Childhood overweight and obesity – identifying early risk factors

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If we know better, we do better…..

Abstract

Background: The incidence of childhood obesity is increasing. Many children become obese during preschool years and stay obese, with lifelong health consequences, such as impaired insulin sensitivity. There is growing evidence that factors during intrauterine life and infancy influence the risk of developing obesity. The aim was to describe early factors in infancy related to childhood obesity.
Research questions: Is waist circumference an auxological variable to be used in early childhood and is this variable related to early metabolic markers? Can biomarkers of bone and nutrition during infancy predict the development of childhood obesity and insulin resistance? How is the gut microbiota established during infancy and influenced by nutrition? Is any gut microbiota pattern during infancy associated with subsequent weight gain or the development of childhood obesity?
Methodology:
Paper 1: A cross-sectional population-based study comprising 4,500 children aged 0-5 years were followed at the child health clinic (CHC) in the County of Halland in 2006. Data on height, weight and waist circumference (WC) were collected from 6-60 months. Reference curves for WC were developed using the Box-Cox-power exponential (BCPE) distribution.
Paper 2: Waist circumferences and BMI were investigated in moderately preterm preschool children (n=154), a group known to have increased risk of impaired insulin sensitivity.
Paper 3 and 4: 388 healthy children were followed from birth to three years of age. Blood and stool samples were collected (cord, at 4, 12 and 36 months). Parents filled in questionnaires regarding hereditary, social factors and feeding preferences and anthropometric data was collected at the CHC. Fecal samples (n=100 at birth, 4 and 12 months) were analyzed with whole genome shotgun sequencing.
Results: Swedish reference curves for WC and waist to height ratio for preschool children were constructed and found comparable to contemporary curves from Germany. Waist to height ratio declined from birth and reached a mean less than 0.5 first at five years of age. A cohort of moderately preterm children was compared to the new reference curves and were found to have an increased WC at 2 years of age despite being lean. In healthy children, multivariate regression analysis showed that neonatal levels of osteocalcin and vitamin D were predictors of body composition at three years of age. Early feeding patterns influenced levels of bone markers and BMI development. The early development of gut microbiota in 100 of the above mentioned children was described from birth to 12 months and compared with the gut microbiome of the mother. The gut microbiota evolved from low abundance to a more adult-like microbiota at one year of age and the early establishment was influenced by feeding patterns.
Conclusion: We investigated the longitudinal development of obesity and found that early nutrition correlated to factors like gut microbiota, bone markers, insulin and leptin sensitivity as well as BMI and WC in early childhood.

Contents

List of publications

This thesis is based on the following papers, referred to in the text by their Roman numerals:

I **Population-based waist circumference and waist-to-height ratio reference values in preschool children**
Josefine Roswall, Stefan Bergman, Gerd Almqvist-Tangen, Bernt Alm, Aimon Niklasson, Andreas F M Nierop, Jovanna Dahlgren
Acta Paediatr. 2009:98(10):1632-36

II **Preschool children born moderately preterm have increased waist circumference at two years of age despite low body mass index**
Josefine Roswall, Ann-Katrine Karlsson, Kerstin Allvin, Gerd Almqvist-Tangen, Stefan Bergman, Aimon Niklasson, Bernt Alm, Jovanna Dahlgren
Acta Paediatr. 2012:101(11):1175-81

III **Low levels of osteocalcin and vitamin D at birth predispose for obesity and impaired insulin sensitivity in early childhood**
Josefine Roswall, Stefan Bergman, Gerd Almqvist-Tangen, Bernt Alm, Jovanna Dahlgren
Submitted to J Clin Endocrinol Metabol

IV **Establishment of the human gut metagenome**
Fredrik Bäckhed, Josefine Roswall, Penquin Yang, Valentina Tremaroli, Petia Kovatcheva, Qiang Feng. Stefan Bergman, Karsten Kristiansen, Jovanna Dahlgren*, Wang Jun* (*contributed equally)
manuscript

Abbreviations

BMI	body mass index
BPCED	Box-Cox-power exponential distribution
CHC	child health centers
CV	coefficient of variation
ECM	extracellular matrix
GA	gestational age
GLP	glucagon-like peptide
GIP	glucose-dependent insuline tropic polypeptide
IOTF	International Obesity Task Force
LGA	large for gestational age
m	month
MC4R	melacortin-4 receptor
25OHD	25-hydroxyvitamin D
PE	paired-end
POMC	proopiomelancortin
PYY	peptide YY
SD	standard deviation
SDS	SD score
SGA	small for gestational age
WC	waist circumference
WtHR	waist-to-height ratio

Introduction

Worldwide obesity has increased dramatically in the last few decades and it is now called the obesity epidemic. In 2008, it was estimated that, 1.4 billion adults were overweight and probably as many as 500 million of them were obese, giving 35% of the adult population are overweight and 11% are obese worldwide (1).
The increasing obesity and the related health risks are associated with almost twice as high productivity losses to society as for normal weight over a lifetime in a Swedish setting (2), and in Europe the calculated direct and indirect cost for obesity in the society was 0.47–0.61% of gross domestic product (3).

In adults, overweight is defined as a body mass index (BMI) (weight (kg)/ height (m^2) of >25 and obesity as >30. These cut-offs are set as they correlate to increased health risks. Obesity has been linked to a number of chronic diseases such as cardiovascular disease, type 2 diabetes mellitus, hypertension and several types of cancers and is linked to shorter life expectancy (4). The cluster of symptoms, such as insulin resistance, dyslipidemia, hypertension and central obesity, related to an increased risk of cardiovascular disease and type 2 diabetes mellitus, are commonly known as the metabolic syndrome.

Childhood obesity

Childhood obesity is also increasing worldwide and has reached epidemic proportions in the last 20 years. The WHO estimates that, globally, over 44 million children under age of five were overweight in 2012 (5). In Sweden about 15% of four-year-old children are overweight and 3-4% are obese (6). Little is known about the risk factors for childhood overweight, but most children who become obese stay obese as adults (7, 8).

Todays epidemic increase in obesity is mainly due to the obesogenic life-style of the modern society, with an almost unlimited exposure for high-energy alimentation, less physical exercise and sedentary life. Some of the previously known genetic factors predisposing for severe early obesity are mutations in the melacortin (MC)4 receptor, fat mass- and obesity-associated (FTO) and leptin genes. However, as little as 7% of obesity can be explained by these monogenic disorders, and the majority of childhood obesity involves polymorphisms of several gene locus, shown by genome wide association studies (GWAS), accounting for 40-60% of the variance in BMI (9).

The last decades, researchers have studied a number of environmental factors during perinatal life or during infancy predisposing for childhood obesity, such as social status, parental smoking, mode of nutrition, essential fatty acids and protein content in infant milk.

Normal weight development during preschool years

The normal weight gain in healthy children follows a pattern of an extreme fat spurt during infancy with a peak around nine to twelwe months of age, and then an extremely lean period until normally the age of seven to eight years. At that point, the BMI reaches a nadir and then increases again. This point is called the adiposity rebound. Many children who become obese exhibit a sudden shift with accelerated weight gain already between two to four years of age and they are then said to have an early adiposity rebound (10).

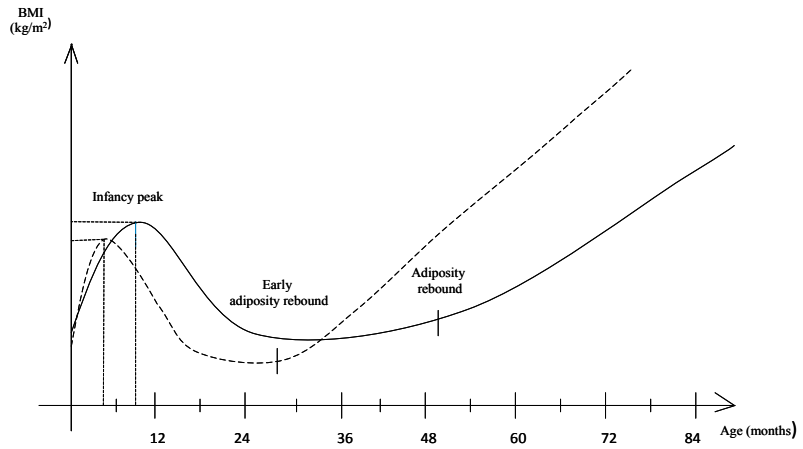

Figure 1. Schematic view of BMI development during childhood, marked with adiposity rebound or the lowest values at 5-7 years.

