

Pediatric Graduate School  
Children's Hospital  
University of Helsinki  
Helsinki University Central Hospital  
Helsinki, Finland

# **ROLE OF BREASTFEEDING IN BONE HEALTH, BODY COMPOSITION AND VASCULAR HEALTH AND THEIR INTER-CONNECTIVITY**

*A Follow-Up Study from Birth to 32 Years of Age*

**Satu Pirilä**

## **ACADEMIC DISSERTATION**

To be presented, with the permission of the Faculty of Medicine,  
University of Helsinki, for public examination  
at the Niilo Hallman Auditorium, Children's Hospital,  
on June 6, 2014, at 12 noon.

Helsinki 2014

**Supervised by****Docent Mervi Taskinen**

Children's Hospital  
Helsinki University Central Hospital  
University of Helsinki  
Helsinki, Finland

**Professor Ulla M. Pihkala**

Children's Hospital  
Helsinki University Central Hospital  
University of Helsinki  
Helsinki, Finland

**Reviewed by****Docent Marjatta Antikainen**

Department of Adolescent Psychiatry  
Helsinki University Central Hospital  
University of Helsinki  
Helsinki, Finland

**Docent Pekka Arikoski**

Department of Paediatrics  
Kuopio University Hospital  
University of Kuopio  
Kuopio, Finland

**Opponent****Docent Harri Niinikoski**

Department of Paediatrics  
Turku University Hospital  
University of Turku  
Turku, Finland

Cover: Sari Kukkasniemi

ISBN 978-952-10-9957-1 (paper back)

ISBN 978-952-10-9958-8 (PDF)

<http://ethesis.helsinki.fi>

Helsinki University Printing House  
Helsinki 2014

*To my grandmother*

# Table of Contents

<b>Abstract</b>	<b>7</b>
<b>Tiivistelmä</b>	<b>9</b>
<b>List of original publications</b>	<b>11</b>
<b>Abbreviations</b>	<b>12</b>
<b>1 Introduction</b>	<b>13</b>
<b>2 Review of the literature</b>	<b>14</b>
2.1 Recommendations regarding infant feeding	14
2.1.1 History of infant feeding recommendations	14
2.1.2 World Health Organization	16
2.1.3 Recommendations in Finland	18
2.2 The frequency of breastfeeding	20
2.2.1 Finland	20
2.2.2 The prevalence of breastfeeding in other Western countries	21
2.3 Infant milk components	22
2.3.1 Breast milk	22
2.3.2 Cow's milk and diluted cow's milk	24
2.3.3 Commercial milk formulas in 1975 and 2010	24
2.4 Influences of breastfeeding	25
2.4.1 Growth, body composition and development	25
2.4.2 Bone health	28
2.4.3 Immunology and allergies	28
2.4.4 Metabolic effects	30
2.4.5 Type 1 diabetes	32
2.4.6 Infant-mother attachment relationship	32
2.5 Potential pathways of infant feeding on subsequent health	32
2.5.1 Nutritional factors	33
2.5.2 Immunology, bacterial colonisation and maturity of gut	34
2.5.3 Mechanical negative pressure	35
2.5.4 Pathogen exposure	35
2.5.5 Epigenetics	36
2.5.6 Adapted life style	36
2.6 Common public health problems: Osteoporosis, overweight and obesity and cardiovascular disease	37
2.6.1 Osteoporosis	37
2.6.2 Central obesity, overweight and metabolic syndrome	37
2.6.3 Cardiovascular disease	38
2.6.4 Possible linkage between bone, central fat and cardiovascular disease	39

<b>3 Aims of the study</b>	<b>40</b>
<b>4 Subjects and methods</b>	<b>41</b>
4.1 The original study cohort	41
4.2 The present study	42
4.2.1 Questionnaire	42
4.2.2 Clinical visit	42
4.2.3 History of dietary intake	43
4.2.4 Dual energy x-ray for bone and body composition	43
4.2.5 Biochemical markers	43
4.2.6 Risk scores	44
4.2.7 Statistical methods	44
4.2.8 Ethical considerations	45
<b>5 Results</b>	<b>46</b>
5.1 Characteristics of the study population (I-IV)	46
5.1.1 Breastfeeding in the analyses	46
5.2 Comparison of backgrounds between participants and non-participants	47
5.3 Life-style factors (I-IV)	48
5.4 The impact of infant feeding on bone health (I)	49
5.5 Determinants of adult body composition (II)	50
5.6 Breastfeeding and later CVD risk factors and metabolic syndrome (III)	52
5.7 The association between CVD risk factors and bone health (IV)	52
<b>6 Discussion</b>	<b>54</b>
6.1 Major public health diseases	54
6.2 Bone health (I)	54
6.3 Body composition (II)	55
6.4 Cardiovascular disease (III)	55
6.5 Bone health and CVD risk factors (IV)	56
6.6 The breastfeeding recommendations	57
6.7 Realisation of recommendations	58
6.8 Problems with the interpretation of research results	59
6.9 Different study designs to control for confounders	60
6.10 Considerations of different eras	61
<b>7 Conclusions and future aspects</b>	<b>62</b>
<b>Acknowledgements</b>	<b>64</b>
<b>References</b>	<b>67</b>



## Abstract

Breast milk is the preferred nutritional choice for supporting the optimal growth of infants. Breastfeeding also allows close skin-to-skin contact that supports the development of the relationship between the mother and the baby. Antibodies are transferred via breast milk to the child to protect against infections in the first few months of the baby's life. The World Health Organization (WHO) recommends exclusive breastfeeding for the first six months and then continued breastfeeding, along with complementary foods, up to two years of age. These recommendations are particularly justified in developing countries that have low levels of hygiene and where alternatives to breast milk may be contaminated and have insufficient nutritional contents.

Several studies have observed the benefits that breastfeeding has on infant, childhood and adolescence health, including prevention of infections and obesity. However, follow-up studies up to adulthood are scarce, with the main limitation being the retrospectively collected data on infant feeding. While most studies have shown that breastfeeding is either beneficial or has no effect, only a few have reported adverse effects on adult health. In addition, the interpretation of the studies is difficult because breastfeeding is associated with a number of confounding factors that relate to health behaviour and obesity, such as the individual's level of education and socioeconomic status.

This study was designed to evaluate whether breastfeeding in infancy influenced adult health. The focus was on diseases and risk factors with public health importance, such as osteoporosis, cardiovascular diseases (CVD) and body composition, particularly central obesity. The unique cohort (n=238) was originally collected in 1975 in the Maternity Hospital of Helsinki University Central Hospital. During the first year of the babies' lives, families were met seven times and thereafter at three, five, 10 and 17 years of age. Mothers were recommended to breastfeed for as long as possible. When breast milk became insufficient, families were given detailed instructions regarding the initiation of additional milk and solid foods. Data on infant milk feeding, body length/height and weight were collected at each visit. Over the period 2006–2009, a total of 158 subjects (66 percent) from the original cohort participated at the age of 32 years in the follow-up visit at the Children's Hospital, Helsinki University Central Hospital to collect data for the present study. At that visit, fasting venous blood samples were drawn and anthropometrics and blood pressure were measured. Data on participants' medical history and lifestyle factors, such as physical activity, smoking and dietary intakes, were collected. Bone mineral density (BMD) and body composition were assessed with dual energy x-ray (DXA), and vascular properties were investigated with an ultrasonic device. Possible confounders were included in data analyses.

The first study focused on bone health. BMD was higher among men who had been breastfed for a shorter duration as babies, but the same finding was not seen in women. This finding was significant after taking into account calcium intake, teenage physical

activity and adult height. One potential explanation for the finding is that the important ingredients for bone accrual calcium, phosphate and protein contents were higher in milk formula and cow's milk dilution than in breast milk.

In the second study, the most important determinants of adult body composition were gender, growth in infancy, current physical activity and maternal BMI. No evidence was found of an association between the duration of breastfeeding and adult body composition, even after controlling for confounders. First year's growth and skin fold thickness at 12 months of age were associated with adult body composition (waist circumference, trunk fat and BMI). Therefore, breastfeeding had an indirect impact on adult fat accumulation.

The third study investigated the impact of the duration of breastfeeding to the later risk of CVD. Individual risk factors of CVD were evaluated (including blood pressure, cholesterol levels, insulin resistance, and carotid artery intima media thickness) and a Framingham risk score (FRS) was calculated, which predicts the risk of coronary heart disease over a period of 10 years. The duration of breastfeeding had no clinical significance on individual risk factors of CVD and no marked effect on the FRS.

The final sub-study focused on the possible association between CVD and bone health. Inverse associations of individual risk factors (glucose, insulin) of CVD with bone status (BMD and bone formation markers) were observed in a crude model, but detailed investigation revealed that this association was due to common confounders (fat mass, physical activity, smoking and alcohol). In addition, BMD was slightly lower among those males who fulfilled the criteria for metabolic syndrome, but again the association disappeared after controlling for fat mass and other common risk factors. We concluded that the association between CVD and bone health is mediated by common risk factors such as fat mass, physical activity, smoking, and alcohol consumption.

In summary, breast milk has an indirect and relatively minor effect on adult body composition and no association with the risk factors of CVD. Therefore, the duration of breastfeeding is unlikely to resolve any public health problems. BMD was lower but still normal among men with extended duration of breastfeeding than in men with shorter duration of breastfeeding, but more studies are needed to confirm this finding. Regardless of these findings, the initiation and continuing of breastfeeding is recommended to all mothers, if possible. Child health clinics frequently monitor infants' growth and development; if breast milk is insufficient to ensure steady growth, earlier introduction of age-appropriate complementary foods should not be limited. This study indicate that more emphasis should be placed also on other healthy lifestyle factors, such as healthy diet, adequate physical activity and non-smoking, all of which are beneficial also for bone reconstruction and weight management. Such advice plays a major role in preventing diseases such as CVD and osteoporosis.



# Tiivistelmä

Rintamaito on parasta ravintoa imeväiselle, ja se useimmiten riittää takaamaan lapsen optimaalisen kasvun. Ravitsemuksellisen merkityksen ohella rintaruokintaan liittyy ihokontakti, joka tukee kiinteän äiti-lapsisuhteen muodostumista. Rintamaidon kautta lapselle siirtyy äidin vasta-aineita suojaamaan infektioilta.

Maailman terveysjärjestö WHO suosittelee yksinomaista rintaruokintaa kuusi ensimmäistä elinkuukautta ja sen jälkeen rintaruokinnan jatkamista kahden vuoden ikään saakka muun ruoan lisänä. Nämä suositukset ovat hyvin perusteltuja kehitysmaissa, joissa hygieniataso on alhainen ja lisäruokavaihtoehdot eivät välttämättä ole riittäviä tai puhtaita. Tutkimuksissa on selvitetty rintamaidon terveysvaikutuksia imeväisikäisille, lapsille ja nuorille, mutta aikuisuuteen jatkuneita tutkimuksia on vähemmän ja niiden yksi suurimpia heikkouksia on ollut luotettavan rintaruokintatiedon olemassaolo. Monet tulokset rintaruokinnan hyödyistä ovat ristiriitaisia: jotkin tulokset tukevat rintaruokintaa, toisissa tutkimuksissa ei ole löytynyt yhteyttä hyötyihin, mutta raporteja terveydelle epäsuotuisista vaikutuksista on vain yksittäisiä. Rintamaitotutkimuksen tulkintaa hankaloittavat monet sekoittavat tekijät, kuten yhteys koulutukseen ja sosiaaliluokkaan, jotka ovat puolestaan yhteydessä terveyskäyttäytymiseen, kuten tupakointiin, ylipainoon ja terveellisen ravitsemuksen toteuttamiseen.

Tässä tutkimuksessa selvitettiin, onko rintamaidolla aikuisikäen jatkuvia terveysvaikutuksia. Tutkimus keskittyi kansanterveydellisesti merkittäviin sairauksiin ja niiden riskitekijöihin kuten osteoporoosiin, sydän- ja verisuonisairauksiin sekä ylipainoon. Käytössä oli ainutlaatuinen aineisto (n=238), joka oli kerätty vuonna 1975 Helsingin Naistenklinikalta. Lapsia oli tavattu ensimmäisen vuoden aikana seitsemän kertaa. Äitejä kehoitettiin imettämään mahdollisimman pitkään ja rintamaidon vähentyessä/loputtua perheille annettiin tarkat ohjeet lisämaidon ja kiinteiden ruokien aloittamisesta. Vuosina 2006–2009 toteutettiin HYKS Lastenklinikalla seurantatutkimus, johon kaikki alkuperäistutkimukseen osallistuneet kutsuttiin. Tähän tutkimukseen osallistui 158 henkilöä, joka on 66 % alkuperäisestä kohortista. Tutkimuskäynnillä otettiin verinäytteitä sekä mitattiin pituus, paino, vyötärönympäryys ja verenpaine. Osallistujilta kerättiin taustatietoja omasta terveydestä ja elintavoista. Lisäksi he pitivät ruokapäiväkirjaa. Luuston mineraalitiheyttä ja kehonkoostumusta kuvattiin DXA-laitteella ja verisuonien seinämiä ultraäänellä.

Ensimmäinen osatyö käsitteli luuston terveyttä. Luuston mineraalitiheys (BMD) oli parempi miehillä, joilla rintaruokinnan kesto oli ollut lyhyempi verrattuna pidempikestoisen rintaruokinnan ryhmään. Naisilla ei löydetty rintaruokinnan keston korreloivia eroja BMD-tasoissa. Analyyseissä huomioitiin tärkeimpinä sekoittavina tekijöinä kalsiumin saanti, liikuntamäärä nuoruudessa ja henkilön pituus. Mahdollinen selitys löydökselle on rintamaidon, äidinmaidon korvikkeiden ja laimennetun

lehmänmaidon kalsium-, fosfaatti- ja proteiinipitoisuuksien väliset erot. Nämä aineet ovat tärkeitä luuston rakennusaineita, ja niiden pitoisuudet ovat alhaisimmat rintamaidossa.

Toisen osatyön tarkasteluissa ei havaittu suoraa yhteyttä rintaruokinnan keston ja aikuisiän kehonkoostumuksen välillä. Ensimmäisen vuoden kasvu ja ihopoimiumittaukset korreloivat aikuisiän kokoon, jota arvioitiin kehon rasvaprosentin, vyötärönympärysmittan sekä painoindeksin avulla. Aikuisiän keskivartalolihavuutta selittivät eniten tämän hetkinen liikunnan määrä ja äidin painoindeksi (perimä/opittu tapa). Kehon rasvattoman massan määrään vaikuttivat eniten lapsuusiän kasvu sekä sukupuoli. Analyyseissä huomioitiin mahdollisimman hyvin sekoittavia elämäntapatekijöitä. Rintaruokinta ei noussut merkittäväksi tekijäksi aikuisiän kehonkoostumukselle.

Kolmannessa osatyössä tarkasteltiin, onko rintaruokinnalla vaikutusta myöhempään sydän- ja verisuonisairauksien riskiin. Yhteyttä tarkasteltiin yksittäisiin riskitekijöihin (verenpaine, veren rasva-arvot, insuliini resistenssi, valtimoseinämän paksuus) sekä muodostettiin Framingham Heart Study -riskilaskurin avulla niin sanottu riskiarvo (Framingham risk score, FRS), jolla arvioidaan henkilön riskiä sairastua sepelvaltimotautiin 10 vuoden kuluessa. Rintaruokinnalla ei ollut yhteyttä yksittäisiin riskitekijöihin eikä FRS-riskiarvoon.

Viimeisessä osatyössä tarkasteltiin verisuonisairauksien riskitekijöiden kuten keskivartalolihavuuden, glukoosiaineenvaihdunnan, veren rasva-arvojen ja verenpaineen tason yhteyttä luuston terveyteen. Glukoosi- ja insuliinitasot olivat korkeammat niillä, joilla luun muodostumista kuvaavat merkkiaineet (osteokalsiini ja PINP) olivat matalat. Tämä yhteys kuitenkin hävisi, kun kehon rasvamassa ja elämäntavat (liikunta, tupakointi ja alkoholin käyttö) huomioitiin. Lisäksi tutkittiin, onko metabolisella oireyhtymällä yhteyttä luuston terveyteen. Luuston mineraalitiheys BMD oli heikompi niillä miehillä, joilla metabolisen oireyhtymän kriteerit täyttyivät, mutta myös tämä yhteys hävisi, kun kehon rasvamassa ja elämäntavat huomioitiin.

Johtopäätöksenä todetaan, että rintamaidolla on epäsuora vaikutus lapsuusiän kehonkoostumuksen välityksellä aikuisen kehonkoostumukseen, mutta rintaruokinnan kestolla ei ole vaikutusta sydän- ja verisuonisairauksien riskiin. Tuore löydös siitä, että luuston mineraalitiheys on heikompi mutta silti normaali pitkään rintaruokituilla miehillä, kaipaa lisävarmennusta uusilla tutkimuksilla. Tämän tutkimuksen löydöksistä huolimatta rintaruokintaa tulee suositella kaikille imeväisille ensimmäisten elinkuukausien aikana. Lapsen kasvua ja kehitystä tulee seurata tarkasti neuvolassa, ja mikäli rintamaito ei riitä takaamaan tasaista kasvua, on aloitettava kiinteiden ruokien tai lisämaidon anto. Neuvolassa ja jatkossa koulussa on korostettava myös muita terveyteen vaikuttavia tekijöitä: terveellistä ruokavaliota, tupakoimattomuutta sekä riittävää liikunnan määrää, jotka ovat merkittäviä sekä luuston vahvistumiselle että painonhallinnalle ja siten tärkeässä roolissa ehkäisemässä monia myöhempiä sairauksia, kuten sydän- ja verisuonisairauksia sekä osteoporoosia.

# List of original publications

This thesis is based on the following publications:

- I Satu Pirilä, Mervi Taskinen, Heli Viljakainen, Maila Turanlahti, Merja Kajosaari, Ulla M. Saarinen-Pihkala and Outi Mäkitie. Infant milk feeding influences adult bone health: a prospective study from birth to 32 years. *PLoS ONE*, 2011 Apr 27;6(4):e19068
- II Satu Pirilä, Ulla M. Saarinen-Pihkala, Heli Viljakainen, Maila Turanlahti, Merja Kajosaari, Outi Mäkitie and Mervi Taskinen. Breastfeeding and determinants of adult body composition: A prospective study from birth to young adulthood. *Horm Res Paediatr*, 2012;77(5):281-90
- III Satu Pirilä, Mervi Taskinen, Heli Viljakainen, Merja Kajosaari, Outi Mäkitie, Ulla M. Saarinen-Pihkala and Maila Turanlahti. Breastfed infants and their later cardiovascular health: a prospective study from birth to age 32 years. *Br J Nutr*, 2014 Mar;111(6):1069-76
- IV Satu Pirilä\*, Mervi Taskinen\*, Maila Turanlahti, Merja Kajosaari, Outi Mäkitie, Ulla M. Saarinen-Pihkala and Heli Viljakainen. Bone health and risk factors of cardiovascular disease– a cross sectional study in healthy young adults. Submitted.

\*equal contribution

The publications are referred to in the text by the above Roman numerals.

These articles were reprinted with the permission of their copyright holders. Some previously unpublished data are also presented.

# Abbreviations

ANOVA	Analysis of variance
BALP	Bone-specific alkaline phosphatase
BFHI	Baby-Friendly Hospital Initiative
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
CHD	Coronary heart disease
CV	Confidence interval
CVD	Cardiovascular disease
DXA	Dual energy x-ray absorptiometry
FAO	Food and Agricultural Organization of the United Nations
FMD	Flow -mediated dilatation
FRS	Framingham risk score
HDL	High-density lipoprotein
HOMA-IR	Homeostasis model assessment insulin resistance
ICTP	Carboxy-terminal telopeptide of type I collagen
IMT	Intima-media thickness
LDL	Low-density lipoprotein
MANCOVA	Multivariate analysis of variance
NCEP ATPIII	National Cholesterol Education Program Adult Treatment Panel III
NTX	Urinary N-terminal telopeptide
OR	Odds ratio
P1NP	Procollagen type I N-terminal propeptide
PROBIT	Promotion of Breastfeeding Intervention Trial
SD	Standard deviation
UNICEF	United Nations Children's Fund
WHO	World Health Organization
25-OHD	25-hydroxy vitamin D

# 1 Introduction

Apart from humans, no other mammalian new-born offspring has any food option other than breast milk. Breast milk is usually sufficient to ensure optimal growth; signs that should be monitored are the baby's satisfaction, good growth in weight and length, and normal hydration reflected by adequate urine volume and good skin tone. While the nutritional composition of breast milk is adequate, it lacks vitamin D and iron; the former should be supplemented to all babies and the latter to babies born smaller than 2500 grams.

Breastfeeding has many well-known beneficial effects. Among these, the intimate contact may be an important factor for infant-mother attachment relationship. Successful breastfeeding provides particular fulfilment for the mother, which may facilitate to a tighter bond with the child. When the mother takes care of her baby, breast milk is readily available and suitably warm. In addition, breastfeeding is an economical way of feeding and does not produce any waste. Particularly in lower hygienic circumstances, breastfeeding offers protection against infections and the prevalence of gastrointestinal and respiratory infections is lower in breastfed infants.

