obesity in childhood is associated to premature death in adulthood (Franks et al. 2010). Childhood obesity has also many psychosocial consequences (Ebbeling et al. 2002). Children who are overweight or obese as young as 5 years of age can have a negative self-image (Davison & Birch 2001) and adolescents more frequently have declined self-esteem, sadness, loneliness and nervousness (Strauss 2000). Moreover, obesity in childhood is associated with lower cognitive abilities in 7-year-old children (Wirt et al. 2015). The prevention of being overweight and obesity in children is a major public health challenge.

2.2 Genetic and prenatal determinants of obesity in children

2.2.1 Prenatal growth and programming

Birth weight is the most frequently used anthropometric measurement to estimate prenatal growth. Also birth length is used as an indicator of prenatal growth although length measurement contains a large amount of error such as difficulty in the extending the infant's leg completely. In Finland, there are population-based references for birth weight, length and head circumference (Sankilampi et al. 2013). Although birth weight is considered a crude indicator of prenatal growth, it is considered more reliable than

birth length (Johnson et al. 1997; Johnson et al. 2012). Prenatal growth is affected by both genetic and environmental factors. Genetic factors are estimated to explain 25 to 40% of birth weight and length, whereas environmental factors are estimated to explain 60 to 75% (Clausson, Lichtenstein, Cnattingius 2000; Lunde et al. 2007). These genetic factors include the sex of the fetus and genes passed on from mothers and fathers to fetus. Environmental factors include maternal nutrition, endocrine factors, parity, stress, infections, placental functioning, smoking and socioeconomic status. Also gestational age affects largely body size at birth.

Newborn infants can be divided to small (SGA), appropriate (AGA) or large (LGA) for gestational age. Small for gestational age (SGA) babies are smaller in size than normal compared to their gestational age. SGA can be a sign of intrauterine growth retardation, but some SGA babies may also be constitutionally small. Newborns with higher weight, length or head circumference than would be expected at their gestational age are referred to as large for gestational age (LGA). LGA is an indicator of high growth rate during prenatal time. SGA and LGA infants are at increased risk of adverse health outcomes later in life, such as obesity and metabolic syndrome (Clayton et al. 2007; Cnattingius et al. 2012).

In the 1970s, Ravelli et al. (1976) observed that famine during the first half of pregnancy resulted in higher rates of obesity, whereas famine during the last trimester of pregnancy resulted in lower obesity rates (Ravelli et al. 1976). Prenatal growth was further studied by Barker and his colleagues who introduced a hypothesis initially called "Barker's theory," and later the "Developmental Origins of Health and Disease (DOHaD) hypothesis," which stated that fetal growth is associated with a number of chronic diseases later in life (Barker & Osmond 1986; Barker et al. 1989). Barker and coworkers showed that there was a strong association between birth weight and ischaemic heart disease (Barker & Osmond 1986; Barker et al. 1989). Since then epidemiological studies have found evidence that low birth weight (<2500 g) (WHO 1995) is related to an increased risk for obesity and metabolic diseases such as type 2 diabetes, metabolic syndrome and cardiovascular diseases as well as to several other diseases (Barker 1995; Fall et al. 2008; McNamara et al. 2012; Whincup et al. 2008). Some of these associations with disease risks have been observed even within the normal birth weight range. While the associations described above were first observed with low birth weight, studies since then have shown that also high birth weight increases the disease risk later in life suggesting that the relationship between birth weight and disease risk is U-shaped (Dabelea et al. 1999; Eriksson et al. 2003; Harder et al. 2007).

A suboptimal environment during prenatal and early postnatal life may influence organ development and functioning. This process is called programming and may have a long-lasting effect on health (Lucas 1998). During energy restriction there are adaptations to ensure nutrient supply to the most vital organs at the expense of other organs. In addition to maternal undernutrition, also maternal overnutrition during pregnancy is associated with adverse health outcomes such as delivering high birth weight babies with higher risk

of metabolic syndrome (Harder et al. 2007) as well as increased incidence of intrauterine growth restriction (Radulescu et al. 2013). Programming may affect disease risk in later life through direct biological effects such as postprandial appetite regulatory hormone responses (Perälä et al. 2013) but also by modifying adult behavior such as physical activity (Andersen et al. 2009) and dietary habits (Kaseva et al. 2013; Perälä et al. 2012).

Changes in maternal diet during critical periods of fetal development can have obesity-risk increasing effects on fetus probably via epigenetic modification of genes involved in key regulatory pathways (Correia-Branco et al. 2015; McMillen & Robinson 2005). These epigenetic modifications are functionally relevant changes that have no effect on DNA sequence but alter gene expression through DNA methylation and histone modification. Epigenetic markers respond to environmental stimuli. A recent study by Lesseur et al. (2014) showed that maternal obesity and gestational diabetes can alter the placental leptin DNA methylation profile (Lesseur et al. 2014). Leptin gene expression is inversely correlated with promoter DNA methylation and has been proposed as a mediator of metabolic programming, because alterations in leptin profile are associated with altered susceptibility to later obesity and metabolic disorders (Marchi et al. 2011; Vickers & Sloboda 2012). Alterations can occur even after birth, because, for example, breastfeeding duration is associated with the degree of methylation of the leptin gene (Obermann-Borst et al. 2013).

Hormones can act as endogenous functional teratogens by malprogramming the neuroendocrine-immune network and thereby leading to developmental disorders and diseases (Dörner 1975). Indeed, maternal glucose metabolism during pregnancy may initiate a cascade of metabolic conditions manifesting in children later in life. Elevated maternal glucose concentration exposes the fetus to higher concentrations of glucose than normal since glucose travels freely from the mother to the fetus. However, maternal circulating insulin concentrations are not passed on to the fetus which increases the fetus' own insulin production (Reece et al. 2009). Pregnancy increases maternal insulin resistance by 40 to 50% (Yogev & Visser 2009). These metabolic adaptations aim to promote fetal growth by shunting metabolic fuels to the fetus instead of to the mother and are also important for the preparation for breastfeeding. Sometimes theses adaptation are, however, exaggerated resulting in impaired glucose tolerance. For children of mothers with gestational diabetes, elevated insulin levels in fetal and perinatal life are pathognomonic. Those children show an increased risk for obesity and type 2 diabetes later in life (Plagemann 2006). The effect may occur irrespectively of the genetic background and independently of birth weight but seems to depend on perinatal insulin levels. Maternal gestational diabetes increases children's birth weight, risk for hypoglycaemia (Langer et al. 2005), risk for early-onset obesity, type 2 diabetes during adolescence and manifestation of metabolic syndrome in early childhood (Yogev & Visser 2009). The effect of maternal BMI and glucose metabolism on the developing fetus is also evident in children of nondiabetic mothers (Knight et al. 2007; Ong et al. 2008) and may be evident even with maternal glucose levels within a normal range (Clausen et

al. 2005). Maternal diet and physical activity during pregnancy affect maternal glucose metabolism and may also have direct or indirect effects on the developing fetus (Reece et al. 2009).

2.2.2 Genetic risk factors

It is known that single gene disorders, such as mutations in leptin or melanocortin 4 receptor genes, account for a very limited fraction of obesity in children but are frequently associated with severe obesity set-on at an early age (Alharbi et al. 2007; Farooqi 2005). The identification of gene variants predisposing to common polygenic forms of obesity has been enabled by the development of genome-wide association studies (GWAS). GWAS enable the assessment of many genetic variants in different individuals. The first GWAS was published in 2005 (Klein et al. 2005) and since then thousands of studies have been conducted. GWAS typically focus on single nucleotide polymorphisms (SNP) and common traits such as type 2 diabetes and obesity. SNPs are small variations in individual nucleotides in the genome that occur in both non-coding and coding regions. GWAS have issues and limitations that need to be carefully considered and discussed. The main issues are adequate sample sizes, study design, control for multiple testing and appropriate statistical methods. Once these are acknowledged, GWAS can provide reliable results on common trait diseases.

Studies have provided evidence for a strong genetic basis for body mass index (BMI) with heritability estimates ranging from 40% to 70% (Rokholm et al. 2011; Wardle et al. 2008). The genetic influence increases with age and explains 50% of the variation in BMI at 4 years of age but almost 80% at 11 years of age (Haworth et al. 2008). On the other hand, it has been shown in monozygotic twin studies that the initially larger twin tended to remain larger thus demonstrating the long-lasting effects of programming via fetal environment on final body size (Pietiläinen et al. 2002). Genetic influence on BMI has increased probably due to the influence of obesogenic environment suggesting that there is a gene-environment interaction (Rokholm et al. 2011; Wardle et al. 2008). BMI and obesity are complex traits with many genetic factors, environmental factors and gene-environment interactions affecting them.

The common variant in the fat mass and obesity associated gene (FTO, rs9939609) has direct effect on BMI and indirect predisposing effect to type 2 diabetes. It was one of the first variants associated with obesity risk (Frayling et al. 2007). FTO is associated with an increase in BMI, the median effect size per allele being approximately 0.36 kg/m^2, which still remains one of the highest effect sizes in the currently identified common variants. Since the identification of FTO, GWAS have identified several loci associated with BMI, weight or obesity risk (Cotsapas et al. 2009; Croteau-Chonka et al. 2011; Fox et al. 2007; Frayling et al. 2007; Jiao et al. 2011; Johansson et al. 2010; Liu et al. 2010b; Loos et al. 2008; Meyre et al. 2009; Scuteri et al. 2007; Speliotes et al. 2010; Thorleifsson et al. 2009; Wang et al. 2011; Willer et al. 2009). Despite substantial heritability, the effects

of individual variants are modest probably due to polygenic nature and complex gene-environment interactions underlying obesity. However, even common SNPs with weak effects alone can jointly result as a large effect and the gene effects can vary with age and be modified by factors such as prenatal development (Mei et al. 2012).

Common variants that increase adult obesity susceptibility are associated with higher weight and BMI from 2 years onwards (Elks et al. 2012). The association between these SNPs and early growth remains still somewhat unknown. Recently, many studies have focused on genetic risk scores (GRS) constructed by combining risk alleles from multiple single SNPs (Chawla et al. 2014; Gonzalez et al. 2014; Mei et al. 2012). Previously Warrington et al. (Warrington et al. 2013) showed that a GRS comprising 32 SNPs associated with BMI in children and Elks et al. (Elks et al. 2010) showed an association between genetic risk score of eight variants and early infancy growth from birth to 6 weeks. The advantage of the GRS lies in creating combined scores from single variants that may jointly exert a large longitudinal effect on weight or BMI.

2.3 Postnatal determinants of obesity in children

2.3.1 Postnatal growth

Postnatal growth is influenced by several factors. Nutrition, i.e., breastfeeding, energy and nutrient intake, influences postnatal growth predominantly. Postnatal growth is affected by genetic factors more than prenatal growth since the genetic factors accounting for the variation of body size become more pronounced with age (Pietiläinen et al. 2002). Moreover, postnatal growth is affected by gestational age, size at birth and parental socioeconomic status (Hindmarsh et al. 2008; Regnault et al. 2010).

Growth is rapid during early infancy and development of tissues and organs continue during this period. The first 1000 days after conception, i.e., from pregnancy to the age of 2 years, is a critical time for programming of health in later life (Koletzko et al. 2014b). Low birth weight and small body size are related to increased risk of metabolic disease, but also large body size at birth and rapid growth during infancy are associated with a subsequent risk of obesity (Baird et al. 2005; Monasta et al. 2010; Zhao et al. 2012). Indeed, rapid weight gain is associated with being overweight and obese later in life irrespective of whether birth weight was average or low (Ong & Loos 2006). Rapid weight gain during infancy is associated with a higher proportion of body fat later in childhood (Ong et al. 2009). The obesity risk is increased especially in children with low birth weight and high weight gain (Oken & Gillman 2003). Rapid weight gain in infancy can also influence metabolic outcomes and associates with metabolic risk factors already in adolescence (Ekelund et al. 2007).

In addition to weight gain during infancy, the adiposity rebound is another critical period for the development of a child becoming overweight and obese. Adiposity rebound is defined as the age when BMI starts to increase again after initial decrease. It takes

place usually between the ages of 5 and 7 years. During the adiposity rebound, both the number and size of the adipocytes increase (Rolland-Cachera et al. 1984). The adiposity rebound occurs earlier in children with higher BMI (Saari et al. 2011). Early adiposity rebound may increase the risk of being overweight and obese in childhood and during adolescence (Lagström et al. 2008; Rolland-Cachera et al. 1984; Rolland-Cachera et al. 2006). A higher weight gain during early childhood predicts later obesity and growth patterns between normal weight children and children who later become obese, and this may differ as early as at the age of 3 years (Lagström et al. 2008).

2.3.2 Early nutritional determinants of obesity

2.3.2.1 Breastfeeding

Human milk is the optimal source of nutrition for infants. In 1892 Ehrlich demonstrated that human milk contains protective factors (Ehrlich 1892). The main human milk immunoglobulin secretory immunoglobulin A was characterized and isolated in 1961 (Ehrlich 1892; Hanson 1961). Since then human milk has been characterized with many beneficial health effects for both mother and child (Gartner et al. 2005). Some of these characteristics include promoting sensory and cognitive development, protecting the infant from infections and chronic diseases and contributes to health and the wellbeing of mothers (Belfort et al. 2013; Gartner et al. 2005; Hanson 1998). In Finland, exclusive breastfeeding is recommended until the age of 6 months and complementary feeding should start at the latest at the age of 6 months (Hasunen et al. 2004). The recommended earliest age at introduction of complementary foods is from 4 to 6 months in Finland. Partial breastfeeding is recommended until the end of the first year. The WHO recommends exclusive breastfeeding for the first six months of life followed by continuing breastfeeding with complementary foods for the first two years of life (WHO, 2002).

In Finland, the majority of the infants are breastfed: 87% of the infants aged 1 month received breastmilk (Uusitalo et al. 2012). However, exclusive breastfeeding rates are lower as shown in Table 1. Moreover, breastfeeding rates in Finland (Uusitalo et al. 2012) are substantially lower compared to other Nordic countries (Hörnell et al. 2013) but slightly higher than those in the United States (US Center for Disease Control and Prevention, 2014). Especially exclusive breastfeeding rates are low in Finland (Uusitalo et al. 2012) when compared to other Nordic countries (Hörnell et al. 2013) or to the US (US Center for Disease Control and Prevention, 2014). Although 99% of Finnish newborns receive breast milk in the maternity ward, most of them (71-80%) receive also supplementary milk (donated milk or formula) (Erkkola et al. 2010; Uusitalo et al. 2012). Indeed, additional milk feeding in the maternity ward is associated with a risk of short duration of exclusive and total breastfeeding (Erkkola et al. 2010). Other risk factors for shorter duration of breastfeeding and early weaning are low parental education, maternal smoking during pregnancy, mother's young age, small gestational age, having no siblings and having a mother who was overweight or obese (Baker et al. 2007; Erkkola et al. 2010; Erkkola et al. 2013; Uusitalo et al. 2012).

Table 1. Reported breastfeeding rates (% exlclusive and any breastfeeding) in Nordic countries excluding Denmark where only limited data was available (modified from Hörnell et al. 2013) and in the United States (US Center for Disease Control and Prevention, 2014).

	1 month		2 months		3 months		4 months		5 months		6 months		9 months	12 months
	Excl	**Any**	**Excl**	**Any**	**Excl**	**Any**	**Excl**	**Any**	**Excl**	**Any**	**Excl**	**Any**	**Any**	**Any**
Finland [1]	46	87	38	80	34	77	23	68	9	66	0	58	39	34
Sweden [2]			67	87			51	76			11	63	34	16
Norway [3]	82	95	73	91	63	88	46	85	25	82	9	80	63	46
Iceland [4]	87	94	80	91	67	86	63	84	35	79	8	74	45	27
USA[5]	53	74	47	69	41	64	33	57	24	52	19	49	34	27

[1] Children born in 2010
[2] Children born in 2010
[3] Children born in 2006
[4] Children born in 2005-2006 and in 2004-2008
[5] Children born in 2011

Breastfeeding has a modest but consistent protective effect against obesity in childhood and adolescence in the majority of the studies (Chivers et al. 2010; Hörnell et al. 2013; Monasta et al. 2010; Owen et al. 2005; Arenz, et al. 2005 ; Harder et al. 2005). The protective effect of breastfeeding against being overweight during childhood and childhood obesity has gained considerable interest and is included in US recommendations to reduce the prevalence of obesity in childhood (US Department of Health and Human Services, Centers for Disease Control and Prevention, 2007). However, there are also studies that have not concluded associations between breastfeeding and lower obesity risk (Kramer et al. 2007; Michels et al. 2007) and some have suggested that the protective effect may be due to socio-economic factors related to longer breastfeeding (Wadsworth et al. 1999). The problem in comparing these studies lies in different methodological aspects: some compare a breastfed subject with formula-feds and some compare subjects with different duration of breastfeeding. Outcomes of the studies vary from childhood obesity to adulthood obesity. Also the definition to determine obesity in children varies between studies. Despite some conflicting results, breast milk is known to have an effect on infant metabolic phenotype and growth and some possible pathways are illustrated in Figure 1.

Human milk contains insulin, leptin and adiponectin (Fields & Demerath 2012; Young et al. 2012), and breastfed infants have lower insulin and leptin levels than formula-fed infants (Lucas et al. 1980; Singhal et al. 2002), which can be protective against later obesity. Leptin and adiponectin are detected in breast milk at concentrations corresponding to maternal circulating concentrations (Young et al. 2012). Interestingly, maternal BMI correlates with milk leptin and adiponectin concentration (Fields & Demerath 2012; Young et al. 2012). Both leptin and adiponectin are associated with infants' weight gain (Young et al. 2012). In addition, the duration of breastfeeding is inversely associated with the degree of methylation of the leptin gene in young children, which may contribute to the protective effect of breastfeeding against obesity (Obermann-Borst et al. 2013). Breastfeeding may also be associated with slower weight gain in infancy, which may further reduce the risk of later being overweight or obesity (de Hoog et al. 2011; Durmus et al. 2011; Hörnell et al. 2013). A longer duration of breastfeeding is associated with later introduction of complementary feeding (Baker et al. 2004), which may further be protective against being overweight and obesity (Schack-Nielsen et al. 2010).

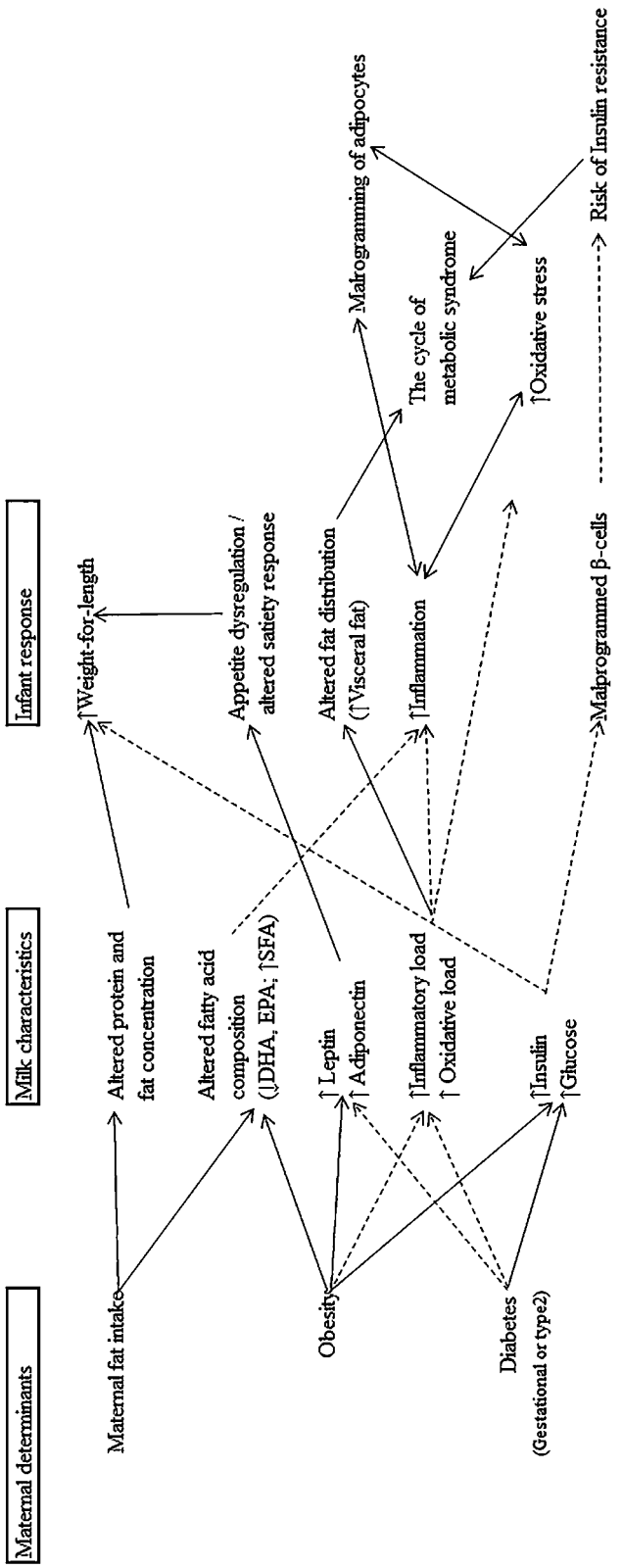


Figure 1. Potential mechanisms how maternal phenotype/behavior may indirectly affect infant metabolic phenotype and growth via alterations in breast milk (modified from Young et al. 2012).

2.3.2.2 Composition of breast milk

The content of nutrients in human milk influences the growth and health of infants (Oddy 2012; Oddy 2002). Human milk contains optimal nutritional composition and bioactive factors that promote survival and healthy development (Oftedal 2012). During the first few days postpartum, the mother produces colostrum that is low in quantity but high in immunological components. Colostrum is low in lactose, indicating its non-nutritional purpose, and levels of sodium, choride and magnesium are higher and potassium and calcium levels lower than in mature milk (Kulski & Hartmann 1981; Pang & Hartmann 2007). Transitional milk has some characteristics from both colostrums and mature milk. Human milk is fully mature at 4 to 6 weeks postpartum. Mature milk remains relatively similar in composition throughout lactation (Ballard & Morrow 2013). Macronutrient composition of mature human milk is estimated to be 0.9-1.2 g/100 ml for protein, 3.2-3.6 g/100 ml for fat and 6.7-7.8 g/100 ml for lactose (Ballard & Morrow 2013). Human mature milk contains energy approximately 65 to 70 kcal/100 ml. The macronutrient composition varies within mothers across lactation but is remarkably conserved across populations, and this indicates its function as an optimal source for infant feeding. The infant's gender can also have an effect on milk composition. In a recent study from the U.S., mothers of male infants produce milk that had as much as 25% greater energy content compared to milk produced by mothers of female infants (Powe et al. 2010). A probable explanation for this lies in evolutionary aspects where greater nutritional investment in sons has been beneficial. Some potential pathways by which maternal determinants may affect milk composition are illustrated in Figure 1.

Human milk proteins can be divided into the whey and casein fractions or complexes with remarkable array of different proteins and peptides (Lönnerdal 2004). Protein levels are higher in colostrum and decrease as milk matures (Bauer & Gerss 2011). The maternal diet does not affect the protein concentration, but high maternal body weight increases the concentration (Nommsen et al. 1991). Also if the mother is producing high amounts of milk, the protein concentration decreases. The carbohydrates are considered the least variable macronutrient in human milk even though some subtle changes can be found according to milk volumes produced (Nommsen et al. 1991). The main carbohydrate in human milk is the disaccharide lactose. Also oligosaccharides are important and comprise approximately 1g/100 ml. However, oligosaccharides are mostly not absorbed and are thus considered as nonnutritive bioactive factors that can act as prebiotics, antiadhesive antimicrobials and immune response modulators (Bode 2012; Jantscher-Krenn & Bode 2012; Musilova et al. 2014).

Fat is the most variable macronutrient in human milk. The total amount of fat varies even during one feeding: foremilk contains 2 to 3 times less fat than hindmilk. Also the individual fatty acid (FA) composition of breast milk varies significantly between lactating mothers (Szabo et al. 2010). Infants who are exclusively breastfed are dependent on essential and long-chain polyunsaturated FAs (LCPUFA) present in the mothers breast milk. The long-term FA composition of the mother's diet reflects the FA composition in breast milk (Del Prado et al. 2001; Jensen 1999) although the mother's

current diet and its FA composition may also influence it (Innis 2007; Nasser et al. 2010). In addition to maternal dietary FAs, breast milk FA composition also reflects mobilization of endogenous stores of FAs and de novo synthesis of FAs by liver or breast tissue. As hypothesized, the FA composition of the breast milk and that of the infant's plasma and tissues correlate strongly with each other (Jensen et al. 2000; Mellies et al. 1979; Pugo-Gunsam et al. 1999). Since maternal diet impacts breast milk FA composition, a maternal diet with low levels of essential FAs can decrease the level of these FAs in breast milk. Indeed, fish oil supplementation during pregnancy increases n-3 LCPUFAs in breast milk and directly impacts the FA status of the erythrocytes of the infants (Dunstan et al. 2007). The FA composition of the infant's diet may also influence early weight gain. It has been hypothesized that especially the ratio between n-6 and n-3 LCPUFA might associate with early weight gain, but the results have been inconsistent (Makrides et al. 2005; Scholtens et al. 2009). Supplementation of infant formula with n-3 LCPUFA may affect infant growth in preterm infants (Carlson et al. 1996; Ryan et al. 1999), but results have been controversial (Gibson et al. 2001; Lapillonne & Carlson 2001; Makrides et al. 2005; Scholtens et al. 2009). The effect of many other fatty acids on early growth or the optimal composition of fatty acids in human milk remains unknown.

In addition to protein, carbohydrates and fat, human milk contains many bioactive components (Ballard & Morrow 2013). For example, human milk contains adiponectin, leptin, insulin, IL-6 and TNF-α, all of which are associated to early growth through energy metabolism or appetite control (Ballard & Morrow 2013; Fields & Demerath 2012). These bioactive components are usually missing from infant formulas, which may be one potential reason for the different growth trajectories between breastfed and formula-fed infants.

2.3.2.3 Infant formulas

Infant formulas are usually based on cow's milk fortified with different vitamins and sometimes also bioactive factors such as prebiotics. Most of the studies suggest that formula-fed children a have higher risk of obesity than breastfed children (Hörnell et al. 2013; Monasta et al. 2010; Oddy 2012; Owen et al. 2005; Arenz et al. 2005; Harder et al. 2005) and grow faster especially during the first months (Imai et al. 2014). This may be due to number of factors such as the inability to self-regulate the amount of milk intake from the bottle (Birch & Fisher 1998; Dewey 2003; Savage et al. 2007) or differences in milk composition (Fields & Demerath 2012).

Formulas contain a higher amount of proteins and lower amount or no bioactive factors compared to breast milk. In 1995 Rolland-Cachera and coworkers proposed that high protein in an infant's diet stimulates growth and increases the likelihood of being overweight in later life (Rolland-Cachera et al. 1995). Since then, many studies have confirmed this association (Brands et al. 2014; Gunnarsdottir & Thorsdottir 2003; Koletzko et al. 2009a; Koletzko et al. 2014a; Öhlund et al. 2010). These results have led to a "Early protein hypothesis," which may partly explain the differences in obesity risk among breastfed and formula-fed infants (Koletzko et al. 2009a; Koletzko et al. 2009b; Koletzko et al. 2014a).

The association with growth may depend on the effect of high protein intake on insulin-like growth factor I (IGF-I), which may result in more rapid growth and increased muscle and fat mass (Michaelsen 2000). Recently it was discovered that high-protein intake may have an effect on saturation of branched-chain amino acid degradation pathway and the initial step of β-oxidation therefore resulting in high early weight gain and body fat deposition (Kirchberg et al. 2015). A recent study with low-protein formulas has shown slower weight gains in infants of overweight mothers fed low-protein formula (Inostroza et al. 2014). Human milk contains also hormones such as leptin and insulin (Fields & Demerath 2012), while formula milk usually lacks these components. Formula-fed infants show higher levels of insulin and leptin than breastfed infants (Lucas et al. 1980; Singhal et al. 2002), therefore possibly predisposing to obesity later in life.