There is also increasing evidence that the timing and tempo of the infancy fat spurt, called the infancy peak, may influence the subsequent risk of obesity. Associations have been found between early weight gain the first five months of life and BMI in early adulthood (11), and weight gain during the first two months of life and body composition at nine to ten years of age (12). Other studies looking at the infancy peak, show relationship with a high infancy BMI peak and reduced risk of obesity (13) or increased lean and not fat mass later in life (14). A recent study modulating both infancy peak and timing of infancy peak found a secular trend over the last 50 years with earlier, but lower infancy peak (15).

Adult BMI cut-offs for overweight and obesity cannot be used since the dynamic growth in children is a continuum until final height is reached, as both length and weight change is part of normal growth. Instead, age-specific cut-offs are constructed corresponding to adult BMI of 25 or 30 according to international obesity task force (IOTF), representing tracking on a specific BMI from childhood to adulthood and are called iso BMI (16). It is important to understand that childhood iso BMI cut-offs account for the risk of remaining obese or overweight as an adult.

Programming

Increasing evidence points to early events during fetal life and infancy influencing subsequent health risks. Based on observational studies performed in the UK "the Barker hypothesis" emerged (17, 18). Children born with low birth weight, even within the normal range, were shown to have increased risk of cardiovascular disease in adulthood. Later on these results have been confirmed from other parts of the world. As a synonym to this, "the thrifty phenotype hypothesis" describes metabolic set-point changes made in utero to prepare the child for environmental challenges (19). Today we know much more about the mechanisms how this is working, as the environment interferes with human genes through epigenetic modulation (20).

Children born small for gestational age (SGA) appear to run at a greater risk of obesity, especially if they have a rapid postnatal catch-up growth, compared with children who grow more slowly (21, 22) although these relationships are complex (23). Not only children with low birth weight (24-27) but also children with high birth weight (28-30) are at increased risk for obesity, atherosclerosis, type 2 diabetes and the metabolic syndrome in adult life. One of the mechanism described is through epigenetic programming by nutrition, for example overfeeding (31).

Historic evidence from children born to women, who became pregnant during the Dutch hunger winter in 1944-45, shows that restricted nutrition during fetal life affects adult health differently compared with what happens when fetal development during pregnancy is impaired (32-34). Children exposed to famine during the first trimester run an increased risk of obesity, atherosclerosis, type 2 diabetes and metabolic disease during adulthood, while children exposed during the third trimester exhibit impaired glucose tolerance and are born SGA. Different mechanism may be involved in fetal restriction and acting differently during different stages of fetal development. Restrained organogenesis, changes in set points of hormonal axes, impact on adipose tissue development and distribution and epigenetic changes have been discussed.

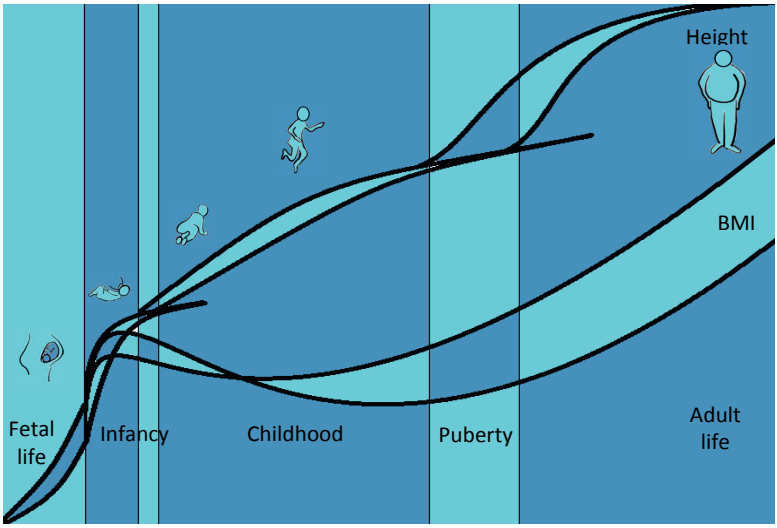

Figure 2. Schematic view of the concept of programming using growth charts in the background.

Early nutritional factors

In animal models, postnatal feeding and the composition of macronutrients have been shown to influence body composition and insulin resistance in the offspring (35). Both protein and total caloric restriction during pregnancy produce epigenetic changes that can be prevented by nutritional interventions during late pregnancy. The postnatal response to feeding depends on the access to nutrients available in fetal life.

In humans, there is currently conflicting evidence relating to the protective effect of breastfeeding on subsequent obesity and the importance of the duration of breastfeeding partly or exclusively (36-38). Interestingly, two studies found that if the mother is obese or diabetic, breastfeeding the child may not be protective (39, 40). However, no randomized studies of different feeding patterns in early infancy can be performed for ethical reasons. A large breastfeeding support study in Russia showed an improvement of increasing breastfeeding rates at four months in the intervention groups to 43.3% compared to 6.4% in the control groups but did not demonstrate a protective effect on subsequent obesity development (41).

Low levels of specific micronutrients like vitamin D status have been associated with the development of the metabolic syndrome (42) and its risk factors (42-44) in adults and older children. Obesity is related to low vitamin-D levels (43, 45). Since vitamin D is stored in body fat the relation between vitamin D and obesity may be more complex (46). Recently, low maternal vitamin-D levels during pregnancy were found to correlate to higher fat mass in the offspring at six years of age (47).

The development of the adipose tissue

Adipocytes emerge from common mesenchymal stem cells with the potential to develop into bone, cartilage or adipose tissue (48). Early in fetal development, adipocytes evolve but are initially sparse and evenly distributed (49). During the second trimester, adipose tissue evolves successively in different depot following vascularization and transforms through hyperplasia and hypertrophy in the third trimester (50). During the postnatal phase adipose tissue expands mainly through extreme hypertrophy which reaches its maximum around 12 months of age (51).

Compared to other species, humans expand adipose tissue to an extreme extent during late fetal life and infancy and 70% of growth expenditure is directed to fat deposition during the early postnatal months. The adipose tissue is thought to have an important protective effect in periods of increased risk of nutrient disruption (ie directly after birth and during weaning) and may also be important for thermoregulation in infancy (52). It can been speculated that the initial infancy BMI peak, its timing and tempo are related to the individual metabolic capacity.

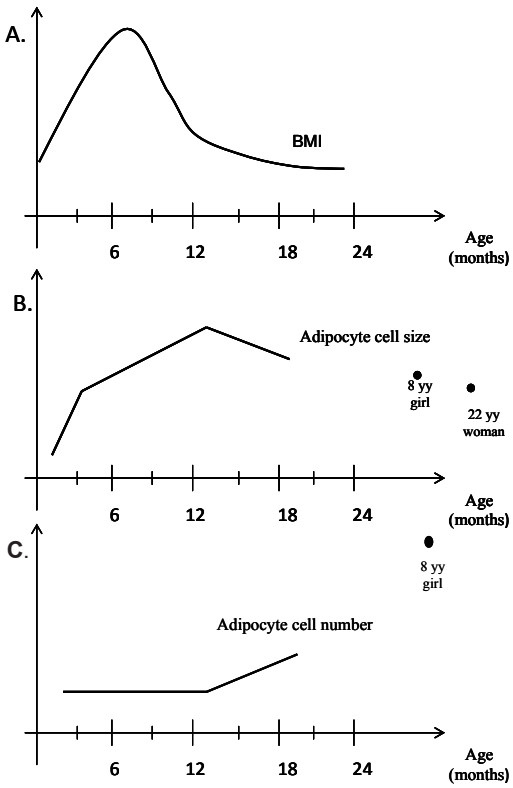

Figure 3. Timing of BMI (A), adipocyte cell size (B) and number (C) development during infancy. Adapted from Häger et al.

The development of unhealthy adipose tissue

Adipose tissue can expand through hyperplasia, ie increased adipocyte number, or hypertrophy, ie increased adipocyte size. When over-nutrition forces the adipose tissue to expand beyond its metabolic capacity, lipids are deposited in ectopic places (for example expanding visceral adiposity instead of subcutaneous fat) and the adipocytes become large. This process induces a lipotoxic state, activating adipose tissue macrophages and thus inducing low-grade inflammation related to adult obesity (53). In adults, it has been shown that visceral adipose tissues strongly linked to insulin resistance, type 2 diabetes, hypertension and dyslipidemia, leading to an increased risk of cardiovascular disease or the metabolic syndrome (54, 55).