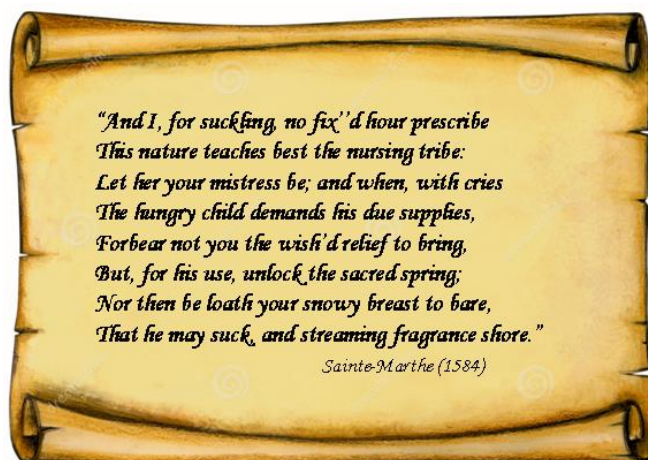
The World Health Organization (WHO) and the United Nations Children's Fund (UNICEF) have issued global guidelines for breastfeeding to ensure the best possible growth, health and life for all children. Although these recommendations are not always met in reality, the tendency has been that the initiation and duration of breastfeeding has increased in recent years. The recommendations and the prevalence of breastfeeding have changed as the knowledge of breast milk, breastfeeding and its benefits have increased remarkably during the last 50 years.

Although breastfeeding has some unquestionable benefits in infancy, the long-term effects of breastfeeding on adult health are uncertain. Obesity, cardiovascular disease (CVD) and osteoporosis are major health issues around the world. Finding ways to prevent these health problems by making early lifestyle choices could make a difference for public health.

## 2 Review of the literature

### 2.1 Recommendations regarding infant feeding

Paedotrophia by Sainte-Marthe (1584):



Adapted from Dr Wickes's doctoral thesis at the University of Cambridge (1951)

#### 2.1.1 History of infant feeding recommendations

Ian G. Wickes' doctoral thesis from 1951 studied the history of infant feeding and found that infant feeding recommendations have varied over the centuries. There have been differences in opinion regarding whether breast milk is sufficient for infants, whether the milk of a cow or another animal is the best alternative, or whether babies should be fed according to the clock or the baby's hunger, and whether the mother should be careful not to overfeed the baby with breast milk. As we can see from the above poem, the poet's conception of baby feeding in 1584 is close to today's recommendations (Wickes 1953).

Infant feeding policies and customs have changed over the last 150 years. In the late 19<sup>th</sup> century, mothers generally weaned their babies mostly to cow's milk between one and three months of age. Ensio Alho reported in Duodecim about infant mortality in Helsinki between 1896 and 1910. The overall prevalence of mortality was high and roughly half of the mortality rate during the first six months of life was caused by acute gastroenteritis (Alho 1915). Alho noted that three-quarters of those babies who died of gastroenteritis were at least partly bottle-fed and the remaining quarter received only breast milk. He believed that breast milk had to have a component that increased resistance towards infections, and because the mortality rate decreased after seven months of age, he concluded that breastfeeding should continue until that time. There was no knowledge of

milk allergies at that time, so Alho also discussed chronic indigestion symptoms and speculated whether these problems were due to cow's milk proteins, fats or carbohydrates.

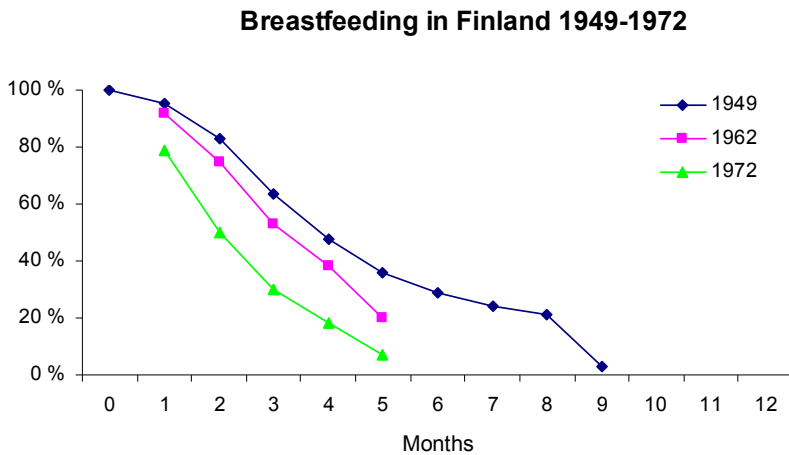
Especially in urban environments, cow's milk was delivered from the countryside, which meant that the milk was warm for long periods of time, and the risk for bacterial contamination was high. Milk was usually boiled before being given to an infant, but this was insufficient to eliminate all bacteria. Physicians were aware of the risk and tried to convince mothers to breastfeed. In the early 20<sup>th</sup> century, attitudes towards breastfeeding changed, along with an increased awareness of the fact that breastfeeding protected the baby from bacterial contaminations of cow's milk.

Prior to the invention of milk formulas, wet nursing was commonly used as an alternative to mother's own milk, especially in higher social classes. In wet nursing, someone other than the baby's own mother (known as a wet nurse) would breastfeed the baby. This method was still in use as recently as the 1960s. Artificial formulas were introduced in Switzerland and Central Europe for the first time in the late 19<sup>th</sup> century. Production expanded in the early 1900s as formula was shown to have saved failure-to-thrive infants in Southern Europe and the US.

Pasteurisation of milk became more common in the 1920s, which reduced bacterial infections caused by contaminated cow's milk, and the infant mortality rate decreased. Therefore, pro-breastfeeding campaigning declined during that time, which reduced the popularity of breastfeeding among mothers (Wolf 2003). In the 1930s, after the introduction of milk pasteurisation the benefits of human milk were forgotten, even by paediatricians, and in the 1930s and 1940s, breastfeeding started to decrease (Vahlquist 1975). During World War II the production of formulas decreased, but increased again soon after the War; it expanded to new continents and, in the 1950s, to the third world. As the infant survival rates improved, formulas were favoured over breast milk, which meant that the initiation of breastfeeding declined for decades; the prevalence of breastfeeding was at its lowest in the 1960s and 1970s (Hultin et al. 1974). In 1973, the Food and Agricultural Organization (FAO) and WHO stated that exclusive breast milk was an inadequate source of energy beyond three or four months of age; based on this estimation, complementary foods were introduced into infants' diets earlier on (FAO/WHO 1973). At the time, only 24 percent of mothers breastfed their babies when discharged from the maternity hospital.

### 2.1.1.1 Infant feeding in Finland from the early 20<sup>th</sup> century until the 1970s

Publications in Finland during the first decades of the 20<sup>th</sup> century all promoted the importance and superiority of breastfeeding. After World War II, the birth rate was very high and mothers were discharged from maternity hospitals early without adequate support and guidance regarding breastfeeding (Hallman et al. 1952). Finland had a particularly good child health clinic system compared with many other countries and doctors in these clinics were educated and informed of the importance of breastfeeding in all professional publications (Hallman et al. 1952, Hirvensalo 1951). However, as the level of hygiene improved and early infections decreased, mothers and health-care providers tended to overlook the importance of breastfeeding. Consequently, the prevalence of breastfeeding decreased in Finland as it had done throughout the Western world. The popularity of breastfeeding reached a new low in Finland in the 1970s (Figure 1), which is when the babies of the present study cohort were born.



**Figure 1.** The prevalence of breastfeeding was at its lowest in the 1970s (Hallman et al. 1952, Hultin et al. 1974).

### 2.1.2 World Health Organization

The 1980s changed overall attitudes towards breastfeeding. WHO and UNICEF emphasised the importance of maintaining the practice of breastfeeding by limiting the marketing, advertising and distribution of infant formulas, and by advising limits to bottle and pacifier use (WHO 1981). In 1990, a WHO meeting was held in Florence, Italy, where the discussions and debates led to a consensus report called: the Innocenti Declaration or “On the protection, promotion and support of breastfeeding with specific instructions for promoting breastfeeding” (Innocenti Declaration. Florence. UNICEF/WHO 1991). Consequently, in 1991 WHO and UNICEF launched the Baby-Friendly Hospital Initiative (BFHI) programme. This programme included a 10-step guideline (Figure 2) for hospitals



and health-care professionals to guide expecting mothers and mothers with new-born babies about the advantages of breastfeeding and support regarding breastfeeding. In 1994, Switzerland introduced the BFHI programme to selected maternity hospitals. The impact of the programme was assessed nine years later, in 2003, when an improvement in the initiation, duration and exclusivity of breastfeeding was observed in the hospitals with BFHI program compared with the hospitals without the programme. Merten et al noticed also that the attitude towards breastfeeding was improved. It was possible that those mothers with previous positive attitudes towards breastfeeding had been more likely to choose a maternity hospital with a BFHI programme and that those mothers who were not interested or not aware of BFHI were more likely to choose their maternity hospital randomly, which could have biased the observed results (Merten et al. 2005).

1. Have a written breastfeeding policy that is routinely communicated to all health-care staff.
2. Train all health-care staff in skills necessary to implement this policy.
3. Inform all pregnant women about the benefits and management of breastfeeding.
4. Help mothers initiate breastfeeding within one half-hour of birth.
5. Show mothers how to breastfeed and maintain lactation, even if they need to be separated from their infants.
6. Give newborn infants no food or drink other than breast milk, unless medically indicated.
7. Practice rooming in; that is, allow mothers and infants to remain together 24 hours a day.
8. Encourage breastfeeding on demand.
9. Give no artificial teats or pacifiers (also called dummies or soothers) to breastfeeding infants.
10. Foster the establishment of breastfeeding support groups and refer mothers to them on discharge from the hospital or clinic.

**Figure 2.** Baby-Friendly Hospital Initiative's 10 steps to successful breastfeeding.

The WHO and UNICEF review was updated in 2001 and the current recommendations are based on this review (Kramer et al. 2002b, WHO 2001). The 2001 recommendation increased the duration of exclusive breastfeeding from four to six months of age to ensure proper growth and development, and to reduce the risk of gastrointestinal infections for all infants in both developed and developing countries. Over time breastfeeding recommendations have been extended for mothers to continue breastfeeding until the age of two along with complementary foods.

### **2.1.2.1 WHO definition of breastfeeding**

According to WHO (2007), definition of breastfeeding is as follows:

- Exclusive breastfeeding: Breast milk is the only source of nutrition, with no additional complementary food, drinks or even water used. Vitamins, minerals and medicines supplements are allowed.
- Predominant breastfeeding: Breast milk is the predominant source of nourishment, allowing the usage of water, water-based drinks and fruit juice, as well as vitamins, minerals and medicines.
- Mixed milk feeding: In addition to breast milk infant milk formula or weaning milk used.
- Complementary feeding: Human milk substitutes and solid, semi-solid or soft food is used while still breastfeeding. Breastfeeding is complemented with solid food such as vegetables, fruit, fruit juices or cereal.

### **2.1.2.2 Other definitions**

The definition of breastfeeding varies considerably across studies, as does the reliability of retrospectively collected information on the duration of breastfeeding; this complicates the interpretation of the results. Some studies have been primarily interested in the impact of exclusive breastfeeding, while others have also included those with mixed milk feeding or have only focused on the duration of exclusive or any breastfeeding, and yet others have compared infants who were never breastfed with those who received some breast milk.

The categorisation/formation of groups in breastfeeding studies also varies widely without any standardised policy. For example, some studies have used four months as the cut-off point (the previous WHO recommendation) or six months (according to the current recommendation).

According to WHO, an infant that has received any foreign food, non-human milk, or solid foods is not considered to have been exclusively breastfed. However, long total duration of breastfeeding with either additional cow's milk-based formula or complementary foods may still provide beneficial effects of human milk.

## **2.1.3 Recommendations in Finland**

### **2.1.3.1 National Institute of Health and Welfare**

The Finnish Ministry of Social Affairs and Health monitors the advertising and information given to families about the health benefits of breast milk and breastfeeding. Health-care professionals are obligated to advise the public to maintain exclusive breastfeeding up to six months and continue breastfeeding while adding solid foods into infant's diet no later than at six months of age (Kansallinen imetyksen edistämisen asiantuntijaryhmä 2009). Five maternity hospitals have been certificated as BFHI hospitals

since 1997. According to BFHI policy, the certification should be renewed every five years; one hospital, Kätilöopisto Maternity Hospital, is the only one to have currently renewed its certification.

### **2.1.3.2 Finnish Paediatric Society**

The Finnish Paediatric Society's recommendation on breastfeeding was updated in 2001 and was announced based on Finnish studies. Breastfeeding is recommended for all children initially; however, most mothers' breast milk does not ensure enough energy and volume to maintain infant's consistent and healthy growth for the first six months of life. Child health clinics provide regular visits to monitor the growth, development and wellbeing of all Finnish children and, when needed, individually advise parents to start feeding complementary foods to the child – either formula milk or solid foods depending on the infant's age. The recommended age to introduce solid foods is between four and six months. In highly hygienic countries such as Finland, no hard evidence has been shown to support the recommendation to breastfeed beyond 12 months of age; this is because, for example, pollution could accumulate into breast milk and even be harmful to the child. Some of a mother's medications or nutrients can be an obstacle for longer breastfeeding. Now breastfeeding is recommended up to 12 months, and should continue thereafter if the infant, mother and family are willing. Solid foods should be added into infant diet no later than six months of age (Suomen lastenlääkäriyhdistys).

# 2.2 The frequency of breastfeeding

## 2.2.1 Finland

A report by the Ministry of Social Affairs and Health of a nationwide survey on infant feeding in Finland was published in 2012 (Uusitalo et al. 2012). The report is based on the information collected from all Finnish child health clinics. The latest data collection involves time period from late 2010 to the first half of 2011, and the participation rate was 60.3 percent, as 5398 families with children aged from two weeks to 12 months attended a regular follow-up visit at child health clinics. These results were compared to the three previous surveys from 1995, 2000 and 2005. The frequency of the intention to breastfeed has been similar during these 15 years; in the 2012 report, 92 percent of the infants received breast milk up to one month of age, compared to 91–97 percent in earlier surveys (Figure 3). The duration of exclusive breastfeeding increased during the survey years (Figure 4). In 1995, 26 percent of infants older than three months of age were exclusively breastfed, while in 2012, the prevalence doubled (to 53 percent). The increase is still clear at five months of age, when corresponding percentages were 3 percent in 1995 and 15 percent in 2012. Also, the number of infants with over six months of breastfeeding increased from 40 percent in 1995 to 55 percent in 2012. Solid foods are introduced by the age of three, four or five months in 16 percent, 55 percent and 83 percent of infants, respectively. The first complementary solid food is commonly vegetables and fruit at four to six months, followed by cereal and meat (Uusitalo et al. 2012).

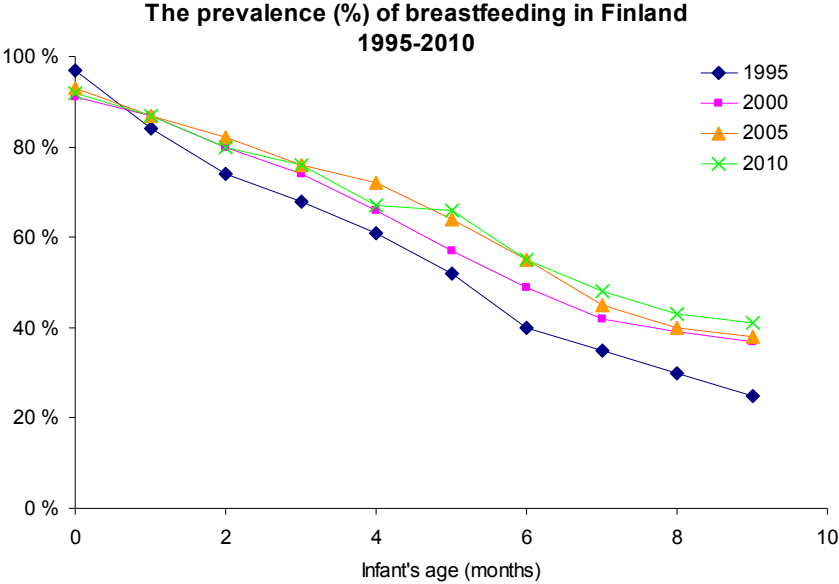
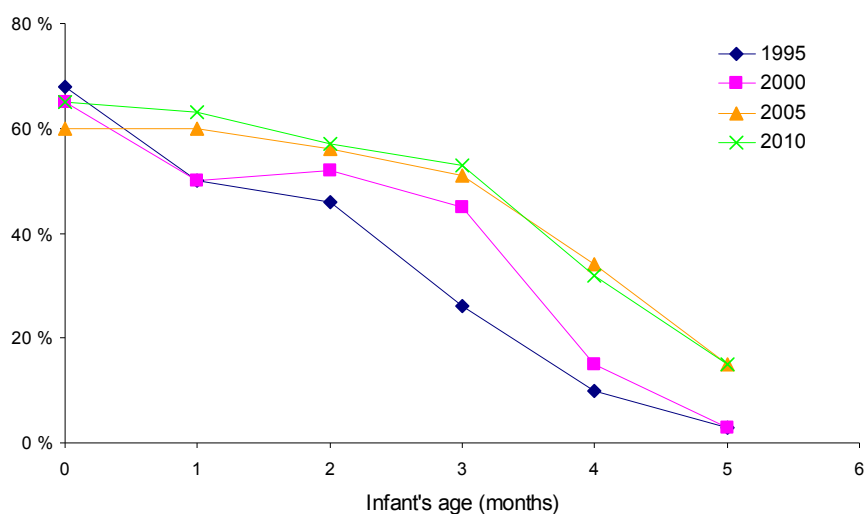


Figure 3. Increase in duration of any breastfeeding from 1995 to 2010 (Uusitalo et al. 2012).

**The prevalence (%) of exclusive breastfeeding in Finland  
1995-2010**



**Figure 4.** Duration of exclusive breastfeeding has increased after the WHO 2001 recommendation changed from four to six months (Uusitalo et al. 2012).

## 2.2.2 The prevalence of breastfeeding in other Western countries

A Canadian study compared local infant feeding practices between 1977–78 and 1984–85 by interviewing 400 mothers. The study concluded that infant feeding in the latter timeframe corresponded closely to the guidelines, and breastfeeding was initiated more often (88 percent vs. 71 percent in 1977–78) and 67 percent of infants were still breastfed at three months of age compared to 40 percent in 1977–78 (Tanaka et al. 1987).

The Euro-Growth Study was a multi-centre study that gathered information on the infant feeding practices of over 2200 healthy term infants born in 1990–93 from 12 European countries. The feeding practices varied considerably between the countries. The study revealed high rates of breastfeeding in Sweden and Greece, and low rates in Ireland, France and the UK. Out of the whole cohort, 52 percent were breastfed exclusively at one month of age and the corresponding percentages were 15 percent at four months and 3 percent at six months (Freeman et al. 2000).

In a 1992 survey from Glasgow, the median age at which solid foods were introduced was 11 weeks (range 4–35 weeks); only 7 percent of infants had not been weaned before they age of four months. Solids were started because mothers had reported that their babies required more food. The authors concluded that mothers in Glasgow followed the recommendations poorly as was reported in other parts of the UK as well (Savage et al.

1998). Less than 1 percent of UK mothers breastfed exclusively up to six months (Bolling et al. 2007).

A relatively recent survey, conducted in 2005 in Sweden, showed that the prevalence of breastfeeding was high, with nearly 98 percent of infants being breastfed and almost 91 percent still receiving breast milk at two months of age. At six months, more than 70 percent and nine months, 40 percent were still receiving breast milk (Official statistics of Sweden, Statistics – Health and Diseases 2007). The UK has had one of the lowest breastfeeding rates in the Western world. However, the intention to breastfeed (81 percent) has increased during the last decade, although the prevalence fell rapidly at six weeks of age, when 55 percent of the infants were breastfed; at six months, only slightly more than a third (34 percent) were still breastfed; and only 1 percent were still exclusively breastfed at six months (Infant feeding survey 2010). In the US, the prevalence of breastfeeding at two months was 64 percent and 50 percent at six months (Grummer-Strawn et al. 2008). In an Australian survey, 69 percent of the infants were still receiving breast milk at four months of age (39 percent exclusively at three months), and 60 percent received breast milk at six months (15 percent were breastfed exclusively at five months) (Australian Institute of Health and Welfare 2011). In Canada, 88 percent of infants were initially breastfed; at six months, 54 percent still received breast milk and at 12 months the prevalence was 16 percent (Health Canada 2013).

## **2.3 Infant milk components**

### **2.3.1 Breast milk**

#### **2.3.1.1 Nutrient components**

The composition of breast milk changes over the duration of breastfeeding: the composition of colostrum is unique and different from later breast milk. Differences have been found in nutritional content at the macro and micro levels and in the content of immunological factors. Differences have also been found in taste and composition between meals, days and mothers (Khan et al. 2012a, Khan et al. 2012b, Savino et al. 2008a).

Breast milk has all the ingredients that a human infant needs to maintain proper and sufficient growth during the first few months of life. Approximately half of the nutritional energy comes from carbohydrates – mainly from lactose, which gives breast milk its sweet taste – and the other half comes from fats. Maternal diet and weight have a major impact on breast milk fatty-acid composition (Mäkelä et al. 2013a). The main proteins in breast

**Table 1.** Nutrient values of infant milks per 100 ml.

Components	Breast milk*	Formula milk Chymos® 1974	Formula milk‡ 2013	Cow's Milk†
Energy, kcal	72	67.5	67	71
Protein, g	1.4 ± 0.2¶	1.5	1.3	3.0
Casein, g	0.3 ± 0.1¶	0.6	0.4	2.6
Whey, g	0.8 ± 0.2¶	0.9	0.9	0.7
Fat, g	4.5	3.5	3.5	4.4
Carbohydrates, mg	6.5	7.2	7.3	4.8
Lactose, mg	6.5	7.2	7.2	4.8
Minerals				
Sodium, mg	20	15	18.6	50
Potassium, mg	50	55	66	106
Calcium, mg	27	38	40	123
Phosphorus, mg	10	26	24.8	90
Magnesium, mg	3	50	5.7	11
Iron, mg	<0.1	11	0.6	<0.1

\* Average human milk during the first few months

‡ Fineli® National Institute of Health and Welfare

† Whole Milk 3.9% <6 months, milk / water mixture 3/2

¶ Khan et al. 2012a

milk are casein and whey. In humans, casein is only produced by mammary glands and consists of calcium, phosphorus and sulphur. Immunoglobulin and albumin are whey proteins. The ratio of casein and whey proteins varies from 10:90 in the early lactation period to 50:50 at the later period (Khan et al. 2012a). During the first few days, when whey protein content is 90 percent, the most important immunoglobulin in breast milk is secretory immunoglobulin A (IgA), when the concentration is 10-fold compared with milk in general. Low levels of secretory IgA have been associated with increased risk of allergies (Iyengar et al. 2012). Breast milk is low in vitamin D and iron, but like most ingredients of breast milk, iron is well-absorbed, which means that the bioavailability is good. Vitamin D supplement is necessary from two weeks of age onwards. Also, minerals such as calcium and zinc have good bioavailability from breast milk (Abrams et al. 1997, Abrams 2010).