The differences in obesity risks among breastfed and formula-fed infants have been explained also by satiety responses because during breastfeeding, infants can more easily control the amount of milk. Signaling of satiation during bottle-feeding is associated with formula composition (Ventura et al. 2014). Also mother's responsiveness seems to have an effect on the amount of milk consumed, i.e., the less responsive the mother is to satiety signals the more milk is consumed. A recent study in Canada showed that while breastfed infants had the lowest obesity risk, a combination feeding containing both breast milk and formula milk was also protective against obesity when compared to formula-fed infants thus demonstrating the protective effect of any breastfeeding (Rossiter et al. 2015).

2.3.2.4 Introduction of complementary feeding

The optimal age for the introduction of complementary foods remains unknown, but according to the WHO recommendations, infants should be exclusively breastfed for the first 6 months of life (World Health Organization 2002). However, the ESPHGAN Nutrition Committee has concluded that in developed countries, there is no scientific evidence that introducing complementary foods to breastfed infants between 4 and 6 months of age is a disadvantage compared to introduction after 6 months (Agostoni et al. 2008; ESPGHAN Committee on Nutrition et al. 2009). The committee has also concluded that complementary foods should not be introduced to the diet of any infant before 17 weeks or should the introduction of complementary foods be delayed after 26 weeks of age (Agostoni et al. 2008). Introducing complementary foods to infants at 4 months may have small positive effect on infants iron status compared to infants who are exclusively breastfed for 6 months (Jonsdottir et al. 2012). In Finland, complementary feeding is advised to start at the latest at the age of 6 months (Hasunen et al. 2004). The Finnish National Allergy Program recommends the introduction of complementary foods between 4 to 6 months of age (Haahtela et al. 2008). In Finland, most of the infants receive complementary foods before the age of 6 months: at the age of 4 months, 55% of infants had received some complementary foods and at 5 months, 83% of infants had received some complementary foods (Uusitalo et al. 2012).

A study by Schack-Nielsen and coworkers showed that later (≥ 4 months) introduction of complementary foods may be protective against being overweight or obese later in life

(Schack-Nielsen et al. 2010). They postulated that the protective effect of longer breastfeeding against obesity derived from the later introduction of complementary foods rather than from longer breastfeeding itself. A recent review concluded that no clear association between the timing of the introduction of complementary foods and a child being overweight or obese was found although evidence indicates that a very early introduction (<4 months) may increase the risk of a child being overweight (Pearce et al. 2013).

Early feeding practices during infancy, such as breastfeeding and introduction of complementary foods, are one potentially modifiable determinant of childhood obesity (Baker et al. 2004). Many different factors associate with feeding practices, for example, maternal age, maternal education level and feeding at the maternity ward influence the timing of introduction of complementary foods (Betoko et al. 2013; Erkkola et al. 2013; Uusitalo et al. 2012). However currently, little is known whether the parent being overweight or obese has an impact on introducing complementary foods to their children. Differences in early feeding practices could partly explain why maternal obesity has such a strong impact on children's weight.

2.3.2.5 Later dietary habits

Many aspects of diet are associated with obesity. Diet plays an important role in the development of being overweight or the development of obesity. There are recommendations for single nutrient intakes as well as food-based dietary guidelines that are promoting the choice of food-items that include the optimal amount of nutrients and are related to decreased disease risk (Nordic Council of Ministers 2013). These guidelines recommend a diet containing high amounts of vegetables, fruits and berries, nuts and wholegrain cereals, regular fish and vegetable oil consumption and the use of soft fats and low-fat dairy products (Nordic Council of Ministers 2013). Early nutritional factors such as breastfeeding are discussed previously in section 2.3.2.

Eating habits and preferences are formed even before birth, because maternal diet during pregnancy influences postnatal food preferences (Mennella et al. 2001). Early infancy has an important role in the development of eating habits and, for example, infrequent fruit and vegetable intake in infancy is associated with an infrequent intake later in childhood (Grimm et al. 2014). Moreover, several eating behavior aspects influence the development of children becoming overweight. For example, the responsiveness to food and satiety, overeating in emotionally challenging situations and enjoyment of food associate with body adiposity measurements whereas satiety responsiveness, slowness in eating, undereating in emotionally challenging situations and food fussiness associate inversely with body adiposity measurements (French et al. 2012).

The nutrient density of the diet decreases after 1 year of age when the children adapt the regular family diet (Kyttälä et al. 2010). Moreover, among 2- to 6-year-old Finnish children, the diet contains too much saturated fat and sucrose and too little polyunsaturated fats (Kyttälä et al. 2010). The type of day care is also associated with

food consumption and nutrient intake in 3-year-old children (Lehtisalo et al. 2010). The study showed that children attending day care outside home consumed more recommended foods, such as fresh vegetables, fruits, berries, rye bread, fish, skimmed milk and vegetable oil based margarines than those who were cared at home and the researchers concluded that the diet of the children attending day care outside home was closer to national recommendations.

Previously it has been shown that consumption of low-quality food, sweets and meats are associated with a risk of being overweight (Nicklas et al. 2003). Foods with high nutritive value relative to their energy content, e.g., fruits and vegetables may have a protective effect against being overweight whereas frequent eating away from home may predispose to being overweight (Ritchie et al. 2005). Also higher protein intake during infancy may increase the risk of later obesity, which is addressed in detail in section 2.3.2.3 (Koletzko et al. 2009a; Koletzko et al. 2009b; Koletzko et al. 2014a). Moreover, high animal protein intake at 12 months of age is associated with accelerated growth and higher BMI at the age of 6 years (Thorisdottir et al. 2014).

2.4 Early environmental determinants of obesity

2.4.1 Parental overweight

Being overweight or obese seems to aggregate in families due to genetic and environmental factors. Moreover, the parent being overweight is a strong predictor of a child being overweight. A birth cohort study in Finland showed that children whose parents were overweight during the entire childhood had a 10- to 15-fold risk of being overweight at 16 years of age when compared to children whose both parents were normal weight during their entire childhood (Jääskeläinen et al. 2011). A study conducted in Germany revealed that children whose parents were overweight or obese had 4.9-fold odds ratio for becoming overweight and 10.2-fold odds ratio for becoming obese compared to children whose parents were normal weight (Kleiser et al. 2009). A Swedish study showed that a parent being overweight was strongly associated with childhood obesity already at the age of 4 years (Mangrio et al. 2010). Reasons for this higher risk of obesity are numerous including genetic and environmental factors as well as programming during pregnancy. Since the parent being overweight is a major risk factor for childhood obesity, preventing intergenerational transmission of obesity is an essential target for public health. This can be done by helping the parents maintain normal weight.

The mother being overweight or obese during pregnancy predisposes the developing fetus to adverse health outcomes. Overweight women have a higher risk for gestational diabetes and other complications after delivery and frequently give birth to heavier babies than women with a normal weight (Sebire et al. 2001; Yogev & Visser 2009). The mother being overweight during pregnancy also predisposes offspring to a disadvantageous body composition later in life (Eriksson et al. 2014; Eriksson et al. 2015). Children

of mothers with gestational diabetes show increased birth weight, higher risk for hypoglycaemia (Langer et al. 2005), increased early-onset obesity risk, type 2 diabetes during adolescence and manifestation of metabolic syndrome during early childhood (Yogev & Visser 2009). The effect of maternal BMI and glucose metabolism on the developing fetus is also evident in children of nondiabetic mothers (Knight et al. 2007; Ong et al. 2008) and in children whose mothers have glucose levels within a normal reference range (Clausen et al. 2005). However, limited data exists on the combined effect of maternal prepregnancy BMI, pregnancy weight gain, glucose metabolism, diet and physical activity on the developing fetus. Interestingly, a recent study showed that siblings born to mothers with prior gestational diabetes after non-diabetic pregnancy had a higher risk of being overweight and obese (Nilsson et al. 2014). This result suggests that the association between gestational diabetes and children's obesity risk may be partly explained by lifestyle habits as well as intrauterine factors.

Familial clustering of obesity is due to both genetic factors and shared environmental factors such as similar eating habits and physical activity (Ritchie et al. 2005). Maternal obesity can influence infant feeding patterns since obese mothers tend to breastfeed for a shorter time (Baker et al. 2007; Kitsantas & Pawloski 2010; Li et al. 2003; Liu et al. 2010a; Mok et al. 2008a; Oddy et al. 2006; Wojcicki 2011). This may be due to a number of factors. Mothers being overweight or obese often have elevated insulin concentrations, which may slow down secretory activation in the mammary gland (Wahlig et al. 2012) and therefore complicate the initiation of breastfeeding. The mother being overweight increases the risk for delayed lactogenesis, which complicates breastfeeding success (Nommsen-Rivers et al. 2010). Overweight mothers may also have difficulty in breastfeeding because of their larger-sized breasts and the difficulties associated with finding good breastfeeding positions. Limited data exists whether overweight or obese mothers introduce complementary food to their infants earlier than normal weight mothers. The duration of breastfeeding, the timing of introduction of complementary foods and maternal prepregnancy BMI influence infant weight gain (Baker et al. 2004).

2.4.2 Physical activity in young children

Higher physical activity is associated with lower body weight (Must & Tybor 2005). Young children are naturally active, but sedentary behavior patterns are quickly acquired. Television viewing is highly prevalent in young children (Council on Communications and Media, 2011) and both cross-sectional and longitudinal studies show the association between children's screen time and being overweight (Jimenez-Pavon et al. 2010; Must & Tybor 2005). A sedentary lifestyle such as high use of electronic media are associated with higher risk of children being overweight (Marshall et al. 2004). Moreover, increases in electronic media time, including TV, DVDs and electronic games, were associated with increased consumption of nutritionally low-quality foods and beverages and decreased consumption of fruit and vegetables (Falbe et al. 2014).

2.4.3 Other environmental factors

A low socio-economic status is more frequent in obese or overweight families than in normal weight families. A family's low socio-economic status is a risk factor for becoming overweight or having an obese child, and it is associated with other risk factors such as less breastfeeding, more electronic media time and less physical activity (Kleiser et al. 2009). A lower educational level in parents also associates with introducing complementary foods early (Erkkola et al. 2013), which predisposes to higher obesity risk (Schack-Nielsen et al. 2010). In a meta-analysis, maternal smoking has been shown to increase obesity risk to 1.5-fold (Oken et al. 2008). However, smoking is more prevalent in lower socioeconomic classes and thus a confounding effect may occur.

Gestational weight gain has also been linked to a higher obesity risk in children. Higher maternal gestational weight gain increases the risk of children being overweight and obese. At 7 years of age the obesity risk increased 3% in children for every 1 kg the mother gained during pregnancy (Wrotniak et al. 2008).

A caesarean section is a known risk factor for obesity in childhood (Bammann et al. 2014; Mueller et al. 2015), and it is more frequent among overweight women than in normal weight women (Gaillard et al. 2013; Papachatzi et al. 2013). A ceasarean section is one factor associated with gut microbiota development. Recently differences in gut microbiota bacteria species have been linked to obesity risk by influencing energy regulation (Nilsson et al. 2014). Moreover, studies have reported differences in the composition of gut microbiota species between overweight and normal weight children (Bervoets et al. 2013; Kalliomäki et al. 2008; Vael et al. 2011). Interestingly, a recent study reported differences in bacterial species of gut microbiota between toddlers of obese and normal weight mothers (Galley et al. 2014).

Chronic insufficiency of sleep during infancy increases obesity risk in childhood (Cespedes et al. 2014; Taveras et al. 2014). An accumulation of obesity risk factors seems to be detrimental since the probability of being overweight at 3 years of age was 29% for children who were exposed to four environmental risk factors compared to 6% of overweight children without these risk factors (Gillman et al. 2008).

2.5 Critical periods in early growth and development of obesity

The current obesity epidemic has the potential to negate the health benefits that have contributed to increased longevity (WHO 2015). Recently the WHO Comission on Ending Childhood Obesity stated that the effectiveness of interventions indicate that novel approaches are required to tackle childhood obesity (WHO 2015). A summary of the early risk factors for obesity is presented in Table 2. The risk factors are numerous and moreover, many of them confound each other, which complicates the effect size assessment of individual risk factors. Intervention studies have been designed based on the identified risk factors, but unfortunately the results have been modest (Monasta et al. 2011).

Table 2. Risk factors for later obesity (modified from Bammann et al. 2014).

Risk factor	Hypotheses
Genetic	
Genetic susceptibility	Risk factor through shared genetic factors: common variants of obesity-susceptibility SNPs, mutations in single genes, epigenetic factors (prenatal) Possible confounder: Gene-environment interactions
Prenatal	
Parental obesity	Risk factor through shared genetic factors, shared environment (diet), fetal programming Possible confounder: socio-economic status
Gestational weight gain	Risk factor through shared genetic factors (weight gain), shared environment (diet), fetal programming Possible confounder: Maternal BMI
Gestational diabetes	Risk factor through shared genetic factors, shared environment, fetal programming Possible confounder: Maternal BMI
Smoking during pregnancy	Risk factor through fetal growth retardation and developmental adaptations Possible confounder: Parental socio-economic status, energy intake
Parental socio-economic status	Risk factor through shared environment Possible confounder: parental obesity, smoking, lower duration of breastfeeding and earlier introduction of complementary foods
Birth weight	Risk factor reflecting fetal growth and programming for lean mass and fat distribution Possible confounder: Maternal BMI, smoking during pregnancy
Ceaserean section	Risk factor through differences in fetal microbial colonization Possible confounder: Maternal BMI, maternal smoking, breastfeeding
Postnatal	
Breastfeeding (initiation, exclusive and partial breastfeeding)	Protective factor through nutritional programming, moderation of genetic effects, reduced risk of over-feeding, optimal macro- and micronutrient composition, bioactive components Possible confounder: Maternal overweight/obesity, maternal smoking during pregnancy, parental socio-economic status
Early introduction of complementary foods	Risk factor through nutritional programming Possible confounder: Breastfeeding, parental socio-economic status

Risk factor	Hypotheses
Infant formula feeding	Risk factor through nutritional programming, suboptimal macronutrient intake (especially high protein intake), increased risk of overfeeding, lack of bioactive components found in breast milk Possible confounder: lower or no breastfeeding, parental socioeconomic status, maternal overweight/ obesity
High weight gain and rapid growth in infancy	Risk factor through excessive cumulation of fat mass and metabolic changes related to that Possible confounder: breastfeeding, infant formula feeding, birth weight
Sedentary behavior, electronic media time	Risk factor through physical inactivity Possible confounder: Parental socio-economic status, maternal BMI
Sleep time	Risk factor through chronic inadequate sleep that predisposes to obesity Possible confounder: Sedentary behavior, electronic media time

Early detection and preventative actions are of vital importance since obesity during an early age tends to be sustained as shown in a recent population-based study from Northern Finland Birth Cohorts (Graversen et al. 2014). Results demonstrated that children, who were above the 90th percentile at 5 years of age, had approximately 12 times higher risk of being overweight at the age 15 than children below the 50th percentile (Graversen et al. 2014). These results highlight the stability between early body size and later being overweight. Indeed, prenatal and postnatal time is a period of rapid growth and the foundation of later health is constructed during that time. Both genetic and environmental factors are known to play a role in early growth and development of obesity, but the interactions between different factors require further studies and the results of which could be utilized in designing new prevention and intervention strategies. Moreover, clustering of obesity risk factors is poorly understood and studies focusing on that could provide novel approaches. Interventions in early life or even before conception are likely to have the greatest positive effects on health (WHO 2015). Due to the complex nature of childhood obesity, effective intervention and prevention strategies will require a combination of scientific research, government support and community partnership.

3. AIMS OF THE STUDY

The general aim of this study was to provide more comprehensive information on genetic and environmental, both prenatal and early postnatal, risk factors associated with weight, weight gain, being overweight and obese in young children. The objectives were:

1. To study how maternal prepregnancy BMI, gestational weight gain, diet and physical activity affected glucose metabolism during pregnancy and assess their impact on prenatal and early postnatal growth (Study I).

2. To study if the mother being overweight was associated with the duration of breastfeeding, breast milk fatty acid composition and the introduction of complementary feeding (Study II, III).

3. To examine if the duration of breastfeeding, introduction of complementary feeding and breast milk fatty acid composition were associated with early postnatal growth (Study II, III).

4. To study the association between genetic risk clustering and weight, BMI, weight-for-length and weight gain from birth to 2 years of age (Study IV).

4. METHODS

4.1 Study design and subjects

Altogether 1797 pregnant women and their spouses (n=1658, 18.3% from eligible cohort) were recruited from maternity clinics or Turku University Hospital to participate in a prospective follow-up study called the STEPS Study (Steps to healthy development) from September 2007 to March 2010 (Figure 2) (Lagström et al. 2013). Follow-up of the families in STEPS study will continue until children are young adults, but this thesis focused on their first 24 months of life.

From all 1797 women participating in the STEPS Study, 144 (8.0%) overweight women had a prepregnancy body mass index (BMI) ≥25kg/m^2 and 108 (6.0%) normal weight women with a prepregnancy BMI <25 kg/m^2 were selected from the STEPS study (Figure 2). The families were selected based on maternal BMI and living area and recruited by sending an information letter and an invitation at 20th gestational weeks to participate in more intensive follow-up study for Early Risk Factors of Childhood Obesity (OBERISK). From these women, 90 (62.5%) overweight women and 73 (67.6 %) normal weight women were willing to participate in this more intensive follow-up study.

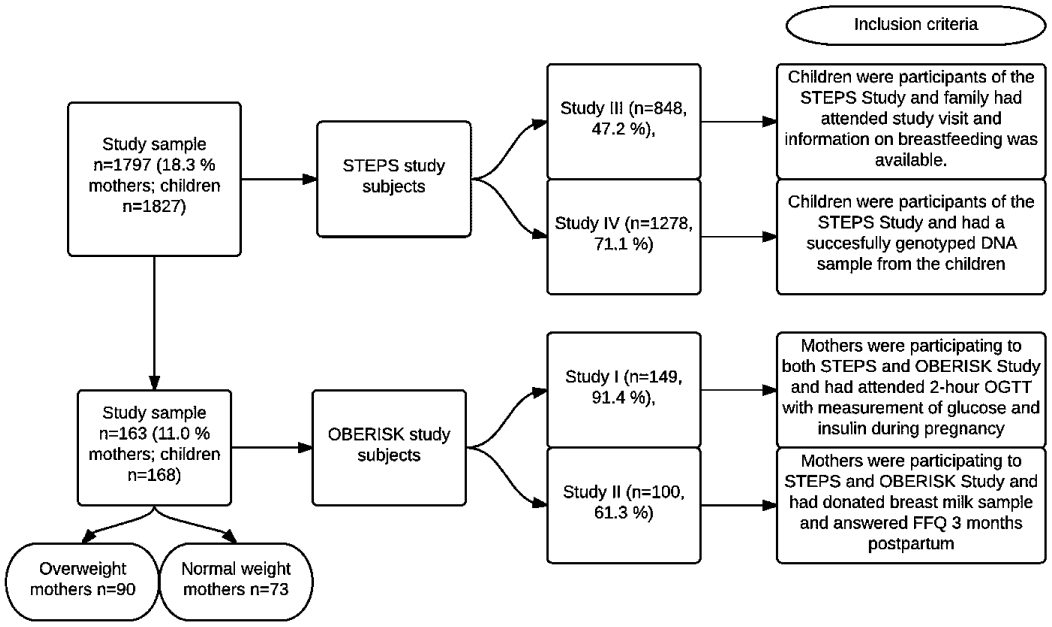


Figure 2. Flow chart of the STEPS and OBERISK Study and inclusion criteria for Studies I-IV.

At 13 months of age, the number of drop-outs in the STEPS Study was 107 (6%), which can be considered small. By the age of 24 months, the number of drop-outs

had increased to 186 (10%). The drop-outs did not differ from participants in any of the studies variables (maternal age, maternal prepregnancy BMI, gestational age, primiparity, socio-economic status, infant's gender or birth anthropometrics). The proportion of non-responders is however larger. At 13 months of age, 519 (28%) and at 24 months of age, 861 (48 %) families did not answer questionnaires or attend study visits. Non-responding was more prevalent in non-primiparious families, in lower occupational class, in rural areas and in families with premature babies. Birth anthropometrics or maternal prepregnancy BMI did not differ between non-responders and responders (Lagström et al. 2013). The non-responders continued in the STEPS Study whereas the drop-outs did not.

4.2 Sampling and data collection

Data in this study were collected from the recruitment at pregnancy until children's age of 2 years. Data collection and sampling is shown in detail in Table 3. Information regarding the course of pregnancy and delivery was obtained from the Longitudinal Census Files and maternity clinics. Delivery was defined as premature if the duration of pregnancy was less than 37 gestational weeks. Information on maternal gestational diabetes, impaired glucose tolerance or chronic diseases were obtained from maternity clinics.

Mothers in the OBERISK study attended a 2-hour oral glucose tolerance test between 26-28 gestational weeks. STEPS study children attended study visits at the ages of 13 months and 2 years. Blood samples were collected at birth (cord blood) and at 13 months and 2 years of age. DNA was extracted from blood samples collected at 13 months or at the 24 months study visit. If neither of these were available, cord blood sample was used. A breast milk sample was collected at the children's 3 months of age. Demographic information, including education, work, household incomes, health, smoking and parity were requested at the time of recruitment and at 13 months and 24 months with questionnaires.

Table 3. Data collection of the STEPS and OBERISK Study from pregnancy to 2 years of age.

		STEPS study (n=1797)			OBERISK study (n=163)	
Time point	**Register data from Longitudinal Census File and Maternity Clinics**	**Questionnaires**	**Study visits**	**Biological samples**	**Questionnaires**	**Biological samples**
Gestational weeks 10-30		Questionnaires for the mother and father: Background information, Self-reported parental weight and height, Index of Diet Quality, Physical activity questionnaire				Weeks 26-28: 2-hour oral glucose tolerance test, blood samples at 0h, 1h and 2h
Whole pregnancy	Maternal weight gain, blood glucose concentration					
Delivery	Birth weight, length and head circumference, gestational age, gender			Cord blood sample		
Mother 3 months postpartum				Breast milk sample	Fat Food Frequency Questionnaire for mother	Breast milk sample
Child 8 months					3-day food record for mother, father and child	
Child 13 months		Background questionnaire for mother, father and child	Health status, weight and length/height	Blood sample	3-day food record for child	
Child 24 months		Background questionnaire for mother, father and child	Health status, weight, height, waist circumference	Blood sample	3-day food record for mother, father and child	
Child 0-24 months		Follow-up diary: breastfeeding and introduction of complementary feeding				

4.3 Anthropometric measurements

4.3.1 Maternal weight, height and weight gain during pregnancy

Maternal weight and height records were obtained from the maternity clinic records throughout pregnancy (weight was measured on average 13 times during pregnancy). Maternal prepregnancy weight was self-reported at the time of recruitment. Maternal weight and height were also measured at study visit 13 and 24 months postpartum. Body mass index (BMI) was calculated as body weight (kg) / body height $(m)^2$. Maternal weight status was classified as overweight if prepregnancy BMI was 25 kg/m^2 or higher and obese if BMI was 30 kg/m^2 or higher. Total gestational weight gain was calculated by subtracting self-reported pre-pregnancy weight from that recorded at the hospital before delivery or at the last visit before delivery at maternity clinics.

4.3.2 Weight, length/height and waist circumference in children

Information regarding birth weights and lengths in children and the course of pregnancy and delivery were obtained from the Longitudinal Census Files and from maternity clinics. To calculate birth weight standard deviation score (SDS), we used population-based references (Sankilampi et al. 2013). At 13 and 24 months of age, children's weights and lengths were measured at the study visits. Weights of the children were measured to the nearest 0.1 kg with an electronic scale (ADE M12010; ADE GmbH & Co Hamburg, Germany) at 13 months of age and thereafter with a Tanita scale (WB110MA, Tanita Corporation. Tokyo, Japan). Lengths of the children before the age of 24 months were recorded recumbent and thereafter standing heights were measured to the nearest millimeter with a wall-mounted Harpenden stadiometer (Holtain, Crymych, UK). Weight gain from birth to 13 months and from birth to 24 months were calculated by extracting birth weight from the weight at 13 or 24 months of age. All anthropometric data was measured by health-care professionals during the study visits. The new Finnish Growth References for Children were used to calculate weight-for-length/height and weight-for-length/height SDS and to determine being overweight or obesity at 2 years of age (Saari et al. 2011). These new growth references for children include age- and sex-specific BMI cut-off points, wherein age is determined by 0.01 year accuracy from 2 years of age onwards.

4.4 Dietary and physical activity data

4.4.1 Breastfeeding and complementary feeding

Information about breastfeeding (BF) and complementary feeding (CF) were collected with a self-administered follow-up diary. The follow-up diary consisted of detailed information about the duration of exclusive BF (date when started and date when ended, EBF, referred in Study III as full BF), total duration of BF (date when started and when ended, total BF) and initiation of CF (solid foods). The follow-up diary included also

information on which specific foods or food groups were given and at what age the foods were first introduced. Also, the use and label of formula milk was recorded. The records were collected real-time to avoid memory-related bias. Exclusive BF was defined as infant receiving no other food or drink than mother's breastmilk, except for water.

4.4.2 Food frequency questionnaire

At breast milk sampling time, 3 months postpartum, the dietary intake of mothers was evaluated with a short food frequency questionnaire. The questionnaire focused on foods rich in different fatty acids or on foods that constitute one of the main sources of certain fatty acids. The questionnaire covered a one week period. The questionnaire contained questions about fish, fish-oil supplements, vegetable oils, spreads, and foods rich in saturated fatty acids (SFA) such as fast food, snacks, sausages, high-fat dairy products and chocolate. The foods included in the questionnaire represented the typical sources of different FAs in the Finnish diet (Paturi et al. 2008). The food frequency questionnaire was answered on the day of the sampling in order to define the intake of FAs during the week before sampling which could partly explain the FA profile of the breast milk samples.

4.4.3 Index of Diet Quality

During the third trimester of pregnancy, maternal diet quality was studied with the Index of Diet Quality (IDQ). IDQ describes the adherence to nutrition recommendations, and it has been previously validated with 7-day food records (Leppälä et al. 2010a). The IDQ consisted of 18 questions with the scoring system ranging from 0 to 15 where the scores between 0 to 9 were defined as non-adherence and those between 10 to 15 as adherence to a health promoting diet and dietary guidelines. Briefly, the higher the points, the better the adherence to nutrition recommendations. A health promoting diet was defined based on Nordic Nutrition Recommendations and scientific knowledge on a health promoting diet. More detailed information about IDQ is described by Leppälä and coworkers (Leppälä et al. 2010). The IDQ enables the assessment of the diet's health-promoting properties taking into account possible synergistic and additive effects of foods when reflected against health outcomes. In the validation study, when compared against 7-day food records, the IDQ reflected the intake of protein, fibre, calcium, iron, vitamin C, saturated fatty acids, the ratio of unsaturated fatty acids to saturated fatty acids and saccharose and is considered to depict the intake of key foods and nutrients associated with health (Leppälä et al. 2010). The IDQ was validated while the 2008 Nordic Nutrition Recommendations were current recommendations and in those recommendations the lowering of consumption of red meat was not highlighted. Therefore the IDQ does not include questions of red meat. Since then, the new Nordic Nutrition Recommendations (Nordic Council of Ministers, 2013) have emphasized the importance of lowering the consumption of red meat.

The consumption and quality of different food groups such as dairy products, vegetables, fruits and berries, fat containing foods (i.e., spreads, oils), fish, whole grain products,

sugar and meal pattern are described by the questions in IDQ. The IDQ is both qualitative- and quantitavive-question-based. Quantitative questions are mainly focused on portion sizes, such as slices of bread, glasses of milk, portions of fruit, whereas the qualitative questions aim is to define more specifically the quality of the different food eaten, such as the amount of fat in dairy products consumed or type of spread mostly used on bread.

4.4.4 Physical activity questionnaire

Physical activity was determined by a self-administered questionnaire about leisure time activities, self-oriented physical activity, and exercise frequency, type of exercise and duration of exercise before and during pregnancy (whole pregnancy time). The questionnaire was answered at the third trimester of pregnancy.