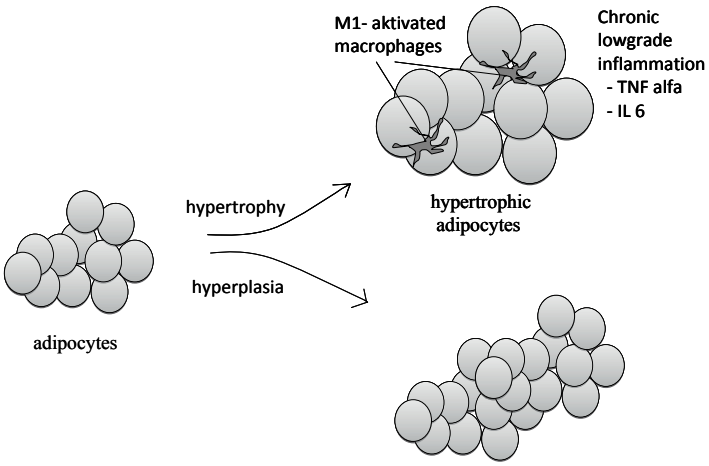

Figure 4. Expansion of the adipose tissue through hyperplasia or hypertrophy.

In children a large amount of the total body fat (90%) is subcutaneous (56) but, despite this, visceral fat disposition in children and adolescents appears to be more strongly related to various cardiovascular and diabetes risk factors than whole-body fat (57). There is currently evidence that children born SGA or extremely prematurely present with a different body composition, with less lean mass and proportionately increased visceral adiposity and insulin resistance already at term age (58-60).

Endocrinology of adipose tissue

It is now well-known that the adipose tissue as a whole acts as an important endocrine organ regulating energy metabolism locally and at distant locations. Adipose hormones and cytokines are produced by the adipose tissue and work in both endocrine and paracrine fashion. The adipokines, leptin and adiponectin, are mainly secreted from the adipocytes themselves but other important adipokines are produced by adipose tissue macrophages or from stromal cells.

Adiponectin

Adiponectin is an adipokine, produced in abundance by mature adipocytes and released into the bloodstream. In adults and older children, adiponectin levels are inversely correlated to BMI, insulin resistance and cardiovascular risk (61-63). In the neonatal period, adiponectin levels are two to three times higher than in adults (64). Children born small, both SGA and preterm children, present with low levels of adiponectin at birth (65). The temporal shift in adiponectin towards the more adult correlation between BMI and adiponectin is thought to occur during the first years of life (66), but the exact age has not been explored in detail. In neonates of normal birth weight, there is no gender dimorphism in adiponectin, whereas in infants born intrauterine growth retarded, adiponectin is lower in the cord blood of boys compared with girls (10).

Leptin

Leptin is secreted from adipocytes as a crucial signal of body energy stores. Serum levels are proportional to fat mass and leptin induces satiety and promotes energy expenditure. Its main actions are mediated through the brain (67) and in particular the hypothalamus is the target as it contains high number of leptin receptors (68). However, new data indicate also the importance of other parts of the brain (69-71). The embryonic expansion of adipose tissue takes place mainly in the second and third trimester and adipose tissue is sparse in early fetal life. Despite this, leptin is one of the first major metabolic hormones to appear during development and it is expressed in many different tissues during embryonic development. Nutritional changes of leptin levels during early life may lead to structural effects on hypothalamic feeding circuits. As an example, in rodents maternal obesity increases leptin levels throughout postnatal life (72) and a high fat diet during pregnancy induces an increased number of orexigenic neurons in the hypothalamus (73). It was previously known that as early as at birth there are gender differences in leptin, with higher cord leptin levels in female newborns (74).

Undernutrition during fetal life and lactation has also been shown in rodents to affect the early development of the hypothalamus, inducing the disrupted organization of proopiomelanocortin (POMC) producing neurons (75), blunting the naturally occurring postnatal leptin surge (76). In humans, cord leptin is found to correlate negatively to weight gain during the first year of life (77), probably due to less dramatic weight gain in those already with big size at birth. However the wide variation in weight gain found during infancy may be pre-set to some extent in utero, mediated through leptin sensitivity. As an example, children born SGA show a transient postnatal overshoot in plasma leptin levels during the catch-up period (78).

Bone tissue and energy metabolism

Bone tissue is responsible for longitudinal growth. It harbors hemophoetic stem cells, regulates calcium and phosphate homeostasis and plays an important role in immunology. Three different bone cell types are involved in bone formation, remodeling and mineral homeostasis: osteoclasts, osteoblasts and osteocytes. Osteoblasts secrete extra cellular matrix (ECM) dominated by type 1 collagen that then becomes min-

eralized. The osteoblast surrounded by ECM matures and forms the osteocyte (79). Osteocytes are thought to sense the distribution and amount of mechanical strength and are involved in mineral homeostasis of calcium and phosphate. Osteoclasts resorb the mineralized ECM.

The involvement of bone tissue in energy metabolism has recently also been demonstrated. Many hormones involved in appetite regulation also affect the short-term regulation of bone tissue and there is increasing evidence of a effect of bone tissue on energy metabolism. Ghrelin, which is an appetite stimulating hormone released from the ventricle (80), regulates together with leptin osteoclast activity (81) and bone mass density (82). Ghrelin is stimulated by fasting and correlates to bone mass in normal weight but not in overweight individuals (83). The reason for the latter may be that overweight or obese individuals are found to have suboptimal levels of Ghrelin (see parallel with Prader Willi syndrome).

Peptide YY (PYY), which is secreted from the distal ileum and proximal colon, regulates satiety through hypothalamic centers (84). This hormone has a direct effect on bone tissue in decreasing osteoblast and increasing osteoclast activity (85).
Leptin, the adipocyte-derived hormone regulating appetite centers in the brain, inhibits adipocyte differentiation. But this hormone promotes also ossification and bone mineralization, and inhibits bone resorption and osteoblast differentiation.

Postprandial hormones also influence bone metabolism. Glucose dependent insulinotropic polypeptide (GIP) receptors are present on osteoblast and osteoclasts, and GIP stimulates bone formation (86, 87). Glucagon like peptide (GLP)-2 reduces markers of bone resorption without any changes in markers of bone formation (84). Osteoblast and osteoclast, both produce adiponectin and express the adiponectin receptor, but there are conflicting results regarding the effect of adiponectin on bone tissue. For example, clinical studies indicate that adiponectin has antiosteogenic effects on the skeleton. There is a negative relationship between serum adiponectin levels and bone mineral density (88), but the mechanism is not known.

Osteocalcin – a marker of bone formation
Osteocalcin is a non-collagenous protein synthesized by osteoblasts and in its carboxylated form, it has a high affinity to hydroxyapatite (89). Osteocalcin levels are increased when osteoblast differentiation is promoted and osteocalcin is therefore a marker of osteoblast activity and bone formation. The level of osteocalcin is higher in cord blood than in maternal blood and is related to gestational age (GA) (90), with a peak at 22-27 weeks of GA. In term newborns, cord blood osteocalcin levels are lower in SGA infants than in normal weight term newborns (91).

Osteocalcin knock-out mice have decreased energy expenditure (92). Mice with osteoblast specific ESP-knock-out, that make them unable to inactivate osteocalcin secretion, have the opposite phenotype with high energy expenditure and resistance to high fat diet induced obesity (92).

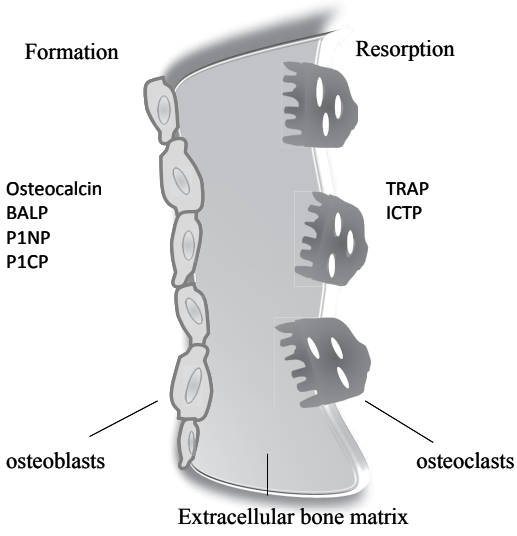

Figure 5. The proteins and the cell types involved in formation versus resorption of bone.