### **2.3.1.2 Biological components**

Human milk is a biologically active substance and contains hormones, growth factors, cytokines, and complements. Satiety hormones, such as leptin, are also found in breast milk but not in cow's milk, and their function in the infant's satiety regulation is also of interest (Savino et al. 2008a). Also, the white blood cells are high in breast milk such as neutrophils, monocytes and lymphocytes that are active in immunologic defence.

### **2.3.2 Cow's milk and diluted cow's milk**

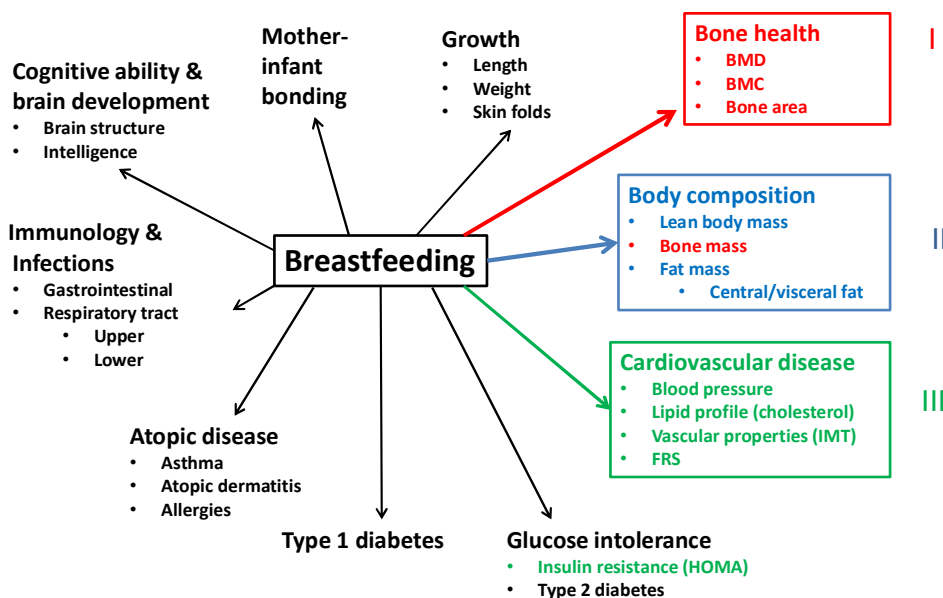
Today, cow's milk as such is rarely given to infants younger than 12 months of age, and thereafter skim milk is recommended for children. The energy content is highly dependent on the amount of fat. Before the 1970s, when commercial milk formula was rare, mothers fed their infants with home-prepared diluted cow's milk, the content of which differed by the mixing ratio of milk and water. Protein content was even higher in whole cow's milk than in milk formulas. Milk used for the preparation was usually whole cow's milk with high fat content. Also other ingredients, such as lactose were added into the dilution.

### **2.3.3 Commercial milk formulas in 1975 and 2010**

The content of infant milk formula has been developed to help infants maintain proper growth and development when breastfeeding is not adequate or successful. The composition of milk formula has been improved with increased knowledge of infant's optimal nutrition and needs of trace elements. However, the content of milk formula has remained similar since 1974, compared with current formula; in terms of protein and lactose content (Table 1). Milk formulas are based on cow's milk proteins, resulting in higher protein content than human milk. In order to determine the optimal protein content for formula milk, growth and body composition have been monitored in and compared between infants fed with formula milk that has either high or low protein content. In addition to the difference in protein content, cow's milk lacks all human immunological and other bioactive components. The composition of formula milk can be manipulated by adding prebiotics, growth factors or secretory IgA (Le Huerou-Luron et al. 2010). Added biologically active components in formula milk are believed to protect against allergies and gastrointestinal infections and to maintain good balance in intestinal system. Vitamins, such as vitamin D, are also added into formulas.



## 2.4 Influences of breastfeeding



**Figure 5.** The influences of breastfeeding are studied widely. The interests in present studies I, II and III are highlighted with colours.

### 2.4.1 Growth, body composition and development

#### 2.4.1.1 Breastfeeding and the growth during childhood

In the early 1970s, there was a general perception that exclusive breastfeeding alone may not be sufficient nutrition for an infant. Studies discussed differences in growth patterns depending on which infant milk was used. There is some evidence to suggest that the first year's growth velocity differs slightly between infants who are exclusively breastfed and fully formula fed (Dewey et al. 1992, Dewey et al. 1993, Dewey et al. 1995, Salmenperä et al. 1985). During the first few months of life, breastfed infants tend to be heavier and accumulate more body fat, but between three and 12 months of age, the proportional weight-for-age falls and the difference between breastfed and formula fed infants disappears (Butte et al. 2000, Dewey et al. 1995, Kramer et al. 2002a, Saarinen et al. 1979b), or even shifts in the opposite direction (Dewey et al. 1993). These results suggest adequate growth for those infants who are fully breastfed for four to six months. However, exclusive breastfeeding beyond six months up to nine months was shown to be insufficient (Salmenperä et al. 1985). Length was comparable (Dewey et al. 1992) or slightly reduced in subjects who were exclusively breastfed for six months, but catch-up growth was then observed by the age of 12 months (Kramer et al. 2002a). Head circumference values were similar between feeding groups in all studies. All infant feeding recommendations emphasise the importance of following up on individual infants and, when needed,

requesting to add complementary feeding to maintain adequate growth and well-being. WHO has published separate growth curves for breastfed infants, allowing slightly slower growth velocity without unnecessary concern about undernourishment (WHO Multicentre Growth Reference Study Group 2006).

#### **2.4.1.2 Body composition overweight and obesity beyond infancy**

Overweight and obesity is a growing problem around the world and causes leading to obesity have been studied to find protective ways to improve prevention. The role of breastfeeding in this prevention is of interest. Studies have attempted to determine whether infant milk feeding affects later obesity. In many studies, exclusive breastfeeding along with a longer duration of breastfeeding seems to have decreased the risk of overweight and obesity, BMI level, percentage body fat, and skin-fold thickness during childhood and adolescents. However, the protective effect attenuated after controlling for confounding factors, such as parental BMI and socioeconomic status. In particular, the extended follow-ups suffer from a lack of reliable data about breastfeeding.

During the early childhood period of 5–10 years of age, more than four months of breastfeeding during infancy was associated with a reduced risk of overweight and obesity measured with weight (Koletzko et al. 2009a), percentage of body fat (Buyken et al. 2008, Karaolis-Danckert et al. 2007, Toschke et al. 2007), skin folds (Hunsberger et al. 2013), weight-for-height (Hunsberger et al. 2013, Scholtens et al. 2008), and BMI (McCrorry et al. 2012, Toschke et al. 2007). Buyden et al found differences only among boys with obese and overweight mothers (Buyken et al. 2008). Promotion of Breastfeeding Intervention Trial (PROBIT) from Belarus compared the risk of obesity between babies who were exclusive breastfed for three months and six months, and found no benefits with longer exclusive breastfeeding at the age of 6.5 years (Kramer et al. 2007b). The 11.5-year follow-up visit of the same cohort showed no difference in the risk of obesity and overweight. The researchers concluded that the duration and the exclusivity of breastfeeding did not predict later fat accumulation (Martin et al. 2013).

A few meta-analyses have been published on the effects of breastfeeding on obesity in children and adolescents (Arenz et al. 2004, Harder et al. 2005, Owen et al. 2005a, Owen et al. 2005b). Arenz et al. conducted a meta-analysis of 69,000 participants aged 5–18 years from nine studies to assess the impact that over six months of breastfeeding had compared with no breastfeeding. Prolonged breastfeeding reduced the risk of obesity; OR 0.79 (95%CI [0.71, 0.85]). Four studies found a reduction in obesity in subjects with longer breastfeeding (Arenz et al. 2004). The meta-analysis performed by Harder et al. studied the risk of obesity in respect of the duration of breastfeeding and found that even one month of breastfeeding can reduce the risk of obesity. The follow-up extended through childhood and adolescence in all but one of the 17 studies. Harder et al. stated that every additional month in the duration of breastfeeding would decrease the risk of overweight by 4 percent (Harder et al. 2005).

The role of breastfeeding in prevention of obesity in adulthood is unclear. Studies on the influence of breastfeeding on adult fat accumulation are scarce and their conclusions vary, although no disadvantages have been reported. These extended follow-up studies are prone to bias caused by numerous lifestyle dependent confounders. In addition, data on the duration of breastfeeding are often collected retrospectively. None of the reports have found that the duration of breastfeeding has any impact on later risk of obesity after adjustments with confounding factors. An Australian study by Shield et al. reported the impact of breastfeeding on BMI in 21-year-old subjects and found a significant reduction in BMI in subjects who were breastfed for over six months compared with those who received breast milk for shorter periods or not at all, but the differences attenuated after adjustments (Shields et al. 2010). A study of nurses in the US aged 24 to 42 years considered both the duration and exclusivity of breastfeeding but found that breastfeeding had no effect on BMI (Michels et al. 2007). Fall et al. reported no evidence that the duration of breastfeeding would protect against later obesity at early and mid-adulthood (aged 15 to 41 years) in a pooled analysis of prospectively collected birth cohorts from low- and middle-income countries (Fall et al. 2011). Parsons et al. reported on a cohort born in England in 1958 that had been followed up several times earlier and finally at 33 years of age. They found a protective effect of breastfeeding, but again it was non-significant after controlling for confounders (maternal BMI, maternal smoking and socioeconomic status) (Parsons et al. 2003). Only one study has found that breastfeeding has a significant benefit on weight control in the long term: the study of a Helsinki cohort including subjects born between 1934 and 1944 in Finland reported a U-shaped association with duration of breastfeeding and obesity assessed by BMI. Breastfeeding for 5–7 months had the most beneficial effect on BMI at the age of 60 years compared to longer and shorter periods of breastfeeding (O'Tierney et al. 2009).

#### **2.4.1.3 Cognitive ability, behaviour and brain development**

Another interesting area of study has been the effect that breastfeeding has had on cognitive development, intelligence and behaviour, each of which has been studied with numerous end points and with particular emphasis on external confounding factors (Jacobson et al. 2014, Walfisch et al. 2013). Several studies have used several different methods (IQ testing, teachers' evaluation, magnetic resonance imaging (MRI) of white matter, and behaviour checklists) and have found that breastfeeding is associated with improved success in intelligence testing and with improved brain development (Kramer et al. 2008, Oddy et al. 2010, Oddy et al. 2011, Deoni et al. 2013, Kafouri et al. 2013). However, the results of these studies can be confounded by the fact that the initiation to breastfeeding and the longer duration of breastfeeding are associated with higher levels of maternal education, standard of living and higher social class. Despite adjustments for these factors, the findings in many studies have remained significant. However, the adjustment specifically for maternal intelligence has been reported in only a few studies (Jacobson et al. 2014, Walfisch et al. 2013). Jacobson et al. found an association between breastfeeding and the child's IQ, which remained after adjusting for social class and

maternal education; however, the association between breastfeeding and childhood intelligence disappeared after adjusting for maternal vocabulary IQ and a parental emotional and intellectual stimulation measure (admitted by interview and observation scoring) (Jacobson et al. 1999).

## **2.4.2 Bone health**

The third component of body composition, in addition to fat mass and lean mass, is bone mass. Studies focusing on the influence of infant milk feeding on bone mass accrual and bone mineral density (BMD) are scarce and the results are controversial. Two studies have shown a positive correlation between breastfeeding and bone health (Jones et al. 2000, Molgaard et al. 2011). According to Jones et al., at eight years of age, both genders had better BMD when breastfeeding continued longer than three months (Jones et al. 2000). Danish adolescents with longer duration of exclusive breastfeeding had better lumbar spine BMD, bone mineral content (BMC) and bone area at 17 years of age (Molgaard et al. 2011). However, one study reported opposite findings: Infants who had been breastfed for over 12 months had lower BMC at two years of age than infants who had been breastfed for less than 12 months and their BMC was lower at 12 months and at two years of age compared with infants who had been exclusively bottle-fed (Butte et al. 2000). Some studies did not find that breastfeeding had any effect on bone health. Children at four years (Harvey et al. 2009, Young et al. 2005) and at 10 years (Fewtrell et al. 2013) of age did not show any association between the duration or exclusiveness of breastfeeding and bone health.

In addition to birth weight, family's socioeconomic status, birth order, infant feeding type has been considered a factor that determines bone health later in life. The major determinants of bone health at 49–51 years of age were adult body size (both weight and height) and lifestyle factors in both genders, but in men early life factors explained 6 percent of the variation of adult BMD. The contribution of early life factors for women's bone health was small (Pearce et al. 2005). However, Koo et al. studied the determinants affecting bone accrual in healthy infants. Similarly, body weight was the most important predictor of bone mineral status, but the type of milk feeding did not correlate with the achieved bone mass during the first year of life (Koo et al. 1998).

## **2.4.3 Immunology and allergies**

### **2.4.3.1 Defence against infections**

In both developed and developing countries, it is impossible to completely avoid diarrhoea and upper and lower respiratory infections. Many studies have observed that breastfeeding has a potentially protective effect against these infections. Again, the results are not convergent and the study setting of the reports differ in terms of exclusivity of breastfeeding, duration of breastfeeding and selection of the control group.

Breastfeeding seems to give a protection against infections early in life, at least in developing countries that have relatively low hygienic circumstances. The findings in a Bangladesh study observed that ongoing breastfeeding halved the risk of cholera infection in both rural and urban environments (Colombara et al. 2013). Strand et al. studied Nepalese children aged 6–35 months with prolonged (> 7 days) diarrhoea and found that the risk for prolonged illness was 9.3-fold greater in non-breastfed infants than in those with any breastfeeding, and the results were not modified by child's age. The cohort in that study only consisted 4 percent of exclusively breastfed children (Strand et al. 2012). The protective effect of exclusive breastfeeding on gastrointestinal infections was also found in industrial society circumstances in the PROBIT study in Belarus. Those children who had exclusive breastfeeding for six months had a significantly lower incidence of gastrointestinal infections during the period from 3–6 months of age than those who were still receiving breast milk but had also received some other foods at three months of age. The same result was found after adjusting for geographic site, urban versus rural location, maternal education, and the number of siblings in the household (Kramer et al. 2003).

Breastfeeding has shown a decrease in the incidence of upper and lower respiratory tract infections, at least during on-going breastfeeding (Abrahams et al. 2011, Duijts et al. 2010, Fisk et al. 2011, Quigley et al. 2007). Some studies have shown an effect even beyond the cessation of breastfeeding up to second or third year of life, even in industrialised countries. In a Finnish study of day-care children, the beneficial effect of breastfeeding on upper respiratory and ear infection extended up to three years of age (Hatakka et al. 2010). Enterovirus infections were less frequent among infants whose mothers had high levels of enterovirus antibodies in serum and in breast milk. The protective effect seemed to be mediated via maternal antibodies in breast milk. Breastfeeding for at least two weeks reduced the risk of enterovirus infection in infants during the first 12 months (Sadeharju et al. 2007). In the UK, exclusive breastfeeding protected against lower respiratory infections and diarrhoea during the breastfeeding period, but after the cessation of breastfeeding the protection weakened immediately against respiratory infections whilst in one month against diarrhoea (Quigley et al. 2007). Similarly, two separate studies in Rotterdam found no decrease in the number of respiratory tract infections beyond six months of age (Duijts et al. 2010) and no decrease in frequency of acute otitis media in the second year of life in babies who were breastfed for at least three months compared with those with shorter duration of breastfeeding (Labout et al. 2011). Some studies have also reported no protection against upper or lower respiratory tract infections (Kramer et al. 2003).

Based on this literature review, it seems that exclusive breastfeeding for six months would provide the best possible protection against infections during the breastfeeding period. However, even two months of any breastfeeding can have protective efficacy against respiratory infections for the child's entire first year, or even up to three years of age. Especially in low-hygiene countries, breastfeeding significantly reduced the risk of gastrointestinal infections.

### **2.4.3.2 Atopic disease and asthma**

Research on the impact of breastfeeding on atopic diseases has been very active. For years there has been a strong belief that breastfeeding protects against allergies (Kull et al. 2005, Saarinen et al. 1995) and asthma (Kull et al. 2010), a belief that was supported by a meta-analysis (van Odijk et al. 2003). However, recent studies have challenged this (Kramer et al. 2007a). There is no unequivocal answer about whether breastfeeding is protective against allergies (Matheson et al. 2012), or actually increases the risk of asthma (Sears et al. 2002) and eczema (Bergmann et al. 2002). The current belief, based on large cohorts (Elliott et al. 2008) and meta-analysis (Kramer et al. 2012), is that breastfeeding does not reduce the risk of atopic eczema, asthma, or other atopic outcomes. Exposure to household pets, family history of atopic diseases, the amount of allergens and allergic cells in breast milk, and the allergy-related individual differences in mothers' breast milks are considered to be possible reasons for conflicting findings (Matheson et al. 2012).

## **2.4.4 Metabolic effects**

### **2.4.4.1 Cardiovascular morbidity and mortality**

CVD include coronary heart disease (CHD) and stroke. The prevalence of these events increases from middle age onwards. A few studies have evaluated whether breastfeeding influences the morbidity and mortality of CVD (Fall et al. 1992, Martin et al. 2004, Martin et al. 2005, Rich-Edwards et al. 2004). All these large cohorts were collected in the UK and included participants born during the first half of 20<sup>th</sup> century, even as early as 1911. The data on breastfeeding was collected retrospectively at middle age from the participants, their mothers or other close female relatives. The end points were CHD, stroke or death caused by a CVD event. Two of these studies found that breastfeeding had a protective effect, especially when continued for more than 9–12 months (Fall et al. 1992, Rich-Edwards et al. 2004). One found no effect (Martin et al. 2004) and another one found an inverse effect as breastfeeding increased the risk of CVD event or mortality (Martin et al. 2005).

### **2.4.4.2 Blood pressure**

A large pool of data of 10,912 subjects aged 15–41 from five low- and middle-income countries (Brazil, Guatemala, India, the Philippines and South Africa) showed no association with longer duration of breastfeeding on blood pressure, obesity or overweight (percentage body fat, waist circumference) (Fall et al. 2011). Few meta-analysis and systematic reviews on the effect of breastfeeding on blood pressure and cholesterol have been published (Martin et al. 2005b, Owen et al. 2003). Most of the above-mentioned studies evaluated the effects of breastfeeding in children and adolescents but not in adults. Pooled systolic blood pressure was 1.4 mm Hg (Martin) and 1.1 mm Hg (Owen) lower in breastfed children than in bottle-fed children. Diastolic blood pressure was not significantly related with infant feeding type in Owen et al.'s study, but Martin et al. found

0.5 mm Hg lower diastolic blood pressure within breastfed subjects. The authors of both of these meta-analyses conclude that the overall possible effect of breastfeeding on blood pressure is modest and has limited clinical or public health relevance (Martin et al. 2005b, Owen et al. 2003).

#### **2.4.4.3 Cholesterol**

A review and pooled meta-analysis of 17 studies concluded that breastfed adults had lower total cholesterol level by 0.04 mmol/l (95% CI: -0.08, 0.00 mmol/l) compared with those who had never received breast milk (Owen et al. 2008). Kajantie et al. studied lipid concentrations at 57–70 years of age in subjects of the Helsinki Birth Cohort and observed an association between adverse lipid profile in adulthood and small body size at birth and slow weight gain during the first two years of life. The length of breastfeeding did not have impact on the adult lipid profile (Kajantie et al. 2008).

#### **2.4.4.4 Vascular properties**

The Cardiovascular Risk in Young Finns Study investigated the association between infant feeding and vascular health later in life with ultrasound by assessing flow-mediated dilatation (FMD), common carotid intima media thickness (IMT) and carotid artery compliance in nearly 1700 young adults aged 24–39 years. Maximal FMD was higher in men who had been breastfed as babies than in those who had been formula-fed, but no differences were observed in women. IMT and carotid artery compliance were not affected by the duration of breastfeeding (Järvisalo et al. 2009). Breastfeeding and formula feeding groups did not differ in respect of mean concentrations of serum lipids, blood pressure or BMI. In a study by Martin et al., breastfeeding was associated with reductions in common carotid and bifurcation IMT and with odds of carotid plaque compared with bottle feeding in 65-year-olds (Martin et al. 2005a). Leeson et al. reported controversial findings in young adults (20–28 years) as those who had been breastfed for four months or more had lower brachial arterial wall distensibility than those who were never breastfed or were breastfed for fewer than four months. However, participants who had never been breastfed had similar distensibility to those who had been exclusively breastfed for less than four months (Leeson et al. 2001).