4.5 Biochemical analyses

4.5.1 Oral glucose tolerance test and glucose and insulin measurements

Two-hour oral glucose tolerance tests (OGTT) were performed at 26-28 gestational weeks at Turku University Hospital Central Laboratories. OGTT with a 75 g dose of glucose included blood samples at baseline and at 1 and 2 hours. Plasma glucose and insulin concentrations were measured at all time points with the Roche Modular PPEE analyzer (Roche Diagnostics GmbH, Mannheim, Germany) on the day of sampling. To evaluate insulin resistance, the homeostasis model assessment (HOMA) index was calculated using a formula suggested by Matthews and coworkers: *Glucose 0h x Insulin 0h / 22.5* (Matthews et al. 1985). The quantitative insulin sensitivity check index (QUICKI) was calculated as described by Katz et al*: 1 / (log Insulin 0h + log Glucose 0h)* (Katz et al. 2000), and the composite insulin sensitivity index (Matsuda ISI) according to Matsuda & DeFronzo: *10000 / (√(Glucose 0h x Insulin 0h) x √(Glucose mean x Insulin mean))* (Matsuda & DeFronzo 1999).

The results of the glucose tolerance tests were considered pathological if the test resulted in one or more abnormal values. The diagnostic pathological values for plasma glucose concentrations were $\geq$ 5.3 mmol/l after fasting, $\geq$ 10.0 mmol/l at 1 hours, and $\geq$ 8.6 mmol/l at 2 hours according to reference values in Finnish current care guidelines and Turku University Central Hospital Laboratories. HOMA index was considered to indicate insulin resistance, and QUICKI and Matsuda ISI indices to reflect insulin sensitivity.

4.5.2 Breast milk fatty acid analysis

Breast milk was collected at 3 months postpartum. The collection procedure was standardized by the following written instructions. The samples were collected by manual expression by the mothers in the morning, first milking a few drops to waste and after that, collecting the actual sample of for-milk (10 ml) into a plastic container.

The mothers brought the samples to the research centre or the samples were collected from their homes on the day of sampling. Samples were frozen and stored at -70ºC until analysis of the FAs.

The total FA composition of the breast milk was analyzed using Triheptadecanoin (Larodan Fine Chemicals, Malmö, Sweden) as an internal standard in breast milk samples. An internal standard was added to the sample, and total lipids were extracted with chloroform : methanol 2 : 1 v/v (Folch, 1957). FA methyl esters were prepared from total lipids of the breast milk with boron trifluoride (Ågren, 1992). The samples were analyzed by gas chromatography (GC-2010 Auto Injector / Auto Sampler, Shimadzu, Japan) with a DB-23 column (60 m x 0.25 mm i.d., 0.25 μm film thickness; Agilent Technologies, DE, USA). The peaks of FAs were identified by comparing them to standard mixtures FAME 37 (Supelco, Bellefonte, PA) and 68D (NuChek Prep, Elysian) and quantified with the internal standard. The FA composition of the total lipids of breast milk was expressed as weight percentage of total FAs.

4.6 SNP genotyping and genetic risk score calculation

Blood samples were collected during study visits at children's age of 13 months and 2 years. DNA was extracted from the blood samples manually using a high salt method. DNA samples were tested spectrophotometrically with NanoDrop. SNPs were genotyped using Sequenom iPlex Gold technology (Sequenom, San Diego, CA, USA). Obesity-related SNPs were selected from the National Institute of Health (NIH) database for genome-wide association studies (www.genome.gov) using the following keywords: BMI, obesity, weight and waist circumference. The search resulted in 133 SNPs which were all selected for further analysis. Genotyping was successfully carried out for 112 SNPs. We excluded 10 SNPs with missing genotypes for ≥ 5% of samples. One SNP was excluded because of a low P-value in the Hardy-Weinberg Equilibrium test ($P \leq 0.0001$) and therefore resulted in a total of 101 SNPs.

The risk score for all 101 SNPs was calculated based on number of risk alleles in a genotype. Single missing genotypes were assigned a value that was equal to two times the effect allele frequency. If all values were missing, then imputing was not done. Sixteen SNPs with linkage disequilibrium (LD) higher than 0.50 were removed from the scores. One SNP was removed because the risk allele was not known and one SNP because it was found to be a duplicate. Total risk score was therefore calculated for 83 SNPs. Based on the results from GWAS, studies risk scores for specific phenotypes of interest were also calculated: BMI, overweight/obesity, waist circumference and weight. BMI risk score was calculated for 51 SNPS, weight risk score for 16 SNPs, obesity score for 21 SNPs and waist circumference score for 13SNPs. To demonstrate the effect size per allele, a sum score without imputing was also calculated. All risk scores were calculated using statistical programming environment R, version 3.0.0 (R Core Team, 2014).

4.7 Statistical analyses

The data were analyzed with SPSS statistical software package (version 16.0; SPSS Inc., Chicago, IL, USA), unless otherwise stated. Preterm infants (<37 gestational weeks) were excluded from the analysis. A T-test for independent samples and a chi-square test were used to compare clinical characteristics between boys and girls participating in the STEPS and OBERISK study. Statistical significance was set to $P<0.05$.

Glucose and insulin metabolism during pregnancy and early growth

A T-test for independent samples was used for comparing maternal glucose and insulin metabolism, diet quality and physical activity in overweight and normal weight women (Figure 3). Pearson correlation coefficients were used in studying the effects of maternal prepregnancy BMI on glucose metabolism and the infant's weight and length. A Spearman's correlation was used to analyze maternal BMI and diet and physical activity. Pregnant women with insulin sensitivity medication were excluded from glucose metabolism analyses. Multivariable regression analysis was used for analyzing the influence of diet quality, physical activity, maternal prepregnancy BMI, maternal weight gain during pregnancy and maternal glycaemia to children early growth and anthropometrics.

Maternal overweight, breast milk fatty acid composition and early growth

The T-test for independent samples was used to study the effect of maternal overweightness on the FA composition of breast milk (Table 9). Pearson correlation coefficients were used to analyze the associations of maternal BMI with breast milk FA composition. Pearson correlation coefficients were also used to examine breast milk FA composition and weight at 13 months and weight gain from birth to 13 months in children. In the children's growth data, we analyzed boys and girls jointly since the numbers of boys and girls were similar in the groups of overweight and normal weight mothers. In the interaction test, no connection between maternal BMI and child gender was found. Pearson correlation coefficients were also used in examining the correlations between breast milk FAs and foods containing high amounts of fat or that are significant sources of certain FAs. Linear regression analysis was used to investigate the association of the breast milk FA composition on child's weight at 13 months, height at 13 months, BMI at 13 months, weight gain from birth to 13 months and change in BMI from birth to 13 months. Stepwise linear regression was also used for studying the effect of maternal socio-economic variables, age and gestational weight gain on breast milk fatty acid composition.

Maternal overweight, infant feeding patterns and early growth

A T-test was used for independent samples to compare the duration of BF and introduction of CF between overweight and normal weight women (Figure 4). ANOVA with Bonferroni corrections was used to study early feeding and weight gain in children (Table 7). To investigate the effect of BF and introduction of CF on children's growth,

we used linear regression analysis (Table 8). These variables were coded as dummy variables with following categories based on breastfeeding recommendations (Hasunen et al. 2004): EBF <4 months, 4-6 months and ≥6 months; total duration of BF <6 months, 6-12 months and ≥12 months; introduction of CF <4 months, 4-6 months and ≥6 months. First, we examined unadjusted associations between EBF, total duration of BF and introduction of CF and children's weight gain from birth to 24 months. We then further combined these variables into model 1. Second, we examined the unadjusted associations between maternal prepregnancy BMI, EBF, total duration of BF and introduction of CF and children's weight gain from birth to 24 months. These variables were combined into model 2. Model 2 variables were used as continuous variables, not like in model 1 where they were computed to dummy variables. After examining these unadjusted associations we examined potentially confounding factors and adjusted the model for the following variables: infant sex, birth weight, maternal prepregnancy BMI (only in model 1), gestational weight gain and duration of pregnancy. We also examined maternal education level, family incomes and maternal smoking during pregnancy as confounding factors, but since these factors did not associate with outcomes or enhance the models statistical power, they were excluded from the final model. The confounding factors were chosen based on scientific literature and the data available in STEPS Study. A repeated measures ANOVA with a Greenhouse-Geisser correction and Bonferroni Post hoc-test was used to examine the weight of children of normal weight and overweight and obese mothers at 13 months and 24 months.

Logistic regression analysis, both unadjusted and adjusted, was used to study the effect of EBF, total duration of BF, introduction of CF and introduction of infant formulas on the risk of overweight or obesity at 24 months of age (Table 13). The analysis were adjusted for sex, gestational age, birth weight, prepregnancy BMI, maternal smoking, maternal education level and maternal age. Survival analysis of total duration of breastfeeding in normal weight and overweight mothers was conducted (Figure 5).The growth trajectories of children later becoming overweight or obese were compared with T-test for independent samples (Figure 8, Figure 9).

SNP and risk score association analysis

The PLINK software version v.1.07 (Purcell et al. 2007) was used to conduct association tests between SNP genotypes and phenotypes of interest. For normally distributed traits, we utilized linear regression for assessing associations. All analysis were adjusted for age and sex and analysis with weight as phenotype also for length/height. For being overweight and obese as phenotypes, a logistic regression analysis was used. Linear regression was used to study the association between total risk scores and weight (birth, 13 months, 2 years), BMI (birth, 13 months, 2 years), weight-for-length/height (birth, 13 months, 2years) and waist circumference (2 years) (Table 10, Table 11). All analysis were adjusted for age and sex, except for analysis with weight-for-length/height since the variable is already calculated based on age and sex. Analyses with weight were

adjusted also for height. For overweight and obesity as a phenotype a logistic regression was used. Weight genetic risk score (WeightGRS) was divided to three groups based on number of risk alleles: low risk (≤12 risk alleles), medium risk (13-17 risk alleles) and high risk (≥ 18 risk alleles). ANOVA with Bonferroni corrections was used to examine the association to weight and weight z-score at birth, at 13 months and at 2 years of age (Table 12). All risk score association analyses were done with SPSS (version 21.0).

Obesity Risk Score and clustering of the obesity risk factors

To examine the combined effect of individual obesity risk factors the Obesity Risk Score was formulated. It was calculated based on nine individual risk factors which were all scored (Table 4). Total scores ranged from 0 to 12. In this study, none of the subjects scored 12 points and the Obesity Risk Score ranged from 0 to 9. The cut-off points for scoring were determined based on the data. Logistic regression analysis was used to examine the individual risk factors and combined Obesity Risk Score against being overweight or obese at 24 months (Table 14). To determine the accuracy and predictive rate of individual obesity risk factors and the Obesity Risk Score and for being overweight or obese at 24 months, we used the ROC analysis (Figure 10). ROC-analysis showed the accuracy at which the individual risk factors and the Obesity Risk Score were able to predict being overweight and obese in 24-month-old children. The analyses were done with SPSS (version 21.0).

Table 4. The calculation of Obesity Risk Score.

Risk factors		**Scoring**
Gestational weight gain	<13.9 kg	0
	13.9-17.5 kg	1
	>17.5 kg	2
Maternal prepregnancy weight	Normal weight (<25 kg/m^2)	0
	Overweight/Obesity (≥25 kg/m^2)	1
Maternal gestational diabetes	No	0
	Yes	1
Birth weight	< 2500 g	1
	2500-4500 g	0
	>4500 g	1
Genetic risk	Low risk (<73 risk alleles)	0
	Medium risk (73-84 risk alleles)	1
	High risk (>84 risk alleles)	2
Maternal smoking	No	0
	Yes	1
Maternal education level	Low (no college degree)	1
	High (at least college degree)	0
Family income	<2000 €/month	1
	≥2000 €/month	0
Total duration of breastfeeding	<4 months	2
	4-8 months	1
	>8 months	0
Total		0-12

4.8 Ethics

The STEPS Study was approved on 27th of February 2007 and OBERISK Study on 19th of February 2008 by the Hospital District of Southwest Finland Ethics Committee. Written informed consent was obtained from all the participants. The children's consent was given by parents. Subjects were free to drop-out from the study at any time without any specific reason. The protocol of the STEPS and OBERISK Study is consistent with the principles of the Declaration of Helsinki.

5. RESULTS

5.1 Clinical characteristics of the STEPS Study subjects

Mothers participating in the STEPS Study (n=1797) were on average 30.7 (4.6) years old with a mean BMI of 24.3 (4.9) and 30.7% were overweight or obese before pregnancy. The mean duration of pregnancy was 39.7 (1.8) weeks and 54.3% were primiparious. Most of them were highly educated, since 22.8% had at least professional occupational class and 60.3% had at least bachelor's degree. Maternal smoking during pregnancy was rare since 52 (2.8%) mothers smoked during pregnancy. At the the child's age of 24 months, 63 (3.4%) mothers smoked. At 13 months of age, 23% of children and at 24 months of age, 54% of children were attending day care outside home. Clinical characteristics of the children participating in the STEPS and the OBERISK Study are presented in Table 5. The clinical characteristics of the subjects included in Studies I through IV are described in detail in the original publications.

Table 5. Clinical characteristics of study children in the STEPS and OBERISK Study. All children in the OBERISK Study are participants of the STEPS Study. Values are represented as means (standard deviation).

	STEPS Study			**OBERISK Study**		
	Boys	Girls	P[1]	Boys	Girls	P[1]
Birth	n=863	n=785		n=81	n=87	
Birth weight (kg)	3.62 (0.54)	3.51 (0.46)	**<0.0001**	3.59 (0.65)	3.48 (0.56)	0.155
Birth length (cm)	51.3 (2.1)	50.5 (2.0)	**<0.0001**	51.0 (2.6)	50.1 (2.1)	**0.012**
BMI (kg/m^2)	13.7 (1.2)	13.8 (1.3)	0.889	13.5 (1.3)	14.1 (1.3)	**0.008**
Weight SDS [2]	-0.08 (0.98)	-0.03 (1.0)	0.396	-0.11 (1.0)	-0.05 (1.1)	0.174
Weight-for-length (%)	-1.6 (8.6)	-0.76 (9.3)	0.164	-2.0 (9.0)	-0.92 (9.7)	0.105
Weight-for-length SDS	-0.23 (1.0)	-0.14 (1.1)	0.241	-0.25 (1.1)	-0.18 (1.2)	0.235
Gestational age (weeks)	40.0 (1.3)	40.0 (1.2)	0.882	39.7 (1.6)	39.4 (1.9)	0.423
Small for gestational age (SGA) [2]	2.6%	3.1%	0.124	2.5%	2.6%	0.282
Large for gestational age (LGA) [2]	2.4%	2.6%	0.445	2.7%	2.9%	0.384
13 months	n=658	n=611		n=75	n=82	
Weight (kg)	10.6 (1.1)	9.9 (1.1)	**<0.0001**	10.7 (1.2)	10.1 (1.0)	**0.002**
Length (cm)	78.1 (2.5)	76.5 (2.6)	**<0.0001**	78.9 (2.7)	76.6 (2.3)	**<0.0001**
BMI (kg/m^2)	17.3 (1.3)	16.9 (1.3)	**<0.0001**	17.2 (1.2)	17.2 (1.3)	0.725
Weight-for-length (%)	+0.90 (7.8)	+0.82 (8.5)	0.877	+0.89 (8.3)	+0.86 (9.2)	0.923
Weight-for-length SDS	+0.03 (0.98)	+0.04 (1.0)	0.741	+0.03 (1.0)	+0.05 (1.0)	0.841
Weight gain (kg) birth to 13 mo	6.9 (1.1)	6.4 (1.0)	**<0.0001**	7.2 (1.0)	6.6 (0.9)	**0.001**
24 months	n=582	n=528		n=70	n=72	
Weight (kg)	13.3 (1.5)	12.7 (1.4)	**<0.0001**	13.4 (1.8)	12.9 (1.6)	**0.001**
Length (cm)	89.3 (3.2)	87.8 (3.1)	**<0.0001**	89.9 (3.2)	87.9 (3.1)	**<0.0001**
BMI (kg/m2)	16.6 (1.3)	16.4 (1.4)	**0.005**	16.7 (1.5)	16.5 (1.5)	**0.011**
BMI SDS	0.22 (1.0)	0.23 (1.1)	0.896	0.23 (1.1)	0.24 (1.0)	0.892
Weight-for-height (%)	+1.5 (7.9)	+1.8 (8.5)	0.656	+1.4 (8.4)	+1.9 (9.2)	0.775
Weight-for-height SDS	+0.10 (1.0)	+0.14 (1.0)	0.601	+0.12 (1.0)	+0.15 (1.1)	0.441
Weight gain (kg) birth to 24 mo	9.6 (1.4)	9.1 (1.4)	**<0.0001**	9.9 (1.6)	9.2 (1.6)	**0.001**
Waist circumference (cm)	48.0 (3.2)	47.7 (3.2)	0.155	48.0 (3.6)	47.9 (3.7)	0.235
Overweight at 24 mo [3]	25.7% [4]	17.3% [4]	**<0.0001**	24.1% [4]	17.8% [4]	**0.022**
Obesity at 24 mo [3]	4.6%	4.2%	0.765	4.4%	4.1%	0.322

[1] T-test for independent samples except for overweight and obesity chi-square-test, P value for comparison against boys and girls

[2] According to new population-based references for birth weight and length (Sankilampi et al. 2013)

[3] According to the new Finnish Growth References for Children BMI-for-age criteria (Saari et al. 2011)

[4] The proportion of overweight children includes also obese children

5.2 Prenatal factors and early growth

5.2.1 Maternal prepregnancy overweight, gestational weight gain and early growth (Study I and unpublished data)

Overweight women in the STEPS study gained almost 2 kg less weight during pregnancy (13.0 kg vs 14.8 kg, P<0.0001) and had a consistently higher BMI (29.9 vs 21.8 kg/m^2, P<0.0001) than normal weight women. Children born to overweight mothers were heavier at birth, at 13 months and at 2 years of age (P<0.05 for all) than children born to normal weight mothers (Table 6). Children of overweight women were also taller at birth than the children of normal weight women (P<0.0001), whereas later no difference in length was discovered. Children of overweight women had higher BMI and weight-for-length at birth, 13 months and 2 years (P<0.05 for all). No differences between boys and girls were observed in these results, and thus the results are presented jointly for boys and girls.

Table 6. Comparison of anthropometrics at birth, 13 months and 24 months of children of normal weight and overweight mothers between normal weight and overweight women and their children's anthropometrics at birth, 13 months and 2 years. Preterms (<37 gestational weeks) were excluded. Values represented as means (standard deviation).

	Normal weight mothers (prepregnancy BMI<25)	Overweight mothers (prepregnancy BMI ≥25)	P [1]
Child characteristics			
Birth	n=1164	n=514	
Gender (male/female)	609/555	268/246	0.958
Gestational age (weeks)	40.0 (1.5)	39.9 (1.6)	0.559
Weight (kg)	3.53 (0.45)	3.67 (0.50)	**<0.0001**
Length (cm)	50.8 (2.0)	51.2 (2.1)	**<0.0001**
Weight SDS [2]	-0.15 (0.95)	0.15 (1.1)	**<0.0001**
Weight-for-length (%)	-1.5 (8.9)	-0.46 (8.9)	**0.026**
Weight-for-length SDS	-0.23 (1.0)	-0.098 (1.0)	**0.022**
BMI (kg/m^2)	13.6 (1.2)	13.9 (1.3)	**<0.0001**
13 months	n=900	n=363	
Weight (kg)	10.2 (1.1)	10.5 (1.2)	**<0.0001**
Length (cm)	77.2 (2.7)	77.5 (2.7)	0.080
Weight-for-length (%)	+0.17 (7.8)	+2.4 (8.7)	**<0.0001**
Weight-for-length SDS	-0.044 (0.98)	0.22 (1.03)	**<0.0001**
BMI (kg/m^2)	17.0 (1.3)	17.3 (1.4)	**<0.0001**
Weight gain (kg) from birth to 13 mo	6.6 (1.1)	6.7 (1.1)	0.110
2 years	n=789	n=315	
Weight (kg)	12.9 (1.44)	13.1 (1.51)	**0.032**
Length (cm)	88.6 (3.2)	88.6 (3.2)	0.647
Weight-for-length (%)	+1.2 (7.9)	+2.6 (8.7)	**0.009**
Weight-for-length SDS	0.070 (1.0)	0.23 (1.0)	**0.019**
BMI (kg/m^2)	16.4 (1.3)	16.7 (1.4)	**0.007**
Weight gain from birth to 24 mo (kg)	9.4 (1.4)	9.5 (1.5)	0.241
Overweight at 2 y (%) [3]	20.1% [4]	25.7% [4]	**0.038**
Obese at 2 y (%) [3]	3.8%	5.8%	0.162

[1] T-test for independent samples, except for gender and overweight/obesity chi-square test

[2] According to new population-based references for birth weight and length (Sankilampi et al. 2013)

[3] According to the new Finnish Growth References for Children BMI-for-age criteria (Saari et al. 2011)

[4] The proportion of overweight children includes also obese children

5.2.2 Glucose and insulin metabolism during pregnancy and early growth (Study I and unpublished data)

A total of 82 overweight women and 67 normal weight women from the OBERISK study completed a 2 hour oral glucose tolerance test (OGTT) with measurement of plasma glucose and insulin. From all 149 pregnant women participating to the oral glucose tolerance test, 16 (10% of all women, 13 overweight and 3 normal weight) were diagnosed with gestational diabetes. As shown in Figure 3, overweight women had higher serum glucose concentrations at 0h, 1h and 2h and higher insulin at 0h than normal weight women (P for all <0.05). Insulin resistance described with HOMA-index was more prevalent in overweight than normal weight women (2.6 vs. 1.8, P=0.022). Overweight women also had lower insulin sensitivity than normal weight women when measured with QUICKI-index (0.34 vs. 0.36, P<0.0001) and Matsuda ISI-index (5.1 vs. 7.0, P=0.001). Women with gestational diabetes or having medication affecting insulin sensitivity were excluded from these analysis.

In a model combining the influence of maternal diet quality, physical activity, maternal prepregnancy BMI, weight gain during pregnancy and glucose and insulin metabolism, the most strongest predictors for an infant's higher birth weight (kg) were maternal plasma glucose at 1h (β=0.09, P=0.01), plasma insulin at 2h (β=0.08, P=0.03), maternal prepregnancy BMI (β=0.11, P=0.001) and diet quality (β=0.06, P=0.03), (R^2=0.15, P=0.02 for the model). No associations between children's weight or BMI at 13 months and glucose and insulin metabolism during pregnancy were seen (data not shown). However, at 2 years of age, maternal fasting glucose correlated positively (Pearson correlation) with children's BMI (r=0.22, P=0.038) and weight-for-length SDS (r=0.22, P=0.042), whereas for weight or length no significant correlation was seen (unpublished data).

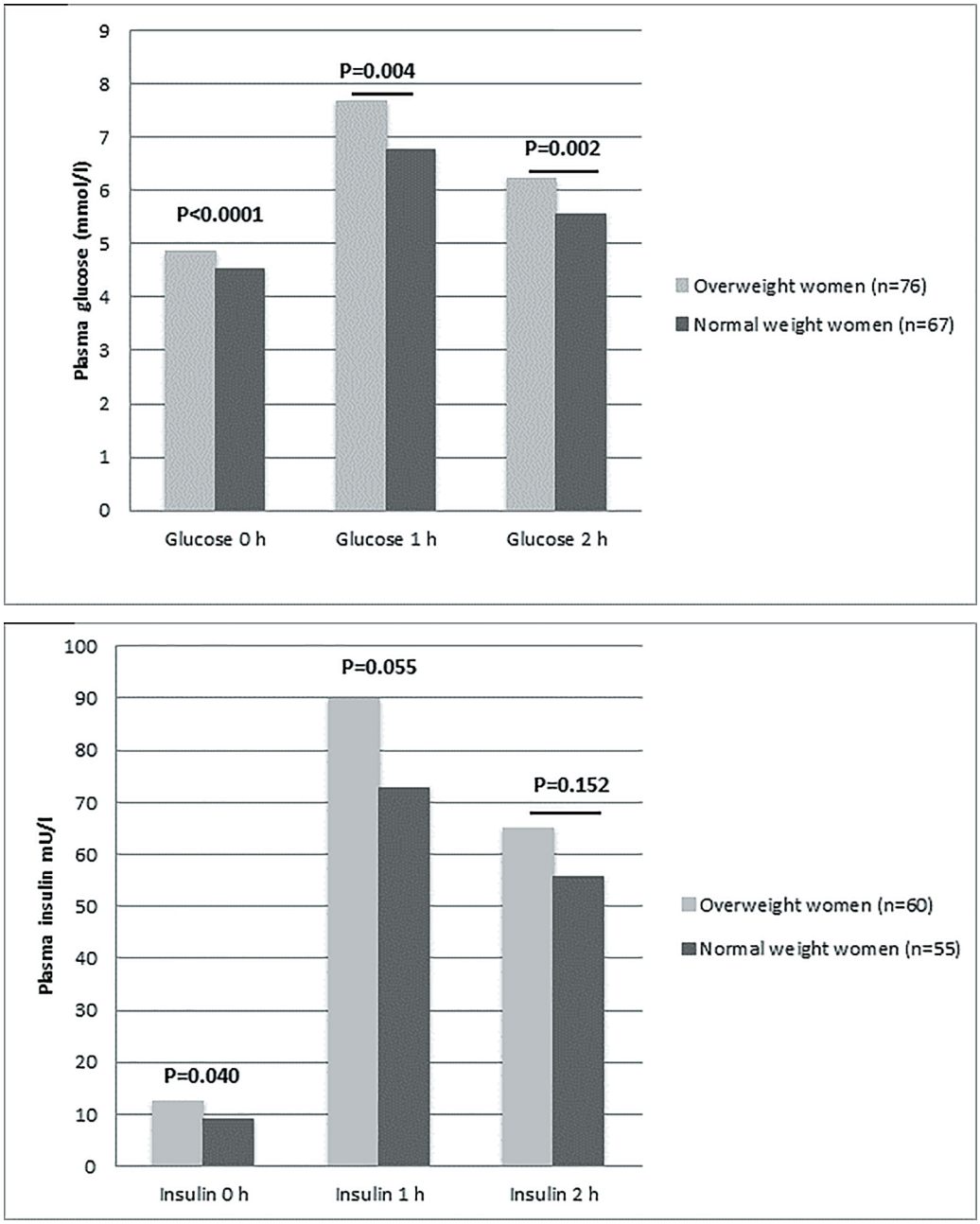


Figure 3. Glucose and insulin concentrations measured with a 2-hour oral glucose tolerance test in pregnant overweight and normal weight women. Women with gestational diabetes or having medication affecting insulin sensitivity were excluded.

5.2.3 Maternal diet quality and physical activity during pregnancy and early growth (Study I, II)

At the third trimester of pregnancy, normal weight women followed a health promoting diet more often than overweight women. A health promoting diet, measured with IDQ, was followed by 75% of normal weight women and 55% of overweight women (P=0.037). Higher maternal prepregnancy BMI correlated with a lower consumption of fish (r=-0.17, P=0.049) and whole grain products (r=-0.21, P=0.017). Overweight women ate less vegetables (P=0.02) and more frequently fast food (P= 0.01) and had a less regular meal pattern (P=0.04) than normal weight women. Physical activity assessed with a questionnaire was similar in overweight and normal weight women before (P=0.31) and during pregnancy (P=0.99). The strongest predictors for an infant's higher birth weight (kg) were maternal plasma glucose at 1h (β=0.09, P=0.01), plasma insulin at 2h (β=0.08, P=0.03), maternal prepregnancy BMI (β=0.11, P=0.001) and diet quality (β=0.06, P=0.03), (R^2=0.15, P=0.02 for the model). For birth length, the strongest predictors were maternal prepregnancy BMI and regular exercise during pregnancy (R^2=0.11, P=0.002).

5.3 Breastfeeding, introduction of complementary feeding and early growth

5.3.1 Duration of breastfeeding and early growth (Study III)

In the STEPS study, exclusive breastfeeding (EBF) continued on average for 2.6 (2.1) months, while the total duration of breastfeeding (total BF) was 8.6 (4.5) months. A longer duration of EBF and total BF was associated with lower weight gain during the first 24 months (Table 7).