Bone tissue and glucose metabolism

In its un- or undercarboxylated form, osteocalcin is released from osteoblast into the circulation and influences glucose metabolism (89). Osteocalcin secretion is regulated by leptin and insulin (93). In otherwise healthy individuals, osteocalcin is reduced in starvation, malnutrition, and anorexia nervosa, resulting in low bone turnover osteoporosis. On the other hand, giving leptin during starvation seems to prevent the typical fall in osteocalcin (94). In young adults, osteocalcin levels have been shown to be inversely related to BMI, WC and systolic blood pressure. Interestingly, carboxylated osteocalcin is reported to correlate to the adipocyte derived insulin-sensitizer adiponectin (95). Moreover, mice lacking osteocalcin display decreased beta-cell proliferation, glucose intolerance, and insulin resistance (96). The effect of osteocalcin on insulin resistance is mediated through increased secretion of adiponectin. However, in a cross-generation study of women, osteocalcin levels did not seem to correlate to adiponectin levels and correlated inversely to leptin in adults but not in children (97).

Waist circumference (WC) as a risk marker of visceral fat accumulation

In adults, WC can be used as a surrogate measure of visceral adiposity and the related health risks (98). In older children and adolescents WC is related to different components of the metabolic syndrome, such as dyslipidemia, intima thickness and insulin resistance (99). By relating waist to height, it is thought to be a measurement that is more independent of size. The relevance of this in childhood or whether the

changing body proportions during childhood overestimate the influence of length in early childhood are still the subject of debate. It has been proposed that a cut-off of 0.5 as a risk variable would work as an easy risk marker of visceral adiposity in the population, but reliable cut-offs related to health risks in children and adolescents are lacking (100).

Reference curves for WC have been developed in many countries for older children (ie from school age) and adolescents, but only a few cover the youngest age group (101-104). Children born extremely prematurely (<32 weeks GA) present with a different body composition already at term (60) with decreased lean mass and a relative increase in visceral adiposity and insulin resistance that is preserved during childhood (59). Moreover, children born SGA present with this change in body composition and insulin sensitivity, especially if the intrauterine growth retardation is followed by rapid catch-up growth (105).

Gut microbiota and obesity

Bacteria can be classified according to the Linnaean classification. Each member can be classified, depending on the depth chosen, into phyla (divisions), class, orders, families, genera and species. These bacteria are more like one another within the same group compared with members of other groups.

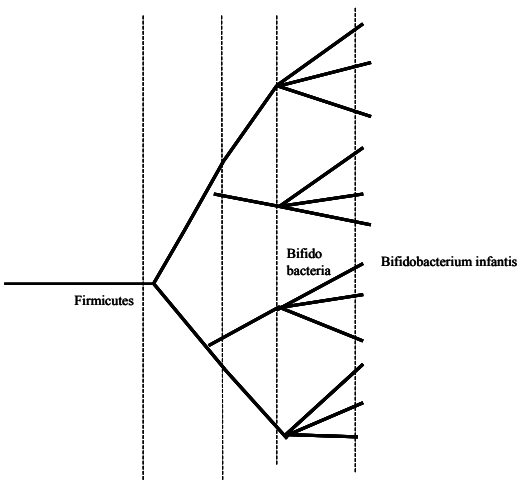

Figure 6. Classification according to the Linnaean concept.

The gut microbiota has evolved together with the human race over a period of thousands of years. The different families of bacteria residing in the gut are carefully selected and have developed over time to match the gut environment and live in symbiosis with the host (106). Nine selected divisions of bacteria are found in the human gut. Bacteroides and Firmicutes are the two dominant divisions, contribut-

ing to 90% of the microbal society of the gut (107, 108). The interplay between the host and the gut microbiota is becoming more and more evident and the host is dependent on several important functions of the gut microbiota for the degradation and uptake of nutrients. The diet has important effects on the structure of the human adult (109, 110) and child (111) microbiota. The gut microbiota has recently been reported to function as an environmental factor that contributes to metabolic (109, 112-115) and inflammatory (116, 117) diseases, which may be the result of microbiota-diet-host gene interactions (117, 118).

New DNA-based sequencing techniques that have evolved during the past 10 years are enabling us to obtain a better understanding of the complex society of bacteria, coexisting with us in the gut. Many bacteria residing in the human gut are strict anaerobes and only about 20% of species can therefore be cultivated using common techniques. Using whole genome shotgun sequencing, the entire metagenome of the human gut can be investigated. Information about the gene content can be assigned to divisions of bacteria, according to their similarity to unknown species (metagenome operational units - mOTUs) down to species (in the case of known species) available in gene catalogues. The function of genes and changes in the quantity of different genes can also be described for the whole bacterial community, thereby providing some idea of the importance of different functions performed by the metagenome over time.

Obese adults are found to differ in their gut microbiota compared with lean individuals, with decreased diversity and, in some studies, a shift in composition between the two major bacterial divisions (112, 119). Transferring the obese human microbiota to a germfree mouse model demonstrated an increase in energy harvesting and the development of obesity (120).

The human infant gut is thought to be more or less sterile in utero and colonized by microorganisms during the passage through the birth canal. Many of the first colonizers of vaginally born children are obtained from the mothers' vagina and feces, whereas children delivered by C-section exhibit a more skin-like microbiota (121, 122). The gut microbiome then transforms from low abundance and diversity to high abundance and diversity during the first year of life first maturing to a more adultlike microbiome at three years of age (123). This early establishment is thought to be influenced by environmental factors, such as type of delivery, antibiotics and feeding, and is more vulnerable to external influences than the more complex adult microbiome (124). Recent focus have been put on cataloguing the adult human microbiota using shotgun sequencing (125, 126), whereas the infant microbiota mainly been restricted to 16S-based profiling and/or small sample sizes (122, 127-129) or investigated influencing factors such as early nutrition or studied its impact on early growth.

Aims and hypotheses

Overall aim

The aim of this thesis was to explore some possible risk factors during infancy and markers of the development of childhood obesity and its metabolic consequences.

Specific aims

To investigate the longitudinal development of WC in a Swedish population of preschool children.

To investigate the WC development in a group of children known to have increased risk of insulin resistance.

To investigate whether early bone and nutritional markers are able to predict the development of childhood obesity and insulin resistance.

To investigate the early establishment of the gut microbiota and how different patterns are influenced by nutrition and related to early child hood obesity.

Hypotheses

1. WC is a better marker than BMI of increased risk to achieve signs of decreased insulin sensitivity during preschool years.

2. Bone and nutritional markers during infancy can predict the development of overweight and obesity later during preschool years.

3. Special patterns of gut microbiota during infancy correlate to feeding patterns, and subsequent early development of childhood obesity.

Methods

Subjects and design

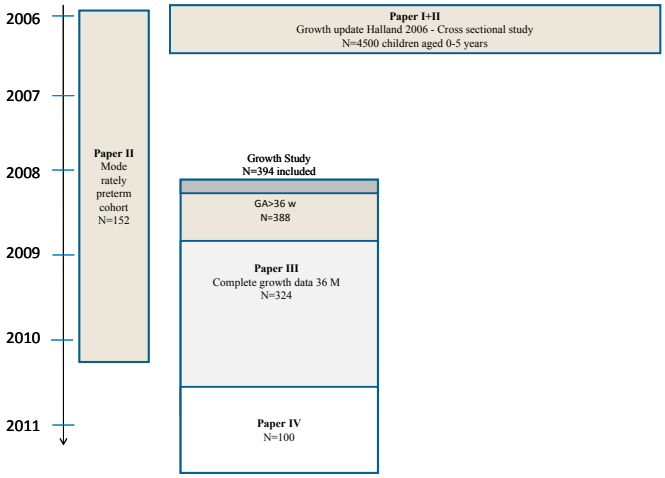

Figure 7. Study subjects included in this thesis.

Paper I

All children coming for their regular CHC visit in Halland in February, May and November 2006 were measured by the regular CHC nurse and data on age, height, weight and WC were collected (n=4,500). The sample represents 25% of the pre-school population in Halland aged 0, 6, 12, 24, 36, 48 and 60 months.

We investigated the mean intra- and interpersonal variance when repeatedly measuring WC in the same child in a subgroup of children (n= 82, 492 measurements). For intra-personal variance a trained CHC nurse repeatedly measured the same child and for inter-personal variance an experienced CHC nurse and a medical student repeatedly measured WC in the same child (see table 1).