#### **2.4.4.5 Type 2 diabetes and insulin resistance**

The association between the duration of breastfeeding and insulin resistance in adulthood has been widely studied. In Pima Indians, who have an extremely high prevalence of type 2 diabetes, a lower rate of diabetes was observed among those people aged 39 or less who had been exclusively breastfed for two months than those who had been exclusively bottle-fed (Pettitt et al. 1997). Similarly, in a Dutch study plasma glucose concentration was lower in adults aged 48–53 years who had been exclusively breastfed than in those who were bottle-fed in infancy (Ravelli et al. 2000). A meta-analysis suggested that breastfeeding was associated with a reduced risk of type 2 diabetes in adulthood compared

with having been bottle-fed (Owen et al. 2006). However, in a recent study of subjects at 23–27 years of age, no association was found between insulin resistance and infant feeding type (Williams et al. 2012).

### **2.4.5 Type 1 diabetes**

The role of breastfeeding in the prevention of type 1 diabetes is uncertain. A recent Finnish study compared breastfed infants with infants who received cow's-milk-based formula milk or hydrolysed milk and found that the prevalence of type 1 diabetes was not dependent on the type of infant feeding. The authors concluded that even very early exposure to cow's milk did not increase the risk of diabetes (Savilahti et al. 2009). Another large interventional study in Finland evaluated the risk of type 1 diabetes among those with elevated diabetes risk in the family. Findings were controversial, but a protective effect of prolonged breastfeeding and a negative effect of an early exposure to foreign protein were observed (Knip et al. 2010).

### **2.4.6 Infant-mother attachment relationship**

It has often been assumed in the scientific literature, without empirical evidence, that breastfeeding strengthens the infant-mother attachment relationship (Jansen et al. 2008). Some evidence has been presented that indicates that infant-mother interaction was higher in quality in breastfeeding infant-mother pairs than in bottle-feeding pairs (Britton et al. 2006). Maltreatment, as a sign of an insecure disorganized attachment, occurred less frequently in pairs with breastfeeding than in bottle-feeding pairs. However, the quality of attachment of infant-mother pairs was independent of the type of infant feeding (Britton et al. 2006). Some studies have considered the possible interaction between the intention to breastfeed and the related maternal care-giving behaviour, when observing the impact that the duration of breastfeeding has on the infant-mother attachment relationship (Britton et al. 2006, Tharner et al. 2012). The differences in the attachment security between breastfed and limited or no breastfeeding infant-mother pairs have been small and bottle feeding has not been shown to harm the relationship (Tharner et al. 2012). Therefore, there is no reason for serious concern about the infant-mother relationship in pairs with limited breastfeeding.

## **2.5 Potential pathways of infant feeding on subsequent health**

Although the mechanisms and potential pathways that could link early nutrition to later health and well-being have been widely discussed and investigated, they are not well understood. Most studies have been observational and the causality between early nutrition and health conditions later in adulthood is confounded by many external aspects and choices in life, many of which interact with each other. During the lactation period breast milk and breastfeeding may affect the infant in several ways. The sensitive time



period at early infancy can induce lasting effects on later health and disease risk. There is no unanimous opinion regarding how and what effects and changes could persist and, thus, permanently programme later health, even up to old age. This section will deal with these theories.

**Table 2.** Potential mechanisms of how breastfeeding may induce early programming for future health.

---

Nutritional

Energy content

Protein

Fats

Mineral content

Immunology, bacterial colonisation and intestinal maturation

Bacterial colonization

Immunology

Bioactive components

Intestinal maturation

Mechanical negative pressure

Pathogen exposure

Epigenetics

Adapted life style

---

### 2.5.1 Nutritional factors

Energy content of breast milk supports infants' self-regulated intake of milk and appetite. During the first months of life, an infant needs approximately  $1/6^{\text{th}}$  of its own weight in milk per day. Overfeeding is considered to be more likely with bottle feeding. Bottle-fed babies may get too much energy from formula milk, as the energy content remains unchanged despite the age of the infant. The energy content in breast milk, on the other hand, changes as the child grows to a certain point, but it is uncertain whether the nutritional needs are met with exclusive breastfeeding up to six months. The energy content of breast milk is at its highest when the child is 3–4 months old and might not be sufficient enough at six months of age. The absolute need for energy increases as the infant grows and gains weight. Hence, early growth patterns may programme and track into adulthood, increasing the risk of obesity (Arenz et al. 2004, Koletzko et al. 2009b).

Protein content is lower in breast milk than in cow's milk. In general, animals reach their mature size by one year of age, which means that their energy and protein needs for mother's milk are different than in human babies. For human babies, excessive protein

intake should be limited, because it can be harmful for the immature kidney, and may increase the risk of overweight (Koletzko et al. 2009b). Koletzko et al. introduced the theory of “early protein hypothesis” and randomised infants to receive two formulas of different protein contents. The formula with lower content of protein was adequate for the growth of infants and resulted in more preferable body composition than the milk with higher protein content. Higher protein and energy intake stimulated insulin secretion and resulted in insulin resistance; the increased tendency continued up to adolescence and adulthood (Koletzko et al. 2009b).

The exposure to foreign milk protein as a risk factor for the onset of allergies (particularly in children with genetically high risk) (Kramer et al. 2007a) and diabetes (Virtanen et al. 2003) has been presented and studied.

The fat content of breast milk differs between mothers and during the lactation (Khan et al. 2012b, Mäkelä et al. 2013a). Fat content is highest during the first months of breastfeeding and breast-fed babies grow faster than formula-fed babies during these months (Saarinen et al. 1979b).

The most important mineral in milk is calcium, which is particularly important for bone mineral accrual and muscles. The content of calcium differs between breast milk and cow’s milk. However, the bioavailability of calcium from breast milk is especially good and the total absorption seems to be adequate to meet bone mineral accretion requirements (Abrams 2006, Abrams 2010).

## **2.5.2 Immunology, bacterial colonisation and maturity of gut**

At birth, the intestine is sterile. The route of delivery and type of infant feeding affects bacterial colonisation in an infant’s intestine, which then increases exponentially from the first hours, days and weeks of life. Intestinal bacteria and their diversity are different depending on the type of infant milk. Oral (Holgerson et al. 2013) and actual intestinal microbial profiles in breastfed babies are different from those of bottle-fed babies. Colonisation of lactobacilli in an infant’s oral cavity and gut is more frequent with breastfeeding than with bottle feeding (Martin et al. 2003, Vestman et al. 2013). Formula feeding induces intestinal hypertrophy and accelerates the maturation of hydrolysis capacities, and also increases intestinal permeability and bacterial translocation (Le Huerou-Luron et al. 2010). The diversity of intestinal bacteria is believed to affect the risk of allergic diseases (Abrahamsson et al. 2012), obesity (Kalliomaki et al. 2008), and other diseases such as diabetes (Vaarala 2013).

Neonatal immunity at birth is completely transferred from the mother. Ingested immunological elements of breast milk affect the absorption of nutrients and other factors in an infant’s gut. These immunologic elements include cells, antimicrobial, anti-inflammatory and immunomodulatory agents and enzymes (M'Rabet et al. 2008). The bioavailability of these active metabolites is variable. Antibodies penetrate through the

placenta during the prenatal period. The maternal-infant immunological link is maintained through breast milk. Maternal immunity continues up to six months. Immunoglobulins transferred through placenta are anti-IgG antibodies; however, the most important maternal antibodies are secretory IgA. The infant's own formation of antibodies begins immediately after birth, when he or she is exposed to foreign antigens and pathogens. The immune system should recognise harmless antigens such as food and gut microbiota: and the failure to do so results in the development of allergy and autoimmune diseases (Iyengar et al. 2012, Vaarala 2013).

Breast milk contains important bioactive compounds regulating satiety and hunger, such as leptin, which is absent in infant formula (Savino et al. 2008a). Leptin is important in angiogenesis, bone metabolism, haematological differentiation, and possibly in brain development. Leptin receptors exist in the small intestine, and leptin acts as a small intestine growth factor. One of the most important aspects of leptin is the modulative effect on the immune system (Locke 2002). Higher leptin levels in human milk may be associated with childhood obesity (Weyermann et al. 2007). The importance breast milk's leptin on the infant's appetite regulation and its bioavailability has not been conclusive. However, leptin concentration was higher in breastfed infants (Savino et al. 2008b).

During the first few months of life, the intestine is immature and prone to plasticity. Larger particles are able to penetrate the intestinal mucosa. Higher permeability has been measured in formula-fed infants compared with breastfed infants (Le Huerou-Luron et al. 2010). In addition, there is evidence that formula milk induces gut hypertrophy, which accelerates maturation of hydrolytic capacities (Le Huerou-Luron et al. 2010). By four months of age, the intestine starts to achieve mature-type absorption features.

### **2.5.3 Mechanical negative pressure**

The mechanical negative pressure while sucking, swallowing and breathing could contribute to a reduced rate of ear infections in breastfed infants (Abrahams et al. 2011, Saarinen 1982). Bottle feeding does not necessarily require the same pressure, because milk flows generally more easily from the artificial nipple. However, this would not explain the reduction beyond the suckling period.

### **2.5.4 Pathogen exposure**

Breast milk offers greater immunological protection, especially in areas with poor sanitation, where foreign antibodies and pathogens may be introduced to the infant through contaminated water, bottles and nipples. Pathogen avoidance is easier in industrialised countries, where the level of hygiene is high and clean water is available.

### **2.5.5 Epigenetics**

Epigenetics is the study of permanent and heritable changes in gene expression without affecting the DNA sequence itself. Epigenetics or programming is a phenomenon in which early external events during foetal life and early neonatal period make permanent changes on molecules that may affect later health (Gluckman et al. 2004, Koletzko et al. 2009b). This theory was originally presented by Professor David Barker and is therefore also known as the Barker's hypothesis. Barker found that impaired prenatal growth and consequent low birth weight is associated with increased risk for later CVD, hypertension or obesity (Barker et al. 1993, Barker 1995). The chemical changes, epigenetic modifications such as DNA methylation or histone modifications take place on the structure proteins on the surface of DNA chain that affect the gene function without modifying the DNA sequence itself. These changes take place during the prenatal and early neonatal sensitive, immature period when a baby's body adapts to external conditions and the changes may last through cell divisions throughout life and even pass to multiple generations (Cedar et al. 2009). The timing of the plasticity period, the role of early nutrition on postnatal programming and epigenetics remain unknown (Wiedmeier et al. 2011).

### **2.5.6 Adapted life style**

Many factors may influence mother's decision to initiate and continue breastfeeding. Mothers in families favouring healthy lifestyle may smoke less, exercise more, keep normal weight and have healthier diets. Healthy lifestyle habits clusters to same individuals, as do unhealthy habits to the others. Those mothers who initially choose to breastfeed are more likely to continue to follow the instructions from health education. Infant feeding patterns are related to maternal education level and age, as more educated and older women were more likely to breastfeed longer and introduce complementary foods later and use home-made foods (Betoko et al. 2013, Gage et al. 2012). Children who had been breastfed longer were less likely to have unhealthy diet and more likely to consume more fruits and vegetables and less white bread, carbonated soft drinks, chocolate bars and fried snacks than those who were formula-fed in infancy. This trend was seen in studies with preschool children (de Lauzon-Guillain et al. 2013, Scholtens et al. 2008) and at middle-age (Robinson et al. 2012). Habits from childhood influence the choices made in that child's own family in adulthood. Overweight and obese mothers tend to breastfeed for shorter periods or do not initiate breastfeeding at all (Krause et al. 2011, Mäkelä et al. 2013b). This could be due to mechanical difficulties with breastfeeding or life priorities, and these habits persist as the child grows. Also, mothers who smoke are less likely to breastfeed and more likely to wean earlier (Amir et al. 2002, Weiser et al. 2009).

## **2.6 Common public health problems: Osteoporosis, overweight and obesity and cardiovascular disease**

Three of the most common diseases causing mortality and morbidity in the Finnish population in general are osteoporosis, cardiovascular disease (CVD), and problems related with overweight and obesity. These are a major burden on public health, resulting in high health-care costs in an aging population. The prevalence of these diseases increases with age in all societies and early prevention can reduce morbidity.

### **2.6.1 Osteoporosis**

Osteoporosis affects approximately 400,000 in Finland and the annual number of osteoporotic fractures is between 30,000 and 40,000, of which more than 7000 are hip fractures. Hip fractures are known to increase mortality (Current Care Summary 2006). The incidence of hip fractures has declined steadily since the late 1990s. However, it has been predicted that the prevalence will increase because of the aging population (Korhonen et al. 2013).

Bone health can be assessed with dual radio imaging X-ray (DXA) to measure bone mineral density (BMD), bone mineral content (BMC) and bone area. BMD values are compared with reference values for healthy same age and sex population and expressed as a Z-score. In clinical practice, a Z-score of 0 SD is equal to the median value and values between -2 SD and +2 SD are considered to be within the normal range. When the Z-score is below -2 SD, the bone is considered to be osteoporotic. Further, the criteria of osteopenia is met when the Z-score is at the lower end of the normal range between -1SD and -2 SD. BMD and BMC strengthen up to 20 or even 30 years of age. With age, bone strength gradually decreases and the prevalence of osteoporosis increases. In females, BMD loss accelerates significantly after menopause. Genetic background determines 80 percent of bone strength, but other well-known determinants are physical load such as body weight and physical activity, dietary calcium and vitamin D intake. Smoking and excessive alcohol consumption are known to weaken bones.

Fractures occur throughout life. It has been shown that a 1-SD decrease in Z-score doubles the risk of fractures. Primary osteoporosis is still rare in childhood, adolescence and young adulthood (Mäyränpää et al. 2011). Fracture incidence increases exponentially with age, particularly over the age of 75 years.

### **2.6.2 Central obesity, overweight and metabolic syndrome**

Overweight and obesity are important risk factors for chronic diseases, including CVD and type 2 diabetes as well as musculoskeletal problems and impaired quality of life. Obesity is a growing problem also in Finland. In 2012, the average BMI of Finnish men was 27.1 kg/m<sup>2</sup> and women 26.0 kg/m<sup>2</sup>. Of men, 66% had BMI level above 25 kg/m<sup>2</sup> and

20% above 30 kg/m<sup>2</sup> and the corresponding percentages for women were 46% and 19% (Männistö et al. 2012). Central obesity is a component of metabolic syndrome and associates with the other risk factors such as glucose intolerance, dyslipidemia and hypertension. According to National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) the criteria of metabolic syndrome is fulfilled when three out of five disturbances are present (Figure 6) (Olufadi et al. 2008). The prevalence of metabolic syndrome is increasing as almost half of the adult population have waist circumference above the cut-off point. The actual criteria of metabolic syndrome are met by 35% of Finnish population aged between 24-75 years (Finrisk 2007). The incidence of dyslipidemia has declined for many years in Finland, but the latest trend showed increase in mean cholesterol levels in both men and women. Currently, 58% of men and 62% of women have cholesterol levels above the recommendations (Vartiainen et al. 2012).

<p><b>Framingham risk score (scale)</b></p> <ul style="list-style-type: none"> <li>• Age (-7 – 16)</li> <li>• HDL cholesterol (-1 – 2)</li> <li>• Total cholesterol (0 – 13)</li> <li>• Blood pressure (0 – 6)</li> <li>• Diabetes (0 – 2)</li> <li>• Smoking (0 – 2)</li> </ul> <p><b>Each risk factor are given points according to the age and gender- specific table and then calculated a single value that estimates the CVD risk</b></p>	<p><b>NCEP ATP III</b></p> <ul style="list-style-type: none"> <li>• Blood pressure (SBP/DBP 130/ 85 mmHg)</li> <li>• Fasting glucose (&gt; 6.4 mmol/l)</li> <li>• Waist circumference (men/women &gt;100/85 cm)</li> <li>• Triglycerides (&gt; 1.5 mmol/l)</li> <li>• HDL cholesterol (men/women &lt; 1.0/1.5 mmol/l)</li> </ul> <p><b>The presence of three or more risk factors are required to fulfill the criteria of metabolic syndrome</b></p>
-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------	------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------

**Figure 6.** The risk factors included in FRS and NCEP ATP III.

### 2.6.3 Cardiovascular disease

The prevalence of CVD has decreased remarkably from 1970 up to 2009, although the prevalence has levelled out or even increased slightly since then, in parallel with cholesterol levels (Vartiainen et al. 2012). CVD is still one of the major causes of death. Since the 1970s, the most important causes for the reduction have been the changes in lifestyle factors such as diet and reduction of smoking. Treatment facilities have also improved. A current concern is the increase in obesity and related diseases, such as type 2 diabetes, which could increase the prevalence of CVD.

CVD develops gradually over the years. The first sign of declined endothelial function is the decrease in vessel wall distensibility, after which vascular intima media starts to thicken. These findings are even measurable in childhood with vascular ultrasonic device by measuring flow mediated dilatation (FMD) and common carotid artery intima media thickness (IMT). Cardiovascular events are still rare at young adulthood, but CVD risk can be estimated with the accumulation of risk factors.

The Framingham risk score (FRS) is one estimate of later CHD. This score is calculated by using measures of CVD risk factors such as age, gender, lipid profile, blood pressure,

smoking, and possible diabetes (Figure 6) (Wilson et al. 1998). The score gives an estimated risk for CHD over a period of 10 years.

#### **2.6.4 Possible linkage between bone, central fat and cardiovascular disease**

Bone health, overweight and CVD risk factors may have direct metabolic links with each other. Recent research has shown that BMI may protect against bone loss and osteoporosis in aging subjects. The load induced by body weight and physical activity are important determinants of improved bone strength and density. At postmenopausal age, higher BMI resulted in a lower risk of fractures (Johansson et al. 2013). In 11-year-old obese children, lean mass was the main predictor of BMC and BMD and fat mass did not contribute on bone health (Afghani et al. 2005). Therefore, body weight alone does not explain bone strength, but lean body mass would be beneficial and adipose tissue could increase inflammation and therefore be even harmful to bone mineralisation (Jeon et al. 2011). Fat accumulation, especially central fat, was associated with impaired bone structure in bone biopsy (Cohen et al. 2013). Women with metabolic syndrome had lower BMD and a higher CRP level as a sign of inflammation (Jeon et al. 2011). In a study of adolescents, BMC was 5.4 percent higher among obese healthy participants than among those who were obese with multiple other metabolic components (Pollock et al. 2011). In addition, Hamann et al. reported a connection between the presence of type 2 diabetes and lower BMD (Hamann et al. 2012), while Shu et al. found that bone turnover markers describing bone formation, osteocalcin and procollagen type I N-terminal propeptide (P1NP) were lower among women with type 2 diabetes referring lower bone turnover than among their healthy counterparts (Shu et al. 2012). Similarly, among adolescent Korean males, insulin resistance was associated with lower BMC in subjects aged 13 to 19 years. Such association was not noticed in females (Lee 2013). Also, CVD events have been associated with low BMD levels (Tanko et al. 2005).

Osteoporosis and CVD both have degenerative and multifactor entities, they increase with age and, in addition to inheritance, the common risk factors include smoking, inadequate physical activity and excessive alcohol consumption.

Findings between bone health and metabolic syndrome thus are controversial (Johansson et al. 2013, Pollock et al. 2011). The mechanisms behind the linkage are not well understood and may differ during different phases of life as the balance of bone remodelling, bone formation and resorption is different during growth, aging and at a steady state in young adulthood before menopause.

### **3 Aims of the study**

The primary aim of this study was to evaluate whether duration of breastfeeding has an impact on the recipient's later health in young adulthood. The study focused on diseases and risk factors such as osteoporosis, overweight and obesity and CVD which are of importance to public health as a major cause of morbidity and mortality.

More specifically, the study investigated the following areas:

1. The impact of the duration of breastfeeding on adult bone mineral density, bone mineral content and bone area as well as bone turn-over markers.
2. The determinants of adult body composition from early infancy, adolescence and current lifestyle, with particular focus on the influence of breastfeeding.
3. The influence that the duration of breastfeeding has on the risk of later CVD and metabolic syndrome.
4. The possible connection between risk factors of cardiovascular disease and bone health, and whether impaired glucose metabolism leads to weaker bones.



## 4 Subjects and methods

### 4.1 The original study cohort

The present study of adult health in subjects at 32 years of age was based on a follow-up cohort originally collected at birth in the Women's Hospital, Helsinki University Central Hospital during the first three months of 1975. The original study included all families (n=256) with healthy, singleton, full-term babies with birth weight exceeding 3000 grams who agreed to participate. Most of the participating families were in the middle and upper-middle social classes, and since the maternity hospital was situated in the city of Helsinki, many participants came from an urban background. Babies were assigned a participation number at the time of the study collection. The purpose of the original studies were to evaluate the role of infant milk type on iron balance in healthy full-term infants (Saarinen et al. 1977a, Saarinen et al. 1977b, Saarinen et al. 1977, Saarinen 1978, Saarinen et al. 1978a, Saarinen et al. 1978b, Saarinen et al. 1979a).

In total, 238 participants completed all seven visits in the Children's Hospital, Helsinki University Central Hospital, at two weeks and then at one, two, four, six, nine and 12 months of age. At each visit, the same physician (Ulla Saarinen-Pihkala) met all the infants and their families, and gathered the data on milk feeding, body length and weight of the infants and measured the thickness of scapular and biceps skin folds at the six-, nine- and 12-month visits. Venous blood was drawn at each visit for haemoglobin, red blood cells indices and iron markers.

The follow-ups continued at three, five, 10, and 17 years of age, when the main purpose of the study was to assess the role of breastfeeding on the prevalence of atopic diseases in childhood and adolescence (Kajosaari et al. 1981, Saarinen et al. 1979, Saarinen et al. 1979, Saarinen et al. 1982, Saarinen et al. 1982, Saarinen et al. 1995). Approximately 160 children participated in these visits and the signs of allergy or atopic diseases were observed. Weight and height were also measured at the three- and five-year visits. The weight and height of the parents were enrolled at the three-year visit.