Linear regression analysis was conducted to investigate the effect of BF and introduction of CF on children's weight gain from birth to 13 months and birth to 24 months (Table 8). A longer duration of EBF and PBF was associated with a lower weight gain from birth to 13 months and birth to 24 months. However, if EBF continued over 6 months and total breastfeeding duration was over 12 months, the effect was diminished. When these variables were combined in model 1 (Table 8), an association was seen for BF and lower weight gain from birth to 13 months and birth to 24 months. We further expanded the analysis by adjusting with possible confounding factors. The confounding factors were gestational age, infant birth weight, infant sex, gestational weight gain and maternal prepregnancy BMI (only in model 1). After adjusting for these confounding factors only EBF remained a statistically significant factor for weight gain from 0 to 13 months and 0 to 24 months (Table 8).

5.3.2 Introduction of complementary feeding and early growth (Study III)

Complementary foods (solid foods) were introduced to infants on average at 4.2 (1.0) months of age. The mean age at introducing infant formulas was 2.7 (3.1) months. The timing of introduction of CF was not significantly associated with weight gain from birth to 2 years (Table 8). The timing when different food groups (vegetables, fruits, grains, meat, fish, dairy) were introduced was not associated with weight gain during the first 2 years (data not shown).

The effect of BF and introduction of CF on children's weight gain from birth to 13 months and from birth to 2 years was studied with linear regression analysis. A longer duration of EBF (β=-0.037, P<0.001) and total BF (β=-0.027, P=0.01) and later introduction of CF (β=-0.057, P=0.0006) were associated with lower weight gain from birth to 13 months. Moreover, a longer duration of EBF (β=-0.039, P=0.0001) and later introduction of CF (β=-0.047, P=0.024) were associated with a slower weight gain from birth to 2 years. The total duration of BF was not associated with weight gain from birth to 2 years (β=-0.19, P=0.17). Interestingly, if EBF continued over 6 months and total breastfeeding duration was over 12 months, the effect was diminished (data not shown). When these variables were combined in model 1 (Table 8), an association was seen between BF and lower weight gain from birth to 13 months and from birth to 24 months but not for the introduction of CF. The analyses in Table 8 were further extended by adjusting with possible confounding factors, such as gestational age, infant birth weight, infant sex, gestational weight gain and maternal prepregnancy BMI (only in model 1). After adjusting for these confounding factors, only EBF remained a statistically significant factor for slower weight gain from birth to 13 months and from birth to 24 months (Table 8).

Table 7. The duration of exclusive (EBF) and total breastfeeding (total BF) and introduction of complementary foods according to weight gain from birth to 24 months was classified in percentiles (10^{th}=7.886 kg; 25^{th}=8.5 kg; 50^{th}=10.4324 kg, 75^{th}=11.4059, 90^{th}=17.65 kg). Values presented as means (SD).

	Weight gain from birth to 24 months					
	10^{th} percentile (n=84)	25^{th} percentile (n=123)	50^{th} percentile (n=433)	75^{th} percentile (n=125)	90^{th} percentile (n=83)	P (between groups)
EBF (months)	3.4 (2.0)	2.8 (2.1)	2.6 (2.0)	2.5 (2.1)	1.8 (1.9)	**0.004**
total BF (months)	10.0 (5.0)	8.7 (5.0)	8.9 (4.7)	7.9 (3.6)	7.9 (4.4)	0.055
Introduction of CF (months)	4.3 (1.0)	4.2 (0.9)	4.1 (0.9)	4.1 (0.9)	4.1 (0.8)	0.807
Introduction of infant formula (months)	2.7 (3.2)	3.0 (3.2)	2.8 (2.9)	3.0 (3.1)	3.0 (3.5)	0.973

Table 8. Multiple linear regression analysis of infant weight gain from birth to 13 months and from birth to 24 months by exclusive breastfeeding (EBF), total duration of breastfeeding (total BF) and introduction of complementary foods (CF).

		Infant weight gain from 0 to 13 months Unadjusted analysis				**Infant weight gain from 0 to 24 months Unadjusted analysis**			
Variable		β	SE	R^2	P	β	SE	R^2	P
Model 1 [1, 2]				0.03	**<0.0001**			0.02	**0.005**
EBF	<4 mo (intercept, n=716)	7.15	0.06		**<0.0001**	9.70	0.08		**<0.0001**
	4-6 mo (n=376)	-0.29	0.10		**0.003**	-0.34	0.12		**0.006**
	>6 mo (n=27)	-0.27	0.29		0.339	-0.24	0.37		0.527
Total BF	6-12 mo (n=606)	0.08	0.09		0.331	0.11	0.11		0.309
	>12 mo (n=171)	-0.12	0.11		0.293	-0.04	0.14		0.798
Introduction of CF	4-6 mo (n=697)	-0.11	0.09		0.212	-0.10	0.12		0.389
	>6 mo (n=74)	-0.31	0.19		0.104	-0.27	0.26		0.306
		Adjusted analysis				**Adjusted analysis**			
		β	SE	adj R^2	P	β	SE	adj R^2	P
Model 1 [1,2]				0.10	**<0.0001**			0.07	**<0.0001**
EBF	< 4 mo (n=716)	12.29	1.01		**<0.0001**	16.58	1.40		**<0.0001**
	4-6 mo (n=376)	-0.25	0.10		**0.012**	-0.33	0.13		**0.012**
	>6 mo (n=27)	-0.23	0.30		0.458	-0.12	0.40		0.773
Total BF	6-12 mo (n=606)	0.07	0.09		0.414	0.15	0.11		0.202
	>12 mo (n=171)	-0.03	0.11		0.807	0.10	0.15		0.495
Introduction of CF	4-6 mo (n=697)	-0.11	0.10		0.235	-0.13	0.13		0.323
	>6 mo (n=74)	-0.15	0.20		0.468	-0.14	0.28		0.615
Model 2 [1]				0.11	**<0.0001**			0.05	**<0.0001**
Prepregnancy BMI		0.006	0.009		0.473	0.005	0.01		0.657
EBF		-0.07	0.03		**0.013**	-0.09	0.04		**0.024**
Total BF		-0.02	0.01		0.063	-0.01	0.02		0.489
Introduction of CF		0.07	0.05		0.231	0.10	0.08		0.218

[1]Models were adjusted for infant sex, infant birth weight, gestational weight gain, gestational age and maternal prepregnancy BMI (only in model 1)
[2] Variables coded as dummy variables

5.3.3 Breast milk fatty acid composition and early growth (Study II)

Higher breast milk SFA correlated positively with weight gain from birth to 13 months (R=0.22, P=0.04) and with an increase in BMI from birth to 13 months (R=0.22, P=0.04). In addition, the ratio of unsaturated to saturated FAs correlated negatively with a child's BMI at 13 months (R=-0.21, P=0.05), weight gain (R=0.23, P=0.04) and increase in BMI (R=-0.24, P=0.03) from birth to 13 months. However, when analyses were adjusted for duration of FBF, the effects were diminished and no statistically significant associations were found between children's growth and breast milk FA composition (data not shown). FA composition of the breast milk did not differ between boys and girls (data not shown).

Maternal diet had an impact on milk FA composition. Higher maternal IDQ points during pregnancy correlated positively with PUFA levels in the breast milk (R=0.25, P=0.012)

but not with those of SFA (R=-0.145, P=0.15), MUFA (R=-0.05, P=0.96) or total fat content (R=-0.177, P=0.078). Maternal current diet measured with food frequency questionnaire (FFQ) correlated with breast milk fatty acids as follows. The use of fatty fish within the last two days before the sampling day associated with a 34% increase in n-3 FAs in breast milk (P=0.007). Breast milk PUFA was higher in women consuming vegetable oil-based spreads than in the other women (15.1% vs. 12.8%, respectively, P=0.001) and especially, the n-6 FA contents were higher in the breast milk of vegetable oil-based spread users (10.5% vs. 12.5%, P<0.0001). The use of high-fat dairy products correlated with the SFA levels in breast milk (R=0.21, P=0.04), while no significant correlations were found for other foods. The use of vegetable oils, butter, cheese, pastries, salad dressing, sausages and fast food did not correlate with breast milk FA distribution.

5.3.4 Maternal overweight, breastfeeding duration, introduction of complementary feeding and breast milk fatty acid composition (Study II, III)

In the STEPS study, 44% of women were overweight or obese before pregnancy (BMI≥25 kg/m^2). Prepregnancy BMI had an effect on breastfeeding duration. Normal weight mothers continued EBF (2.8 months vs. 2.2 months, P<0.0001) and total BF (9.1 months vs. 7.4 months, P<0.0001) longer than overweight mothers. Seventy-one percent of overweight and 62% of normal weight mothers discontinued EBF before infants reached the age of 4 months (P<0.05 between overweight and normal weight mothers, Figure 4). Less than 3% of normal weight women and less than 2% of overweight women continued full breastfeeding for 6 months as recommended by the WHO (2002). The total duration of BF was almost 2 months longer in normal weight mothers than in overweight mothers (9.1 months vs. 7.4 months, P<0.0001). Eighty-two percent of overweight women and 76% of normal weight women discontinued breastfeeding before infants reached the age of 12 months (P<0.001). Survival analysis of total duration of breastfeeding in normal weight and overweight mothers is presented in Figure 5.

The mean age when complementary foods were first introduced to infants was 4.2 (1.0) months. Normal weight women introduced CF to their infants 0.2 months later than overweight women (4.3 vs. 4.1 months, P=0.008). Thirty-seven percent of overweight women and 30% of normal weight women introduced CF before the age of 4 months (P<0.05; Figure 4). Shorter duration of BF associated with earlier introduction of CF. Duration of full and total BF correlated positively with age at the introduction of CF (R=0.55; R= 0.41, respectively, P<0.001). The mean age at introducing infant formulas was 2.7 (3.1) months. Normal weight mothers introduced infant formulas to their infants later than overweight mothers (3.1 vs. 2.0 months, P<0.0001). When exclusive breastfeeding ceased, the reasons were: 41% started CF and 59% started using formula milk. Therefore exclusive breastfeeding was more commonly terminated by the introduction of infant formula than by the introduction of complementary foods (solid foods). Most of the mothers (70.3%) gave infant formulas to their infants during the first four months of life.

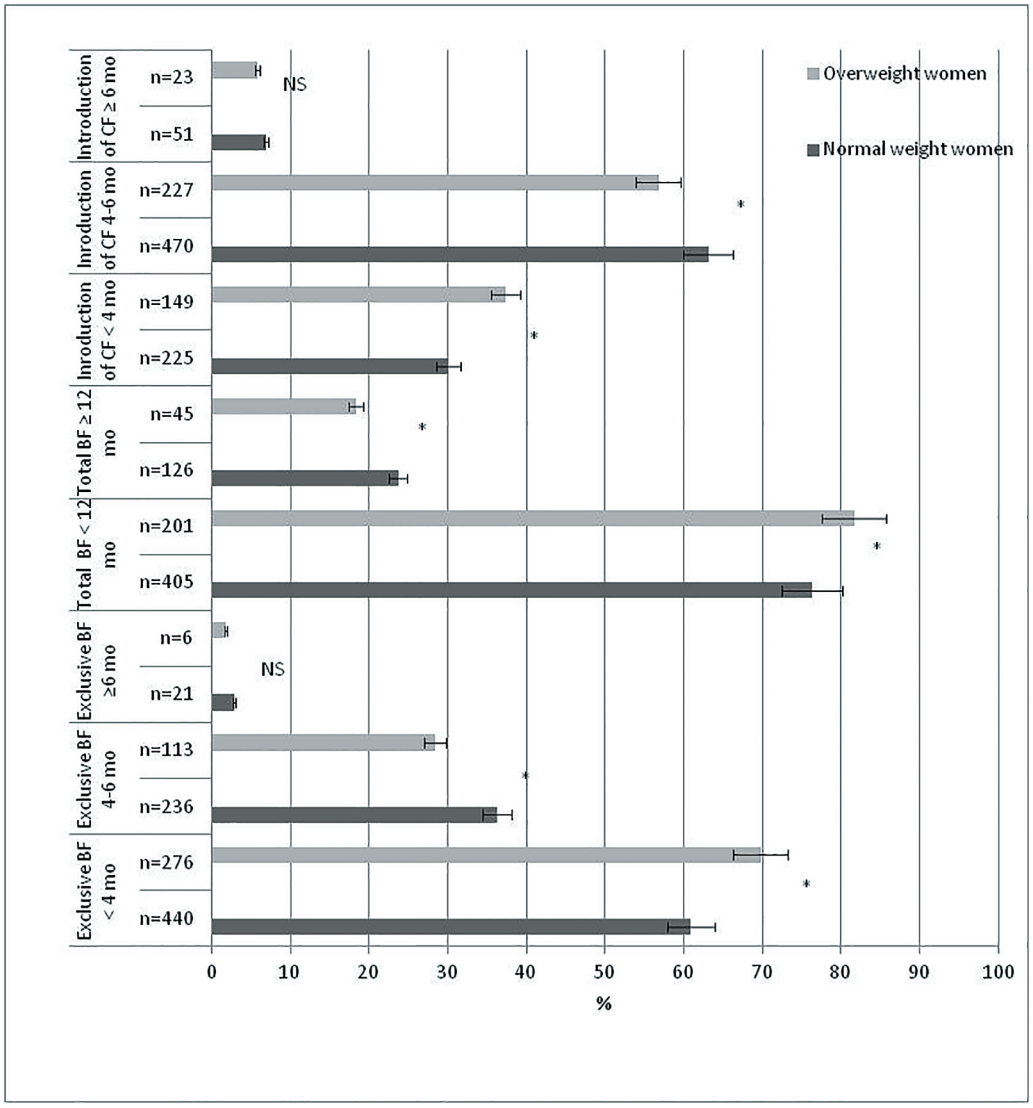


Figure 4. Exclusive breastfeeding (BF), total duration of BF and introduction of complementary foods (CF) in normal weight women (nw) and overweight women (ow). *P<0.05, NS = non-significant.

The total fat content of the milk did not differ between overweight and normal weight women (P=0.625) as shown in Table 9. The breast milk of normal weight women was significantly lower in SFA (P=0.012) and higher in n-3 FAs (P=0.010) and the ratio of unsaturated to saturated FAs and n-6 FAs to n-3 FAs was lower (P<0.05 for both) than in overweight women. The total fat content of the milk correlated positively to PUFA in the milk (R=0.21, P=0.032) and, especially, n-3 FAs (R=0.21, P=0.038). We also studied with stepwise linear regression analysis whether maternal age, education, household incomes or gestational weight gain would affect breast milk fatty acid composition. From the 100 women studied here, no one smoked during pregnancy

and therefore smoking was not included in the analysis as a confounding variable. Gestational weight gain was a significant predictor of breast milk SFA concentration (R^2=0.24, P=0.025) demonstrating again the effect of maternal weight status on fatty acid composition. For other factors, no significant associations were found. The same pattern was found with the MUFA concentration wherein gestational weight gain was a significant predictor (R^2=0.23, P=0.031), but other variables were not. For total PUFA, n-6 or n-3 fatty acids, no significant associations were found between maternal age, education, household incomes or gestational weight gain. Furthermore, maternal weight gain during pregnancy or changes in weight from prepregnancy to 13 months postpartum were not associated with the amount of fat in the breast milk (data not shown).

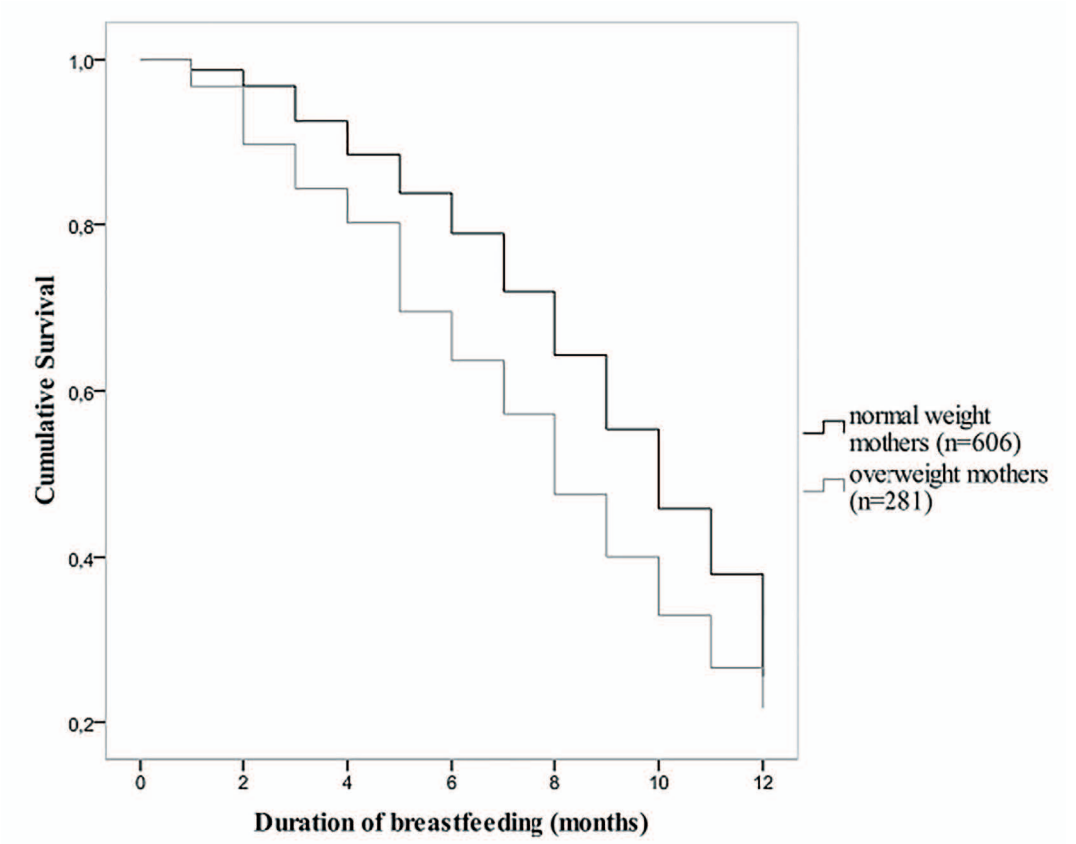


Figure 5. Survival analysis of total duration of breastfeeding between normal weight and overweight mothers.

Table 9. Breast milk fatty acid composition in overweight and normal weight women. Values presented as percentages from all fatty acids (mean (SD)) unless otherwise stated.

	Overweight women (n=51)	**Normal weight women (n=49)**	**P[1]**
Total fat (mg/ml)	31.3 (15.7)	30.0 (10.8)	0.63
Saturated fatty acids (SFA%)	46.3 (4.4)	43.6 (6.0)	**0.012**
Monounsaturated fatty acids (MUFA%)	38.8 (3.6)	40.1 (4.0)	0.080
Polyunsaturated fatty acids (PUFA%)	13.6 (2.7)	14.8 (4.0)	0.068
n-6 PUFA (%)	11.4 (2.1)	12.1 (3.4)	0.18
18:2 n-6 (Linoleic acid %)	10.5 (2.1)	11.1 (3.2)	0.22
18:3 n-6 (Gamma-linolenic acid %)	0.07 (0.06)	0.09 (0.06)	0.087
20:2 n-6 (Eicosadienoic acid %)	0.20 (0.09)	0.22 (0.08)	0.18
20:3 n-6 (Dihomo-gamma-linolenic acid %)	0.28 (0.09)	0.30 (0.10)	0.50
20:4 n-6 (Arachidonic acid %)	0.37 (0.06)	0.39 (0.09)	0.14
n-3 PUFA (%)	2.2 (0.79)	2.7 (1.1)	**0.010**
18:3 n-3 (Alpha-linolenic acid %)	1.9 (0.7)	2.0 (0.7)	0.19
20:3 n-3 (Eicosatrienoic acid %)	0.024 (0.044)	0.024 (0.046)	0.96
20:5 n-3 (Eicosapentaeonic acid %)	0.10 (0.13)	0.18 (0.22)	**0.028**
22:6 n-3 (Docosahexaeonic acid %)	0.22 (0.29)	0.46 (0.54)	**0.008**
trans fatty acids (%)	0.31 (0.18)	0.32 (0.12)	0.69
unsaturated / saturated fatty acid ratio	1.1 (0.2)	1.3 (0.4)	**0.008**
MUFA / SFA ratio	0.85 (0.15)	0.95 (0.22)	**0.011**
PUFA / SFA ratio	0.30 (0.08)	0.36 (0.16)	**0.022**
PUFA / MUFA ratio	0.35 (0.08)	0.37 (0.10)	0.31
n-6 / n-3 ratio	5.7 (1.8)	4.9 (1.6)	**0.031**

[1] Statistical test used: T-test for independent samples.

5.4 Genetic factors for weight gain and obesity (Study IV)

Genetic risk score (GRS) was calculated from 83 SNPs (GRS83) associated with weight, BMI, obesity or waist circumference in previous genome-wide association studies. The mean value for GRS83 was 78.6 (5.2), and the sum of scores ranged from 64 to 97. GRS83 was associated with a higher weight at 13 and 24 months, higher weight-for-length/height at 13 and 24 months and also with higher weight gain from birth to 13 months and from birth to 24 months (Table 10, $P \leq 0.05$). GRS83 was not associated with BMI at birth, 13 months and 2 years or waist circumference at 2 years of age ($P > 0.05$). Weight-for-length/height and BMI SDS were statistically significantly associated to GRS83 at 2 years. No difference was seen between weighted or unweighted risk scores (data not shown), and here we focused on unweighted risk scores.

Table 10. Results of linear regression analysis of unweighted and weighted risk scores for all 83 SNPs and anthropometric measurements at birth, at 13 months and 2 years of age.

		GRS83	
		Beta	**P**
Birth [1]	n=1278		
	Weight (kg)	0.027	0.530
	Length (cm)	0.007	0.827
	BMI (kg/m^2)	0.017	0.559
	Weight-for-length (%)	0.011	0.691
	Weight-for-length SDS	0.014	0.630
13 months [2]	n=958		
	Weight (kg)	0.080	**0.015**
	Length/height (n=954) (cm)	0.070	**0.046**
	BMI (n=954) (kg/m^2)	0.052	0.092
	Weight-for-length (n=954) (%)	0.053	0.084
	Weight-for length SDS (n=954)	0.053	0.086
	Weight gain from birth to 13 months (kg)	0.069	**0.036**
2 years [2]	n=842		
	Weight (kg)	0.080	**0.017**
	Length/height (cm)	0.059	0.090
	BMI (kg/m^2)	0.060	0.064
	BMI SDS	0.068	**0.045**
	Weight-for-height (%)	0.065	**0.046**
	Weight-for-height SDS	0.074	**0.022**
	Weight gain from birth to 24 months (kg)	0.074	**0.028**
	Waist circumference (n=789) (cm)	0.043	0.227

[1]Adjusted for gestational age and sex. Birth weight adjusted also for birth length.
[2]Adjusted for age and sex.

Sixteen SNPs associated with weight as a phenotype in GWAS were used to calculate a genetic risk score for weight. WeightGRS was associated with higher weight at 13 and 24 months (Table 11, $P \leq 0.05$ for both) but not with birth weight ($P > 0.05$). All analyses were adjusted for age and sex, except for weight-for-length/height. The beta for WeightGRS was 0.081 for weight at 13 months of age (P=0.014) and 0.086 for weight at 2 years of age (P=0.0011). This result indicated that one risk allele increased weight for 81 grams at the age of 13 months and 86 grams at the age of 2 years. The number of weight risk alleles in our study subjects varied between 8 and 22. Therefore, the effect on weight varied from 0.65 kg to 1.79 kg at 13 months of age and from 0.69 kg to 1.89 kg at 2 years of age. The association between the risk alleles and weight at children's age of 2 years is illustrated in Figure 6. WeightGRS was also statistically significantly associated with weight-for-length at 13 months, whereas at 2 years the association was borderline (P=0.055).

Table 11. Results of linear regression analysis of genetic risk score of 16 SNPs for weight and weight-for-length/height at birth, at 13 months and 24 months of age [1]

		WeightGRS	
		Beta	**P**
Birth (n=1278)	Weight (kg) [1]	0.034	0.422
	Weight-for-length (%)	0.025	0.371
13 months (n=958)	Weight (kg) [1]	0.081	**0.014**
	Weight-for-length (%)	0.068	**0.028**
2 years (n=842)	Weight (kg) [1]	0.086	**0.011**
	Weight-for-height (%)	0.062	0.055

[1]adjusted for age or gestational age and sex

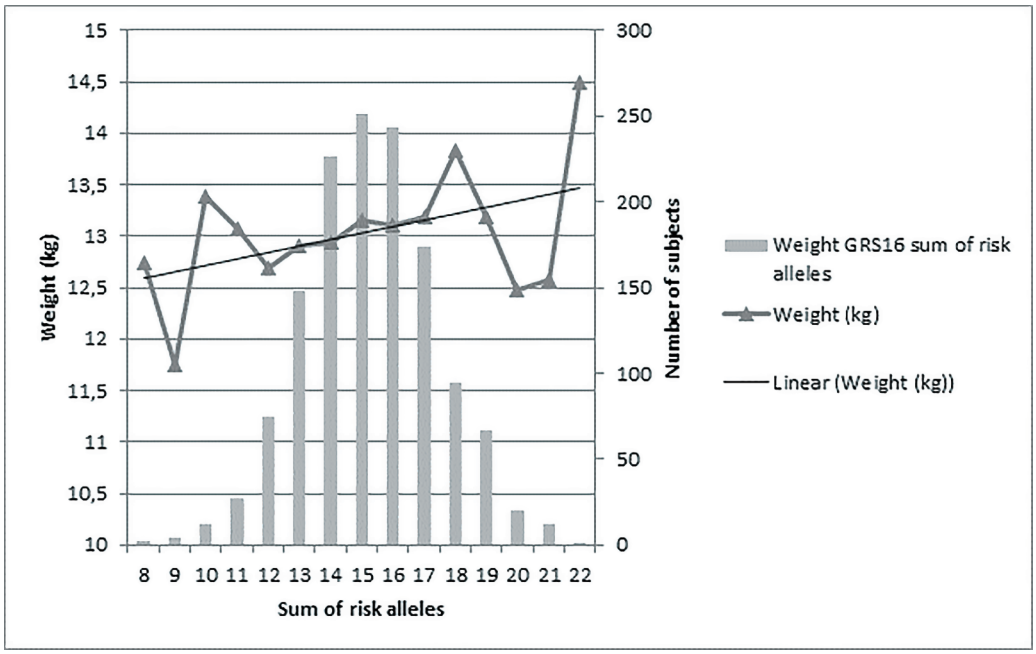


Figure 6. Association between Weight genetic risk score sum of alleles (16 SNPs) and weight at 2 years of age (P≤0.05).

Since an association between WeightGRS and weight was seen, we further expanded our analysis and divided children into three groups based on sum of risk alleles: 1) children with low genetic risk for high body weight (≤12 weight risk alleles), 2) children with medium genetic risk for high body weight (13-17 weight risk alleles) and 3) children with high genetic risk for high body weight (≥18 weight risk alleles). The cut-off points were defined based on the data being analyzed. When their anthropometrics were compared, a statistically significant difference in weight, height and weight gain from birth to 2 years was detected (Table 12). Children with high genetic risk for high body weight weighted

on average 0.6 kg more than children with low genetic risk at 2 years of age (P=0.023). The different growth trajectories are illustrated in Figure 7.

Table 12. Children with low risk (≤12 weight risk alleles) and medium risk (13-17 weight risk alleles) compared to children with high risk (≥18 weight risk alleles) for high body weight.