Table 1. Intra- and interpersonal variance when measuring waist circumference in pre-school children.

	mean	range	CV	p-value
Intrapersonal variance (cm)	0.45	0-1.5	0.42%	ns
interpersonal variance (cm)	0.79	0-2.93	1.2%	<0.01

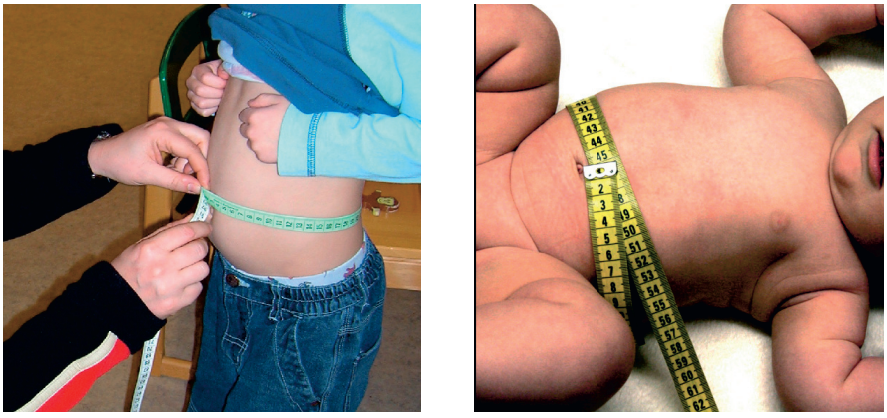

Figure 8. Measuring waist circumference in infants (right) and preschool children (left).

Paper II

Data from Paper I were validated against a population of children born moderately preterm (GA week 32-37) in Gothenburg in 2006-2007, followed longitudinally with WC at 18, 24, 54 and 60 months (n=153).

The longitudinal development of WC in the moderately preterm cohort was described and the mean WC and BMI at age 24, 54 and 60 months were compared cross-sectional between the two cohorts using ANOVA.

Paper III

394 healthy children were recruited in a longitudinal prospective birth cohort in south-western Sweden. The children and parents were recruited before birth on arrival at the Halmstad delivery ward and consented to be followed up with questionnaires and growth data on nine occasions (1, 4, 6, 12, 18, 24, 36, 48 and 60 months) during the preschool years and blood, breast milk and stool sampling as well a food diary at birth, 4, 12, 36 and 60 months of age. 388 of the above children were born at GA >36 weeks and 324 of them with complete growth data at 36 months were used in the analysis of this paper.

Paper IV

100 children with a complete stool sample series during the first year of life (mother, newborn, 4, 12 months) were selected from the longitudinal prospective cohort described in Paper III.

Statistics

Paper I

The cross-sectional data on length and WC were transformed to reference curves for WC and WtH ratio using Box-Cox-power exponential (BCPE) distribution.

(130). References for age were constructed for WC and for WtH ratio using the with curve smoothing by cubic splines and presented as standard deviation (SD) lines according to WHO recommendations (131). The BCPE accommodates various kinds of distribution, from normal to skewed or kurtotic. The BCPE method summarizes the distribution of the data by four spline curves; mu (l), sigma (r), nu (m) and tau (s), which may be interpreted as relating to the median, the coefficient of variation, skewness and kurtosis, that vary in time. The data were limited to ±3 SD, after mirroring the data above and below the median according to recommendations (131) , and then fitted to a corresponding BCPE model. All fittings were performed with the GAMLSS R-package (132). As a diagnostic tool, the worm plot (133) was used to detect possible biases in estimated z-score. Our reference curves were compared with a few other existing reference curves of WC in other countries and with preterm children.

Paper II

The constructed reference curves were validated with a new cohort, and on this occasion the children were moderately preterm. Waist circumference and WtH ratio in the longitudinal cohort of moderately preterm children were translated into SDS according to the reference curve, using Matlab and the logarithm described Paper I (134). Weight, height and BMI were compared after being transformed to SDS according to logarithms of national references (135-137). Wilcoxon signed rank test was used for analysis of longitudinal changes of WC, weight, height and BMI. Mean WC, WtH ratio and BMI in the two populations were compared using Student's t-test using SPSS statistics (PAWS statistics 18.0.0, SPSS Inc, Chicago, IL, USA). The chi-square test was used to compare proportions in the two populations. The data were presented as the mean ± SD (range) and a p-value of <0.05 was considered statistically significant.

We applied a linear random coefficient mixed model with a general covariance structure with corrected age as fixed effect (slope) and patient's corrected age as random effect, to analyse a linear trend from two to five years and to investigate the influence of covariates at birth.

Paper III

We constructed predictive models for BMI, WtH ratio and adiponectin levels, all three at 36 month of age. We allowed only univariate significant variables into the models (gender, GA, mother´s age, pre- and post-pregnancy weight, weight gain during pregnancy, child weight, length and waist SDS during the first year of life, feeding pattern at four months of age, levels of adiponectin, leptin, osteocalcin and vitamin D during the first year of life). The data were presented as the mean (SD) and a p-value of <0.05 was considered statistically significant.

Paper IV

Whole genome shotgun sequencing was performed on individual fecal DNA samples. Sequenced contigs within each sample were rearranged using a metagenome

linage method according to similarity to the known genetic expression of different phyla, families, orders and genera of bacteria. We obtained an average of 3.99 gigabase (Gb) (39.9 million) paired-end (PE) reads for each, totaling 1516.58 Gb of high-quality data that were free from human DNA and adapter contaminants. DNA library construction was performed and a one-PE library with an insert size of 350bp for each sample was built and sequenced with PE100bp. Adapter contamination, low-quality reads and host-contaminating reads were removed from the raw sequencing reads sets. As a result, on average 39.9 million high-quality reads per sample were generated for further analyses. On average, the proportion of high-quality reads among all raw reads from each sample is about 86.7% (384 samples in total). The de novo construction of MetaOTUs and gene catalogs was performed. In addition, an estimation of relative abundance, the identification of signature species/genera, reporter score computation and statistical analyses of influencing factors with PERMANOVA and constrained analysis of principal coordinates were performed.

Ethical considerations

The longitudinal healthy cohort study was approved by the Regional Ethical Review Board in Lund (44/2008) and the longitudinal cohort of moderately preterm children was approved by the Regional Ethical Review Board in Gothenburg (Ö562-01).

Results

1. Waist circumference as a surrogate marker of metabolic risk

Reference curves for WC and WtH ratio were constructed for preschool boys and girls. In this cross-sectional study, 15% were overweight and a subgroup of 2.5% were obese, as defined by iso BMI > 25 and >30 according to IOTF. Waist circumference increased with age (r=0.80, p<0.001). After adjustment to the individual height, expressed as WtH ratio, there was an inverse correlation to age during the first five years of life (r=0.87, p<0.001). In a subsequent cohort, longitudinal measurements of WC in children born moderately preterm were compared with our reference curve. We found that WC in the preterm children changed from a mean of +0.9±1.2 SDS (-2.9 to 2.6) at two years of age, to a mean of +0.2±1.2 SDS (-3.2 to 3.7) at five years of age. Mean WC differed significantly between preterm infants and the reference population (51.3 cm vs 48.5 cm, p<0.001) at two years of age. At five years of age, the preterm cohort presented with a slight decrease in mean WC (53.2 cm vs 54.0 cm, p<0.05) but, at the same time, a significantly lower mean BMI (15.1 vs 16.0, p<0.001).

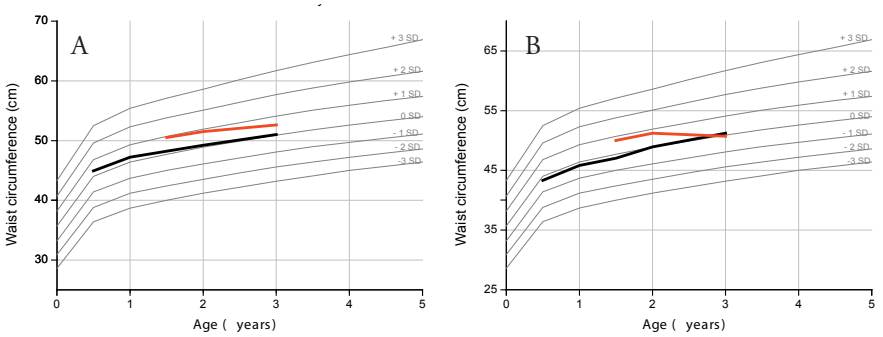

Figure 9. Reference charts for waist circumference for boys (A) and girls (B). Compared in black median waist circumference in longitudinal cohort (n=394) and in red preterm cohort (n=152) for boys (A) and girls (B)

2. Bone markers influencing obesity development

We evaluated early factors influencing BMI and WC development as well as adiponectin, as an indirect marker of insulin resistance. Serum osteocalcin and 25 OHD during infancy and early childhood showed the same pattern, in terms of low levels in cord blood, with a steep peak at four months of age, and then declining. Cord levels of leptin and osteocalcin did not correlate to future level, but cord adiponectin levels correlated to subsequent adiponectin levels during preschool years (r=0.38 to 0.71, p<0.01 to 0.001).