Breastfeeding was initially recommended for all babies at the maternity hospital and personal support was given to mothers; breastfeeding was encouraged to continue for as long as possible, and when breast milk became insufficient, instructions were given to start providing either home-prepared diluted cow's milk or commercial milk formula (Bona® Chymos, Lappeenranta, Finland) according to the given participation number. Data on the duration of breastfeeding, additional milk introduction and the duration of exclusive breastfeeding were available for the present study. Solid foods were introduced to all infants at 3.5 months with detailed instructions (Figure 7), after which exclusive breastfeeding was considered to have ceased, according to WHO's definition. Vegetables

were provided first, then cereals were added at five months, and meat and eggs were introduced at six months, and normal mixed food after nine months of age. In accordance to recommendations in 1975, vitamin D was supplemented to all from two weeks of age (1000 IU/day).

## **4.2 The present study**

The present study began in 2006 after Helsinki Ethics committee approved the study protocol. The contact information for the entire original cohort was traced through the Population Register of Finland. The invitation letters were sent between late 2006 and early 2009. Those who did not respond to the first letter were sent a reminder letter. Finally, those with available contact information were phoned and were informed about the study. In total, 188 individuals were contacted and eventually 158 attended the 32-year follow-up visit. Those who were contacted, but did not participate had either no time or interest. Data on breastfeeding, weight, length and scapular skin folds of the first year measures, and parental weight and height from the three year visit were available for the present analysis from the entire original cohort, both from participants and non-participants.

### **4.2.1 Questionnaire**

A questionnaire was sent to all participants to gather information about their health, diagnosed illnesses, medication, and possible fractures. Also, in order to survey lifestyle habits, the questionnaire included questions about teenage and current physical activity, tobacco usage and alcohol consumption, dietary habits, overweight problems, remarkable weight gains or losses. The medical history of parents and siblings was also registered. This included notes on illnesses, medication, fractures, blood pressure, cholesterol level, known allergies or the possible cause and age of death. This questionnaire was completed as an interview during the visit.

### **4.2.2 Clinical visit**

The clinic took place between 2006 and 2009 in the outpatient clinic of Children's Hospital at Helsinki University Central Hospital. During the visits, the physician (SP) was blinded to the information of the duration of breastfeeding. The examination day and the circumstances were standardised for all participants. Each participant arrived after fasting overnight and were abstaining from the use of tobacco or caffeine for at least 4 hours prior to the visit. Blood pressure was assessed twice within five minutes after a 10-minute supine rest period with an automatic device Dinamap (Critikon; GE Medical systems) and with a cuff size appropriate to the participant's left arm. The average of two measurements was calculated. The participants weight and height wearing light clothing was assessed to the closest 0.1 kg and 0.1 cm, respectively. Hip and waist circumferences were measured to the closest 0.1 cm. All the participants underwent physical examination.

Vascular measurements for the right common carotid artery IMT were assessed using a B-mode ultrasound imager (Vivid 7; GE Vingmed AS) with 12L vascular probe (GE Medical Systems). Electrocardiogram was monitored simultaneously. Three subsequent diastolic images were stored and the average IMT of these was used for analysis. Measurements were obtained from all participants but due to a technical problem, only 69 (44 percent) images were available for data analysis. A semi-automatic image analysing software package (AMS II, version 1.1331, Chalmers University of Technology, Gothenburg, Sweden) was used to analyse the digitally stored data.

### **4.2.3 History of dietary intake**

A food record, with structured instructions attached, was sent to all participants to register all food and drinks consumed over a three-day period, including one weekend day. The record was completed by 146 participants and then analysed by a licensed dietician with software designed specifically for this purpose (AIVO 2000-Diet32, version 1.4.6.2; AIVO Finland Ltd). We used calcium and phosphorus intake in the bone study (I), the proportion of proteins in diet in the body composition study (II), and the proportion of fats and carbohydrates in diet in the cardiovascular study (III).

### **4.2.4 Dual energy x-ray for bone and body composition**

DXA scanning was assessed to measure BMD and body composition with dual energy x-ray absorptiometry (Hologic Discovery A, software version 12.4:3, Waltham, MA, USA) in all but one participant, who was pregnant. Data of BMD ( $\text{g}/\text{cm}^2$ ), BMC (g) and bone area ( $\text{cm}^2$ ) of lumbar spine (L1-L4), femoral neck and whole body were obtained. In addition, DXA was used for a variety of measures that describe lean body mass and the distribution of body fat: fat mass, percentage body fat and trunk fat. The percentage of body fat was further transformed in Z-scores to control for differences caused by gender by using age-, gender-, and ethnicity-appropriate reference tables (Kelly et al. 2009).

### **4.2.5 Biochemical markers**

Lipid levels of fasting samples from venous blood for total, LDL and HDL cholesterol, and triglycerides were determined by photometric enzymatic analysis. Insulin (immuno-fluorimetry) and glucose (photometric) were used to determine homeostatic model assessment of insulin resistance (HOMA-IR) using the following formula:  $\text{fasting plasma insulin} \times \text{glucose} / 22.5 \text{ mmol/L}$ . Bone turnover markers, osteocalcin (electrochemiluminescence immunoassay) and PINP (radioimmunoassay) describing remodelling, and bone resorption markers carboxy-terminal telopeptide of type I collagen (ICTP) and urinary N-terminal telopeptide (NTX) (enzyme-linked immunosorbent assay) were used in analysis. Serum vitamin D (25-hydroxy vitamin D, 25-OHD) (HPLC method) and bone-specific alkaline phosphatase (BALP) (agarose gel electrophoresis) and leptin (ELISA) were assessed.

## 4.2.6 Risk scores

Calculated risk scores are designed to predict the risk of later CVD. The FRS (Figure 6), which is based on the Framingham Heart Study, estimates the risk of CHD over a 10-year period (Wilson et al. 1998). Individual risk factors used in FR scoring include gender, age, blood pressure, cholesterol and LDL cholesterol levels, the presence of diabetes and smoking. The FRS provides a calculated value that combines various risk factors into one variable (range -17 to 5). In the present study, metabolic syndrome – defined by the NCEP ATPIII (Figure 6) – was based on the accumulation of risk factors (blood pressure, LDL cholesterol, HDL cholesterol, waist circumference, fasting glucose level) in the same individual (Olufadi et al. 2008) and was formed by giving a value of 0 when a risk was not present and 1 when the risk limit was exceeded, and then adding up all risks to form up a single value (range 0 to 5). None of the participants in this cohort had been diagnosed for CVD or type 2 diabetes prior to the study. Therefore, the risk scores were used to evaluate the impact of the duration of breastfeeding on the risk of CVD.

## 4.2.7 Statistical methods

The distribution of the variables was inspected visually for normality and logarithmic transformations were used when needed. Primary assumption testing was conducted before analysis in order to check for normality, linearity, and outliers and for homogeneity of variance matrices and no violations were noted. The Student's *t*-test were utilised when comparing continuous variables between two groups and the Chi square if variables were categorized. For test for crude associations between continuous variables, Pearson correlation was utilised and partial correlation was applied when adjusting needed. For selection of covariates after the noticed associations, linear regression was used to prioritise the confounding factors. A possible mediator/confounder was also chosen by estimating the effect before and after adding a new variable into the model and approved when the model was improved (=increase in adjusted  $R^2$  and decrease in standard error estimate). Path model (study II) was built to clarify and illustrate the links between multiple determinants of body composition and their interaction.

Analysis of variance (ANOVA) (studies I, II) was used to compare the mean values and their variance of outcome variables between three breastfeeding groups, and multivariate analysis of covariance (MANCOVA) (study I) was used when adjusted for covariates. Univariate analysis of covariance (ANCOVA) (studies II, IV) was utilised when evaluating the impact of breastfeeding on body composition or whether bone health was associated with metabolic syndrome. Linear and logistic models (study III) were fitted to evaluate the impact of duration of breastfeeding on individual risk factors of CVD or combined risk scores in different models. Additive models were constructed to introduce a covariate in each step, for example model 1: unadjusted, model 2: gender, and model 3: lifestyle factors.

To control for the differences between men and women, the analyses were done after stratification (study I) or by controlling for gender in models (studies II, III, IV) or using Z-score transformations (study II). Adjunct professor Hannu Rita (Department of Forest Resource Management, University of Helsinki) statistically advised studies II–IV. SPSS Statistics Versions 17–19 (IBM) were used in all analyses. A P-value <0.05 was considered statistically significant.

#### **4.2.8 Ethical considerations**

This study was approved by the Ethical Committee of Helsinki and Uusimaa and a written consent was obtained from all participants.

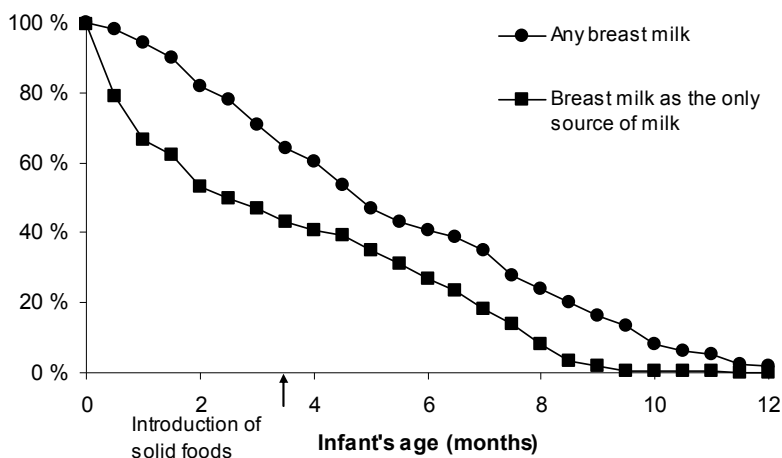
## 5 Results

### 5.1 Characteristics of the study population (I-IV)

One hundred and fifty-eight subjects of the original cohort participated in this study, representing a participation rate of 66 percent. From the original cohort, 61.1 percent of men and 73.0 percent of women attended the 32-year follow-up study, representing a total study population of 76 men and 82 women. The mean age of those attending was 32.6 years (range 31.7–34.0 years). All of the participants in the present study had been breastfed for at least two weeks and some were still breastfed at 12 months of age (Figure 7). The mean duration of breastfeeding was 5.2 (SD 3.2) months and there were no significant differences between boys and girls (Student's *t*-test,  $P=0.29$ ) nor between participants and non-participants (Student's *t*-test,  $P=0.53$ ). Weaning milk was introduced at an average of 3.6 (SD 3.1) months of age for the participants and 3.4 (SD 3.1) months of the non-participants (Student's *t*-test,  $P=0.77$ ). Of the participants, partial breastfeeding continued for an average of 1.5 months after the initiation of weaning milk (Figure 7) and the total duration of breastfeeding was associated strongly with the age of the initiation of weaning milk (Pearson,  $P<0.001$ ). Among the participants at adulthood, 82 percent were still breastfeeding at 2 months, 60 percent at four months, 40.5 percent at six months, 16.5 percent at nine months and 2 percent were still breastfeeding at the 12 month visit. Breast milk was the only source of milk in 80 percent of participants at two weeks, 55 percent at two months, 40 percent at four months, and 18 percent at seven months of age (Figure 7).

#### 5.1.1 Breastfeeding in the analyses

For the analysis we used the total duration of breastfeeding both as a continuous variable (studies II and III) and as a categorised variable (studies I and II). The groups were formed by dividing the cohort into three equal size groups (tertiles) depending on the duration of breastfeeding. The cut-off points were at  $\leq 3$  and  $\geq 7$  months and the groups were named accordingly: short, intermediate and prolonged breastfeeding groups. The sizes of the groups were 55, 48 and 55, respectively.



**Figure 7.** The proportion of breastfed infants during the first year of life in those who participated in the 32-year study.

Socioeconomic status at infancy was determined by the fathers' level of education and the original cohort consisted mostly of middle- and upper-middle-class families, as 68.2 percent of fathers were at least high-school graduates. At the 32-year follow-up visit, 77.8 percent of participants had graduated from high school. The clinical cohort characteristics are presented in Table 3.

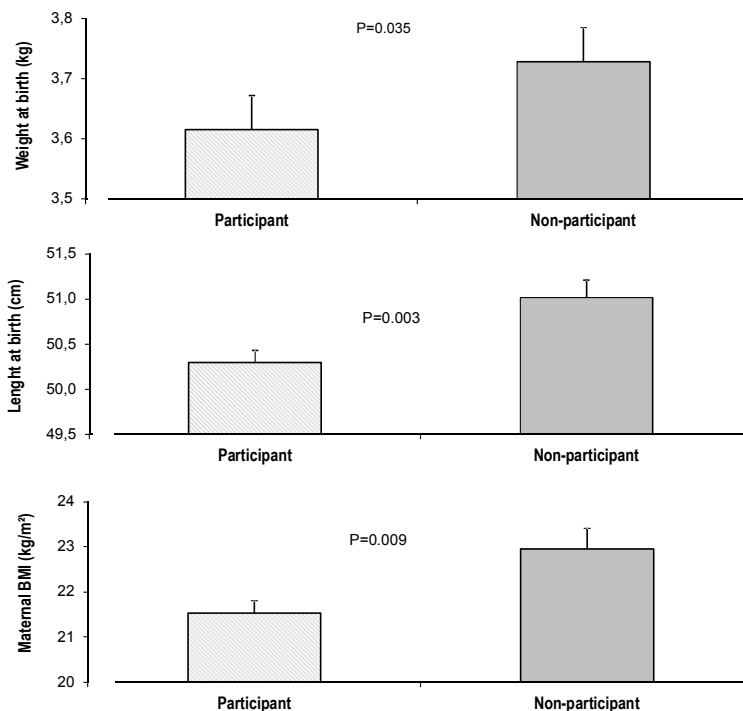
**Table 3.** Cohort characteristics of the participants in 32-year visit.

	Women		Men		All	
		SD		SD		SD
Age, years, range	32.6	31.7–34.0	32.6	31.7–33.9	32.5	31.7–34.0
Weight, kg	67.2	11.1	83.7	12.4	75.1	14.2
Height, m	167.5	6.1	180.3	6.5	173.6	9.0
BMI, kg/m <sup>2</sup>	24.0	4.0	25.7	3.3	24.8	3.8
Waist circumference, cm	78.5	10.3	89.9	10.4	84.0	11.8
Hip circumference, cm	96.4	9.1	100.0	6.4	97.9	8.0
Waist-to-hip ratio	0.8	0.1	0.9	0.1	0.9	0.1

## 5.2 Comparison of backgrounds between participants and non-participants

Differences were observed in the birth measurements of the participants and non-participants. Firstly, participants' birth weight and length were significantly lower than

those of non-participants (Figure 8). Men were overrepresented among those who did not participate, although the differences remained significant after controlling for the gender. Secondly, parental height did not differ, but maternal BMI (Student's *t*-test,  $P=0.031$ ) and weight ( $P=0.020$ ) were higher in non-participants than in participants. Those individuals who were reached but declined to participate ( $n=20$ ) had higher mean maternal BMI than the participants ( $23.7 \text{ kg/m}^2$  compared with  $21.5 \text{ kg/m}^2$ , respectively) (Figure 8). The participants' maternal BMI associated with the participants own BMI at 32 years (Pearson,  $R=0.29$ ,  $P=0.001$ ).



**Figure 8.** Differences in birth size and maternal BMI between participants and those who did not attend the study at the 32-year visit.

### 5.3 Life-style factors (I–IV)

During teenage years, 82 percent of participants were physically active and 75 percent currently exercised at least twice a week. No differences were observed between the genders. Alcohol consumption was mild or moderate (less than twice a day) in 82 percent of the men and 92 percent of the women. Current regular smoking was reported by 32 percent of participants and 21 percent had quit smoking.

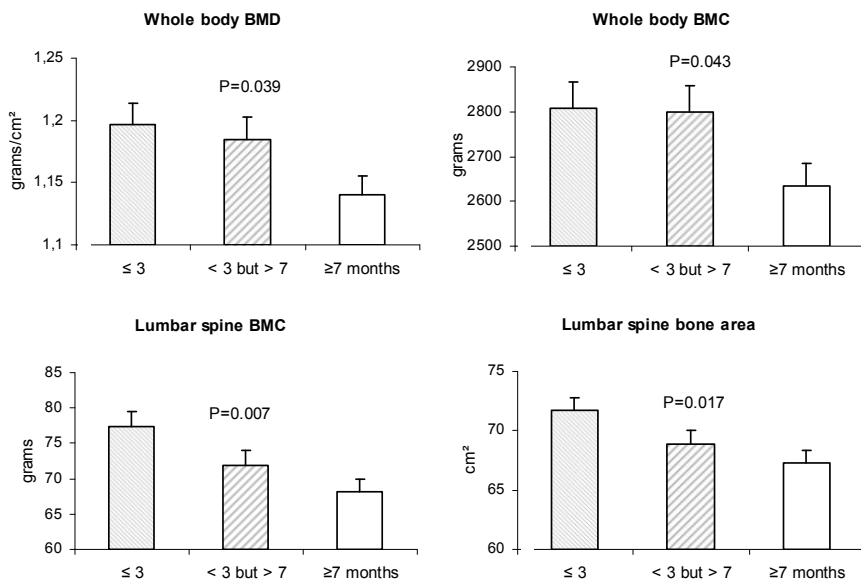


Food records were completed by 146 participants. The proportion of protein from total energy intake was within the recommended levels in 81 percent of all participants. The participants' average daily intake of calcium was 1220 mg (SD 500 mg) and average daily intake of vitamin D was 47 ug/day (SD 15 ug). The duration of breastfeeding in infancy was related to current calcium intake as it was higher in the short breastfeeding group compared with the other breastfeeding groups ( $\chi^2$ ,  $P=0.020$ ). Mothers who breastfed longer were leaner and their BMI was lower than those who weaned early (Pearson,  $P=0.001$ ).

## 5.4 The impact of infant feeding on bone health (I)

Early infant feeding seems to be related to adult bone health in men. Males who had been breastfed up to three months of age had 4.3 percent higher whole-body BMD than those who were breastfed for more than seven months. This is a clinically significant finding. Lumbar spine BMC and bone area were significantly higher in the participants in short breastfeeding group (MANCOVA;  $P=0.007$  and  $P=0.017$ , respectively) than in those in prolonged breastfeeding group. The same trend was observed in whole-body BMD, and BMC was higher in the short breastfeeding group (MANCOVA;  $P=0.039$  and  $0.043$ , respectively). These findings were only seen in men. In women, no differences were observed between the breastfeeding groups.

Bone metabolism measured with bone formation markers, osteocalcin and P1NP, and bone resorption markers, ICTP and NTX, was not associated with the duration of breastfeeding.

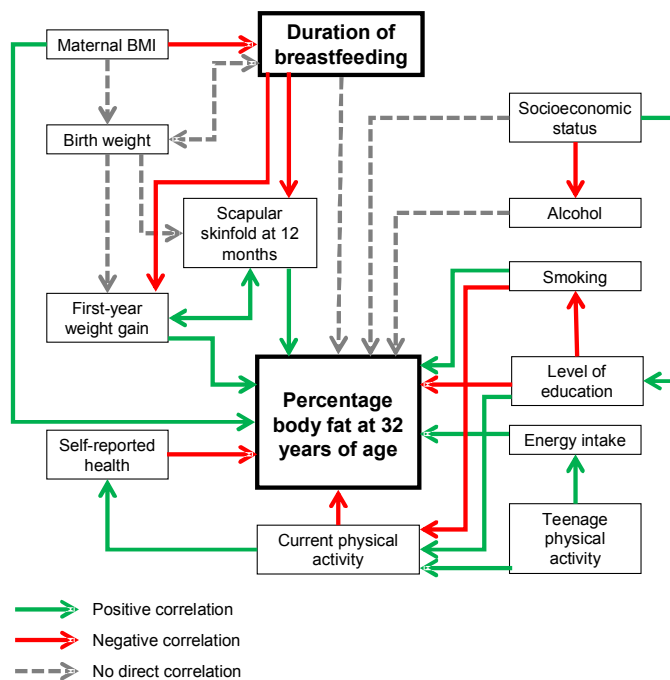


**Figure 9.** Differences of bone parameters between breastfeeding groups in males.

## 5.5 Determinants of adult body composition (II)

The determinants of adult body composition were evaluated from birth to young adulthood. Figure 10 of the path model illustrates the associations between early life span determinants and body composition in infancy and adulthood, and the associations between current lifestyle and adult body composition, as well as the interactions between the determinants. The path model was prepared using data based on the recent statistical associations obtained from partial correlation analysis while controlling for gender, and using data from previous publication of this cohort in infancy (Saarinen et al. 1979b). Statistically significant associations are shown in colour (red/green) and dashed lines represent non-significant relations.