		≤12 weight risk alleles	**13-17 weight risk alleles**	**≥18 weight risk alleles**	**P [1]**	**P Group comparisons [2]**
Birth		n=114	n=938	n=182		
	Weight (kg)	3.65 (0.46)	3.57 (0.46)	3.59 (0.45)	0.210	0.266; 0.998
	Length (cm)	51.3 (1.9)	51.0 (2.1)	51.0 (1.9)	0.243	0.321; 0.999
	BMI (kg/m^2)	13.8 (1.3)	13.7 (1.2)	13.7 (1.2)	0.598	0.984; 0.994
	Weight SDS	-0.29 (1.0)	-0.43 (1.0)	-0.19 (0.98)	0.195	0.581; 0.230
	Weight-for-length	-1.1 (9.0)	-1.4 (8.8)	-1.2 (8.6)	0.898	1.000; 1.000
	Weight-for-length SDS	-0.35 (1.1)	-0.18 (1.0)	-0.06 (1.0)	0.181	0.748; 0.345
13 months		n=79	n=730	n=133		
	Weight (kg)	10.3 (1.1)	10.5 (1.2)	10.6 (1.2)	0.235	0.798; 0.276
	Length (cm)	77.9 (2.8)	78.0 (2.7)	78.1 (2.6)	0.831	1.000; 1.000
	BMI (kg/m^2)	17.0 (1.3)	17.1 (1.3)	17.3 (1.3)	0.165	0.704; 0.185
	Weight-for-length (%)	-0.6 (7.4)	+0.9 (8.2)	+1.6 (8.6)	0.144	0.354; 0.149
	Weight-for-length SDS	0.06 (0.91)	0.01 (1.0)	0.03 (1.1)	0.932	0.991; 0.852
	Weight gain from 0 to 13 months (kg)	6.7 (1.0)	6.9 (1.0)	7.0 (1.1)	0.190	0.384; 0.212
2 years		n=70	n=645	n=113		
	Weight (kg)	12.8 (1.1)	13.1 (1.5)	13.4 (1.5)	0.017	0.564; **0.023**
	Height (cm)	87.7 (2.8)	88.6 (3.2)	88.9 (3.3)	0.045	0.111; **0.042**
	BMI (kg/m^2)	16.6 (1.1)	16.6 (1.4)	16.9 (1.3)	0.070	0.428; 0.065
	BMI SDS	0.19 (1.2)	0.18 (1.1)	0.26 (1.0)	0.800	0.832; 0.645
	Weight-for-length (%)	+0.94 (7.0)	+1.8 (8.6)	+2.8 (7.8)	0.272	1.000; 0.368
	Weight-for-length SDS	0.05 (1.2)	0.08 (1.1)	0.15 (1.0)	0.760	0.832; 0.231
	Weight gain from 0 to 2 y (kg)	9.2 (1.0)	9.5 (1.4)	9.8 (1.4)	0.022	0.125; **0.021**

[1] ANOVA

[2] The group comparison (low risk vs. medium risk; low risk vs. high risk) is given Bonferroni corrected

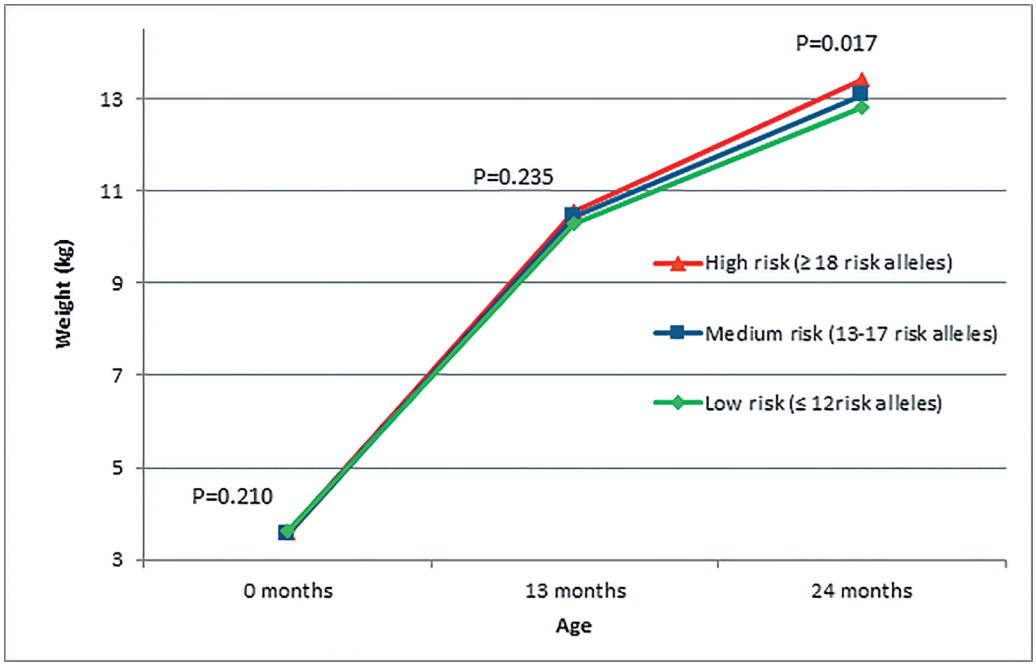


Figure 7. Children with low, medium and high genetic risk for higher body weight and weight development from birth to 2 years of age.

5.5 Being overweight and obese at 24 months (Study III, IV and unpublished data)

At the age of 24 months 25.7% of boys and 17.3% of girls were overweight or obese and 4.6% and 4.2% obese, respectively (Table 5). Being overweight and obese was defined according to the Finnish Growth References for Children age- and sex-specific BMI-criteria (Saari et al. 2011). Children born to overweight mothers were more likely to be overweight or obese at 24 months than children born to normal weight mothers (25.7% vs. 20.1%, P=0.038, Table 6). Children of overweight women were heavier (13.1 kg vs. 12.9 kg, P=0.032), had higher BMI (16.7 vs. 16.4, P=0.007), had higher weight-for-length (2.6 % vs. 1.2 %, P=0.009, Table 6) at 24 months of age than children of normal weight women. A repeated measures ANOVA with a Greenhouse-Geisser correction determined that mean weight of children of normal weight, overweight or obese mothers at 13 months and 2 years differed statistically significantly between time points (F=6.489, P<0.0001). Post hoc tests using the Bonferroni correction revealed that the differences in weight between children of normal weight, overweight or obese mothers were statistically significantly different at all time points and children of overweight women were heavier than children of normal weight women (P<0.0001 for all).

Overweight and obese children showed different growth trajectories early in life. Children who were overweight or obese at 24 months had a higher BMI at birth and at 13 months of age when compared to children who were normal weight at the age of 24 months (P<0.05 for all, Figure 8). Children who were overweight or obese at 24 months had also a higher weight-for-length SDS at 13 months and 24 months of age when compared to children who were normal weight at the age of 24 months (P<0.05 for all, Figure 9). In girls, there was also a statistically significant difference in weight-for-length SDS at birth (P=0.007), whereas in boys, there was only a tendency to the same direction (P=0.07). However, birth weight SDS was higher in boys who were overweight or obese at 2 years of age compared to boys who were normal weight (0.23 vs. -0.18, P<0.0001), and a similar association was seen in girls also (-0.06 vs. 0.24, P=0.002, respectively).

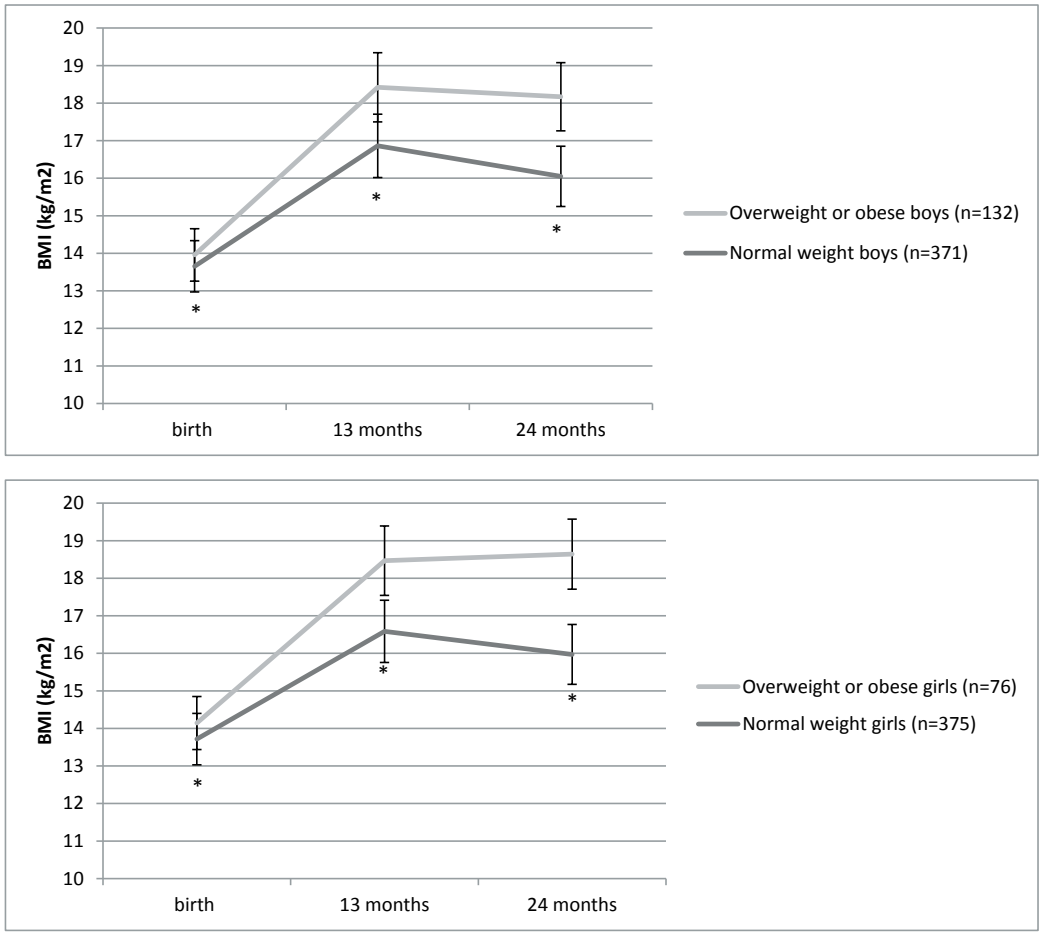


Figure 8. BMI (SE) from 0 to 2 y in boys and girls who were normal weight or overweight/obese at 2 y of age. *P≤0.05.

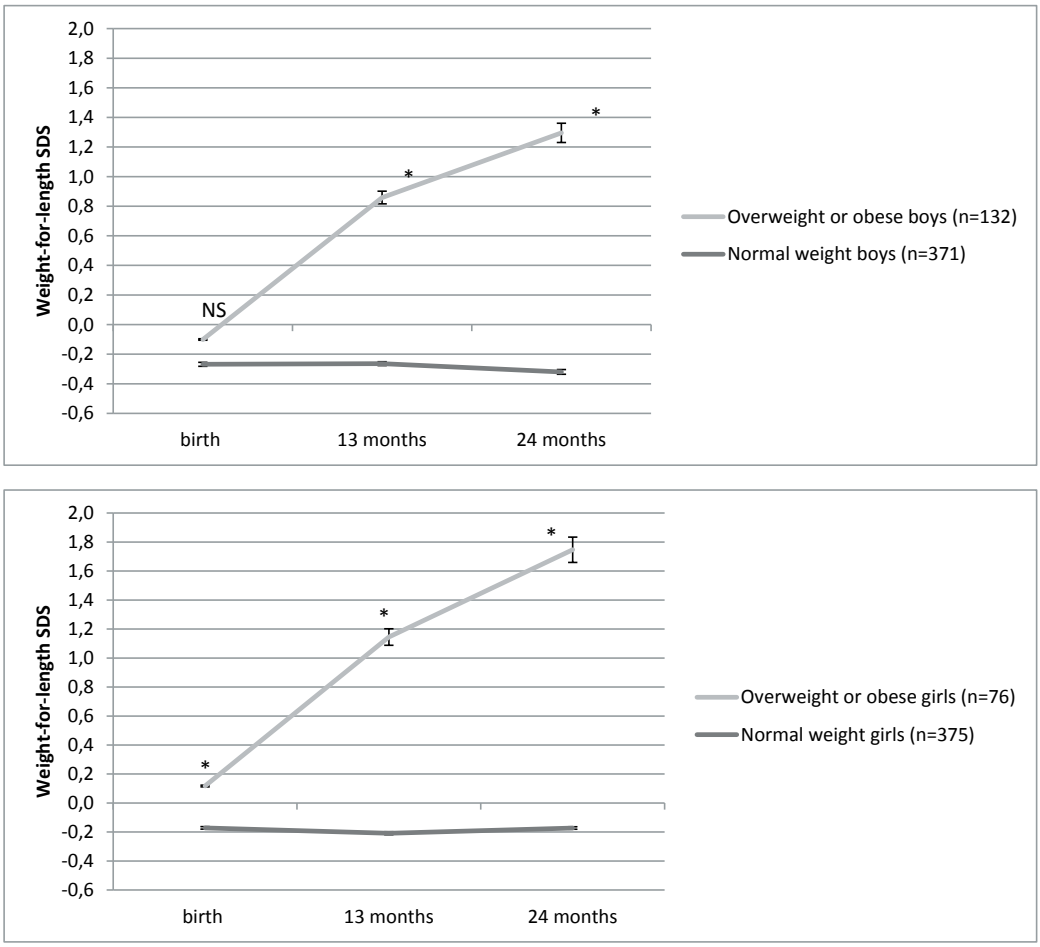


Figure 9. Weight-for-length SDS from 0 to 24 mo in boys and girls who were normal weight or overweight/obese at 24 months of age. Differences in weight-for-length SDS between overweight or obese and normal weight boys were statistically significant at 13 and 24 months ($P<0.0001$) but not at birth ($P=0.07$). In girls, all differences were statistically significant ($P\leq0.05$). Only boys and girls with all weight and length measurements are included. * $P\leq0.05$, NS= non significant.

Early nutritional factors, a longer duration of BF and later introduction of CF were protective against obesity at 24 months but not for being overweight in unadjusted models (Table 13). However, in the adjusted models, none of them were statistically significantly protective. The association between electronic media time and sleep time with anthropometrics at 2 years were also studied but no association was found (data not shown).

Table 13. Results from unadjusted and adjusted logistic regression analyses with being overweight or obesity at 2 y as outcomes

	Unadjusted				Adjusted[1]			
	Overweight		Obesity		Overweight		Obesity	
	OR	P	OR	P	OR	P	OR	P
EBF	1.01	0.784	0.86	**0.047**	0.82	0.099	0.72	0.056
Total BF	0.96	0.079	0.91	**0.024**	0.99	0.327	0.92	0.117
Introduction of CF	1.03	0.751	0.69	**0.025**	1.08	0.436	0.73	0.092
Introduction of infant formulas	0.99	0.759	1.01	0.644	1.03	0.833	1.02	0.609

[1]Analyses of all four models (EBF, total BF, introduction of CF and introduction of infant formulas) adjusted for sex, gestational weight gain, gestational age, birth weight, maternal prepregnancy BMI, maternal age, maternal smoking and maternal education.

5.6 Combined effect of genetic, prenatal and postnatal risk factors on being overweight and obese at 24 months (unpublished data)

Prenatal, postnatal and genetic obesity risk factors and being overweight and obesity at 24 months of age were studied with logistic regression analysis (Table 14). The examination of individual risk factors revealed that a higher gestational weight gain (>13.9 kg), low (<2500 g) or high (>4500 g) birth weight, higher genetic risk (>84 risk alleles) and a lower duration of breastfeeding (<4 months) were statistically significantly associated with a higher risk of being overweight and obese at 24 months. When all the individual risk factors presented in Table 14 were combined to Obesity Risk Score (Table 4) and scores calculated, a significant increase in odds ratios was discovered. The Obesity Risk Score ranged in these subjects from 0 to 9 and the mean value was 2.8 (1.8) points. Compared to subjects with 0 points, the ones with the highest points (9) had almost six times higher obesity risk (P<0.0001).

To evaluate the accuracy of the calculated Obesity Risk Score, we conducted ROC analysis (Fig 10). The ROC analysis of individual risk factors resulted in area under curve (AUC) between 0.49-0.54 and none of the individual risk factors were statistically significant. However, when these risk factors were combined in Obesity Risk Score, the AUC was 0.82 (95% CI, 0.77-0.87) and showed high accuracy in predicting overweight and obesity at 24 months compared to individual risk factors.

Table 14. Logistic regression analysis on overweight and obesity at 24 months of age and potential risk factors. Preterms (<37 gestational weeks) excluded.

		Overweight or obesity at 24 months [1]	
		OR	**95% CI**
Gestational weight gain	<13.9 kg (n=515)	1.00	
	13.9-17.5 kg (n=270)	**1.40 ***	1.01-1.94
	>17.5 kg (n=247)	**1.41 ***	1.01-1.97
Maternal prepregnancy weight	Normal weight (n=849)	1.00	
	Overweight/Obesity (n=328)	1.28	0.97-1.69
Maternal gestational diabetes	No (n=1040)	1.00	
	Yes (n=144)	1.24	0.85-1.80
Birth weight	<2500 g (n=38)	**1.39 ***	1.13-2.01
	2500-4500 g (n=1117)	1.00	
	>4500 g (n=27)	**2.77 ***	1.29-5.96
Genetic risk	Low risk (<73 risk alleles, n=170)	1.00	
	Medium risk (73-84 risk alleles, n=593)	1.11	0.69-1.78
	High risk (>84 risk alleles, n=149)	**1.83 ***	1.57-2.21
Maternal smoking	No (n=752)	1.00	
	Yes (n=30)	1.97	0.94-4.14
Maternal education level	Low (no college degree, n=458)	1.13	0.89-1.43
	High (at least college degree, n= 727)	1.00	
Family income	<2000 €/month (n=237)	1.20	0.80-1.50
	≥2000 €/month (n=948)	1.00	
Total duration of breastfeeding	< 4 months (n=110)	**1.36 ***	1.12-1.98
	4-8 months (n=170)	1.32	0.94-2.09
	>8 months (n=372)	1.00	
Obesity Risk Score [2]	0 points (n=101)	1.00	
	1 points (n=152)	1.06	0.54-2.01
	2 points (n=224)	1.05	0.85-2.00
	3 points (n=274)	**1.38 ***	1.12-2.15
	4 points (n=190)	**1.59 ***	1.21-3.10
	5 points (n=147)	**1.90***	1.29-3.22
	6 points (n=97)	**2.61 ***	1.65-3.90
	7 points (n=90)	**3.97 ***	2.27-4.41
	8 points (n=76)	**4.47 ***	3.12-5.26
	9 points (n=57)	**5.92 ***	4.32-6.90

[1] According to the new Finnish Growth References for Children BMI-for-age criteria (Saari et al. 2011)

[2] Obesity Risk Score calculated from risk factors presented in the table previously, scores ranging from 0 to 12 (in these subjects 0 to 9).

*P≤0.05

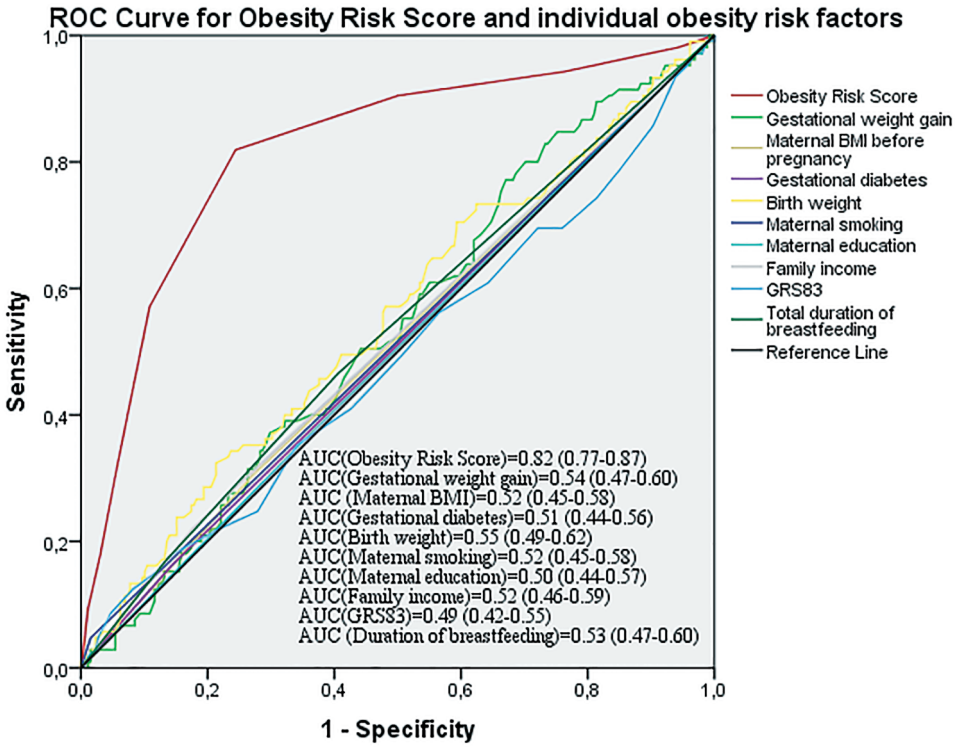


Figure 10. Accuracy of individual obesity risk factors and combined Obesity Risk Score in 24-month-old overweight and obese children. Area under curve (AUC) results and associated 95% CIs are shown.

6. DISCUSSION

6.1 Main findings

The main finding of this thesis was that a clustering of obesity risk factors predisposed children to higher rates of being overweight and obesity already at 24 months of age. Whilst single prenatal, postnatal and genetic obesity risk factors showed low predictive rates for being overweight and obese, the combined Obesity Risk Score highlighted the effect of clustering of these risk factors. The growth trajectories for children who were overweight at 24 months were already different at the age of 13 months and for girls even at birth. Many of the risk factors were confounding to each other and tended to cluster within families. A longer total duration of breastfeeding was protective against excessive weight gain, high BMI, high body weight and high weight-for-length SDS during the first 24 months of life. Children of overweight mothers were more often overweight at 24 months of age when compared to children of normal weight mothers. Overweight mothers also breastfed for a shorter time and introduced complementary foods earlier, thus predisposing the children to a higher risk of being overweight. However, the introduction of complementary foods was mostly within the recommendations even among overweight mothers. Moreover, genetic risk factor clustering had an effect on body weight at 2 years of age.

6.2 The effect of prenatal obesity determinants on weight and weight gain

Children of overweight mothers were heavier and had higher BMI and weight-for-length/height at birth, at 13 months and 2 years of age compared to children of normal weight mothers. According to Perinatal Statistics in the Nordic Countries (National Research and Development Centre for Welfare and Health 2012), the mean birth weight in Finland in 2010 was 3.49 kg. In our study, the mean birth weight in children of overweight mothers was 3.67 kg and 3.53 kg in children of normal weight women. This result suggests that infant's birth weight is increased when maternal prepregnancy BMI exceeds 25 kg/m^2. Indeed, parental size and infant birth weight are related through various genetic and environmental mechanisms, and the link between maternal and fetal size may represent contributions of the intrauterine environment (Griffiths et al. 2007). In addition to a higher obesity risk, high birth weight is associated to increased disease risk later in life (Dabelea et al. 1999; Harder et al. 2007). The effect may be partly explained by maternal glucose metabolism and raised fetal insulin levels, but it was also discovered that maternal BMI is also an independent predictor of birth size.

In this study otherwise healthy pregnant overweight women had higher plasma glucose and insulin concentrations, increased prevalence of insulin resistance and lower insulin sensitivity than normal weight women. This may contribute to health risks and result in adverse health outcomes for both the mother and the fetus. The hypothesis that increased

glucose concentrations, even before reaching levels traditionally considered as gestational diabetes, are associated with accelerated fetal growth (Ong et al. 2008) is supported by these results, because these effects were seen in these healthy women. Glucose transported from mother to the fetus may raise fetal insulin levels resulting in fetal overgrowth, which may have long-term health effects. Previous results show that increased size at birth is associated with an increased likelihood of adiposity in later life and with alterations in glucose metabolism and β-cell function (Catalano 2007). Moreover, maternal hyperglycaemia is associated with childhood obesity and glucose intolerance later in life (Hillier et al. 2007). This is in accordance with the results from this study, wherein overweight women who had elevated plasma glucose and insulin concentrations gave birth to infants with increased birth size compared to infants of normal weight women. Moreover, the prevalence of being overweight at 2 years of age was higher in children of overweight mothers than in children of normal weight mothers in the STEPS Study.

Previously a strong relation between maternal BMI and birth weight, fetal adiposity and hyperinsulinism was demonstrated by the researchers in the HAPO Study (HAPO Study Cooperative Research Group 2009; HAPO Study Cooperative Research Group 2010). They showed that both maternal BMI and glycaemia have strong independent associations with a range of clinically important pregnancy outcomes. Maternal BMI may influence the offspring's weight and length rather than fetal adiposity, which is more influenced by maternal hyperglycaemia (Ong et al. 2008). Results in this study were in accordance with this hypothesis since maternal plasma glucose at 1h, plasma insulin at 2h, maternal prepregnancy BMI and diet quality influenced mostly the infant's birth weight. Interestingly, in the regression analysis, the strongest predictors for maternal glucose metabolism were prepregnancy BMI and maternal weight gain during pregnancy, whereas lifestyle factors had smaller influence. However, lifestyle factors impact BMI and weight gain during pregnancy, and this result emphasizes the indirect effects of lifestyle factors on maternal glucose and insulin metabolism.

Normal weight women adhered better to nutrition recommendations than did normal weight women when measured with IDQ. However, during lactation, there were no significant differences in diet between normal weight and overweight women. This may be explained by the fact that many mothers improve their diet quality during pregnancy and lactation period. This also demonstrates that pregnancy can be a successful time for health-related interventions (Gresham et al. 2014). During pregnancy, 2.8% of mothers smoked, which is lower than the 15% prevalence of smoking during pregnancy reported previously in Finland (Tikkanen 2008). At a children's age of 2 years 3.4% of mothers smoked.

6.3 Early nutritional determinants

6.3.1 Breastfeeding and introduction of complementary foods

In the STEPS Study, the mean duration of exclusive breastfeeding was 2.6 months and total breastfeeding 8.6 months, which is in concordance with previous results reported in

Finland (Hasunen et al. 2005). However, compared to a more recent survey of over 5000 families in Finland (Uusitalo et al. 2012), at 4 months of age breastfeeding was more prevalent in our study sample (81% in our study vs. 68% in nationwide survey) as was exclusive breastfeeding (30% in our study vs. 23% in nationwide survey).

However there were differences in data collection between the STEPS Study and the nationwide survey, and the impact of different data collection methods on the results cannot be excluded. Consistently, the STEPS Study mothers breastfed longer when compared to type 1 Diabetes Prediction and Prevention (DIPP) project in Finland wherein the duration of exclusive breastfeeding was 1.8 months and total breastfeeding 7.0 months (Erkkola et al. 2005). Compared to breastfeeding rates in other Nordic countries (Hörnell et al. 2013), Finland and this study have significantly lower breastfeeding rates. The lower breastfeeding rates in Finland can be due to various reasons such as lack of systematic breastfeeding counseling or differences in maternity ward breastfeeding practices. In our study, the mean age at introduction of CF was 4.2 months, which is in concordance with a nationwide survey (Uusitalo et al. 2012). Infant formulas were introduced on average at 2.7 (3.1) months of age.

Overweight women introduced complementary foods and infant formulas earlier than normal weight women. To our knowledge, this was the first study to have reported this phenomenon. Previously, maternal age, smoking and parental education have been found to be associated with timing of introducing complementary foods (Betoko et al. 2013; Erkkola et al. 2013; Uusitalo et al. 2012). These results show that the mother being overweight impacts infant feeding patterns since women who were overweight before pregnancy also breastfed for shorter time, which is in accordance with previous results (Baker et al. 2007; Kitsantas and Pawloski 2010; Li et al. 2003; Liu et al. 2010a; Mok et al. 2008b; Oddy et al. 2006; Wojcicki 2011). Normal weight women had better adherence to breastfeeding recommendations by the WHO than did overweight women. Previous studies have shown that a reduced duration of breastfeeding (Chivers et al. 2010; Hörnell et al. 2013; Monasta et al. 2010) and/or earlier introduction of complementary foods (Huh et al. 2011; Schack-Nielsen et al. 2010) may predispose children to being overweight or obese.