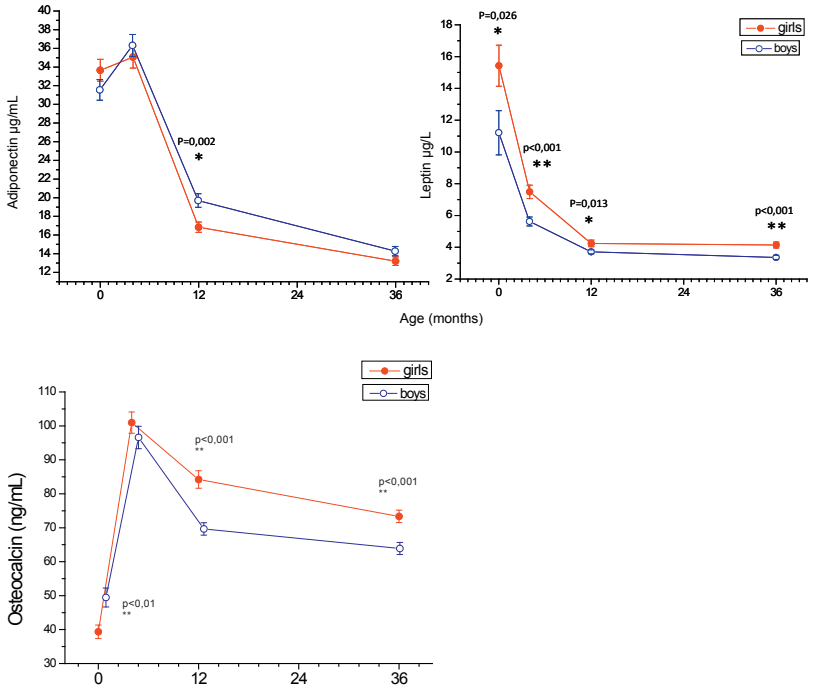

Figure 10. Longitudinal development of adiponectin (A), leptin (B) and osteocalcin (C) for girls (red) and boys (blue).

Breastfeeding was associated with higher mean (SD) osteocalcin levels, 103 (37) ng/mL versus 75 (19) ng/mL ($p<0.001$), at four months but not to insulin sensitivity at any point. There were gender differences for serum levels of osteocalcin, leptin and adiponectin (see Figure 1 and 2). Males had significantly higher osteocalcin levels in cord blood ($p<0.05$), but after the age of four months a shift was found, with higher levels in females ($p<0.001$). Contrary to the pattern of osteocalcin and 25OHD, mean adiponectin and leptin levels were higher in cord blood and at four month ($p<0.001$), compared with a lowering thereafter, with significantlylower adiponectin levels in females at 12 months. Leptin levels were higher at all measured time-points in males ($p<0.05$-0.01).
There was no gender difference for serum 25OHD or for background variables such as social status, the mother´s age, her weight, height and BMI at delivery as well as the infants´ GA and feeding patterns (i.e. breast fed/mixed fed/formula fed).

Multivariate regression analysis revealed cord levels of osteocalcin and 25OHD as predictors of body composition. Low cord osteocalcin predicted a high BMI at 36 months ($p<0.001$). Low 25OHD in cord blood predicted impaired insulin sensitivity at 36 months ($p<0.01$).

3. Microbiota influencing obesity development

The establishment of the gut microbiota

We investigated the early establishment of the gut microbiota, by whole genome shot-gun deep sequencing. During the first week of life, the microbiota was mainly dominated by three phyla; Bacteroidetes, Actinobacteria and Proteobacteria. The dominant genera were Bacteroides (average relative abundance 26.0%), Bifidobacterium (average relative abundance 19.6%) and Escherichia/Shigella (average relative abundance 19.5%).

The taxonomic composition was dominated by Bacteroides (30/96), Bifidobacterium (21/96) or Escherichia/Shigella (21/96) in 72 of 96 samples.
Interestingly, the genera of the microbiome of newborns dominated by Escherichia/Shigella tended to be sampled earlier (mean 2.6 days after birth) compared with Bacteroides mean 3.6 days or Bifidobacterium (mean 5.4 days).
At four months of age, the genera of the microbiota were dominated by either Bifidobacterium (phylum Actinobacteria) or Bacteroides (phylum Bacteroidetes) in the majority (77/96) of the infants and the levels of Bifidobacterium exceeded those of Bacteroides. At 12 months of age, the microbiome increased in complexity and was dominated by Bacteroides in the majority of the infants (64 out of 96) , but Roseburia (phylum Firmicutes) and Prevotella (phylum Bacteroidetes) were also abundant.

At genus level, signature taxa in the newborn gut microbiome belonged to Enterococcus, Staphylococcus, Escherichia/Shigella and Streptococcus and may reflect a relative aerobic environment in the gut during the first week of life. Roseburia and Ruminococcus were signature genera of 12-months-old infants. The signature taxa of adults are far more extended than those of infants, including many genera associated with important metabolic functions in the gut such as Bilophila, Desulfovibrio (barely found during the first year of life), Eubacerium and Faecalibacterium.

Specific Bacteroides strains may be selected due to unique environmental shifts at different developmental stages. Lactobacillus delbrueckii and Lactobacillus ruminisare are found to be the signature MetaOTU in adult samples, whereas MetaOTUs annotated as Lactobacillus rhamnosus, Lactobacillus paracasei, Lactobacillus casei, Lactobacillus johnsonii amd Lactobacillus gasseri were enriched in four month infants but rarely found in adults. Similar observation can be found within the Streptococcus genus, which is a signature genus of newborns, whereas S. gordonii and S. mutans turn out to be most enriched in mothers samples and are rarely found in infants' samples.

Succession of the gut microbiota

We found that the majority of MetaOTUs (144 of 198) with known taxonomic annotation were present in both the newborns and their mothers, suggesting a direct transmission from mother to child.

Nutrition and the gut microbiota

During the first week of life the sampling time correlated significantly with the gut microbiome at both compositional and functional level, but breastfeeding reported at one week of age did not. Reported feeding patterns (exclusive breastfeeding, exclusive formula feeding or mixed feeding) at four months influenced the establishment of gut microbiota at both genus level and MetaOTU level (genus_JSD p= 0.021) but not at functional level (KO_JSD p= 0.1476).

The alpha diversity (Shannon Index using genus, MetaOTU and KO profile) of exclusively breastfed infants at four months is significantly lower than that of infants fed exclusively with formula, while the mixed-fed infants cluster in between. Using effect size calculated using Cohen's d, we compared the exclusively breastfed infants with infants exclusively fed formula. We found that the genera that are most different between the two groups, Collinsella, Enterococcus, Citrobacter, Clostridium and Eggerthella, are the top five genera that are most enriched in exclusively formula-fed infants. On the other hand , Lactobacillus, Bacteroides, Bifidobacterium, Sutterella and Haemophilus are the top five genera that are most enriched in exclusively breastfed infants.

We found that many of the MetaOTUs that are most enriched in exclusively formula-fed infants are assigned to species that have been reported as pathogens or opportunity pathogens. The introduction of small amounts of solid food (taste portions) at four months did not significantly alter the gut microbiota composition (p=0.470, with genus JSD, p-value= 0.384) but the cessation of breastfeeding at 12 months did (p-value= 0.0007 genus JSD test p-value=0.002). The potential external influence on microbiome examined in this study explained 13% of the sample-to-sample variation and the single influencing factors never exceeded 5% of the explained variation.

Gut microbiota, growth and obesity

37 % of the mothers included in the mapping of the early establishment of the gut microbiota were overweight at the start of pregnancy defined as a BMI of >25. The mean BMI on the first visit to the maternity health clinic at the beginning of pregnancy differed significantly ($p<0.05$), according to the mothers´ dominant phylum at the time of delivery. Mothers presenting with Firmicutes as dominant phylum had a mean weight of 70.5 kg, while women presenting with Bacteroidetes as the dominant phylum had a mean weight of 66 kg.