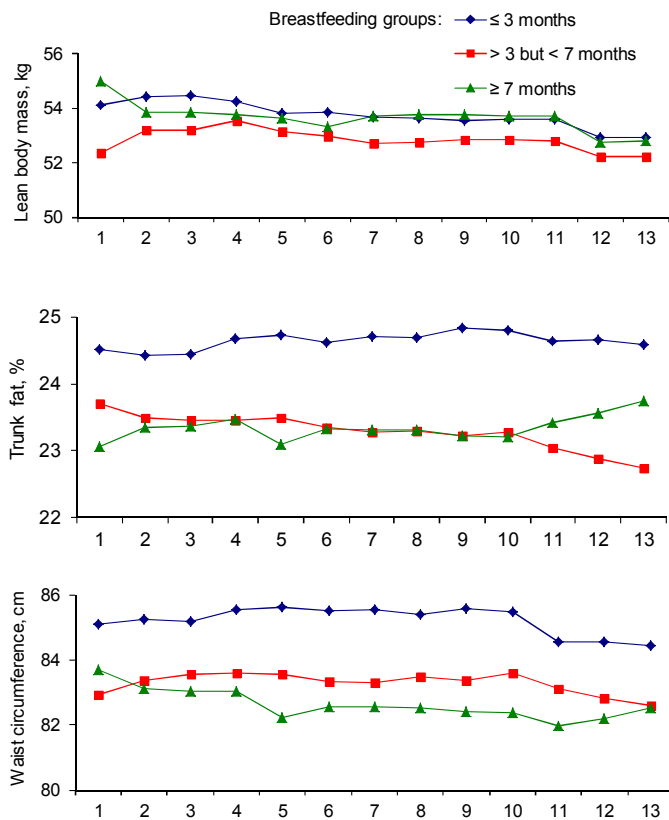
Of all the determinants, the main interest was whether the duration of breastfeeding had an impact on adult body composition. From all factors in infancy, first years' weight gain was positively associated with adult BMI, percentage trunk and body fat, waist circumference and lean body mass. Scapular skin fold thickness at 12 months of age tracked to adult trunk fat ( $R=0.227$ ,  $P=0.005$ ), body fat ( $R=0.183$ ,  $P=0.023$ ), and waist circumference ( $R=0.174$ ,  $P=0.031$ ). Breastfeeding was not directly associated with adult body composition in any measured variable. Teenage physical activity still had an impact on adult percentage body fat ( $R=-0.179$ ,  $P=0.025$ ).



**Figure 10.** Path model of the determinants of adult body composition. Green (positive) and red (negative) lines show statistically significant associations. Modified from a figure used in study II.

With linear regression analysis, physical activity emerged as the most important of all the evaluated determinants of body composition at adult life in terms of reducing the percentage of body and trunk fat and the level of BMI. Leptin levels were also reduced when physical activity level was higher. BMI and waist circumference were lower among those with higher education level and higher among smokers. Maternal BMI, measured at the three-year visit, did not correlate with infant growth during the first year or with fat accumulation at 12 months of age, however, it did strongly correlate with the BMI, waist circumference as well as fat and lean body mass at 32 years of age.

In summary, many lifestyle and genetic factors are determinants of adult body composition. To evaluate whether breastfeeding had an independent effect on body composition, we built up a model to control for multiple confounding factors in the analysis. This is illustrated in Figure 11. Breastfeeding did not have direct effect on any parameters even after all confounders were included (P for all >0.05).



**Figure 11.** The influence of breastfeeding on adult body composition. Covariates added on horizontal axis 1=unadjusted, 2=gender, 3=birth weight, 4=maternal BMI, 5= socioeconomic status, 6=first-year weight gain, 7=teenage physical activity, 8=smoking, 9=alcohol, 10=level of education, 11=proportion of protein in diet, 12=current physical activity, 13= self-reported health.

## 5.6 Breastfeeding and later CVD risk factors and metabolic syndrome (III)

Pearson's correlation showed no associations between the duration of breastfeeding and FRS, NCEP-ATP III scoring or individual CVD risk factors ( $P > 0.05$  for all). Linear regression analysis showed that the change in the duration of breastfeeding had only a minor effect on FRS, blood pressure, cholesterol concentrations, insulin resistance (HOMA-IR), and CCA-IMT. In Table 4, the coefficients characterise the impact of one-month change in the duration of breastfeeding on outcome variables. The duration of breastfeeding showed no significant effects on any variables before adjustment (model 1), and the results remained unchanged after adjustments were made for the following covariates: gender (model 2), smoking, alcohol consumption, physical activity and intake of vitamin C and the proportion of fats in diet (model 3). Logistic regression showed that the duration of breastfeeding did not have an impact on the risk of developing metabolic syndrome at 32 years of age.

**Table 4.** Cardiovascular risk variables.

Exposure: Duration of breastfeeding (months)	FRS (Range -17, 5)	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)
<b>MODEL 1</b>			
$\beta$ (95% CI)	0.06 (-0.24, 0.48)	-0.02 (-0.78, 0.67)	-0.05 (-0.57, 0.33)
P	0.50	0.85	0.60
<b>MODEL 2</b>			
$\beta$ (95% CI)	-0.02 (-0.18, 0.11)	-0.05 (-0.90, 0.37)	-0.07 (-0.61, 0.24)
P	0.62	0.47	0.40
<b>MODEL 3</b>			
$\beta$ (95% CI)	-0.02 (-0.19, 0.09)	-0.06 (-0.90, 0.40)	-0.07 (-0.63, 0.23)
P	0.47	0.42	0.36

## 5.7 The association between CVD risk factors and bone health (IV)

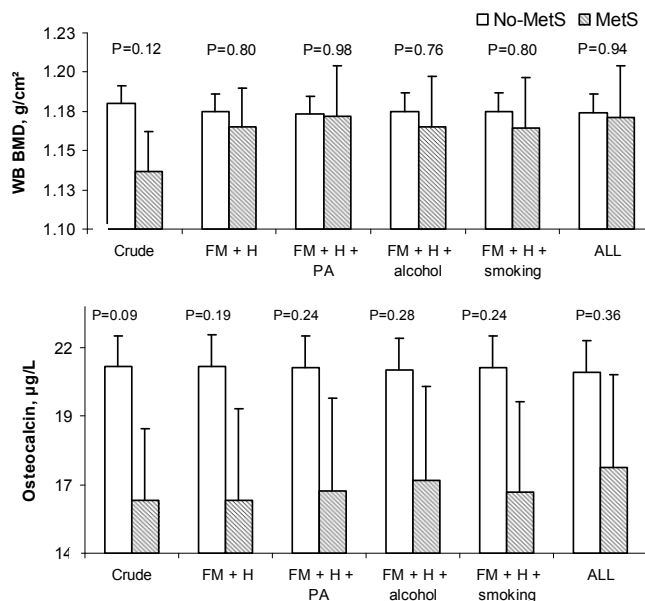
Impaired glucose tolerance, measured with fasting glucose and insulin, was associated with bone formation markers osteocalcin ( $R = -0.213$ ,  $P = 0.009$  and  $R = -0.243$ ,  $P = 0.003$ , respectively) and P1NP ( $R = -0.190$ ,  $P = 0.019$  and  $R = -0.187$ ,  $P = 0.021$ , respectively). However, after controlling for gender (model 1), gender, height and fat mass (model 2) and all of the above plus lifestyle factors (physical activity, smoking and alcohol consumption) (model 3) the association attenuated between glucose/insulin and osteocalcin (model 3:  $R = -0.099$ ,  $P = 0.240$  and  $R = -0.162$ ,  $P = 0.053$ , respectively) and P1NP (model 3:  $R = -0.133$ ,  $P = 0.110$  and  $R = -0.093$ ,  $P = 0.266$ , respectively). An association was also seen between plasma glucose and bone resorption marker ICTP ( $R = -0.232$ ,  $P = 0.011$ )

in unadjusted model but the association disappeared after the adjustments. Plasma glucose or insulin levels did not correlate with the achieved bone strength in terms of BMD. Other risk factors of CVD were not associated with bone turnover markers. Unexpectedly, triglyceride level was inversely correlated with whole-body BMD, which remained after adjustments. Parallel findings were not observed with waist circumference or other plasma lipids.

CVD and bone health have common risk factors and the connection between insulin/glucose and bone resorption markers disappeared after the adjustments; this finding suggests that the connection is mediated by the common risk factors.

We also evaluated whether metabolic syndrome was associated with bone parameters (BMD and bone turnover markers) by comparing these variables between men with fulfilled criteria for metabolic syndrome (MetS) (n=12) and those who were healthy (no-MetS) (n=60) (Figure 12). Bone formation markers, osteocalcin and P1NP tended to be lower in men with MetS, but the difference attenuated after adjustments were made for common risk factors. Similarly, BMD tended to be lower in men with MetS than men in the no-MetS group, but this difference disappeared after the adjustments.

In conclusion, P1NP was associated with glucose and insulin in the same way as the previously reported osteocalcin. These associations may be explained by the common risk factors that modify both bone and glucose metabolism.



**Figure 12.** Whole body bone mineral density (BMD) and osteocalcin level between healthy men (No-MetS) and those with metabolic syndrome (MetS) (NCEP  $\geq 3$ ). Covariates attenuated the difference: FM, fat mass; H, height; PA, physical activity. Modified from figure used in study IV.

## **6 Discussion**

### **6.1 Major public health diseases**

Osteoporosis, cardiovascular disease (CVD) and obesity are major public health problems, major causes of premature death and involve expensive treatments. Therefore, it is important to find ways to reduce the incidence of these conditions and to find ways that individuals can modify own lifestyle choices to affect the risk of developing later disease. Breast milk may have a preventive influence on overweight and a possible reduction of blood pressure. However, previous findings have not been conclusive. The present study population represents a unique, prospectively collected cohort with extended follow-up to adulthood which has enabled the evaluation of the possible preventive effects of breastfeeding on the risk factors of these conditions and diseases.

### **6.2 Bone health (I)**

Studies on the effect of breastfeeding on adult bone are rare. Most studies on bone health have only extended into childhood (Butte et al. 2000, Harvey et al. 2009, Jones et al. 2000) or adolescence (Molgaard et al. 2011a, Pollock et al. 2011), times at which when bone maturation is still in process and peak bone mass has not been achieved. The only cohort in which participants reached middle age was a retrospective study that combined the impact of breastfeeding with other infant-related factors and reached no conclusions on the individual effect of breastfeeding (Pearce et al. 2010). The cohort used in this study had a unique prospective setting: the age of 32 years, when the peak bone mass is achieved but age-related demineralisation has not yet started. The main finding was that men who were weaned early had higher whole-body BMD and BMC than those who were breastfed longer. Women did not show such differences between infant feeding groups. In women, many life-dependent determinants on bone health such as hormonal changes, contraceptive pills (Scholes et al. 2010), parity and lactation (Karlsson et al. 2001, Pesonen et al. 2005) may be more important factors than the duration of breastfeeding and therefore overrule the possible effects of infant feeding on bone health. No signs were found on the impact of infant feeding on bone turn-over markers. Compared to cow's milk, breast milk has lower content of calcium, phosphorus and protein (Table 1); which are critical components of bone remodelling and bone mass accrual. The differences between these infant milks could be a possible explanation for the findings in the present study, as the higher mineral and protein intake was started earlier age in those who were weaned younger. The findings showed lower but normal BMD in men with longer breastfeeding, although further studies are required in order to confirm this finding. Regardless of these findings, breastfeeding should be recommended for infants because of the many other beneficial effects. However, during childhood growth, it is crucial to emphasise the importance of adequate calcium intake (Greer et al. 2006, Hoppe et al.

2000, Laitinen et al. 2005) as well as healthy nutrition and appropriate physical activity in order to achieve proper good bone structure.

## **6.3 Body composition (II)**

There are high hopes regarding the potential preventive effects of breastfeeding on later obesity. The second study focused on the determinants of adult body composition, specifically those of central obesity. A wide range of variables describing body composition was available and a special interest was in the unfavourable/unhealthy adipose tissue distribution on central body, which also represents visceral obesity. In infancy, longer breastfeeding resulted in leaner body composition. Skin folds were measured initially to ensure adequate growth with exclusive breastfeeding at a time when there were doubts about whether breast milk was sufficient. Saarinen et al. reported that exclusively breastfed babies grew faster during the first three months, but by 12 months of age were slightly smaller than infants fed with two other weaning milks. No undernourishment was observed (Saarinen et al. 1979b). Longer duration of breastfeeding resulted in thinner scapular skin folds and lower BMI at 12 months. These measurements were associated with adult body composition (percentage of trunk and body fat and waist circumference). However, the duration of breastfeeding did not have a direct impact on adult body composition as the most important determinants were physical activity, growth during infancy and gender. The adjustment for multiple confounding factors did not change these results. Early growth has emerged to affect adult body size also in other studies, as weight gain during childhood has been positively associated with adult fat mass (Leunissen et al. 2009, Wells et al. 2007). Juonala et al. on the other hand have reported that only weight in adulthood has an impact on CVD risk, independent of infant, childhood or adolescent weight; therefore, normalisation of body weight is beneficial at any age (Juonala et al. 2011).

In conclusion, the duration of breastfeeding is indirectly associated with adult body composition because it affects first-year growth, which in turns influences adult body composition. According to our study, the most important factors affecting adult body composition appear to be physical activity, genes and learned life-style habits.

## **6.4 Cardiovascular disease (III)**

Obesity, particularly central obesity, is a risk factor for CVD. In the third study, the focus was on the other risk factors of CVD and the calculated CVD risk score, FRS, even though it is conventionally used as an outcome variable. Increase of the FRS-value indicates the growing risk of CVD. Accordingly, we could combine a number of different risk factors into a single value. Our cohort consisted of participants of the same age. An average male at 32 years of age has a 3 percent risk of CHD over a 10-year period, compared to a less than 1 percent risk for a female of the same age (Wilson et al. 1998).

Whether the duration of breastfeeding may affect the risk of metabolic syndrome illustrated by NCEP ATP III was also evaluated. Such an approach is novel in breastfeeding studies. We observed that the effect of the duration of breastfeeding on any of the risk factors of CHD/CVD was minor.

Other studies have reported conflicting findings between breastfeeding and later cardiovascular health and the reported differences of effect size in larger studies were small (Martin et al. 2005b). In some earlier studies (Lawlor et al. 2004, Lawlor et al. 2005) and meta-analyses (Martin et al. 2005b, Owen et al. 2003), systolic blood pressure level was on average 1 mm Hg lower among the breastfed than with those who were bottle fed; and it was unclear whether this would be of clinical significance in respect of CVD morbidity and mortality. To evaluate the effect of a one-unit decrease in population average of systolic blood pressure, we estimated the proportion of individuals whose systolic blood pressure exceeds 130 mmHg. The calculations were based on normal distribution, which was well met in our data. We used the observed averages and standard deviations separately for men and women. The proportion of men whose systolic blood pressure exceeded 130 mmHg was 39.3 percent (original average) and 36.3 percent (average decreased with 1 mmHg); and in women it was 7.69 and 6.50 percent, respectively. This means that there would be 30 fewer men per 1 000 individuals (from 393 to 363; 7.6 percent less) and 12 fewer women (from 77 to 65; 15.6 percent less) with systolic blood pressure above 130 mmHg; correspondingly, their FRS would decrease by one point (Hannu Rita personal communication). This is equivalent to 1 percentage reduction in the risk for CHD in the period of 10 years. This is in line with our findings. As shown in Table 4, the coefficient  $\beta$  for FRS representing the effect of a 1-month change in the duration of breastfeeding was minimal -0.02. The lower end point of confidence interval (95% CI) of FRS was 10-fold (-0.19), which would correspond to the finding that the duration of breastfeeding should continue five months longer, so that the FRS would decrease by one point, which again corresponds to 1 percent change in the risk for CHD over a 10-year period. In conclusion, our findings are in line with large meta-analysis where the effect of the duration of breastfeeding on the risk of CVD is minor.

## **6.5 Bone health and CVD risk factors (IV)**

In studies I, II and III, the effect of breastfeeding on bone health, body composition and the risk factors of CVD was evaluated. Their interconnectivity is an interesting topic that has been studied recently in different ways. Animal studies found an inverse correlation between osteocalcin and glucose intolerance (Ferron et al. 2010, Lee et al. 2007). Human studies have not been inconclusive, as subjects with known type 2 diabetes (Kanazawa et al. 2009) or CVD (Bao et al. 2011) have shown a parallel association between glucose intolerance and impaired bone remodelling markers, although the causality has remained unclear. A similar association was reported in teenagers, although the association disappeared after the adjustment with BMI, which could mean that the relation is



explained and mediated by fat mass (Polgreen et al. 2012). The present study revealed inverse correlations between glucose/insulin and bone turnover markers, but the adjustments with fat mass and lifestyle factors attenuated this connection. Therefore, it is suggested that the association is at least partly explained by the common risk factors. Also, the fact that P1NP reacts with glucose and insulin in the same way as osteocalcin could support the idea that osteocalcin does not have an independent role in the link with glucose intolerance.

No clear single explanation was found for the association between the risk factors of CVD and bone health. The most important determinants for both diseases were shared risk factors which might explain the relationship between these disorders. The causality should be evaluated with intervention and longitudinal follow-up settings. The development and the first signs of adult diseases, such as CVD, osteoporosis, originate early, even during prenatal life (Barker et al. 1993, Barker 1995, Barker 2007). Therefore, the importance of healthy lifestyle begins in infancy and childhood (Oranta et al. 2013). In developed countries, the average life expectancy may not rise much higher. The best way to prevent diseases including CVD and osteoporosis is to maintain normal weight by following a healthy diet and engage in regular physical activity.

## **6.6 The breastfeeding recommendations**

WHO recommends exclusive breastfeeding for six months (Kramer et al. 2012). In 2001, the WHO recommendation increased from four to six months of age to ensure longer protection against infections, particularly in developing countries. Infant's immunity is transferred from the mother via placenta during foetal life; after birth, the protective effect is maintained via breast milk. This protective effect is especially important in countries with poor sanitation, where gastrointestinal infections are a major cause of morbidity and childhood mortality. In Finland and other Western countries, the prevalence and mortality of these serious infections is small. It should be emphasised that maternal immunoglobulin protects only against infections that the mother herself is protected against.

In industrialised countries with high hygienic levels, the extended exclusive breastfeeding might not be as essential as in developing countries, where the alternative foods for breast milk might not meet the nutritional requirements and hygiene standards (Khanal et al. 2013). Most benefits and protective effects are gained and transferred during the first few months of life. Solid foods may be started at four to six months of age or even as early as three months in preterm infants, to ensure adequate nutrition and growth (Reilly et al. 2005a, Reilly et al. 2005b). However, if breastfeeding is successful and sufficient for the infant, and if the mother is willing to continue exclusive breastfeeding, it is appropriate up to six months of age. No harm has been reported due to the introduction of solid foods at four months of age (Grimshaw et al. 2013). In turn, postponed introduction of solids beyond seven months of age has been reported to increase the prevalence of celiac disease

(Olsson et al. 2008), iron deficiency (Salmenperä et al. 1985), and allergies (Nwaru et al. 2013). In fact, the optimal duration of exclusive breastfeeding in industrialised countries is not known, nor is there any unequivocal information as to whether exclusive breastfeeding has benefits over mixed milk feeding. The introduction of complementary foods at four months while continuing breastfeeding seems to be beneficial over exclusive breastfeeding for the prevention of the development of allergies and asthma (Nwaru et al. 2013). In 1975, the nutritional guidance of the present cohort was modern and resembled the current recommendations in relation to the general practice at the time. Breastfeeding was recommended to be continued for as long as possible and the introduction of solid foods was postponed to 3.5 months of age and salt restriction was advised for the first year of life.

Supportive guidance to initiate breastfeeding is important in maternity hospitals and child health clinics. However, as was observed in the current study, the most important determinants of body composition and obesity in adulthood were physical activity and childhood growth. Therefore, it is important to share the information about dietary recommendations and emphasise the importance of regular exercise to families with children throughout childhood (Sääkslahti et al. 2004). The impact of these guidelines may even extend beyond childhood. The information should be given to school-age children themselves, which could have far-reaching effects (Hakanen et al. 2010). Health education also includes the importance of adequate sleep and the dangers of smoking, excessive alcohol consumption and drug abuse (Simell et al. 2009).

## **6.7 Realisation of recommendations**

The influence of breastfeeding on adult health may be minor. Breastfeeding has benefits in early infancy and therefore is recommended for all newborn children. If breast milk is not sufficient, it is acceptable and advisable to start age-appropriate solid foods or formula milk when necessary. Nutritional and immunity advantages are most likely achieved during the first four to six months of life also with partial breastfeeding. Compliance with current WHO recommendations of exclusive breastfeeding is only partly met in Finland and other industrialised countries (Figure 4) (Uusitalo et al. 2012). If breastfeeding is not possible for any reason, or if breast milk is insufficient, it is important to emphasise to the mother and family that a good mother-baby relationship can develop without breastfeeding (Jansen et al. 2008, Larsen et al. 2013). In addition, fathers in Finland, and other countries, participate in infant care more actively than in the past. Thus, regardless of the type of infant feeding, it is equally important to ensure steady growth, a high standard of hygiene, and to provide health education. Complementary foods and formula milk should be given in appropriate amounts in order to avoid excessive weight gain. It is important that all recommendations are based on empirical findings and it should be emphasised that if breastfeeding is not successful, infant formulas usually meet the infant's nutritional needs as closely as possible. Information regarding the protective

effects of breastfeeding should be based on well-documented evidence. Unjustified promises of the benefits of breast milk should not be given, and mothers who choose to bottle feed their infant might even need extra support rather than negative criticism (Jansen et al. 2008, Larsen et al. 2013). It should also be noted that no harm is caused when infants are given formula.

## **6.8 Problems with the interpretation of research results**

Measuring the dosage of breastfeeding is difficult and, particularly in partial breastfeeding, the dose is highly variable and the appropriate and adequate dose is difficult to assess. Research design is clearer when comparing exclusive breastfeeding to exclusive bottle feeding. In addition, mothers who choose to breastfeed might also differ in other health habits from those who prefer exclusive bottle feeding. Therefore, the independent impact of breastfeeding can be biased.