A longer duration of breastfeeding (≥ 4 months) and later introduction of complementary foods (≥ 4 months) were protective against obesity at 24 months of age in an unadjusted analysis, but not after adjusting the analysis for possible confounding factors. Most previous studies have found a protective association between breastfeeding and obesity risk (Chivers et al. 2010; Hörnell et al. 2013; Monasta et al. 2010; Owen et al. 2005; Arenz,et al. 2005; Harder et al. 2005), whereas others have not (Martin et al. 2013; Michels et al. 2007). This may be due to the high complexity of different variables that affect weight gain and the development of obesity in children, which also explains the modest predictive rates of the models. Also the end point investigated here, 24 months, is rather early for determining obesity and some of the associations may not be seen yet. Moreover, breastfeeding is confounded by socio-economic factors, which are known

to affect obesity risk (Wadsworth et al. 1999). Exclusive breastfeeding was associated with children's lower weight gain from birth to 13 and 24 months. Previous studies have also shown that slower weight gain associates with breastfeeding and is protective against later obesity (de Hoog et al. 2011; Durmus et al. 2011; Hörnell et al. 2013). Even though overweight women introduced CF to their children earlier than normal weight women, it was not associated with weight, weight gain, BMI or weight-for-length/height. However, even the overweight women introduced complementary foods within national recommendations limit, which could explain why no association to early growth was seen. Previous studies have shown that a longer duration of breastfeeding is associated with later introduction of complementary foods (Baker et al. 2004), which may further protect against obesity (Huh et al. 2011; Schack-Nielsen et al. 2010).

A longer duration of exclusive breastfeeding was associated with lower weight gain from birth to 2 years. Indeed, a lower weight gain during infancy may reduce the risk of later obesity (de Hoog et al. 2011; Durmus et al. 2011). Interestingly, in our study, the total duration of breastfeeding or timing of introduction of complementary foods did not have such a clear effect on weight gain from birth to 2 years of age as did the duration of exclusive breastfeeding. However, a longer total duration of breastfeeding and later introduction of complementary foods showed a small protective effect against obesity at 2 years of age. Also other studies have found this association (Chivers et al. 2010; Monasta et al. 2010; Owen et al. 2005). Previous results show that infant weight gain is associated with the duration of BF and timing of CF introduction together with maternal prepregnancy BMI (Baker et al. 2004). Based on these results, the researchers concluded that prepregnancy BMI should be taken into account when examining the association between breastfeeding and later growth. Therefore also we included maternal prepregnancy BMI in our models as a covariate even though we found no association between maternal prepregnancy BMI and child's risk of obesity at 24 months of age in our study.

6.3.2 Breast milk fatty acid composition

The breast milk fatty acid (FA) composition differed between overweight and normal weight women. Overweight women had a significantly higher amount of total saturated fatty acids (SFA) and lower amount of n-3 FAs, especially 20:5 n-3 and 22:6 n-3, in their breast milk than normal weight women. In addition, the ratio of unsaturated to saturated FAs was significantly lower and the ratio of n-6 to n-3 was higher in overweight women than in normal weight women. These results suggest that normal weight women have more essential FAs and other unsaturated FAs in their breast milk than overweight women. The dietary intake of FAs was similar between overweight and normal weight mothers during the week before sampling. The mean total fat content of the breast milk in this study was 3.1%, which is similar with previous results (fat 3.22% ± 1%) from banked donor milk analysis in the USA (Wojcik et al. 2009). Maternal weight gain during pregnancy and the amount of fat in her breast milk were not associated or were

the mother's weight change from pre-pregnancy to 13 months postpartum associated with the amount of fat in the breast milk.

Two previous studies have investigated breast milk PUFA and maternal weight. One found no association between maternal BMI and breast milk fatty acid composition (Scholtens et al. 2009) and the other conducted in vulnerable population found an association between maternal obesity and higher PUFA concentration (Marin et al. 2005). These somewhat conflicting findings can be explained by the fact that in a vulnerable population, higher BMI may actually be a sign of higher accessibility to foods containing polyunsaturated fatty acids. In a previous study by Powe and coworkers (Powe et al. 2010), infant gender was found to influence the energy content of the breast milk. They showed that male gender was associated with higher breast milk energy content. In this study, infant gender had no effect on breast milk fat content or FA distribution. This suggests that the association discovered by Powe and coworkers may be related to an increase in breast milk carbohydrate or protein content instead of fat content.

Weak associations were detected between breast milk FA composition and the child's growth from birth to 13 months of age, but these effects of FA composition on growth were diminished after adjusting for total duration of breastfeeding. These results are in accordance with previous research (Scholtens et al. 2009) and meta-analyses (Gibson et al. 2001; Lapillonne & Carlson 2001; Makrides et al. 2005) where no clear associations between n-3 and n-6 LCPUFA and weight and length gain in term infants were found. On the other hand, some previously conducted experimental studies suggest that the main n-6 LCPUFA (arachidonic acid, 20:4n-6) may promote adipose tissue development, whereas n-3 LCPUFA could reduce an excessive increase in adipose tissue (Ailhaud et al. 2006). The content of n-6 PUFAs in breast milk of US women has increased steadily from 6 to 7% to as high as 15 to 16% of total FAs between 1945 and 1995, whereas the main n-3, α-linolenic acid (18:3 n-3), has remained unchanged at approximately 1% (Wojcik et al. 2009). Thus, the ratio of n-6 to n-3 has increased during these years whereas at the same time, childhood obesity has reached epidemic levels. Whether these two phenomenons are linked together remains currently unknown. In our study, the ratio of n-6 to n-3 in the breast milk was not associated with children's growth. However, we further expanded the analysis to other FAs and discovered that a higher content of SFA in milk correlated with higher weight gain in children from birth to 13 months. To our knowledge, no previous studies have reported this phenomenon. However, since no data on total energy intake exists and the sample size and thus the power of analysis is relatively small, no conclusions can be drawn from this. Therefore, further studies with larger number of subjects are needed to investigate this interesting hypothesis.

6.4 Genetic risk factors of obesity and weight gain

Genetic risk score calculated from 83 SNPs (GRS83) previously identified in GWASs is associated with children's weight at 13 months and 24 months of age. These results indicate

that increased genetic risk for higher body weight may be manifested as early as 13 months of age. Warrington et al. (2013) showed that some adult BMI increasing alleles have an effect on childhood growth as early as the age of 1 year (Warrington et al. 2013). Findings in this study support this theory since no association was seen between GRS83 and birth weight, and the association manifested at 13 months of age and remained significant at the age of 24 months. GRS83 was also statistically significantly associated with weight SDS at 13 months and 24 months. Indeed, it has been postulated that the genetic influence on adiposity emerges during childhood and heritability of BMI and the influence of SNPs associated with obesity increase over time (Llewellyn et al. 2014) probably as a result of an obesogenic environment (Rokholm et al. 2011). Recently a study showed an association between genetic risk score and newborn adiposity and large-for-gestational-age at birth, therefore demonstrating possible genetic effect also on birth size outcome (Chawla et al. 2014). In our study no such association was seen, which may be explained by the fact that in our study infants had birth weights within the normal range for gestational age.

In addition to GRS83, weightGRS was calculated from the 16 SNPs associated with weight in GWASs and found results in concordance with those of GRS83. These results showed no association with birth weight, but a significant association with weight at 13 months and 24 months. WeightGRS was associated also with higher weight gain from birth to 24 months possibly reflecting faster accumulation of adipose tissue. The effect size per allele in WeightGRS varied from 0.095 kg to 0.109 kg which is in accordance with previous studies (Elks et al. 2012; Warrington et al. 2013). WeightGRS was also associated with greater length in children, a phenomenon which has been discovered also in other studies (Elks et al. 2010; Elks et al. 2012) and possibly reflects a well-known effect of excess weight gain on growth in length. When children were divided into three groups based on their genetic risk for higher body weight, we discovered that at 24 months of age, children with high risk were 600 grams heavier than children with low risk. The difference was 300 grams at 13 months of age, which demonstrates that the genetic effect accumulates as children grow older. These results suggest that the genetic factors influence weight and growth early in life and may accumulate over the life course. However, growth and obesity development are complex traits and depend on many environmental factors as well as gene-environment interactions (Rokholm et al. 2011; Wardle et al. 2008; Warrington et al. 2013).

Interestingly, we found no association between GRS83 and BMI at birth, 13 months or 24 months of age or obesity at 24 months of age. This may be due to the fact that early onset obesity leads also to an increase in length/height (Ong 2006) in which case the effect on BMI is diminished at first. Recently, Warrington et al. (2013) discovered an association between BMI and genetic risk score in children (Warrington et al. 2013). Of the studied two cohorts, one found the association at 1 year of age and the other at 2.5 years of age. Therefore, it may be that the effect was not yet manifested in our study at 24 months of age. Indeed, the genetic background of BMI and other complex traits are known to differ according to ethnicity and age (Klimentidis et al. 2011).

6.5 Being overweight and obese in young children and clustering of obesity risk factors

Twenty-six percent of boys and 17% of girls were overweight and from these 5% and 4%, respectively, were obese at 24 months of age according to new national Finnish Growth References for Children BMI-for-age criteria (Saari et al. 2011). These growth references are based on measurements of over 70000 Finnish children born in years 1983 to 2008 and can be considered as valid growth standard in Finnish children. According to New Finnish Growth References for Children, using a BMI-criteria, 22% of boys and 12% of girls were overweight and 4% and 2% were obese in Finland, respectively (Saari et al. 2011; Working group appointed by the Finnish Paediatric Society 2013). Thus, in this study, the rates of being overweight and obese in 24-month-old children were somewhat higher.

Children who were overweight or obese at 24 months of age had higher BMI at birth, at 13 months and at 24 months of age when compared to children who were normal weight at 24 months of age. Overweight or obese children also had higher weight-for-length/height and higher weight-for-length SDS already at 13 months of age. These results suggest that the growth trajectories of children later becoming overweight or normal weight may differ already at the age of 13 months. More importantly, being overweight at 24 months of age may predict being overweight later in childhood (Lagström et al. 2008).

A study by Danielzik and colleagues (Danielzik et al. 2002) showed that the parent being overweight increases the risk of having an overweight or obese child. From severely obese children, 80% had at least one parent overweight or obese and 25 to 30% had parents who were both overweight and/or obese. In this study, children of overweight mothers were more likely to be overweight or obese at 24 months of age than children of normal weight mothers. Children, whose mothers were overweight, were heavier and had a higher BMI at 24 months of age than children whose mothers were normal weight. Children of overweight women gained more weight from birth to 24 months of age compared to children of normal weight women, but this association was not seen in linear regression analysis. All these factors can indicate a tendency for a higher obesity risk at a later age. A previous study has reported that obese women had heavier infants at 1 year of age (Edwards et al. 1978), which supports our findings.

Longer duration of breastfeeding and later introduction of complementary foods were protective against obesity at 24 months in the unadjusted analysis, but after adjustments for confounding factors the protective effect diminished. This may be due to many factors associated with breastfeeding such as socio-economic class, maternal overweight and smoking. Indeed, it has been postulated that the protective effect of breastfeeding may be partially related to these other factors (Wadsworth et al. 1999). On the other hand, in this study a longer duration of exclusive breastfeeding was associated with lower weight gain from birth to 24 months, which may reduce the risk of later obesity (de Hoog et al. 2011; Durmus et al. 2011).

In addition to early nutritional obesity determinants, also other determinants have shown relatively small effect sizes on weight development and obesity risk (Bammann et al. 2014; Monasta et al. 2011). This is probably due to complex nature of obesity development which is affected by many genetic and environmental factors. The aim of this thesis was to produce more comprehensive knowledge on clustering of obesity risk factors. Therefore the Obesity Risk Score was calculated and it showed how clustering of different risk factors increase the risk of overweight and obesity by almost six-fold as early as 24 months of age. The ROC analysis of the Obesity Risk Score revealed that it had a good accuracy in predicting overweight and obesity at 24 months of age. No previous studies using similar risk factor clustering methods were found. The factors for the Obesity Risk Score were chosen based on the data available and scientific knowledge on factors contributing to obesity development in childhood (Bammann et al. 2014; Monasta et al. 2011).

6.6 Strengths and limitations

Study design and subjects

The main strength of the studies in this thesis was the use of a well-characterized follow-up study cohort representing children born between 2008 and 2010 in Southwestern Finland (Lagström et al. 2013). In addition, birth data and data related to pregnancy and delivery were obtained from the Longitudinal Census Files, which is a reliable data source. One major strength of the study is that it began already during pregnancy and it has enrolled the whole family: mothers, spouses and children. The multidisciplinary study design and study methods enabled the investigation of obesity risk clustering and calculation of Obesity Risk Score, which is one of the major strengths in this thesis.

A limitation of this study is that only 1797 (18%) pregnant women from the total of 9811 participated in the STEPS Study. However, the drop-out analysis has been carried out with the help of birth cohort register data and showed no significant selection bias in terms of outcomes studied in this thesis (Lagstrom et al. 2013). In follow-up studies like the STEPS Study, loss-to-follow-up needs to be carefully taken into account. Unfortunately, we have no data on reasons why families dropped-out. However, at 13 months of age, the number of drop-outs was 107 (6%), which can be considered small. The drop-outs did not differ from participants in any of the variables investigated in this study. The proportion of non-responders is however larger. At 13 months of age, 28% of families did not answer questionnaires or attend study visits and at 24 months, the proportion of non-responders had increased to 48%. When comparison against participants and non-participants was done, it revealed that non-responding was more prevalent in non-primiparious families, in lower occupational class, in rural areas and in families with premature babies. Birth anthropometrics or maternal prepregnancy BMI did not differ between non-responders and responders.

The selection bias regarding this study can be considered relatively small, since key variables (birth anthropometrics and maternal prepregnancy BMI) did not differ between participants and non-participants. However, the non-responding was more prevalent in a lower occupational class, which is a known risk factor for childhood obesity and thus the effect of this selection bias cannot be totally excluded. Moreover, the families participating in the STEPS Study were more educated than the general population in Finland, which affected the analysis regarding sociodemographic factors and variables related to them such as breastfeeding and obesity risk. Smoking during pregnancy was rare (<3%), which limited the use of this confounding factor in some statistical analysis. The STEPS Study follow-up cohort was restricted to families in Southwestern Finland. Our study subjects were ethnically from the same origin (Caucasians), which is an advantage since ethnic origin is known to influence some genetic associations.

Anthropometric measurements

A major strength in this study is that the weights and lengths of the children and mothers were measured by health care professionals, except for the maternal prepregnancy weight, which was self-reported at the time of recruitment. The measurement done by health care professionals prevented possible under-reporting or over-reporting. The study protocol was the same for all children irrespectively of their mothers' weight status. We had anthropometrics on children only at birth, 13 months and 24 months of age, which can be considered as a limitation. However, in the future as the study children grow, these analyses will be expanded to older children. The new Finnish Growth References for Children were used to determine being overweight or obesity at 24 months of age and to calculate weight-for-length/height and weight-for-length/height standard deviation score (SDS) (Saari et al. 2010). These new growth references for children include age- and sex-specific BMI cut-off points, wherein age is determined by 0.01 year accuracy from 24 months of age onwards and can be considered as a reliable reference for growth in Finnish children.

Infant feeding data and maternal dietary measurements

Breastfeeding and complementary feeding data were collected with a follow-up diary, which was self-administered real-time in order to avoid recall bias. Out of 1797 families participating in the STEPS Study, 848 children had data on breastfeeding and attended study visits for anthropometric measurements. This non-responding is a limitation to this study, since we have no data why others did not provide information regarding breastfeeding or complementary feeding or attend study visits. Furthermore, it can be hypothesized that breastfeeding duration was lower in non-responding mothers and therefore the information was not provided. No reliable data on feeding in the maternity ward was available, which is a limitation to this study, since feeding in the maternity ward and especially milk supplementation is known to have an effect on breastfeeding duration (Erkkola et al. 2010).

The mother's dietary quality during pregnancy was measured with validated Index of Diet Quality (IDQ) that describes the adherence to nutrition recommendations (Leppälä et al. 2010). The main advantages of IDQ are that it enables the assessment of the health-promoting properties of the diet taking into account possible synergistic and additive effects of foods when reflected against health outcomes, and that it reflects the intake of key foods and nutrients associated with health. However, the IDQ does not provide data on specific nutrient intakes. Moreover, the IDQ does not reflect the consumption of red meat, since it was validated before lowering red meat consumption was in Nordic Nutrition Recommendations.

The mother's diet at breast milk sampling time (infant's age: 3 months) was studied with a short food frequency questionnaire (FFQ). The questionnaire focused on foods rich in different fatty acids or on foods that constitute one of the main sources of certain fatty acids, and it covered a one week period. The foods included in the questionnaire represented the typical sources of different FAs in the Finnish diet (Paturi et al. 2008). However, the FFQ has not been validated against food records, and therefore it cannot be concluded whether it depicts the intake of different fatty acids. Moreover, the intake of fat is especially hard to depict with FFQs, since the sources are numerous. Since the FFQ correlated relatively well with breast milk fatty acids, it can be argued that it may depict the intake at least on some level. Moreover, in this study, the purpose of the FFQ was to provide background information explaining some of the individual variations in breast milk fatty acid composition, and as such, it provided similar information on all subjects which could be then compared with each other.

Samples

The golden standard of milk collection is sampling all milk expressed over 24 hours with multiple collections from the same individuals (Bauer & Gerss 2011; Nommsen et al. 1991). However, it is neither feasible nor ethical in most of the studies. In this study, one breast milk sample at the age of 3 months was collected. At 3 months of age, breast milk is matured and is relatively unchanged regarding nutrient composition (Nommsen et al. 1991). All breast milk samples were collected at the same age of the children, and the sampling was standardized. However, sample collection was done by mothers themselves, and possible differences in manual expression may result in some differences in total fat amount, since the amount of fat in milk increases from for- to hind-milk. Since mean fat content was similar between overweight and normal weight women, the sample collection can be considered as valid in this aspect. Breast milk fatty acids were analyzed by gas chromatography using both internal and external standards, which is considered an accurate method for fatty acid composition measurements (Jensen 1999).

DNA extraction from blood samples was done manually to ensuring adequate amount and quality of DNA and SNP genotyping was done with standardized method. Gene variants used in this study were selected from the National Institute of Health (NIH)

database for genome-wide association studies. Genetic risk scores were calculated following international guidelines (Che & Motsinger-Reif 2012). The results were carefully studied using bioinformatics assessment tools in order to provide accurate and reliable results.

Statistical methods

All statistical methods used in this thesis were chosen based on the data available and previously published research. Well-known and standardized parametrical and non-parametrical tests which were chosen together with the statistician. Use of covariates in adjusting the models were based on previous research and the data. If some covariates lowered the explanationary level, they were excluded from the analysis. Thus, the selection of adjusting variables were first made based on previous results from other studies and secondly based on the data studied here. Interactions between variables included in same model was tested. Multicollinearity between variables studied in this thesis exists and may affect the results. Multicollinnearity may however weaken the associations and effect sizes and not enhance them. Therefore, the results can be treated as valid, as they may only weaker the associations rather than strengthen them. Results are thus rather underestimated than overestimated.

6.7 Implications for further research

This study showed that children who become later overweight show different growth trajectories as early as 13 months of age. Therefore, prevention of a child being overweight should be targeted to families already during pregnancy or soon after birth. Longitudinal cohort studies are needed to investigate whether these growth trajectories formed during the first years are sustained in later childhood. The mother being overweight increased the children's body weight, BMI and weight-for-length possibly reflecting the first signs of later overweight. Indeed, these children might be on a pathway to obesity even though not currently defined as being overweight or obese by BMI-for-age. The WHO has stated in their recent report that longitudinal data is needed to better understand the development of individual's BMI trajectory, and the results in this study are in concordance with it (WHO 2015).

The mother being overweight was associated with lower breastfeeding rates and less-favorable breast milk fatty acid composition, and this should be focused in breastfeeding counseling. Together with the mother being overweight being a risk factor, lower breastfeeding further predisposes these children to higher risk of being overweight and obesity. Studies should be targeted to study the possible ways by which the risk factor clustering could be prevented. Future intervention studies should be directed to families with identified risk factors as early as possible even before or during pregnancy since interventions early in life are most likely to have greatest positive effects on health (Hanson and Gluckman 2014).

Breastfeeding rates are significantly lower in Finland than in other Nordic countries. Research should be directed to investigate this phenomenon further to reveal the reasons behind these lower breastfeeding rates. Identification of the problems would allow tools for the maternity and child welfare clinics to enhance and support breastfeeding in Finland, which would also have positive effects on preventing childhood obesity.

Genetic risk clustering was shown to predict higher body weight as early as 24 months of age. Future studies are needed to show how this evolves later in childhood and how obesogenic environment influences these associations. Moreover, genetic risk scores will provide a useful tool to assess the effect of multiple SNPs to weight development, early growth and risk of the children being overweight and obese. Follow-up of children with high genetic risk for overweight would reveal protective environmental factors as well as environmental risk factors for adverse weight development in childhood.

The effect of risk factor clustering was well-demonstrated in this study. The accuracy of the Obesity Risk Score in predicting overweight and obesity was good and highlighted the importance of examining many risk factors simultaneously rather than examining only single determinants. Further research should be directed towards studying the clustering of obesity risk factors. The results achieved by this kind of approach could be utilized in prevention and intervention strategies targeted for the whole family. The findings could be utilized in maternity clinics for providing more intensive guidance to families with highest risk of childhood obesity. The results in this thesis show that many of the factors associated with overweight and obesity risk at 24 month of age are actually prenatal or early postnatal determinants. The prevention and intervention strategies should be directed to families planning pregnancy or during the first half of the pregnancy in order to gain the most positive effects for children's later health. In addition, exposure to an obesogenic environment should be reduced significantly especially in children.

7. CONCLUSIONS

The results shown in this thesis provide evidence that genetic, prenatal and early postnatal determinants are associated with early excessive weight gain and overweight and obesity at 24 months of age. This study showed that many of the risk factors tend to cluster within children and families. These results highlight the importance of early detection of infants at risk of obesity and recognition of unfavorable growth trajectories for preventing later obesity and obesity-related diseases.

The main conclusions of this thesis can be summarized as follows:

1) Maternal overweight had an impact on birth weight and early growth from birth to 24 months of age. Children later becoming overweight show different growth trajectories as early as 13 months of age.

2) Overweight mothers breastfed for a shorter time and introduced complementary foods earlier than normal weight mothers. A longer duration of breastfeeding was protective against excessive weight gain, high BMI, high body weight and high weight-for-length SDS during the first 24 months of life.

3) The breast milk fatty acid composition differed between overweight and normal weight mothers. Overweight women had higher amount of saturated fatty acids and lower amount of n-3 fatty acids in breast milk compared to normal weight women.

4) Genetic risk clustering increased a children's body weight at 24 months of age and thus possibly predisposes to later obesity in an obesogenic environment.

5) Genetic, prenatal and postnatal obesity risk factor clustering increased children's risk of overweight and obesity at 24 months of age substantially.

8. ACKNOWLEDGEMENTS

The work for this thesis was carried out at the Department of Paediatrics, University of Turku and Turku Institute for Child and Youth Research, University of Turku and University of Turku Doctoral Program of Clinical Investigation, during the years 2009-2015 as a part of the Steps to healthy development study (the STEPS Study). I wish to acknowledge the multidisciplinary scientific community for initiating the STEPS Study.

I owe my deepest gratitude to my supervisors, Adjunct Professor Hanna Lagström, PhD and Professor Harri Niinikoski, MD. They both excel in their field of expertise and played a significant role in supervising my studies. I warmly thank Hanna for her continuous guidance and support and the opportunity to study in the STEPS Study group. Thank you for always having your door open for me and sharing your knowledge. I acknowledge Harri for his endless encouragement and support during these years. Thank you for sharing your expertise and challenging me to do my best.

Adjunct Professor Maijaliisa Erkkola, PhD and Adjunct Professor Ulla Sankilampi, MD are kindly acknowledged for reviewing this thesis. Your valuable and constructive comments improved this work substantially. I want to acknowledge the members of the follow-up group of my thesis, Adjunct Professor Paula Hakala, PhD and Adjunct Professor Marko Kalliomäki, MD.

I am grateful to all co-authors for your contribution to this work. I thank you for all your efforts and enjoyable collaboration. Adjunct Professor Kaisa Linderborg, PhD and Professor Baoru Yang are acknowledged for their expertise, continuous guidance and help in the laboratory analyses and for giving me the opportunity to conduct the analyses in your lab. Professor Olli Simell is acknowledged for his expertise in pediatrics. Niina Pitkänen, PhD, is thanked for her excellent skills in the area of genetics. I acknowledge your endless patience and help throughout our collaboration. Teemu Kuulasmaa, MSc is thanked for performing the genetic analysis. Professor Markku Laakso, MD is acknowledged for his excellent knowledge in genetics and providing the opportunity to analyze the samples in his lab. Jenni Vaarno MSc, MPHN is thanked for all our enjoyable collaboration and conversations. It has been a great privilege to do the studies with you. The statistical analysis in this thesis would not have succeeded without Anne Kaljonen. My deepest thanks for always helping me.

I am grateful to all my coworkers and colleagues in CYRI and the STEPS Study team. It has been great working with you! My special thanks go to Sanna Talvia, PhD, Leena Haanpää, PhD, Piia af Ursin, M.Ed. and Tanja Matarma, MSc for all the wonderful conversations about science and everything else. From the STEPS Study team I would especially like to thank Adjunct Professor Mari Sandell, PhD and Professor Ville Peltola, MD for your expertise and guidance throughout my studies.

I thank all the children and families participating to the STEPS Study for your time and effort. You have made this study possible.

I wish to express my gratitude to research nurses Niina Lukkarla, Petra Rajala and Anne-Mari Pieniniemi and bioanalyst Mira Katajamäki who have done invaluable clinical work.

Robert M Badeau, PhD, of Aura Professional English Consulting, is thanked for revising the thesis linguistically.

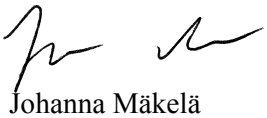
My special thanks go to my friends Laura, Laura-Maria, Suvi, Liisa, Maria, Viivi, Hannamaija and Elina and their families. We've had the privilege of growing up together and you have always supported me in everything. Tiina Saanijoki, MSc and Johanna Jaakkola, PhD are especially thanked for supportive conversations and delightful moments during our studies.

I want to warmly thank my mother and father and their spouses who have taught me to believe in myself and helped me in so many ways. My brother Anton and his family, thank you for your endless support and interest towards my studies. I thank all my relatives and in-laws for their support.

Finally, words fail to describe my gratitude for my dear husband Paavo, for loving me, supporting me and being my best friend and our sons Väinö and Mauno for being my inspiration, the joy of my life and reminding me of life outside work.

In appreciation for financial support for this work, I thank the Alfred Kordelin Foundation, Juho Vainio Foundation and University of Turku Doctoral Program of Clinical Investigation.

Tammela, August 2015



Johanna Mäkelä

9. REFERENCES

Agostoni C, Decsi T, Fewtrell M, Goulet O, Kolacek S, Koletzko B, Michaelsen KF, Moreno L, Puntis J, Rigo J, et al. 2008. Complementary feeding: A commentary by the ESPGHAN committee on nutrition. J Pediatr Gastroenterol Nutr 46(1):99-110.

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

Alberti KG, Zimmet P, Shaw J, IDF Epidemiology Task Force Consensus Group. 2005. The metabolic syndrome--a new worldwide definition. Lancet 366(9491):1059-62.

Alharbi KK, Spanakis E, Tan K, Smith MJ, Aldahmesh MA, O'Dell SD, Sayer AA, Lawlor DA, Ebrahim S, Davey Smith G, et al. 2007. Prevalence and functionality of paucimorphic and private MC4R mutations in a large, unselected european british population, scanned by meltMADGE. Hum Mutat 28(3):294-302.

Andersen LG, Angquist L, Gamborg M, Byberg L, Bengtsson C, Canoy D, Eriksson JG, Eriksson M, Jarvelin MR, Lissner L, et al. 2009. Birth weight in relation to leisure time physical activity in adolescence and adulthood: Meta-analysis of results from 13 nordic cohorts. PLoS One 4(12):e8192.

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

Baker JL, Michaelsen KF, Sorensen TI, Rasmussen KM. 2007. High prepregnant body mass index is associated with early termination of full and any breastfeeding in danish women. Am J Clin Nutr 86(2):404-11.