Infancy mean weight or length did not differ between infants with different dominant phyla during the first week, but head circumference at four, six and 12 months ($p<0,05$) and WtHR at six months did ($p<0.01$).

Table 2. Differences in growth, body composition and adipose and bone tissue markers according to dominating phylum during first week of life.

Phylum	age	Firmicutes	Bacteroidetes	Proteobacteria	Actinobacteria	p-value
genera		Streptococcus Enterococcus Clostridium Ruminococcus	Bacteroides Parabacteroi-des Prevotella	Escheria Enterobacter Haemophilus	Bifidobacterum Collinsella	
N		16	32	24	22	
maternal weight (kg)		70.5	65	70	71	<0.05
head circum-ference (cm)	4M	41	41.5	41	42.3	<0.01
	6M	43.8	44	43	44	<0.05
	12M	46.7	46.5	46	47	<0.05
WtHR (SDS)	6M	-0.39	-0.03	-0.26	0.43	<0.01
S-osteocalcin (ng/ml)	36M	68.7	64.9	71	55.7	<0.05
S-adiponectin (µg/ml)	36M	17.5	14.2	11.2	16.4	<0.05
M=months. P-value according to Mann-Whitney U-test.						

Discussion

The obesity epidemic among children and the health risks related to it pose new challenges for society. Over the last decade, our knowledge of adipose tissue as an important endocrine organ and its cross-talk with other tissues has been enhanced. Once established, obesity is difficult to treat and there is currently unsufficient knowledge about what drives childhood obesity. We lack specific markers to identify children with increased health risks related to obesity at an early stage.
The new findings in this thesis are the nutritional factors influencing body composition and markers of energy metabolism from bone tissue and adipose tissue. Breastfeeding is beneficial not only because of higher osteocalcin levels but also because of the modulation of microbiota establishment.

Intrauterine and early postnatal factors

Increasing evidence points to intrauterine and early postnatal factors influencing body composition and insulin resistance(59, 60). It has previously been found that, during pregnancy, leptin, insulin and adiponectin act in an autocrine fashion both in the placenta and in adipose tissue, playing a role in the maternal-fetal interface and contributing to fetal development (138). We found that cord levels of biomarkers involved in energy metabolism were related to body composition and insulin resistance at three years of age. Low cord levels of osteocalcin predicted high BMI SDS and WtH ratio SDS at three years of age. Low levels of cord vitamin D appear to influence the metabolic status during preschool years, but we were unable to confirm the previous finding of low maternal vitamin-D levels correlating to higher BMI in preschool years (47).
We also observed that the gut microbiota is initially characterized by low alpha and high beta diversity. A large proportion of the initial microbiota of normal births may therefore originate from the mothers´ vaginal and fecal microbiota (122) and it is initially characterized by Proteobacteria, which is subsequently replaced by Bifidobacteria and Bacteroides. The dominant infant phylum during the first week of life was related to differences in the mother´s weight at the beginning of pregnancy, which may reflect the level of succession during this time. The dominant phylum during the first week was also related to differences in WtH ratio during the early infancy peak but not to other growth parameters except head circumference This needs to be confirmed, in more complex models adjusting for influential factors, such as feeding and genetic predisposition.

Influence of nutrition

Feeding patterns reported during the first week of life did not significantly influence the gut microbiome during the first 12 days of life. This lack of correlation may be due to the fact that the exact timing of the establishment of feeding in relation to fecal sampling could not be addressed properly in the current study and also reflects the highly evolving bacterial community during this time.
At four months of age, feeding patterns significantly influenced the gut microbiome genera but not the functional level, reflecting the importance of gene function at dif-

ferent stages of development. Formula-fed infants tended to harbor more pathogens and feeding patterns were also related to specific species within the same genera. We hypothesize that this may be a clue to the relationship between different growth patterns and feeding in infancy. Interestingly, it is not the introduction of solid food (taste portions) but the cessation of breastfeeding at twelve months of age that produces a shift in the gut microbiota toward a more adult-like one. Further long-term studies of the evolvement of the gut microbiota and subsequent health related to the influence of feeding patterns is warranted in order to understand the effect of this.
Hormonal status is known to be influenced by feeding patterns and formula feeding has been shown to predispose to rapid weight gain and obesity (139). In the present study, the formula-fed infants were found to have lower osteocalcin levels, potentially leading to a reduction in bone mass later in life. Despite normal osteocalcin levels at a later stage, we speculate that the impact of breastfeeding on bone mass will persist to adulthood. A recent study revealed lower osteocalcin levels at six months of age and subsequently less bone mineral density in 17-year-old adolescents who had previously been formula fed (140). The low osteocalcin levels in formula-fed infants may also predispose to impaired glucose metabolism, but, in the present study, lower adiponectin levels were not observed in three-year-old children who were formula fed compared with breast fed.

Longitudinal changes in body composition

The intense infancy fat spurt and BMI peak may reflect the subsequent metabolic capacity of the adipose tissue and they appear to relate differently to subsequent obesity depending on intrauterine growth.

In this project, we explored different aspects of WC in early childhood, as a possible simple health marker that is easy to transfer to the effective infrastructure within CHC in Sweden. We constructed the first Swedish reference curves for WC for preschool children, based on highly representative cross-sectional material for more than 4,000 children. Waist circumference is similar between genders but increases with age and it should therefore be age adjusted. After adjustment to the individual height, expressed as the WtH ratio, there was an inverse correlation to age during the first five years of age. The mean WtH ratio did not reach less than 0.5 until five years of age and adult cut-offs for increased metabolic risk cannot therefore be applied. In adults, WC acts as an indirect marker of visceral adiposity and a WtH ratio of less than 0.5 has been proposed as a simple health message. However, in older children, conflicting results have been published. The WtH ratio has been identified as a better (141), similar (142) or poorer (143) indicator of cardiovascular risk than WC in various studies. In another study, the WtH ratio was found to be a more accurate predictor of cardiovascular risk factors than BMI in children aged 4–17years (27). The usefulness of WC as risk marker in young children has not yet been fully evaluated. Our study confirms the results of others (144) that a WtH ratio of more than 0.5 is not a relevant cut-off in early childhood and age-specific reference charts in this age group must be used in a clinical context. Moreover, there is a risk that the WtH ratio overestimates the dependence of WC on height in preschool children (145).

Our reference curves are comparable with contemporary curves from Germany and, compared with a Dutch reference curve based on children born in 1996-97, the mean in our reference population was consistently 2 cm larger, possibly describing a secular trend towards increasing obesity(103). Comparing our cross-sectional reference curve with the longitudinal cohort of 394 children born in Halland in 2008, there were no significant differences in mean WC at any age (0-4 y).

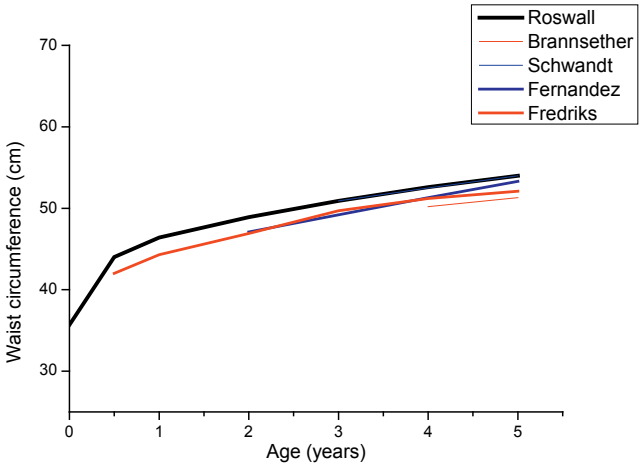

Figure 11. Median waist cirumference in different reference populations compared to the reference curve in paper I.

Comparing longitudinal measurements of WC in a cohort of moderately preterm children revealed that they presented with increased WC, despite being lean at two years of age but not later during preschool years. This indicates a possible difference in body composition during early infancy that is not detected later during the lean period of early childhood. Because preschool children normally have this lean period between three and seven years of age, WC may fail to identify children at risk during this period. We postulate that late infancy and early childhood (i.e. 1.5-2 years of age) may therefore present a window of opportunity to identify children at risk.