The retrospective data collection of duration and/or exclusiveness of breastfeeding in some infant feeding studies are a major source of bias. Data may have been collected years or even decades after the actual feeding. In one South African study, for example, data about infant feeding was collected both prospectively and retrospectively at 6–9 months of age, and the authors found a significant difference between the actual duration and the memory-based duration of breastfeeding. They concluded that the memory-based duration was longer than in reality (Bland et al. 2003). The particular strength of the current study is the prospective setting. The original study design of the cohort allowed the comparison between babies who were exclusively breastfed, exclusively formula-fed and fed exclusively with diluted cow's milk, because those participants with partial breastfeeding were excluded. The previous studies with the same cohort investigated the differences of iron absorption and growth between different milk feeding groups (Saarinen 1978, Saarinen et al. 1979b).

Because it was not possible in the present investigation to use the original study groups due to restricted group size, the focus was instead on the total duration of breastfeeding. However, a participation rate of 66 percent could be considered good in such a long follow-up period. With a larger cohort size, smaller effects could be detected in analyses, which mean that the cohort size can be considered as a limitation of the study. However, the current cohort size made it possible for all participants to be met by the same physician (U.S.-P.) in infancy; and one physician (S.P.) was responsible for the data collection and physical examinations. Another limitation was in study III where, due to a technical problem, the images only from an unselected group of 69 (44 percent) were available for data analysis. However, the backgrounds of the groups with and without ultrasonic measurement did not differ. Underreporting in food records was another limitation of the

study. Because the proportions of energy of proteins, carbohydrates and fats were close to the recommendations, they were used in analysis.

## **6.9 Different study designs to control for confounders**

Many health-related determinants affect both the focused outcome variables and the mother's decision to initiate and continue breastfeeding. Most breastfeeding studies are observational and the controlling for confounders is assessed by mathematical statistical methods. Intervention studies aim to manage the selection and the effects of confounding factors on the outcome variable. Intervention studies on breastfeeding are rare. PROBIT from Belarus was one of the few such studies where maternity hospitals and related policlinics were randomly selected either to introduce BFHI program or to continue the previous practice. This intervention resulted in increased exclusive breastfeeding in the areas of these intervention hospitals and policlinics. Consequently, families with similar backgrounds (such as socioeconomic status) differed in the duration of exclusive breastfeeding, which meant it was possible to evaluate the impact of breastfeeding on health-related variables without the confounding effect of socioeconomic status (Kramer et al. 2003, Kramer et al. 2009) Another way to control for the effect of socioeconomic status was used in a study that compared two cohorts from different backgrounds of living conditions, one from England, and the other from Pelotas, Brazil, which involved a cohort from a low- and middle-income area. These studies evaluated the effect of breastfeeding on intelligence quotient (IQ), and found that, despite the effect of social class, breastfeeding may have causal effects on IQ (Brion et al. 2011).

The most important confounding and mediating factors are socioeconomic status, level of education, parental (especially maternal) overweight, dietary habits, physical activity, smoking and alcohol consumption. Many of these factors also interact with each other. The majority of the present cohort was from the middle and upper-middle classes, which may result in double selection bias as, on average, those families with higher education levels are more interested in health, both at accrual to the original cohort and now as participants themselves. In turn, this can be considered as a strength since the confounding effect of socioeconomic status on the duration of breastfeeding was not seen in this homogenous cohort. However, the findings may not be applied to all social classes. An interesting finding was that participants who did not attend were significantly larger at birth, both in terms of length and weight, and their maternal BMI was also significantly higher than those who participated (Figure 8). These parameters are associated with adult weight and BMI of the participants, which means that the non-participants could, on average, have been more overweight and even have more metabolic disturbances. This type of selection in participation is natural and a well-known bias in voluntary studies. As maternal BMI is associated with the offspring's BMI at 32 years of age, this can be

considered as both a reflection of genetic and learnt life-style habit, and is therefore a confounding factor in the association between breastfeeding and later health.

## **6.10 Considerations of different eras**

Long-term follow-ups reflect life circumstances and nutritional recommendations that are very different from those of the present time. Standards of living, the availability of formula milks, dietary shortage and habits, and overall health awareness have all changed over the years. Some of the cohorts were recruited in mid-20<sup>th</sup> century, both during and shortly after World War II, when commercial formula milks were not universally available or the quality of food did not meet current standards, and many families had a shortage of food. The effects of the types of infant feeding were likely to have been confounded by very different factors than in studies nowadays. In the 1970s, when the current cohort was originally collected, food and health habits were different from the current ones among the nursing mothers themselves; however, food supply was generally sufficient for families. Health awareness on smoking and salt restriction was different from today. Therefore, not all of the effects may be directly transferable to the modern world.

## 7 Conclusions and future aspects

Study I. In men, early infant milk feeding may have a significant impact on adult bone health. Early formula feeding, over prolonged breastfeeding, associated with higher adult bone mass in males beyond the age of peak bone mass attainment. In women, the duration of breastfeeding did not reveal any differences in bone mass. The possible explanation may be the higher concentrations of calcium, phosphate, and protein in formula milk and cow's milk dilution compared with breast milk. Further studies are needed to confirm the impact of infant milk feeding on bone development.

Study II. The most important factors that influence adult body composition and fat accumulation are current physical activity, growth in infancy, maternal BMI (obtained from the child's three-year visit) and gender. Prolonged breastfeeding led to moderate growth and leaner body composition during the first year of life, which was associated with body composition at 32 years of age. Thus, breastfeeding had an indirect effect on adult body fat accumulation by affecting early growth and body adiposity.

Study III. The association between the duration of breastfeeding and cardiovascular risk factors in adulthood was very weak. Considering current lifestyle factors, including physical activity, dietary habits, smoking and alcohol consumption, did not change the findings. The male gender seems to have the single-most important impact on increased risk of cardiovascular disease. The clinical significance of the effects of breastfeeding on the risk factors of cardiovascular diseases is questionable, both at the individual and public health levels.

Study IV. In young adults, the inverse association between bone formation markers (osteocalcin, P1NP) and fasting glucose and insulin seemed to be mainly mediated by fat mass. However, triglycerides remained, independently of fat mass, and these were inversely associated to bone mineral density. Men with metabolic syndrome tended to have lower bone turn-over markers and whole-body bone mineral density than men without metabolic syndrome, but the differences disappeared after considering fat mass in the analysis, which also proposed that fat mass would be a common explanation for this connection. Prospective studies are needed in order to further evaluate the pathophysiology and causality between bone and energy metabolism, and to specify the role of common risk factors, such as lifestyle habits and fat mass, in the prevention of osteoporosis and cardiovascular diseases.

In conclusion, the long-term effects of breastfeeding on adult obesity, body composition and cardiovascular risk are minor and unlikely to resolve any public health problems. Bone mineral density was lower in men who had been breastfed for longer as infants. Genetic and lifestyle factors appeared to be stronger determinants of public health problems than infant feeding. However, breastfeeding has beneficial effects and health-care professionals at maternity hospitals and child health clinics should encourage mothers

to initiate and continue breastfeeding. Growth and well-being are frequently monitored by public health nurses and age-appropriate complementary foods are required if breast milk becomes insufficient or breastfeeding is not successful. Health-care professionals should also emphasise the importance of adequate calcium intake and healthy nutrition in general, and should encourage physical activity in order to achieve the best possible bone strength and maintain weight management. These guidelines apply beyond childhood and help prevent future morbidity, including later cardiovascular disease, osteoporosis and obesity.

# Acknowledgements

This study was carried out in 2006-2014 at the Children's Hospital, Helsinki University Central Hospital.

I wish to express my sincere gratitude to Docent Jari Petäjä, Director of the Department of Gynecology and Pediatrics, Professor Mikael Knip, Docent Eero Jokinen, Children's Hospital, Helsinki University Central Hospital for providing excellent research facilities. I also want to thank Professor Markku Heikinheimo, Head of the Institute of Clinical Medicine and former Director of the Pediatric Graduate School and Docent Jussi Merenmies, the present Director of the Pediatric Graduate School for their interest in educating young researchers and for creating a supportive research environment. During these years with Markku, we have participated in several sporting activities alongside with science.

I am indebted to all the "Aikuistuneet mallilapsi" - participants, who kindly took part in this study and thus made this thesis possible.

I am most grateful to my supervisors, Docent Mervi Taskinen and Professor Ulla Pihkala, who have inspired me on the path to the research world and kindly supported and encouraged me during these years. Ulla, I am grateful for getting a chance to be the latest extension of your successful group of students. I am pleased that I was asked to work with this unique cohort, which you originally collected. Mervi, I would like to thank you for sharing your time with me in your office. Thank you for the friendship we have developed. I have always admired your efficiency at work, and now I got to experience it even closer during our long shared sessions.

I want to thank all my co-authors. Merja Kajosaari, I have adopted you as my mentor and I'm happy to share you with my "older and bigger" sister Usvis. You are a dear friend and your warm heart and understanding have saved me and many of my days. Maila Turanlahti, you taught me the first secrets of ultrasound and we have also shared some nice times and activities outside of science. Outi Mäkitie, I admire your ability to make scientific text readable and user friendly. You are also interested in and willing to keep up good team spirit. I am grateful that you have kindly included me in your "Skeletal Girls" research group, which also deserves special thanks for support and fun times. Heli Viljakainen, this thesis would not be completed yet without your help. The storylines and statistics of all the papers have been dependent on your guidance. I have learned so much from you, I really hope that our friendship and scientific cooperation will continue here after. Hannu Rita, I am very happy that Heli introduced us. I got more confidence in our work by having our long, interesting and fruitful discussions. Our discussions made me feel like a real scientist.



Thank you to the members of my thesis committee, Docent Päivi Miettinen and Docent Marjatta Antikainen, for your time and support throughout this project.

I am most grateful to the official reviewers Docent Marjatta Antikainen and Docent Pekka Arikoski for your valuable comments and for the help in improving this thesis. I am thankful for our discussions, and I enjoyed and learned a lot from you.

I gratefully acknowledge research nurses Minna Savolainen, Merja Helske and Tiina Järvinen, physiotherapist Miiru Kurimo, and licenced nutrician Ulla Vesala for their help in gathering the data during the first few years.

My fellow researchers at the sixth floor office in Biomedicum 2, thank you for walking beside me with all the ups and downs of this project. I love the atmosphere we have and I appreciate all the scientific discussions, practical advice and our refreshing breaks. Most importantly, you all understand the joys and frustrations scientific work brings along. Thank you to all you who have worked with me during the past years: thank you to my room mates Usvis, Anu, and Ilkka for putting up with me – and sorry. Hopefully you got something done in between our chats. My gratitude goes also to Anne N., Anne S, Annika, Elisa, Hanna, Hanne, Helena O., Helena V., Jenni, Johanna, Juuso, Laura, Maarit, Matti, Mervi, Pirjo, Satu, Silja, Sonja and all others during these years.

After working at the Children's Hospital for almost 20 years, I have had the privilege to form great relationships with wonderful colleagues and other professionals, who have made an impact on how I enjoy my job. I want to thank you all. Special thanks to my dear friend Elisa Ylinen, who I can always trust to be there for me and with whom I share day-to-day life matters also outside of work. Thank you to my closest colleague Anna Siina Kotiranta and the entire team in the Adolescents' Department for understanding and supporting me. I promise to spend more time with you in the future. Thank you to all of you who shared the years with me during our specialization: Ilkka, Laura, Mirka, to name a few and all others. I also want to thank Anna, Jarmo, Karoliina and Petteri for the unforgettable congress trips.

My oldest and dearest friends, who also had a contribution in helping me to improve my theses, Sari Kukkasniemi, Lulu Williams and Susanna Niemi, thank you for your invaluable help and unconditioned friendship. You have a permanent place in my heart.

I also want to thank all the friends in Suomen Lääkärien Tennisseura where I have spent a great part of my leisure time. We have organized events, won championships and enjoyed tennis. Thank you to Micaela, Jaana, Pekka, Lotta, Leena, Ritva, Mikko, Kristiina and all others on our team.

Also other tennis friends have definitely been a big and an important part of my life outside of work. Maybe this thesis would have been completed earlier, but I would not be happier without having spent all those hours enjoying this marvellous sport. I want thank

you all, but just to name a few Karoliina, Anna, Kati, Minnamaria, Leena, Hannu, Susa, and Erja.

Thank you to my other group of friends, whom I have met in Pickala through activities - with and without golf. Thank you to Satu for sharing the joys of science, dogs, golf and children's activities.

I formed a big family with my friends from the medical school as we grew up together both chronologically and professionally. I came particularly close to Maija, Maarit and Kirsi and our friendship has remained even if we do not meet as often I would like to.

I am particularly grateful to my grandmother, who has led the way as a successful, academic woman and a very important person in my life. She always took my thesis for granted. Thank you to my beloved parents, who have been very supportive and helped me in every possible way. Thank you to my father Pekka, whom I can contact almost any time of the day online and from whom I can get help to almost any of my problems. He has a well-known reputation amongst my friends and even neighbours suggesting I should ask advice from him. My wonderful sister, Outi and brother Vesa, I can always count on you.

Finally, to my amazing family - I could not have done this without you. Our children, Jesse and Jenna, you are the love of my life. I am very proud of you and love you. I promise to be there in the future. And my beloved husband, Jari, thank you for being there for me. You have always supported me and believed in me. I am grateful for your encouragement and unconditional love.

This study was financially supported by the grants from Foundation for Paediatric Research, Päivikki and Sakari Solhberg Foundation, Yrjö Jahnsson Foundation, Ida Montin Foundation and the Paediatric Graduate School and the National Graduate School of Clinical Investigation.

*Satu Piriä*

Helsinki, May 2014

## References

- Abrahams SW, Labbok MH.** Breastfeeding and otitis media: a review of recent evidence. *Curr Allergy Asthma Rep* 2011 Dec;11(6):508-512.
- Abrahamsson TR, Jakobsson HE, Andersson AF, Bjorksten B, Engstrand L, Jenmalm MC.** Low diversity of the gut microbiota in infants with atopic eczema. *J Allergy Clin Immunol* 2012 Feb;129(2):434-40, 440.e1-2.
- Abrams SA.** Building bones in babies: can and should we exceed the human milk-fed infant's rate of bone calcium accretion? *Nutr Rev* 2006 Nov;64(11):487-494.
- Abrams SA.** Calcium absorption in infants and small children: methods of determination and recent findings. *Nutrients* 2010 Apr;2(4):474-480.
- Abrams SA, Wen J, Stuff JE.** Absorption of calcium, zinc, and iron from breast milk by five- to seven-month-old infants. *Pediatr Res* 1997 Mar;41(3):384-390.
- Afghani A, Cruz ML, Goran MI.** Impaired glucose tolerance and bone mineral content in overweight latino children with a family history of type 2 diabetes. *Diabetes Care* 2005 Feb;28(2):372-378.
- Alho E.** Pienten lasten kuolevaisuus vatsatauteihin Helsingissä vv. 1896-1910 sekä muutamia teorioita vatsatautien patogenesisista. *Duodecim; lääketieteellinen aikakauskirja* 1915;31:1-24.
- Amir LH, Donath SM.** Does maternal smoking have a negative physiological effect on breastfeeding? The epidemiological evidence. *Birth* 2002 Jun;29(2):112-123.
- Arenz S, Ruckerl R, Koletzko B, von Kries R.** Breast-feeding and childhood obesity - a systematic review. *Int J Obes Relat Metab Disord* 2004 Oct;28(10):1247-1256.
- Australian Institute of Health and Welfare 2011.** 2010 Australian National Infant Feeding Survey: indicator results. Canberra: AIHW.
- Bao Y, Zhou M, Lu Z, Li H, Wang Y, Sun L, Gao M, Wei M, Jia W.** Serum levels of osteocalcin are inversely associated with the metabolic syndrome and the severity of coronary artery disease in Chinese men. *Clin Endocrinol (Oxf)* 2011 Aug;75(2):196-201.
- Barker DJ.** Fetal origins of coronary heart disease. *BMJ* 1995 Jul 15;311(6998):171-174.
- Barker DJ.** The origins of the developmental origins theory. *J Intern Med* 2007 May;261(5):412-417.
- Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS.** Fetal nutrition and cardiovascular disease in adult life. *Lancet* 1993 Apr 10;341(8850):938-941.
- Bergmann RL, Diepgen TL, Kuss O, Bergmann KE, Kujat J, Dudenhausen JW, Wahn U; MAS study group.** Breastfeeding duration is a risk factor for atopic eczema. *Clin Exp Allergy* 2002 Feb;32(2):205-209.
- Betoko A, Charles MA, Hankard R, Forhan A, Bonet M, Saurel-Cubizolles MJ, de Lauzon-Guillain B; the EDEN Mother-Child Cohort Study Group.** Infant feeding patterns over the first year of life: influence of family characteristics. *Eur J Clin Nutr* 2013 Jun;67(6):631-637.
- Bland RM, Rollins NC, Solarsh G, Van den Broeck J, Coovadia HM, Child Health Group.** Maternal recall of exclusive breast feeding duration. *Arch Dis Child* 2003 Sep;88(9):778-783.
- Bolling K, Grant C, Hamlyn B, Thornton A.** Infant Feeding Survey 2005 UK: The information Centre, Government Statistical Service. 2007.
- Brion MJ, Lawlor DA, Matijasevich A, Horta B, Anselmi L, Araujo CL, Menezes AM, Victora CG, Smith GD.** What are the causal effects of breastfeeding on IQ, obesity and blood pressure? Evidence from comparing high-income with middle-income cohorts. *Int J Epidemiol* 2011 Jun;40(3):670-680.

**Britton JR, Britton HL, Gronwaldt V.** Breastfeeding, sensitivity, and attachment. *Pediatrics* 2006 Nov;118(5):e1436-43.

**Butte NF, Wong WW, Hopkinson JM, Smith EO, Ellis KJ.** Infant feeding mode affects early growth and body composition. *Pediatrics* 2000 Dec;106(6):1355-1366.

**Buyken AE, Karaolis-Danckert N, Remer T, Bolzenius K, Landsberg B, Kroke A.** Effects of breastfeeding on trajectories of body fat and BMI throughout childhood. *Obesity* (Silver Spring) 2008 Feb;16(2):389-395.

**Cedar H, Bergman Y.** Linking DNA methylation and histone modification: patterns and paradigms. *Nat Rev Genet* 2009 May;10(5):295-304.

**Cohen A, Dempster DW, Recker RR, Lappe JM, Zhou H, Zwahlen A, Muller R, Zhao B, Guo X, Lang T, Saeed I, Liu XS, Guo XE, Cremers S, Rosen CJ, Stein EM, Nickolas TL, McMahon DJ, Young P, Shane E.** Abdominal fat is associated with lower bone formation and inferior bone quality in healthy premenopausal women: a transiliac bone biopsy study. *J Clin Endocrinol Metab* 2013 Jun;98(6):2562-2572.

**Colombara DV, Cowgill KD, Faruque AS.** Risk Factors for Severe Cholera among Children under Five in Rural and Urban Bangladesh, 2000-2008: A Hospital-Based Surveillance Study. *PLoS One* 2013;8(1):e54395.

**Current Care Summary, Osteoporosis.** Osteoporosis: Current Care Guidelines, Working group set up by the Finnish Medical Society Duodecim and the Finnish Endocrinology Society and the Finnish Gynecology Society. Finnish Medical Society Duodecim, 2000 (referred 11 October 2006) Available online at: [www.kaypahoito.fi](http://www.kaypahoito.fi) 2006.

**de Lauzon-Guillain B, Jones L, Oliveira A, Moschonis G, Betoko A, Lopes C, Moreira P, Manios Y, Papadopoulou NG, Emmett P, Charles MA.** The influence of early feeding practices on fruit and vegetable intake among preschool children in 4 European birth cohorts. *Am J Clin Nutr* 2013 Sep;98(3):804-812.

**Deoni SC, Dean DC, 3rd, Piryatinsky I, O'Muircheartaigh J, Waskiewicz N, Lehman K, Han M, Dirks H.** Breastfeeding and early white matter development: A cross-sectional study. *Neuroimage* 2013 May 28;82C:77-86.

**Dewey KG, Heinig MJ, Nommsen LA, Peerson JM, Lonnerdal B.** Growth of breast-fed and formula-fed infants from 0 to 18 months: the DARLING Study. *Pediatrics* 1992 Jun;89(6 Pt 1):1035-1041.

**Dewey KG, Heinig MJ, Nommsen LA, Peerson JM, Lonnerdal B.** Breast-fed infants are leaner than formula-fed infants at 1 y of age: the DARLING study. *Am J Clin Nutr* 1993 Feb;57(2):140-145.

**Dewey KG, Peerson JM, Brown KH, Krebs NF, Michaelsen KF, Persson LA, Salmenperä L, Whitehead RG, Yeung DL.** Growth of breast-fed infants deviates from current reference data: a pooled analysis of US, Canadian, and European data sets. World Health Organization Working Group on Infant Growth. *Pediatrics* 1995 Sep;96(3 Pt 1):495-503.

**Duijts L, Jaddoe VW, Hofman A, Moll HA.** Prolonged and exclusive breastfeeding reduces the risk of infectious diseases in infancy. *Pediatrics* 2010 Jul;126(1):e18-25.

**Elliott L, Henderson J, Northstone K, Chiu GY, Dunson D, London SJ.** Prospective study of breast-feeding in relation to wheeze, atopy, and bronchial hyperresponsiveness in the Avon Longitudinal Study of Parents and Children (ALSPAC). *J Allergy Clin Immunol* 2008 Jul;122(1):49-54, 54.e1-3.

**FAO/WHO.** Energy and protein requirements. 1973: Rome: FAO. 52: FAO Nutritional meetings.

**Fall CH, Barker DJ, Osmond C, Winter PD, Clark PM, Hales CN.** Relation of infant feeding to adult serum cholesterol concentration and death from ischaemic heart disease. *BMJ* 1992 Mar 28;304(6830):801-805.