Baker JL, Michaelsen KF, Rasmussen KM, Sorensen TI. 2004. Maternal prepregnant body mass index, duration of breastfeeding, and timing of complementary food introduction are associated with infant weight gain. Am J Clin Nutr 80(6):1579-88.

Ballard O and Morrow AL. 2013. Human milk composition: Nutrients and bioactive factors. Pediatr Clin North Am 60(1):49-74.

Bammann K, Peplies J, De Henauw S, Hunsberger M, Molnar D, Moreno LA, Tornaritis M, Veidebaum T, Ahrens W, Siani A, et al. 2014. Early life course risk factors for childhood obesity: The IDEFICS case-control study. PLoS One 9(2):e86914.

Barker DJ. 1995. Fetal origins of coronary heart disease. BMJ 311(6998):171-4.

Barker DJ and Osmond C. 1986. Infant mortality, childhood nutrition, and ischaemic heart disease in england and wales. Lancet 1(8489):1077-81.

Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ. 1989. Weight in infancy and death from ischaemic heart disease. Lancet 2(8663):577-80.

Bauer J and Gerss J. 2011. Longitudinal analysis of macronutrients and minerals in human milk produced by mothers of preterm infants. Clin Nutr 30(2):215-20.

Belfort MB, Rifas-Shiman SL, Kleinman KP, Guthrie LB, Bellinger DC, Taveras EM, Gillman MW, Oken E. 2013. Infant feeding and childhood cognition at ages 3 and 7 years: Effects of breastfeeding duration and exclusivity. JAMA Pediatr 167(9):836-44.

Bervoets L, Van Hoorenbeeck K, Kortleven I, Van Noten C, Hens N, Vael C, Goossens H, Desager KN, Vankerckhoven V. 2013. Differences in gut microbiota composition between obese and lean children: A cross-sectional study. Gut Pathog 5(1):10,4749-5-10.

Betoko A, Charles MA, Hankard R, Forhan A, Bonet M, Saurel-Cubizolles MJ, Heude B, de Lauzon-Guillain B. 2013. Infant feeding patterns over the first year of life: Influence of family characteristics. Eur J Clin Nutr 67(6):631-7.

Birch LL and Fisher JO. 1998. Development of eating behaviors among children and adolescents. Pediatrics 101(3 Pt 2):539-49.

Bode L. 2012. Human milk oligosaccharides: Every baby needs a sugar mama. Glycobiology 22(9):1147-62.

Brands B, Demmelmair H, Koletzko B, EarlyNutrition Project. 2014. How growth due to infant nutrition influences obesity and later disease risk. Acta Paediatr 103(6):578-85.

Carlson SE, Werkman SH, Tolley EA. 1996. Effect of long-chain n-3 fatty acid supplementation on visual acuity and growth of preterm infants with and without bronchopulmonary dysplasia. Am J Clin Nutr 63(5):687-97.

Catalano PM. 2007. Management of obesity in pregnancy. Obstet Gynecol 109(2):419-33.

Centers for Disease Control and Prevention. US Department of Health and Human Services. Physical activity and good nutrition: essential elements to prevent chronic diseases and obesity 2007 [Internet]

[cited 2015 02/03/2015]. Available from: http://www.cdc.gov/chronicdisease/resources/publications/aag/obesity.htm

Centers for Disease Control and Prevention. Breastfeeding among US children born 2001-2011, CDC National Immunization Survey. Available at: www.cdc.gov/breastfeeding/data/NIS_data/index.htm [Internet] [cited 2015 02/26].

Cespedes EM, Rifas-Shiman SL, Redline S, Gillman MW, Pena MM, Taveras EM. 2014. Longitudinal associations of sleep curtailment with metabolic risk in mid-childhood. Obesity (Silver Spring) 22(12):2586-92.

Chawla R, Badon S, Rangarajan J, Reisetter A, Armstrong LL, Lowe LP, Urbanek M, Metzger BE, Hayes MG, Scholtens DM, et al. 2014. A genetic risk score for prediction of newborn adiposity and large for gestational age birth. J Clin Endocrinol Metab : 99(11):E2377-86.

Che R and Motsinger-Reif AA. 2012. A new explained-variance based genetic risk score for predictive modeling of disease risk. Stat Appl Genet Mol Biol 11(4):Article 15. doi: 10.1515/1544-6115.1796

Chivers P, Hands B, Parker H, Bulsara M, Beilin LJ, Kendall GE, Oddy WH. 2010. Body mass index, adiposity rebound and early feeding in a longitudinal cohort (raine study). Int J Obes (Lond) 34(7):1169-76.

Clausen T, Burski TK, Oyen N, Godang K, Bollerslev J, Henriksen T. 2005. Maternal anthropometric and metabolic factors in the first half of pregnancy and risk of neonatal macrosomia in term pregnancies. A prospective study. Eur J Endocrinol 153(6):887-94.

Clausson B, Lichtenstein P, Cnattingius S. 2000. Genetic influence on birthweight and gestational length determined by studies in offspring of twins. BJOG 107(3):375-81.

Clayton PE, Cianfarani S, Czernichow P, Johannsson G, Rapaport R, Rogol A. 2007. Management of the child born small for gestational age through to adulthood: A consensus statement of the international societies of pediatric endocrinology and the growth hormone research society. J Clin Endocrinol Metab 92(3):804-10.

Cnattingius S, Villamor E, Lagerros YT, Wikstrom AK, Granath F. 2012. High birth weight and obesity--a vicious circle across generations. Int J Obes (Lond) 36(10):1320-4.

Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. 2000. Establishing a standard definition for child overweight and obesity worldwide: International survey. BMJ 320(7244):1240-3.

Correia-Branco A, Keating E, Martel F. 2015. Maternal undernutrition and fetal developmental programming of obesity: The glucocorticoid connection. Reprod Sci 22(2):138-45.

Cotsapas C, Speliotes EK, Hatoum IJ, Greenawalt DM, Dobrin R, Lum PY, Suver C, Chudin E, Kemp D, Reitman M, et al. 2009. Common body mass index-associated variants confer risk of extreme obesity. Hum Mol Genet 18(18):3502-7.

Council on Communications and Media and Strasburger VC. 2011. Children, adolescents, obesity, and the media. Pediatrics 128(1):201-8.

Croteau-Chonka DC, Marvelle AF, Lange EM, Lee NR, Adair LS, Lange LA, Mohlke KL. 2011. Genome-wide association study of anthropometric traits and evidence of interactions with age and study year in filipino women. Obesity (Silver Spring) 19(5):1019-27.

Dabelea D, Pettitt DJ, Hanson RL, Imperatore G, Bennett PH, Knowler WC. 1999. Birth weight, type 2 diabetes, and insulin resistance in pima indian children and young adults. Diabetes Care 22(6):944-50.

Danielzik S, Langnase K, Mast M, Spethmann C, Muller MJ. 2002. Impact of parental BMI on the manifestation of overweight 5-7 year old children. Eur J Nutr 41(3):132-8.

Davison KK and Birch LL. 2001. Weight status, parent reaction, and self-concept in five-year-old girls. Pediatrics 107(1):46-53.

de Hoog ML, van Eijsden M, Stronks K, Gemke RJ, Vrijkotte TG. 2011. The role of infant feeding practices in the explanation for ethnic differences in infant growth: The amsterdam born children and their development study. Br J Nutr 106(10):1592-601.

Del Prado M, Villalpando S, Elizondo A, Rodriguez M, Demmelmair H, Koletzko B. 2001. Contribution of dietary and newly formed arachidonic acid to human milk lipids in women eating a low-fat diet. Am J Clin Nutr 74(2):242-7.

Dewey KG. 2003. Is breastfeeding protective against child obesity? J Hum Lact 19(1):9-18.

Dörner G. 1975. Perinatal hormone levels and brain organization. Anatomical Neuroendocrinology 1:245-52.

Dörner G. 1975. Problems and terminology of functional teratology. Acta Biol Med Ger 34:1093,1094, 1095.

Dunstan JA, Mitoulas LR, Dixon G, Doherty DA, Hartmann PE, Simmer K, Prescott SL. 2007. The effects of fish oil supplementation in pregnancy on breast milk fatty acid composition over the course of lactation: A randomized controlled trial. Pediatr Res 62(6):689-94.

Durmus B, van Rossem L, Duijts L, Arends LR, Raat H, Moll HA, Hofman A, Steegers EA, Jaddoe VW. 2011. Breast-feeding and growth in children until the age of 3 years: The generation R study. Br J Nutr 105(11):1704-11.

Ebbeling CB, Pawlak DB, Ludwig DS. 2002. Childhood obesity: Public-health crisis, common sense cure. Lancet 360(9331):473-82.

Edwards LE, Dickes WF, Alton IR, Hakanson EY. 1978. Pregnancy in the massively obese: Course, outcome, and obesity prognosis of the infant. Am J Obstet Gynecol 131(5):479-83.

Ehrlich P. 1892. Ueber immunitaet durch vererbung un saegung (about inherited immunity and immunity through suckling) (in german). Zeitschrift Fur Hygiene Und Infektionskrankheiten 12:183-203.

Ekelund U, Ong KK, Linne Y, Neovius M, Brage S, Dunger DB, Wareham NJ, Rossner S. 2007. Association of weight gain in infancy and early childhood with metabolic risk in young adults. J Clin Endocrinol Metab 92(1):98-103.

Elks CE, Loos RJ, Hardy R, Wills AK, Wong A, Wareham NJ, Kuh D, Ong KK. 2012. Adult obesity susceptibility variants are associated with greater childhood weight gain and a faster tempo of growth: The 1946 british birth cohort study. Am J Clin Nutr 95(5):1150-6.

Elks CE, Loos RJ, Sharp SJ, Langenberg C, Ring SM, Timpson NJ, Ness AR, Davey Smith G, Dunger DB, Wareham NJ, et al. 2010. Genetic markers of adult obesity risk are associated with greater early infancy weight gain and growth. PLoS Med 7(5):e1000284.

Eriksson JG, Forsen TJ, Osmond C, Barker DJ. 2003. Pathways of infant and childhood growth that lead to type 2 diabetes. Diabetes Care 26(11):3006-10.

Eriksson JG, Sandboge S, Salonen M, Kajantie E, Osmond C. 2015. Maternal weight in pregnancy and offspring body composition in late adulthood: Findings from the helsinki birth cohort study (HBCS). Ann Med 47(2):94-9.

Eriksson JG, Sandboge S, Salonen MK, Kajantie E, Osmond C. 2014. Long-term consequences of maternal overweight in pregnancy on offspring later health: Findings from the helsinki birth cohort study. Ann Med 46(6):434-8.

Erkkola M, Pigg HM, Virta-Autio P, Hekkala A, Hypponen E, Knip M, Virtanen SM. 2005. Infant feeding patterns in the finnish type I diabetes prediction and prevention nutrition study cohort. Eur J Clin Nutr 59(1):107-13.

Erkkola M, Salmenhaara M, Nwaru BI, Uusitalo L, Kronberg-Kippila C, Ahonen S, Veijola R, Knip M, Virtanen SM. 2013. Sociodemographic determinants of early weaning: A finnish birth cohort study in infants with human leucocyte antigen-conferred susceptibility to type 1 diabetes. Public Health Nutr 16(2):296-304.

Erkkola M, Salmenhaara M, Kronberg-Kippila C, Ahonen S, Arkkola T, Uusitalo L, Pietinen P, Veijola R, Knip M, Virtanen SM. 2010. Determinants of breast-feeding in a finnish birth cohort. Public Health Nutr 13(4):504-13.

ESPGHAN Committee on Nutrition, Agostoni C, Braegger C, Decsi T, Kolacek S, Koletzko B, Michaelsen KF, Mihatsch W, Moreno LA, Puntis J, et al. 2009. Breast-feeding: A commentary by the ESPGHAN committee on nutrition. J Pediatr Gastroenterol Nutr 49(1):112-25.

Falbe J, Willett WC, Rosner B, Gortmaker SL, Sonneville KR, Field AE. 2014. Longitudinal relations of television, electronic games, and digital versatile discs with changes in diet in adolescents. Am J Clin Nutr 100(4):1173-81.

Fall CH, Sachdev HS, Osmond C, Lakshmy R, Biswas SD, Prabhakaran D, Tandon N, Ramji S, Reddy KS, Barker DJ, et al. 2008. Adult metabolic syndrome and impaired glucose tolerance are associated with different patterns of BMI gain during infancy: Data from the new delhi birth cohort. Diabetes Care 31(12):2349-56.

Farooqi IS. 2005. Genetic and hereditary aspects of childhood obesity. Best Pract Res Clin Endocrinol Metab 19(3):359-74.

Fields DA and Demerath EW. 2012. Relationship of insulin, glucose, leptin, IL-6 and TNF-alpha in human breast milk with infant growth and body composition. Pediatr Obes 7(4):304-12.

Folch J, Lees M, Sloane Stanley GH. 1957. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 226(1):497-509.

Fox CS, Heard-Costa N, Cupples LA, Dupuis J, Vasan RS, Atwood LD. 2007. Genome-wide association to body mass index and waist circumference: The framingham heart study 100K project. BMC Med Genet 8 Suppl 1:S18.

Franks PW, Hanson RL, Knowler WC, Sievers ML, Bennett PH, Looker HC. 2010. Childhood obesity, other cardiovascular risk factors, and premature death. N Engl J Med 362(6):485-93.

Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, Perry JR, Elliott KS, Lango H, Rayner NW, et al. 2007. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science 316(5826):889-94.

French SA, Epstein LH, Jeffery RW, Blundell JE, Wardle J. 2012. Eating behavior dimensions. associations with energy intake and body weight. A review. Appetite 59(2):541-9.

Gaillard R, Durmus B, Hofman A, Mackenbach JP, Steegers EA, Jaddoe VW. 2013. Risk factors and outcomes of maternal obesity and excessive weight gain during pregnancy. Obesity (Silver Spring) 21(5):1046-55.

Galley JD, Bailey M, Kamp Dush C, Schoppe-Sullivan S, Christian LM. 2014. Maternal obesity is associated with alterations in the gut microbiome in toddlers. PLoS One 9(11):e113026.

Gartner LM, Morton J, Lawrence RA, Naylor AJ, O'Hare D, Schanler RJ, Eidelman AI, American Academy of Pediatrics Section on Breastfeeding. 2005. Breastfeeding and the use of human milk. Pediatrics 115(2):496-506.

Gibson RA, Chen W, Makrides M. 2001. Randomized trials with polyunsaturated fatty acid interventions in preterm and term infants: Functional and clinical outcomes. Lipids 36(9):873-83.

Gillman MW, Rifas-Shiman SL, Kleinman K, Oken E, Rich-Edwards JW, Taveras EM. 2008. Developmental origins of childhood overweight: Potential public health impact. Obesity (Silver Spring) 16(7):1651-6.

Gonzalez JR, Estevez MN, Giralt PS, Caceres A, Perez LM, Gonzalez-Carpio M, Ballester F, Sunyer J, Rodriguez-Lopez R. 2014. Genetic risk profiles for a childhood with severely overweight. Pediatr Obes 9(4):272-80.

Graversen L, Sorensen TI, Petersen L, Sovio U, Kaakinen M, Sandbaek A, Laitinen J, Taanila A, Pouta A, Jarvelin MR, et al. 2014. Stability of the associations between early life risk indicators and adolescent overweight over the evolving obesity epidemic. PLoS One 9(4):e95314.

Griffiths LJ, Dezateux C, Cole TJ. 2007. Differential parental weight and height contributions to offspring birthweight and weight gain in infancy. Int J Epidemiol 36(1):104-7.

Grimm KA, Kim SA, Yaroch AL, Scanlon KS. 2014. Fruit and vegetable intake during infancy and early childhood. Pediatrics 134 Suppl 1:S63-9.

Gunnarsdottir I and Thorsdottir I. 2003. Relationship between growth and feeding in infancy and body mass index at the age of 6 years. Int J Obes Relat Metab Disord 27(12):1523-7.

Haahtela T, von Hertzen L, M, M., Hannuksela M, Allergiatyöryhmä: Erhola M, Kaila M, Kauppinen R, Killström L, Klaukka T, Korhonen K, et al. 2008. Kansallinen allergiaohjelma 2008-2018: Aika muuttaa suuntaa. Suomen Lääkärilehti 63(14):9-21.

Hanson LA. 1998. Breastfeeding provides passive and likely long-lasting active immunity. Ann Allergy Asthma Immunol 81(6):523,33; quiz 533-4, 537.

Hanson LA. 1961. Comparative immunological studies of the immune globulins of human milk and of blood serum. Int Arch Allergy Appl Immunol 18:241-67.

Hanson MA and Gluckman PD. 2014. Early developmental conditioning of later health and disease: Physiology or pathophysiology? Physiol Rev 94(4):1027-76.

HAPO Study Cooperative Research Group. 2009. Hyperglycemia and adverse pregnancy outcome (HAPO) study: Associations with neonatal anthropometrics. Diabetes 58(2):453-9.

HAPO Study Cooperative Research Group. 2010. Hyperglycaemia and adverse pregnancy outcome (HAPO) study: Associations with maternal body mass index. BJOG 117(5):575-84.

Harder T, Rodekamp E, Schellong K, Dudenhausen JW, Plagemann A. 2007. Birth weight and subsequent risk of type 2 diabetes: A meta-analysis. Am J Epidemiol 165(8):849-57.

Hasunen K. RS. 2005. Infant feeding in finland 2005. Reports of the Ministry of Social Affairs and Health.

Hasunen K, Kalavainen M, Keinonen H, Lagström H, Lyytikäinen A, Nurttila A, Peltola T, Talvia S. 2004. Nutrition recommendations for infants and young children as well as pregnant and breastfeeding mothers. 4th edition ed. Helsinki: Publications of the Ministry of Social Affairs and Health, ISSN 1236-2050, 2004:11, ISBN 952-00-1528-0.

Haworth CM, Carnell S, Meaburn EL, Davis OS, Plomin R, Wardle J. 2008. Increasing heritability of BMI and stronger associations with the FTO gene over childhood. Obesity (Silver Spring) 16(12):2663-8.

Hillier TA, Pedula KL, Schmidt MM, Mullen JA, Charles MA, Pettitt DJ. 2007. Childhood obesity and metabolic imprinting: The ongoing effects of maternal hyperglycemia. Diabetes Care 30(9):2287-92.

Hindmarsh PC, Geary MP, Rodeck CH, Kingdom JC, Cole TJ. 2008. Factors predicting ante- and postnatal growth. Pediatr Res 63(1):99-102.

Hörnell A, Lagström H, Lande B, Thorsdottir I. 2013. Breastfeeding, introduction of other foods and effects on health: A systematic literature review for the 5th nordic nutrition recommendations. Food Nutr Res 57:10.3402/fnr.v57i0.20823.

Huh SY, Rifas-Shiman SL, Taveras EM, Oken E, Gillman MW. 2011. Timing of solid food introduction and risk of obesity in preschool-aged children. Pediatrics 127(3):e544-51.

Imai CM, Gunnarsdottir I, Thorisdottir B, Halldorsson TI, Thorsdottir I. 2014. Associations between infant feeding practice prior to six months and body mass index at six years of age. Nutrients 17(6):1608-17.

Innis SM. 2007. Human milk: Maternal dietary lipids and infant development. Proc Nutr Soc 66(3):397-404.

Inostroza J, Haschke F, Steenhout P, Grathwohl D, Nelson SE, Ziegler EE. 2014. Low-protein formula slows weight gain in infants of overweight mothers. J Pediatr Gastroenterol Nutr 59(1):70-7.

Jääskeläinen A, Pussinen J, Nuutinen O, Schwab U, Pirkola J, Kolehmainen M, Järvelin MR, Laitinen J. 2011. Intergenerational transmission of overweight among finnish adolescents and their parents: A 16-year follow-up study. Int J Obes (Lond) 35(10):1289-94.

Jantscher-Krenn E and Bode L. 2012. Human milk oligosaccharides and their potential benefits for the breast-fed neonate. Minerva Pediatr 64(1):83-99.

Jensen CL, Maude M, Anderson RE, Heird WC. 2000. Effect of docosahexaenoic acid supplementation of lactating women on the fatty acid composition of breast milk lipids and maternal and infant plasma phospholipids. Am J Clin Nutr 71(1 Suppl):292S-9S.

Jensen RG. 1999. Lipids in human milk. Lipids 34(12):1243-71.

Jiao H, Arner P, Hoffstedt J, Brodin D, Dubern B, Czernichow S, van't Hooft F, Axelsson T, Pedersen O, Hansen T, et al. 2011. Genome wide association study identifies KCNMA1 contributing to human obesity. BMC Med Genomics 4:51,8794-4-51.

Jimenez-Pavon D, Kelly J, Reilly JJ. 2010. Associations between objectively measured habitual physical activity and adiposity in children and adolescents: Systematic review. Int J Pediatr Obes 5(1):3-18.

Johansson A, Marroni F, Hayward C, Franklin CS, Kirichenko AV, Jonasson I, Hicks AA, Vitart V, Isaacs A, Axenovich T, et al. 2010. Linkage and genome-wide association analysis of obesity-related phenotypes: Association of weight with the MGAT1 gene. Obesity (Silver Spring) 18(4):803-8.

Johnson TS, Engström JL, Gelhar DK. 1997. Intra- and interexaminer reliability of anthropometric measurements of term infants. J Pediatr Gastroenterol Nutr 24(5):497-505.

Johnson W, Wright J, Cameron N. 2012. The risk of obesity by assessing infant growth against the UK-WHO charts compared to the UK90 reference: Findings from the born in bradford birth cohort study. BMC Pediatr 12:104,2431-12-104.

Jonsdottir OH, Thorsdottir I, Hibberd BL, Fewtrell MS, Wells JC, Palsson GI, Lucas A, Gunnlaugsson G, Kleinman RE. 2012. Timing of the introduction of complementary foods in infancy: a randomized controlled trial. Pediatrics 130(6):1038-45.

Juliusson PB, Roelants M, Nordal E, Furevik L, Eide GE, Moster D, Hauspie R, Bjerknes R. 2013. Growth references for 0-19 year-old norwegian children for length/height, weight, body mass index and head circumference. Ann Hum Biol 40(3):220-7.

Kalliomäki M, Collado MC, Salminen S, Isolauri E. 2008. Early differences in fecal microbiota composition in children may predict overweight. Am J Clin Nutr 87(3):534-8.

Kaseva N, Wehkalampi K, Hemiö K, Hovi P, Järvenpää AL, Andersson S, Eriksson JG, Lindström J, Kajantie E. 2013. Diet and nutrient intake in young adults born preterm at very low birth weight. J Pediatr 163(1):43-8.

Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, Quon MJ. 2000. Quantitative insulin sensitivity check index: A simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab 85(7):2402-10.

Kelly T, Yang W, Chen CS, Reynolds K, He J. 2008. Global burden of obesity in 2005 and projections to 2030. Int J Obes (Lond) 32(9):1431-7.

Kirchberg FF, Harder U, Weber M, Grote V, Demmelmair H, Peissner W, Rzehak P, Xhonneux A, Carlier C, Ferre N, et al. 2015. Dietary protein intake affects amino acid and acylcarnitine metabolism in infants aged 6 months. J Clin Endocrinol Metab 100(1):149-58.

Kitsantas P and Pawloski LR. 2010. Maternal obesity, health status during pregnancy, and breastfeeding initiation and duration. J Matern Fetal Neonatal Med 23(2):135-41.

Klein RJ, Zeiss C, Chew EY, Tsai JY, Sackler RS, Haynes C, Henning AK, SanGiovanni JP, Mane SM, Mayne ST, et al. 2005. Complement factor H polymorphism in age-related macular degeneration. Science 308(5720):385-9.

Kleiser C, Schaffrath Rosario A, Mensink GB, Prinz-Langenohl R, Kurth BM. 2009. Potential determinants of obesity among children and adolescents in germany: Results from the cross-sectional KiGGS study. BMC Public Health 9:46,2458-9-46.

Klimentidis YC, Chen GB, Lopez-Alarcon M, Harris JJ, Duarte CW, Fernandez JR. 2011. Associations of obesity genes with obesity-related outcomes in multiethnic children. Arch Med Res 42(6):509-14.

Knight B, Shields BM, Hill A, Powell RJ, Wright D, Hattersley AT. 2007. The impact of maternal glycemia and obesity on early postnatal growth in a nondiabetic caucasian population. Diabetes Care 30(4):777-83.

Koletzko B, Chourdakis M, Grote V, Hellmuth C, Prell C, Rzehak P, Uhl O, Weber M. 2014a. Regulation of early human growth: Impact on long-term health. Ann Nutr Metab 65(2-3):101-9.

Koletzko B, Brands B, Chourdakis M, Cramer S, Grote V, Hellmuth C, Kirchberg F, Prell C, Rzehak P, Uhl O, et al. 2014b. The power of programming and the EarlyNutrition project: Opportunities for health promotion by nutrition during the first thousand days of life and beyond. Ann Nutr Metab 64(3-4):187-96.

Koletzko B, von Kries R, Closa R, Escribano J, Scaglioni S, Giovannini M, Beyer J, Demmelmair H, Gruszfeld D, Dobrzanska A, et al. 2009a. Lower protein in infant formula is associated with lower weight up to age 2 y: A randomized clinical trial. Am J Clin Nutr 89(6):1836-45.

Koletzko B, von Kries R, Monasterolo RC, Subias JE, Scaglioni S, Giovannini M, Beyer J, Demmelmair H, Anton B, Gruszfeld D, et al. 2009b. Infant feeding and later obesity risk. Adv Exp Med Biol 646:15-29.

Korsten-Reck U, Kromeyer-Hauschild K, Korsten K, Baumstark MW, Dickhuth HH, Berg A. 2008. Frequency of secondary dyslipidemia in obese children. Vasc Health Risk Manag 4(5):1089-94.

Kramer MS, Matush L, Vanilovich I, Platt RW, Bogdanovich N, Sevkovskaya Z, Dzikovich I, Shishko G, Collet JP, Martin RM, et al. 2007. Effects of prolonged and exclusive breastfeeding on child height, weight, adiposity, and blood pressure at age 6.5 y: Evidence from a large randomized trial. Am J Clin Nutr 86(6):1717-21.

Kulski JK and Hartmann PE. 1981. Changes in human milk composition during the initiation of lactation. Aust J Exp Biol Med Sci 59(1):101-14.

Kyttälä P, Erkkola M, Kronberg-Kippila C, Tapanainen H, Veijola R, Simell O, Knip M, Virtanen SM. 2010. Food consumption and nutrient intake in finnish 1-6-year-old children. Public Health Nutr 13(6A):947-56.

Lagström H, Hakanen M, Niinikoski H, Viikari J, Rönnemaa T, Saarinen M, Pahkala K, Simell O. 2008. Growth patterns and obesity development in overweight or normal-weight 13-year-old adolescents: The STRIP study. Pediatrics 122(4):e876-83.

Lagström H, Rautava P, Kaljonen A, Räihä H, Pihlaja P, Korpilahti P, Peltola V, Rautakoski P, Österbacka E, Simell O, et al. 2013. Cohort profile: Steps to the healthy development and well-being of children (the STEPS study). Int J Epidemiol 42(5):1273-84.

Langer O, Yogev Y, Most O, Xenakis EM. 2005. Gestational diabetes: The consequences of not treating. Am J Obstet Gynecol 192(4):989-97.

Lapillonne A and Carlson SE. 2001. Polyunsaturated fatty acids and infant growth. Lipids 36(9):901-11.

Lehtisalo J, Erkkola M, Tapanainen H, Kronberg-Kippila C, Veijola R, Knip M, Virtanen SM. 2010. Food consumption and nutrient intake in day care and at home in 3-year-old finnish children. Public Health Nutr 13(6A):957-64.

Leppälä J, Lagström H, Kaljonen A, Laitinen K. 2010. Construction and evaluation of a self-contained index for assessment of diet quality. Scand J Public Health 38(8):794-802.

Lesseur C, Armstrong DA, Paquette AG, Li Z, Padbury JF, Marsit CJ. 2014. Maternal obesity and gestational diabetes are associated with placental leptin DNA methylation. Am J Obstet Gynecol .