Longitudinal changes in bonemarkers and insulin sensitivity

We found that, with increasing changes in BMI from the age of four months to the peak in BMI at 12 months, there was a dramatic drop in the levels of adiponectin and leptin during late infancy. These findings were already known, but the pattern has not been studied in detail. The decrease in adiponectin is not surprising in the context of increased fat mass and may reflect the status of insulin resistance found in late infancy. Periods of rapid growth, during pubertal years, for example, have been associated with similar findings. In the present study, the increase in BMI was not paralleled by an increase in leptin levels, as might have been expected based on experience in adults (146). The drop in leptin is remarkable and may reflect the

physiological up-regulation of leptin sensitivity in the brain early in infancy. Based on our findings, we speculate that the set point of leptin sensitivity centrally and insulin sensitivity peripherally is already determined to a large degree either during perinatal life or no later than during infancy.

Longitudinal changes in gut microbiota

At four months of age the gut microbiota was dominated by the two genera Bifidobacteria and Bacteroides. We observed that, if the sample obtained during the first week was dominated by Bifidobacteria, it was also likely to be dominated by Bifidobacteria at four months.
Bifidobacteria and Bacteroides had strong negative correlation at four months, when Bifidobacteria was the most abundant genus, and the number of Bifidobacteria was reduced after 12 months. The difference in early feeding patterns may play an important role in determining the early microbiota.

Gender differences

We found substantial gender differences in the serum levels of osteocalcin and leptin already at birth. The higher levels of osteocalcin in newborn males could have been due to lower maternal weight and BMI, as there was a significant negative correlation between cord osteocalcin and maternal weight or maternal BMI in a previous study (147). However, in the present study, maternal weight or maternal BMI did not differ between male and female pregnancies. The higher leptin levels found in females, who are found to have significantly lower weights at birth, despite no significant differences in maternal weight, come as some surprise. As with osteocalcin, this finding is also a paradox, but, like the drop in leptin found during infancy, it may support the concept that leptin during infancy is a marker of much more than just the amount of fat mass.

We were unable to find any gender differences in adiponectin, excluding significantly lower adiponectin levels at 12 months of age in females. This could be a transient finding or it could coincide with the subsequent BMI peak in females at 12 months compared with the earlier BMI peak at nine months in males. In another cohort of children, no clear relationship between adiponectin and BMI or insulin sensitivity was seen at eight years of age in boys, but it was seen in girls (148). A different study found a relationship between cord levels of adiponectin and body composition measured as skinfold thicknesses at three years of age (149), but in the present study we did not find that cord adiponectin had any impact on body composition measured as BMI or WtH ratio during infancy or at three years of age. There is still a need for further studies investigating the longitudinal evolvement of adiponectin during childhood and its relationship with insulin sensitivity and body composition later in life.

The strength of this thesis is the design with a large population-based cohort of full-term-born children followed prospectively and longitudinally from birth with blood, breast milk and stool samples

The limitations, on the other hand, are first of all, the low explained variances of insulin sensitivity if only data at birth was entered in the multivariate models. The low number of preterm children followed longitudinally can be regarded as a weakness and maybe not representative of the preterm population. Therefore, new validating studies into other preterm populations are needed

To conclude, we found that markers related to intrauterine life such as cord levels of osteocalcin and early postnatal influences, such as the early establishment of the gut microbiota, are influenced by early nutrition and are related to measurements of body composition, such as BMI and WC in early childhood. We also explored WC in early childhood and further studies are needed to look more closely at infancy growth patterns and subsequent obesity risk.

Future perspectives

Much remains to be done when it comes to exploring WC as an early risk marker of childhood obesity. Further studies should address the longitudinal follow-up of WC from infancy to adulthood and its relationship to the metabolic syndrome. Studies addressing WC compared with more direct measurements of visceral adiposity with MRI at the time of infancy peak may elucidate whetherthis period represents an estimate of the individual metabolic capacity but also body composition later in life. The dynamic relationships between weight gain, longitudinal growth, BMI and WC, especially during the infancy peak, warrant further investigation and the development of effective statistical methods to compare the complexity of different longitudinal growth patterns.
This thesis elucidates the complex interactions between early feeding and factors influencing subsequent obesity risks. Other nutritional factors, such as essential fatty acids, have been related to increased metabolic risks in adult and, in rodents, the dietary ratio between the two essential fatty acids aflalinoleic acid and lineolic acid, is related to increased metabolic risks.

It remains to be investigated whether the early establishment of the gut microbiome relates to childhood obesity. Data generated in our cohort of children may provide clues to the protective effect of certain gut microbiome development during early childhood. Transferring gut microbiomes from different stages of development to germ-free mice can help elucidate the mechanism involved in gut microbiome development. In more distant future, increasing our knowledge of the early establishment of the gut microbiota could provide us with a tool to create normal healthy gut colonization for children at risk, such as those born prematurely or by cesarean. We may acquire increased knowledge of how to compensate for the influence of antibiotics, for example, or understand the complex interaction between gut microbiota and the host genetics and immunology.

Populärvetenskaplig sammanfattning

Vi har undersökt riskmarkörer och tidiga påverkansfaktorer för fetma under förskoleåldern. Mycket talar idag för att faktorer tidigt i livet, som foster och under första levnadsåret har stor betydelse för hälsorisker som vuxen. Att födas liten, antingen tillväxthämmad eller för tidigt, är kopplat till ökad risk för fetma och hjärtkärlsjukdom och diabetes. Att redan under första levnadsåret identifiera barn som har ökad risk utgör basen för möjligheter att förebygga barnfetma. Det är framför allt bukfetma som är kopplad till ökad sjuklighet och hos vuxna finns definierade gränsvärden för bukomfång som korrelerar till risk. I Sverige har vi saknat referenskurvor för bukomfång för barn. ettväven producerar flera hormon såsom leptin och adiponektin, som reglerar aptit och energiomsättning i kroppen. Även skelettet producerar ämnen såsom osteocalcin som påverka insulinkänsligheten. När vi föds koloniseras vår tarm av bakterier i samband med och efter födelsen. Dessa bakterier är noga utvalda för att fungera i tarmens miljö och hjälper tillgodogöra oss vissa näringsämnen. Feta vuxna har visat sig ha en annorlunda sammansättning mellan de vanligaste bakteriegrupperna och en minskad mångfald i tarmfloran. Om feta personers tarmflora överförs till möss uppfödda i helt steril miljö så blir mössen överviktiga, trots att de inte äter mer än vanligt. Den tidiga kosten som för spädbarnet är bröstmjölk eller ersättning är en viktig påverkansfaktor för tarmbakterierna. Vissa studier har visat att amning verkar skyddande mot övervikt men resultaten är inte entydiga.

Vi undersöker i denna avhandling flera möjliga riskfaktorer för barnfetma såsom bukomfång, uppfödning och tarmflora, och hur dessa samspelar med markörer från ben- och fettväv samt insulinkänslighet. Vi konstruerade de första svenska referenskurvorna för bukomfång för förskolebarn i Sverige och testade kurvorna mot en grupp barn födda förtidigt. Dessa barn hade förhöjt bukomfång jämfört med referensen speciellt under slutet av spädbarnstiden trots att de var smala. Senare under förskoletiden försvann dessa skillnader. Vi har även undersökt specifika faktorer i kosten s som vitamin D och dess relation till benmarkörer, kroppssammansättning och insulinkänslighet vid 3 års ålder. Låga nivåer av D-vitamin och benmarkören osteocalcin i navelsträngsprov kunde förutsäga högt BMI och bukomfång vid 3 årsåldern. Amning påverkade nivåer av osteocalcin under första levnadsåret. Det fanns en stor grad av succession av bakterier mellan mor och barn i nyföddhetsperioden och tarmfloran påverkades av om barnet ammades eller inte. När barnet var ett år gammalt så blev tarmfloran mer vuxenlik, framförallt hos de barn som nu slutat att amma.

Sammanfattningsvis Vi fann konkreta förklaringar till att kosten har betydelse för fetmautvecklingen under förskoleåren. Amning påverkar BMI och bukomfångsutveckling genom att bland annat påverka tarmfloran och benmarkörer som har betydelse för insulinkänslighet. Det behövs fortfarande mer forskning för att kunna avgöra om bukomfång fungerar som en riskmarkör i tidig barndom. Kanske kan den uttalade fettspurten tidigt under första levnadsåret, som är mer uttalad hos pojkar, utgöra en kritisk tidpunkt där barn med risk för bukfetma skiljer ut sig.

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