Li R, Jewell S, Grummer-Strawn L. 2003. Maternal obesity and breast-feeding practices. Am J Clin Nutr 77(4):931-6.

Liu J, Smith MG, Dobre MA, Ferguson JE. 2010a. Maternal obesity and breast-feeding practices among white and black women. Obesity (Silver Spring) 18(1):175-82.

Liu JZ, Medland SE, Wright MJ, Henders AK, Heath AC, Madden PA, Duncan A, Montgomery GW, Martin NG, McRae AF. 2010b. Genome-wide association study of height and body mass index in australian twin families. Twin Res Hum Genet 13(2):179-93.

Llewellyn CH, Trzaskowski M, Plomin R, Wardle J. 2014. From modeling to measurement: Developmental trends in genetic influence on adiposity in childhood. Obesity (Silver Spring) 22(7):1756-61.

Lönnerdal B. 2004. Human milk proteins: Key components for the biological activity of human milk. Adv Exp Med Biol 554:11-25.

Loos RJ, Lindgren CM, Li S, Wheeler E, Zhao JH, Prokopenko I, Inouye M, Freathy RM, Attwood AP, Beckmann JS, et al. 2008. Common variants near MC4R are associated with fat mass, weight and risk of obesity. Nat Genet 40(6):768-75.

Lucas A. 1998. Programming by early nutrition: An experimental approach. J Nutr 128(2 Suppl):401S-6S.

Lucas A, Sarson DL, Blackburn AM, Adrian TE, Aynsley-Green A, Bloom SR. 1980. Breast vs bottle: Endocrine responses are different with formula feeding. Lancet 1(8181):1267-9.

Lunde A, Melve KK, Gjessing HK, Skjaerven R, Irgens LM. 2007. Genetic and environmental influences on birth weight, birth length, head circumference, and gestational age by use of population-based parent-offspring data. Am J Epidemiol 165(7):734-41.

Makrides M, Gibson RA, Udell T, Ried K, International LCPUFA Investigators. 2005. Supplementation of infant formula with long-chain polyunsaturated fatty acids does not influence the growth of term infants. Am J Clin Nutr 81(5):1094-101.

Mangrio E, Lindström M, Rosvall M. 2010. Early life factors and being overweight at 4 years of age among children in malmo, sweden. BMC Public Health 10:764,2458-10-764.

Marchi M, Lisi S, Curcio M, Barbuti S, Piaggi P, Ceccarini G, Nannipieri M, Anselmino M, Di Salvo C, Vitti P, et al. 2011. Human leptin tissue distribution, but not weight loss-dependent change in expression, is associated with methylation of its promoter. Epigenetics 6(10):1198-206.

Marin MC, Sanjurjo A, Rodrigo MA, de Alaniz MJ. 2005. Long-chain polyunsaturated fatty acids in breast milk in la plata, argentina: Relationship with maternal nutritional status. Prostaglandins Leukot Essent Fatty Acids 73(5):355-60.

Marshall SJ, Biddle SJ, Gorely T, Cameron N, Murdey I. 2004. Relationships between media use, body fatness and physical activity in children and youth: A meta-analysis. Int J Obes Relat Metab Disord 28(10):1238-46.

Martin RM, Patel R, Kramer MS, Guthrie L, Vilchuck K, Bogdanovich N, Sergeichick N, Gusina N, Foo Y, Palmer T, et al. 2013. Effects of promoting longer-term and exclusive breastfeeding on adiposity and insulin-like growth factor-I at age 11.5 years: A randomized trial. JAMA 309(10):1005-13.

Matsuda M and DeFronzo RA. 1999. Insulin sensitivity indices obtained from oral glucose tolerance testing: Comparison with the euglycemic insulin clamp. Diabetes Care 22(9):1462-70.

Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. 1985. Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28(7):412-9.

Mattsson N, Rönnemaa T, Juonala M, Viikari JS, Raitakari OT. 2008. Childhood predictors of the metabolic syndrome in adulthood. the cardiovascular risk in young finns study. Ann Med 40(7):542-52.

McMillen IC and Robinson JS. 2005. Developmental origins of the metabolic syndrome: Prediction, plasticity, and programming. Physiol Rev 85(2):571-633.

McNamara BJ, Gubhaju L, Chamberlain C, Stanley F, Eades SJ. 2012. Early life influences on cardio-metabolic disease risk in aboriginal populations--what is the evidence? A systematic review of longitudinal and case-control studies. Int J Epidemiol 41(6):1661-82.

Mei H, Chen W, Jiang F, He J, Srinivasan S, Smith EN, Schork N, Murray S, Berenson GS. 2012. Longitudinal replication studies of GWAS risk SNPs influencing body mass index over the course of childhood and adulthood. PLoS One 7(2):e31470.

Mellies MJ, Ishikawa TT, Gartside PS, Burton K, MacGee J, Allen K, Steiner PM, Brady D, Glueck CJ. 1979. Effects of varying maternal dietary fatty acids in lactating women and their infants. Am J Clin Nutr 32(2):299-303.

Mennella JA, Jagnow CP, Beauchamp GK. 2001. Prenatal and postnatal flavor learning by human infants. Pediatrics 107(6):E88.

Meyre D, Delplanque J, Chevre JC, Lecoeur C, Lobbens S, Gallina S, Durand E, Vatin V, Degraeve F, Proenca C, et al. 2009. Genome-wide association study for early-onset and morbid adult obesity identifies three new risk loci in european populations. Nat Genet 41(2):157-9.

Michaelsen KF. 2000. Are there negative effects of an excessive protein intake? Pediatrics 106(5):1293.

Michels KB, Willett WC, Graubard BI, Vaidya RL, Cantwell MM, Sansbury LB, Forman MR. 2007. A longitudinal study of infant feeding and obesity throughout life course. Int J Obes (Lond) 31(7):1078-85.

Mok E, Multon C, Piguel L, Barroso E, Goua V, Christin P, Perez MJ, Hankard R. 2008a. Decreased full breastfeeding, altered practices, perceptions, and infant weight change of prepregnant obese women: A need for extra support. Pediatrics 121(5):1319-24.

Mok E, Multon C, Piguel L, Barroso E, Goua V, Christin P, Perez MJ, Hankard R. 2008b. Decreased full breastfeeding, altered practices, perceptions, and infant weight change of prepregnant obese women: A need for extra support. Pediatrics 121(5):1319-24.

Monasta L, Batty GD, Cattaneo A, Lutje V, Ronfani L, Van Lenthe FJ, Brug J. 2010. Early-life determinants of overweight and obesity: A review of systematic reviews. Obes Rev 11(10):695-708.

Monasta L, Batty GD, Macaluso A, Ronfani L, Lutje V, Bavcar A, van Lenthe FJ, Brug J, Cattaneo A. 2011. Interventions for the prevention of overweight and obesity in preschool children: A systematic review of randomized controlled trials. Obes Rev 12(5):107-18.

Mueller NT, Whyatt R, Hoepner L, Oberfield S, Dominguez-Bello MG, Widen EM, Hassoun A, Perera F, Rundle A. 2015. Prenatal exposure to antibiotics, cesarean section and risk of childhood obesity. Int J Obes (Lond) 39(4):665-70 .

Musilova S, Rada V, Vlkova E, Bunesova V. 2014. Beneficial effects of human milk oligosaccharides on gut microbiota. Benef Microbes 5(3):273-83.

Must A and Tybor DJ. 2005. Physical activity and sedentary behavior: A review of longitudinal studies of weight and adiposity in youth. Int J Obes (Lond) 29 Suppl 2:S84-96.

Must A, Jacques PF, Dallal GE, Bajema CJ, Dietz WH. 1992. Long-term morbidity and mortality of

overweight adolescents. A follow-up of the harvard growth study of 1922 to 1935. N Engl J Med 327(19):1350-5.

Nasser R, Stephen AM, Goh YK, Clandinin MT. 2010. The effect of a controlled manipulation of maternal dietary fat intake on medium and long chain fatty acids in human breast milk in saskatoon, canada. Int Breastfeed J 5:3.

National Research and Development Centre for Welfare and Health (STAKES). 2012. Nordic perinatal staistics 2010. National Institute for Health and Welfare. Report nr ISSN: 1798–0887.

Nguyen QM, Srinivasan SR, Xu JH, Chen W, Kieltyka L, Berenson GS. 2010. Utility of childhood glucose homeostasis variables in predicting adult diabetes and related cardiometabolic risk factors: The bogalusa heart study. Diabetes Care 33(3):670-5.

Nicklas TA, Yang SJ, Baranowski T, Zakeri I, Berenson G. 2003. Eating patterns and obesity in children. the bogalusa heart study. Am J Prev Med 25(1):9-16.

Nilsson C, Carlsson A, Landin-Olsson M. 2014. Increased risk for overweight among swedish children born to mothers with gestational diabetes mellitus. Pediatr Diabetes 15(1):57-66.

Nommsen LA, Lovelady CA, Heinig MJ, Lonnerdal B, Dewey KG. 1991. Determinants of energy, protein, lipid, and lactose concentrations in human milk during the first 12 mo of lactation: The DARLING study. Am J Clin Nutr 53(2):457-65.

Nommsen-Rivers LA, Chantry CJ, Peerson JM, Cohen RJ, Dewey KG. 2010. Delayed onset of lactogenesis among first-time mothers is related to maternal obesity and factors associated with ineffective breastfeeding. Am J Clin Nutr 92(3):574-84.

Nordic Council of Ministers. 2013. Nordic nutrition recommendations 2012. 5th edition ed. Copenhagen: Nordic Council of Ministers.

Obermann-Borst SA, Eilers PH, Tobi EW, de Jong FH, Slagboom PE, Heijmans BT, Steegers-Theunissen RP. 2013. Duration of breastfeeding and gender are associated with methylation of the LEPTIN gene in very young children. Pediatr Res 74(3):344-9.

Oddy WH. 2012. Infant feeding and obesity risk in the child. Breastfeed Rev 20(2):7-12.

Oddy WH. 2002. The impact of breastmilk on infant and child health. Breastfeed Rev 10(3):5-18.

Oddy WH, Li J, Landsborough L, Kendall GE, Henderson S, Downie J. 2006. The association of maternal overweight and obesity with breastfeeding duration. J Pediatr 149(2):185-91.

Oftedal OT. 2012. The evolution of milk secretion and its ancient origins. Animal 6(3):355-68.

Ogden CL, Carroll MD, Kit BK, Flegal KM. 2014. Prevalence of childhood and adult obesity in the United States, 2011-2012. JAMA 311(8):806-14.

Oken E and Gillman MW. 2003. Fetal origins of obesity. Obes Res 11(4):496-506.

Oken E, Levitan EB, Gillman MW. 2008. Maternal smoking during pregnancy and child overweight: Systematic review and meta-analysis. Int J Obes (Lond) 32(2):201-10.

Ong KK. 2006. Size at birth, postnatal growth and risk of obesity. Horm Res 65 Suppl 3:65-9.

Ong KK and Loos RJ. 2006. Rapid infancy weight gain and subsequent obesity: Systematic reviews and hopeful suggestions. Acta Paediatr 95(8):904-8.

Ong KK, Emmett P, Northstone K, Golding J, Rogers I, Ness AR, Wells JC, Dunger DB. 2009. Infancy weight gain predicts childhood body fat and age at menarche in girls. J Clin Endocrinol Metab 94(5):1527-32.

Ong KK, Diderholm B, Salzano G, Wingate D, Hughes IA, MacDougall J, Acerini CL, Dunger DB. 2008. Pregnancy insulin, glucose, and BMI contribute to birth outcomes in nondiabetic mothers. Diabetes Care 31(11):2193-7.

Owen CG, Martin RM, Whincup PH, Smith GD, Cook DG. 2005. Effect of infant feeding on the risk of obesity across the life course: A quantitative review of published evidence. Pediatrics 115(5):1367-77.

Pang WW and Hartmann PE. 2007. Initiation of human lactation: Secretory differentiation and secretory activation. J Mammary Gland Biol Neoplasia 12(4):211-21.

Papachatzi E, Dimitriou G, Dimitropoulos K, Vantarakis A. 2013. Pre-pregnancy obesity: Maternal, neonatal and childhood outcomes. J Neonatal Perinatal Med 6(3):203-16.

Paturi M, Tapanainen H, Reinivuo H, Pietinen P, editor. 2008. The national FINDIET 2007 survey. B23/2008. Helsinki, Finland: Nutrition Unit, National Public Health Institute.

Pearce J, Taylor MA, Langley-Evans SC. 2013. Timing of the introduction of complementary feeding and risk of childhood obesity: A systematic review. Int J Obes (Lond) 37(10):1295-306.

Peneau S, Salanave B, Maillard-Teyssier L, Rolland-Cachera MF, Vergnaud AC, Mejean C, Czernichow S, Vol S, Tichet J, Castetbon K, et al. 2009. Prevalence of overweight in 6- to 15-year-old children in central/western france from 1996 to 2006: Trends toward stabilization. Int J Obes (Lond) 33(4):401-7.

Perälä MM, Kajantie E, Valsta LM, Holst JJ, Leiviska J, Eriksson JG. 2013. Early growth and postprandial

appetite regulatory hormone responses. Br J Nutr 110(9):1591-600.

Perälä MM, Männisto S, Kaartinen NE, Kajantie E, Osmond C, Barker DJ, Valsta LM, Eriksson JG. 2012. Body size at birth is associated with food and nutrient intake in adulthood. PLoS One 7(9):e46139.

Pietiläinen KH, Kaprio J, Räsänen M, Rissanen A, Rose RJ. 2002. Genetic and environmental influences on the tracking of body size from birth to early adulthood. Obes Res 10(9):875-84.

Pietrobelli A, Faith MS, Allison DB, Gallagher D, Chiumello G, Heymsfield SB. 1998. Body mass index as a measure of adiposity among children and adolescents: A validation study. J Pediatr 132(2):204-10.

Pirkola J, Tammelin T, Bloigu A, Pouta A, Laitinen J, Ruokonen A, Tapanainen P, Järvelin MR, Vaarasmaki M. 2008. Prevalence of metabolic syndrome at age 16 using the international diabetes federation paediatric definition. Arch Dis Child 93(11):945-51.

Plagemann A. 2006. Perinatal nutrition and hormone-dependent programming of food intake. Horm Res 65 Suppl 3:83-9.

Powe CE, Knott CD, Conklin-Brittain N. 2010. Infant sex predicts breast milk energy content. Am J Hum Biol 22(1):50-4.

Pugo-Gunsam P, Guesnet P, Subratty AH, Rajcoomar DA, Maurage C, Couet C. 1999. Fatty acid composition of white adipose tissue and breast milk of mauritian and french mothers and erythrocyte phospholipids of their full-term breast-fed infants. Br J Nutr 82(4):263-71.

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, et al. 2007. PLINK: A tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81(3):559-75.

R: A language and environment for statistical computing. [Internet]; c2014 [cited 2014 10/06]. Available from: http://www.R.project.org/ .

Radulescu L, Munteanu O, Popa F, Cirstoiu M. 2013. The implications and consequences of maternal obesity on fetal intrauterine growth restriction. J Med Life 6(3):292-8.

Raitakari OT, Porkka KV, Rönnemaa T, Knip M, Uhari M, Åkerblom HK, Viikari JS. 1995. The role of insulin in clustering of serum lipids and blood pressure in children and adolescents. the cardiovascular risk in young finns study. Diabetologia 38(9):1042-50.

Ravelli GP, Stein ZA, Susser MW. 1976. Obesity in young men after famine exposure in utero and early infancy. N Engl J Med 295(7):349-53.

Reece EA, Leguizamon G, Wiznitzer A. 2009. Gestational diabetes: The need for a common ground. Lancet 373(9677):1789-97.

Regnault N, Botton J, Forhan A, Hankard R, Thiebaugeorges O, Hillier TA, Kaminski M, Heude B, Charles MA. 2010. Determinants of early ponderal and statural growth in full-term infants in the EDEN mother-child cohort study. Am J Clin Nutr 92(3):594-602.

Ritchie LD, Welk G, Styne D, Gerstein DE, Crawford PB. 2005. Family environment and pediatric overweight: What is a parent to do? J Am Diet Assoc 105(5 Suppl 1):S70-9.

Rokholm B, Silventoinen K, Tynelius P, Gamborg M, Sorensen TI, Rasmussen F. 2011. Increasing genetic variance of body mass index during the swedish obesity epidemic. PLoS One 6(11):e27135.

Rolland-Cachera MF, Deheeger M, Maillot M, Bellisle F. 2006. Early adiposity rebound: Causes and consequences for obesity in children and adults. Int J Obes (Lond) 30 Suppl 4:S11-7.

Rolland-Cachera MF, Deheeger M, Akrout M, Bellisle F. 1995. Influence of macronutrients on adiposity development: A follow up study of nutrition and growth from 10 months to 8 years of age. Int J Obes Relat Metab Disord 19(8):573-8.

Rolland-Cachera MF, Deheeger M, Bellisle F, Sempe M, Guilloud-Bataille M, Patois E. 1984. Adiposity rebound in children: A simple indicator for predicting obesity. Am J Clin Nutr 39(1):129-35.

Rossiter MD, Colapinto CK, Khan MK, McIsaac JL, Williams PL, Kirk SF, Veugelers PJ. 2015. Breast, formula and combination feeding in relation to childhood obesity in nova scotia, canada. Matern Child Health J .

Ryan AS, Montalto MB, Groh-Wargo S, Mimouni F, Sentipal-Walerius J, Doyle J, Siegman JS, Thomas AJ. 1999. Effect of DHA-containing formula on growth of preterm infants to 59 weeks postmenstrual age. Am J Hum Biol 11(4):457-67.

S Arenz, R Rückerl, B Koletzko, R von Kries. Breast-feeding and childhood obesity—a systematic review. London:Nature Publishing Group. .

Saari A, Sankilampi U, Hannila ML, Kiviniemi V, Kesseli K, Dunkel L. 2011. New finnish growth references for children and adolescents aged 0 to 20 years: Length/height-for-age, weight-for-length/height, and body mass index-for-age. Ann Med 43(3):235-48.

Salanave B, Peneau S, Rolland-Cachera MF, Hercberg S, Castetbon K. 2009. Stabilization of overweight prevalence in french children between 2000 and 2007. Int J Pediatr Obes :1-7.

Sankilampi U, Hannila ML, Saari A, Gissler M, Dunkel L. 2013. New population-based references for birth

weight, length, and head circumference in singletons and twins from 23 to 43 gestation weeks. Ann Med 45(5-6):446-54.

Savage JS, Fisher JO, Birch LL. 2007. Parental influence on eating behavior: Conception to adolescence. J Law Med Ethics 35(1):22-34.

Schack-Nielsen L, Sorensen TI, Mortensen EL, Michaelsen KF. 2010. Late introduction of complementary feeding, rather than duration of breastfeeding, may protect against adult overweight. Am J Clin Nutr 91(3):619-27.

Schmidt Morgen C, Rokholm B, Sjoberg Brixval C, Schou Andersen C, Geisler Andersen L, Rasmussen M, Nybo Andersen AM, Due P, Sorensen TI. 2013. Trends in prevalence of overweight and obesity in danish infants, children and adolescents--are we still on a plateau? PLoS One 8(7):e69860.

Scholtens S, Wijga AH, Smit HA, Brunekreef B, de Jongste JC, Gerritsen J, Seidell JC. 2009. Long-chain polyunsaturated fatty acids in breast milk and early weight gain in breast-fed infants. Br J Nutr 101(1):116-21.

Scuteri A, Sanna S, Chen WM, Uda M, Albai G, Strait J, Najjar S, Nagaraja R, Orru M, Usala G, et al. 2007. Genome-wide association scan shows genetic variants in the FTO gene are associated with obesity-related traits. PLoS Genet 3(7):e115.

Sebire NJ, Jolly M, Harris JP, Wadsworth J, Joffe M, Beard RW, Regan L, Robinson S. 2001. Maternal obesity and pregnancy outcome: A study of 287,213 pregnancies in london. Int J Obes Relat Metab Disord 25(8):1175-82.

Singh AS, Mulder C, Twisk JW, van Mechelen W, Chinapaw MJ. 2008. Tracking of childhood overweight into adulthood: A systematic review of the literature. Obes Rev 9(5):474-88.

Singhal A, Farooqi IS, O'Rahilly S, Cole TJ, Fewtrell M, Lucas A. 2002. Early nutrition and leptin concentrations in later life. Am J Clin Nutr 75(6):993-9.

Sinha R, Fisch G, Teague B, Tamborlane WV, Banyas B, Allen K, Savoye M, Rieger V, Taksali S, Barbetta G, et al. 2002. Prevalence of impaired glucose tolerance among children and adolescents with marked obesity. N Engl J Med 346(11):802-10.

Speliotes EK, Willer CJ, Berndt SI, Monda KL, Thorleifsson G, Jackson AU, Lango Allen H, Lindgren CM, Luan J, Magi R, et al. 2010. Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. Nat Genet 42(11):937-48.

Strauss RS. 2000. Childhood obesity and self-esteem. Pediatrics 105(1):e15.

Szabo E, Boehm G, Beermann C, Weyermann M, Brenner H, Rothenbacher D, Decsi T. 2010. Fatty acid profile comparisons in human milk sampled from the same mothers at the sixth week and the sixth month of lactation. J Pediatr Gastroenterol Nutr 50(3):316-20.

T Harder, R Bergmann, G Kallischnigg, and A Plagemann. 2005. Duration of breastfeeding and risk of overweight: A meta-analysis. American Journal of Epidemiology 162(5):397-403.

Taveras EM, Gillman MW, Pena MM, Redline S, Rifas-Shiman SL. 2014. Chronic sleep curtailment and adiposity. Pediatrics 133(6):1013-22.

Thorisdottir B, Gunnarsdottir I, Palsson GI, Halldorsson TI, Thorsdottir I. 2014. Animal protein intake at 12 months is associated with growth factors at the age of six. Acta Paediarica 103(5):512-7.

Thorleifsson G, Walters GB, Gudbjartsson DF, Steinthorsdottir V, Sulem P, Helgadottir A, Styrkarsdottir U, Gretarsdottir S, Thorlacius S, Jonsdottir I, et al. 2009. Genome-wide association yields new sequence variants at seven loci that associate with measures of obesity. Nat Genet 41(1):18-24.

Uusitalo L, Nyberg H, Pelkonen M, Sarlio-Lähteenkorva S, Hakulinen-Viitanen T, Virtanen S. 2012. Imeväisikäisten ruokinta suomessa 2010. Helsinki: National Institute for Health and Welfare.

Vael C, Verhulst SL, Nelen V, Goossens H, Desager KN. 2011. Intestinal microflora and body mass index during the first three years of life: An observational study. Gut Pathog 3(1):8,4749-3-8.

Ventura AK, Inamdar LB, Mennella JA. 2014. Consistency in infants' behavioural signalling of satiation during bottle-feeding. Pediatr Obes .

Vickers MH and Sloboda DM. 2012. Leptin as mediator of the effects of developmental programming. Best Pract Res Clin Endocrinol Metab 26(5):677-87.

Vuorela N, Saha MT, Salo MK. 2011a. Change in prevalence of overweight and obesity in finnish children - comparison between 1974 and 2001. Acta Paediatr 100(1):109-15.

Vuorela N, Saha MT, Salo MK. 2011b. Toddlers get slimmer while adolescents get fatter--BMI distribution in five birth cohorts from four decades in finland. Acta Paediatr 100(4):570-7.

Vuorela N, Saha MT, Salo M. 2009. Prevalence of overweight and obesity in 5- and 12-year-old Finnish children in 1986 and 2006. Acta Paediatr 98(3):507-12.

Wadsworth M, Marshall S, Hardy R, Paul A. 1999. Breast feeding and obesity relation may be accounted for by social factors. BMJ 319(7224):1576.

Wahlig JL, Bales ES, Jackman MR, Johnson GC, McManaman JL, Maclean PS. 2012. Impact of high-fat diet and obesity on energy balance and

fuel utilization during the metabolic challenge of lactation. Obesity (Silver Spring) 20(1):65-75.

Wang K, Li WD, Zhang CK, Wang Z, Glessner JT, Grant SF, Zhao H, Hakonarson H, Price RA. 2011. A genome-wide association study on obesity and obesity-related traits. PLoS One 6(4):e18939.

Wardle J, Carnell S, Haworth CM, Plomin R. 2008. Evidence for a strong genetic influence on childhood adiposity despite the force of the obesogenic environment. Am J Clin Nutr 87(2):398-404.

Warrington NM, Howe LD, Wu YY, Timpson NJ, Tilling K, Pennell CE, Newnham J, Davey-Smith G, Palmer LJ, Beilin LJ, et al. 2013. Association of a body mass index genetic risk score with growth throughout childhood and adolescence. PLoS One 8(11):e79547.

Weiss R and Kaufman FR. 2008. Metabolic complications of childhood obesity: Identifying and mitigating the risk. Diabetes Care 31 Suppl 2:S310-6.

Whincup PH, Kaye SJ, Owen CG, Huxley R, Cook DG, Anazawa S, Barrett-Connor E, Bhargava SK, Birgisdottir BE, Carlsson S, et al. 2008. Birth weight and risk of type 2 diabetes: A systematic review. JAMA 300(24):2886-97.

Whitaker RC, Wright JA, Pepe MS, Seidel KD, Dietz WH. 1997. Predicting obesity in young adulthood from childhood and parental obesity. N Engl J Med 337(13):869-73.

Willer CJ, Speliotes EK, Loos RJ, Li S, Lindgren CM, Heid IM, Berndt SI, Elliott AL, Jackson AU, Lamina C, et al. 2009. Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. Nat Genet 41(1):25-34.

Wirt T, Schreiber A, Kesztyus D, Steinacker JM. 2015. Early life cognitive abilities and body weight: Cross-sectional study of the association of inhibitory control, cognitive flexibility, and sustained attention with BMI percentiles in primary school children. J Obes 2015:534651.

Wojcicki JM. 2011. Maternal prepregnancy body mass index and initiation and duration of breastfeeding: A review of the literature. J Womens Health (Larchmt) 20(3):341-7.

Wojcik KY, Rechtman DJ, Lee ML, Montoya A, Medo ET. 2009. Macronutrient analysis of a nationwide sample of donor breast milk. J Am Diet Assoc 109(1):137-40.

Working Group appointed by Finnish Pediatric Society. Current Care Guideline in Finland: Obesity (children) [Internet]; c2013 [cited 2014 11/21]. Available from: http://www.kaypahoito.fi/web/kh/suositukset/suositus?id=hoi50034.

World Health Organization (WHO). 2015. Interim report of the commission on ending childhood obesity. Geneva, Switzerland: WHO. Report nr WHO/NMH/PDN/ECHO/15.1.

Worls Health Organization (WHO), Multicentre Growth Reference Study Group. 2006. WHO child growth standards: Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: Methods and development.
. Geneva, Switzerland: World Health Organization.

World Health Organization (WHO). 2002. Infant and young child nutrition: Global strategy on infant and young child feeding. WHO. Report nr A55/15.

World Health Organization (WHO) Expert Committee on Physical Status. 1995. The newborn infant. In: Pgysical status: The use and interpretation of anthropometry. World Health Organization. 121 p.

Wrotniak BH, Shults J, Butts S, Stettler N. 2008. Gestational weight gain and risk of overweight in the offspring at age 7 y in a multicenter, multiethnic cohort study. Am J Clin Nutr 87(6):1818-24.

Yogev Y and Visser GH. 2009. Obesity, gestational diabetes and pregnancy outcome. Semin Fetal Neonatal Med 14(2):77-84.

Young BE, Johnson SL, Krebs NF. 2012. Biological determinants linking infant weight gain and child obesity: Current knowledge and future directions. Adv Nutr 3(5):675-86.

Zhao Y, Wang SF, Mu M, Sheng J. 2012. Birth weight and overweight/obesity in adults: A meta-analysis. Eur J Pediatr 171(12):1737-46.

Ågren JJ, Julkunen A, Penttilä I. 1992. Rapid separation of serum lipids for fatty acid analysis by a single aminopropyl column. J Lipid Res 33(12):1871-6.

Öhlund I, Hernell O, Hörnell A, Stenlund H, Lind T. 2010. BMI at 4 years of age is associated with previous and current protein intake and with paternal BMI. Eur J Clin Nutr 64(2):138-4