- Fall CH, Borja JB, Osmond C, Richter L, Bhargava SK, Martorell R, Stein AD, Barrons FC, Victora CG; COHORTS group.** Infant-feeding patterns and cardiovascular risk factors in young adulthood: data from five cohorts in low- and middle-income countries. *Int J Epidemiol* 2011 Feb;40(1):47-62.
- Ferron M, Wei J, Yoshizawa T, Del Fattore A, DePinho RA, Teti A, Ducy P, Karsenty G.** Insulin signaling in osteoblasts integrates bone remodeling and energy metabolism. *Cell* 2010 Jul 23;142(2):296-308.
- Fewtrell MS, Kennedy K, Murgatroyd PR, Williams JE, Chomtho S, Lucas A.** Breast-feeding and formula feeding in healthy term infants and bone health at age 10 years. *Br J Nutr* 2013 Feb 7:1-7.
- Fisk CM, Crozier SR, Inskip HM, Godfrey KM, Cooper C, Roberts GC, Robinson SM; Southampton Women's Survey Study Group.** Breastfeeding and reported morbidity during infancy: findings from the Southampton Women's Survey. *Matern Child Nutr* 2011 Jan;7(1):61-70.
- Freeman V, van't Hof M, Haschke F.** Patterns of milk and food intake in infants from birth to age 36 months: the Euro-growth study. *J Pediatr Gastroenterol Nutr* 2000;31 Suppl 1:S76-85.
- Gage H, Williams P, Von Rosen-Von Hoewel J, Laitinen K, Jakobik V, Martin-Bautista E, Schmid M, Egan B, Morgan J, Decsi T, Campoy C, Koletzko B, Raats, M.** Influences on infant feeding decisions of first-time mothers in five European countries. *Eur J Clin Nutr* 2012 Aug;66(8):914-919.
- Gluckman PD, Hanson MA.** The developmental origins of the metabolic syndrome. *Trends in Endocrinology and Metabolism* 2004 5;15(4):183-187.
- Greer FR, Krebs NF, American Academy of Pediatrics Committee on Nutrition.** Optimizing bone health and calcium intakes of infants, children, and adolescents. *Pediatrics* 2006 Feb;117(2):578-585.
- Grimshaw KE, Maskell J, Oliver EM, Morris RC, Foote KD, Mills EN, Roberts G, Margetts BM.** Introduction of Complementary Foods and the Relationship to Food Allergy. *Pediatrics* 2013 Nov 18.
- Grummer-Strawn LM, Scanlon KS, Fein SB.** Infant feeding and feeding transitions during the first year of life. *Pediatrics* 2008 Oct;122 Suppl 2:S36-42.
- Hakanen M, Lagstrom H, Pahkala K, Sillanmaki L, Saarinen M, Niinikoski H, Raitakari OT, Viikari J, Simell O, Rönnemaa T.** Dietary and lifestyle counselling reduces the clustering of overweight-related cardiometabolic risk factors in adolescents. *Acta Paediatr* 2010 Jun;99(6):888-895.
- Hallman N, Nevanlinna E, Tuuteri L.** The significance of maternal education and certain social factors in breast feeding: a review of studies made in Helsinki and Uusimaa during the summer of 1950. *Duodecim* 1952;68(10):802-817.
- Hamann C, Kirschner S, Gunther KP, Hofbauer LC.** Bone, sweet bone--osteoporotic fractures in diabetes mellitus. *Nat Rev Endocrinol* 2012 Jan 17;8(5):297-305.
- Harder T, Bergmann R, Kallschnigg G, Plogemann A.** Duration of breastfeeding and risk of overweight: a meta-analysis. *Am J Epidemiol* 2005 Sep 1;162(5):397-403.
- Harvey NC, Robinson SM, Crozier SR, Marriott LD, Gale CR, Cole ZA, Inskip HM, Godfrey KM, Cooper C; Southampton Women's Survey Study Group.** Breast-feeding and adherence to infant feeding guidelines do not influence bone mass at age 4 years. *Br J Nutr* 2009 Sep;102(6):915-920.
- Hatakka K, Piirainen L, Pohjavuori S, Poussa T, Savilahti E, Korpela R.** Factors associated with acute respiratory illness in day care children. *Scand J Infect Dis* 2010 Sep;42(9):704-711.
- Health and Social Care Information Centre.** Infant Feeding Survey 2010, IFF Research; 2012.

- Health Canada.** Trends in Breastfeeding Practices in Canada (2001-2008), <http://www.hc-sc.gc.ca/fn-an/surveill/nutrition/commun/prenatal/trends-tendances-eng.php>.
- Hirvensalo M.** Lastenneuvoiloissa annettavien ohjeiden yhdenmukaistamisesta. *Suomen Lääkärilehti* 1951;7:250-255.
- Holgerson PL, Vestman NR, Claesson R, Ohman C, Domellof M, Tanner AC, Hernell O, Johansson I.** Oral microbial profile discriminates breast-fed from formula-fed infants. *J Pediatr Gastroenterol Nutr* 2013 Feb;56(2):127-136.
- Hoppe C, Molgaard C, Michaelsen KF.** Bone size and bone mass in 10-year-old Danish children: effect of current diet. *Osteoporos Int* 2000;11(12):1024-1030.
- Hultin H, Opas R, Sarna S.** Breast feeding of babies in Finland in the seventies. *Kätilölehti* 1974 Oct;79(10):365-372.
- Hunsberger M, Lanfer A, Reeske A, Veidebaum T, Russo P, Hadjigeorgiou C, Moreno LA, Molnar D, De Henauw S, Lissner L, Eiben G.** Infant feeding practices and prevalence of obesity in eight European countries - the IDEFICS study. *Public Health Nutr* 2013 Feb;16(2):219-227.
- Iyengar SR, Walker WA.** Immune factors in breast milk and the development of atopic disease. *J Pediatr Gastroenterol Nutr* 2012 Dec;55(6):641-647.
- Jacobson SW, Carter RC, Jacobson JL.** Breastfeeding as a proxy for benefits of parenting skills for later reading readiness and cognitive competence. *J Pediatr* 2014 Mar;164(3):440-442.
- Jacobson SW, Chiodo LM, Jacobson JL.** Breastfeeding effects on intelligence quotient in 4- and 11-year-old children. *Pediatrics* 1999 May;103(5):e71.
- Jansen J, Weerth C, Riksen-Walraven JM.** Breastfeeding and mother-infant relationship - a review. *Dev Rev* 2008;28:503-521.
- Jeon YK, Lee JG, Kim SS, Kim BH, Kim SJ, Kim YK, Kim IJ.** Association between bone mineral density and metabolic syndrome in pre- and postmenopausal women. *Endocr J* 2011;58(2):87-93.
- Johansson H, Kanis JA, Oden A, McCloskey E, Chapurlat RD, Christiansen C, Cummings SR, Diez-Perez A, Eisman JA, Fujiwara S, Gluer CC, Goltzman D, Hans D, Khaw KT, Krieg MA, Kroger H, Lacroix AZ, Lau E, Leslie WD, Mellstrom D, Melton LJ 3rd, O'Neill TW, Pasco JA, Prior JC, Reid DM, Rivadeneira F, van Staa T, Yoshimura N, Zillikens MC.** A meta-analysis of the association of fracture risk and body mass index in women. *J Bone Miner Res* 2013 Jun 17.
- Jones G, Riley M, Dwyer T.** Breastfeeding in early life and bone mass in prepubertal children: a longitudinal study. *Osteoporos Int* 2000;11(2):146-152.
- Juonala M, Magnussen CG, Berenson GS, Venn A, Burns TL, Sabin MA, Srinivasan SR, Daniels SR, Davis PH, Chen W, Sun C, Cheung M, Viikari JS, Dwyer T, Raitakari OT.** Childhood adiposity, adult adiposity, and cardiovascular risk factors. *N Engl J Med* 2011 Nov 17;365(20):1876-1885.
- Järvisalo MJ, Hutri-Kähönen N, Juonala M, Mikkilä V, Räsänen L, Lehtimäki T, Viikari J, Raitakari OT.** Breast feeding in infancy and arterial endothelial function later in life. The Cardiovascular Risk in Young Finns Study. *Eur J Clin Nutr* 2009 May;63(5):640-645.
- Kafouri S, Kramer M, Leonard G, Perron M, Pike B, Richer L, Toro R, Veillette S, Pausova Z, Paus T.** Breastfeeding and brain structure in adolescence. *Int J Epidemiol* 2013 Feb;42(1):150-159.
- Kajantie E, Barker DJ, Osmond C, Forsen T, Eriksson JG.** Growth before 2 years of age and serum lipids 60 years later: the Helsinki Birth Cohort study. *Int J Epidemiol* 2008 Apr;37(2):280-289.

- Kajosaari M, Saarinen UM.** Evaluation of laboratory tests in childhood allergy. Total serum IgE, blood eosinophilia and eosinophil and mast cells in nasal mucosa of 178 children aged 3 years. *Allergy* 1981 Jul;36(5):329-335.
- Kalliomaki M, Collado MC, Salminen S, Isolauri E.** Early differences in fecal microbiota composition in children may predict overweight. *Am J Clin Nutr* 2008 Mar;87(3):534-538.
- Kanazawa I, Yamaguchi T, Yamamoto M, Yamauchi M, Kurioka S, Yano S, Sugimoto T.** Serum osteocalcin level is associated with glucose metabolism and atherosclerosis parameters in type 2 diabetes mellitus. *J Clin Endocrinol Metab* 2009 Jan;94(1):45-49.
- Kansallinen imetyksen edistämisen asiantuntijaryhmä.** Imetyksen edistäminen Suomessa, Toimintaohjelma 2009–2012. 32 | 2009 ed. Helsinki: Yliopistopaino; 2009.
- Karaolis-Danckert N, Gunther AL, Kroke A, Hornberg C, Buyken AE.** How early dietary factors modify the effect of rapid weight gain in infancy on subsequent body-composition development in term children whose birth weight was appropriate for gestational age. *Am J Clin Nutr* 2007 Dec;86(6):1700-1708.
- Karlsson C, Obrant KJ, Karlsson M.** Pregnancy and lactation confer reversible bone loss in humans. *Osteoporos Int* 2001;12(10):828-834.
- Kelly TL, Wilson KE, Heymsfield SB.** Dual energy X-Ray absorptiometry body composition reference values from NHANES. *PLoS One* 2009 Sep 15;4(9):e7038.
- Khan S, Casadio YS, Lai CT, Prime DK, Hepworth AR, Trengove NJ, Hartmann PE.** Investigation of short-term variations in casein and whey proteins in breast milk of term mothers. *J Pediatr Gastroenterol Nutr* 2012a Aug;55(2):136-141.
- Khan S, Hepworth AR, Prime DK, Lai CT, Trengove NJ, Hartmann PE.** Variation in Fat, Lactose, and Protein Composition in Breast Milk over 24 Hours: Associations with Infant Feeding Patterns. *J Hum Lact* 2012b Jul 13.
- Khanal V, Sauer K, Zhao Y.** Exclusive breastfeeding practices in relation to social and health determinants: a comparison of the 2006 and 2011 Nepal Demographic and Health Surveys. *BMC Public Health* 2013 Oct 14;13:958-2458-13-958.
- Knip M, Virtanen SM, Åkerblom HK.** Infant feeding and the risk of type 1 diabetes. *Am J Clin Nutr* 2010 May;91(5):1506S-1513S.
- Koletzko B, von Kries R, Closa R, Escribano J, Scaglioni S, Giovannini M, Beyer J, Demmelmair H, Anton B, Gruszfeld D, Dobrzanska A, Sengier A, Langhendries JP, Rolland Cachera MF, Grote V.** Can infant feeding choices modulate later obesity risk? *Am J Clin Nutr* 2009a May;89(5):1502S-1508S.
- Koletzko B, von Kries R, Monasterolo RC, Subias JE, Scaglioni S, Giovannini M, Beyer J, Demmelmair H, Anton B, Gruszfeld D, Dobrzanska A, Sengier A, Langhendries JP, Cachera MF, Grote V; European Childhood Obesity Trial Study Group.** Infant feeding and later obesity risk. *Adv Exp Med Biol* 2009b;646:15-29.
- Koo WW, Bush AJ, Walters J, Carlson SE.** Postnatal development of bone mineral status during infancy. *J Am Coll Nutr* 1998 Feb;17(1):65-70.
- Korhonen N, Niemi S, Parkkari J, Sievänen H, Palvanen M, Kannus P.** Continuous decline in incidence of hip fracture: nationwide statistics from Finland between 1970 and 2010. *Osteoporos Int* 2013 May;24(5):1599-1603.
- Kramer MS, Aboud F, Mironova E, Vanilovich I, Platt RW, Matush L, Igumnov S, Fombonne E, Bogdanovich N, Ducruet T, Collet JP, Chalmers B, Hodnett E, Davidovsky S, Skugarevsky O, Trofimovich O, Kozlova L, Shapiro S; Promotion of Breastfeeding Intervention Trial (PROBIT) Study Group.** Breastfeeding and child cognitive development: new evidence from a large randomized trial. *Arch Gen Psychiatry* 2008 May;65(5):578-584.

**Kramer MS, Guo T, Platt RW, Sevkovskaya Z, Dzikovich I, Collet JP, Chalmers B, Shapiro S, Chalmers B, Hodnett E, Vanilovich I, Mezen I, Ducruet T, Shishko G, Bogdanovich N.** Infant growth and health outcomes associated with 3 compared with 6 mo of exclusive breastfeeding. *Am J Clin Nutr* 2003 Aug;78(2):291-295.

**Kramer MS, Guo T, Platt RW, Shapiro S, Collet JP, Chalmers B, Hodnett E, Sevkovskaya Z, Dzikovich I, Vanilovich I, PROBIT Study Group.** Breastfeeding and infant growth: biology or bias? *Pediatrics* 2002a Aug;110(2 Pt 1):343-347.

**Kramer MS, Kakuma R.** Optimal duration of exclusive breastfeeding. *Cochrane Database Syst Rev* 2002b;(1)(1):CD003517.

**Kramer MS, Kakuma R.** Optimal duration of exclusive breastfeeding. *Cochrane Database Syst Rev* 2012 Aug 15;8:CD003517.

**Kramer MS, Matush L, Bogdanovich N, Aboud F, Mazer B, Fombonne E, Collet JP, Hodnett E, Mironova E, Igumnov S, Chalmers B, Dahhou M, Platt RW.** Health and development outcomes in 6.5-y-old children breastfed exclusively for 3 or 6 mo. *Am J Clin Nutr* 2009 Oct;90(4):1070-1074.

**Kramer MS, Matush L, Vanilovich I, Platt R, Bogdanovich N, Sevkovskaya Z, Dzikovich I, Shishko G, Collet JP, Martin RM, Davey Smith G, Gillman MW, Chalmers B, Hodnett E, Shapiro S PROBIT Study.** Effect of prolonged and exclusive breast feeding on risk of allergy and asthma: cluster randomised trial. *BMJ* 2007a Oct 20;335(7624):815.

**Kramer MS, Matush L, Vanilovich I, Platt RW, Bogdanovich N, Sevkovskaya Z, Dzikovich I, Shishko G, Collet JP, Martin RM, Davey Smith G, Gillman MW, Chalmers B, Hodnett E, Shapiro S, PROBIT Study Group.** 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* 2007b Dec;86(6):1717-1721.

**Krause KM, Lovelady CA, Ostbye T.** Predictors of breastfeeding in overweight and obese women: data from Active Mothers Postpartum (AMP). *Matern Child Health J* 2011 Apr;15(3):367-375.

**Kull I, Böhme M, Wahlgren CF, Nordvall L, Pershagen G, Wickman M.** Breast-feeding reduces the risk for childhood eczema. *J Allergy Clin Immunol* 2005 Sep;116(3):657-661.

**Kull I, Melen E, Alm J, Hallberg J, Svartengren M, van Hage M, Pershagen G, Wickman M, Bergström A.** Breast-feeding in relation to asthma, lung function, and sensitization in young schoolchildren. *J Allergy Clin Immunol* 2010 May;125(5):1013-1019.

**Labout JA, Duijts L, Lebon A, de Groot R, Hofman A, Jaddoe VV, Verbrugh HA, Hermans PW, Moll HA.** Risk factors for otitis media in children with special emphasis on the role of colonization with bacterial airway pathogens: the Generation R study. *Eur J Epidemiol* 2011 Jan;26(1):61-66.

**Laitinen J, Kiukaanniemi K, Heikkinen J, Koironen M, Nieminen P, Sovio U, Keinänen-Kiukaanniemi S, Järvelin MR.** Body size from birth to adulthood and bone mineral content and density at 31 years of age: results from the northern Finland 1966 birth cohort study. *Osteoporos Int* 2005 Nov;16(11):1417-1424.

**Larsen JS, Kronborg H.** When breastfeeding is unsuccessful--mothers' experiences after giving up breastfeeding. *Scand J Caring Sci* 2013 Dec;27(4):848-856.

**Lawlor DA, Najman JM, Sterne J, Williams GM, Ebrahim S, Davey Smith G.** Associations of parental, birth, and early life characteristics with systolic blood pressure at 5 years of age: findings from the Mater-University study of pregnancy and its outcomes. *Circulation* 2004 Oct 19;110(16):2417-2423.

**Lawlor DA, Riddoch CJ, Page AS, Andersen LB, Wedderkopp N, Harro M, Stansbie D, Smith GD.** Infant feeding and components of the metabolic syndrome: findings from the European Youth Heart Study. *Arch Dis Child* 2005 Jun;90(6):582-588.



- Le Huerou-Luron I, Blat S, Boudry G.** Breast- v. formula-feeding: impacts on the digestive tract and immediate and long-term health effects. *Nutr Res Rev* 2010 Jun;23(1):23-36.
- Lee K.** Sex-specific relationships between insulin resistance and bone mineral content in Korean adolescents. *J Bone Miner Metab* 2013 Mar;31(2):177-182.
- Lee NK, Sowa H, Hinoi E, Ferron M, Ahn JD, Confavreux C, Dacquin R, Mee PJ, McKee MD, Jung DY, Zhang Z, Kim JK, Mauvais-Jarvis F, Ducy P, Karsenty G.** Endocrine regulation of energy metabolism by the skeleton. *Cell* 2007 Aug 10;130(3):456-469.
- Leeson CP, Kattenhorn M, Deanfield JE, Lucas A.** Duration of breast feeding and arterial distensibility in early adult life: population based study. *BMJ* 2001 Mar 17;322(7287):643-647.
- Leunissen RW, Stijnen T, Hokken-Koelega AC.** Influence of birth size on body composition in early adulthood: the programming factors for growth and metabolism (PROGRAM)-study. *Clin Endocrinol (Oxf)* 2009 Feb;70(2):245-251.
- Locke R.** Preventing obesity: the breast milk-leptin connection. *Acta Paediatr* 2002;91(9):891-894.
- Martin R, Langa S, Reviriego C, Jiminez E, Marin ML, Xaus J, Fernandez L, Rodriguez JM.** Human milk is a source of lactic acid bacteria for the infant gut. *J Pediatr* 2003 Dec;143(6):754-758.
- Martin RM, Ben-Shlomo Y, Gunnell D, Elwood P, Yarnell JW, Davey Smith G.** Breast feeding and cardiovascular disease risk factors, incidence, and mortality: the Caerphilly study. *J Epidemiol Community Health* 2005 Feb;59(2):121-129.
- Martin RM, Davey Smith G, Mangtani P, Tilling K, Frankel S, Gunnell D.** Breastfeeding and cardiovascular mortality: the Boyd Orr cohort and a systematic review with meta-analysis. *Eur Heart J* 2004 May;25(9):778-786.
- Martin RM, Ebrahim S, Griffin M, Davey Smith G, Nicolaidis AN, Georgiou N, Watson S, Frankel S, Holly JM, Gunnell D.** Breastfeeding and atherosclerosis: intima-media thickness and plaques at 65-year follow-up of the Boyd Orr cohort. *Arterioscler Thromb Vasc Biol* 2005a Jul;25(7):1482-1488.
- Martin RM, Gunnell D, Smith GD.** Breastfeeding in infancy and blood pressure in later life: systematic review and meta-analysis. *Am J Epidemiol* 2005b Jan 1;161(1):15-26.
- Martin RM, Patel R, Kramer MS, Guthrie L, Vilchuck K, Bogdanovich N, Sergeichick N, Gusina N, Foo Y, Palmer T, Rifas-Shiman SL, Gillman MW, Smith GD, Oken E.** 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* 2013 Mar 13;309(10):1005-1013.
- Matheson MC, Allen KJ, Tang ML.** Understanding the evidence for and against the role of breastfeeding in allergy prevention. *Clin Exp Allergy* 2012 Jun;42(6):827-851.
- McCrary C, Layte R.** Breastfeeding and risk of overweight and obesity at nine-years of age. *Soc Sci Med* 2012 Jul;75(2):323-330.
- Merten S, Dratva J, Ackermann-Liebrich U.** Do baby-friendly hospitals influence breastfeeding duration on a national level? *Pediatrics* 2005 Nov;116(5):e702-8.
- Michels KB, Willett WC, Graubard BI, Vaidya RL, Cantwell MM, Sansbury LB, Forman MR.** A longitudinal study of infant feeding and obesity throughout life course. *Int J Obes (Lond)* 2007 Jul;31(7):1078-1085.
- Molgaard C, Larnkjaer A, Mark AB, Michaelsen KF.** Are early growth and nutrition related to bone health in adolescence? The Copenhagen Cohort Study of infant nutrition and growth. *Am J Clin Nutr* 2011a Dec;94(6 Suppl):1865S-1869S.
- M'Rabet L, Vos AP, Boehm G, Garssen J.** Breast-feeding and its role in early development of the immune system in infants: consequences for health later in life. *J Nutr* 2008 Sep;138(9):1782S-1790S.

**Mäkelä J, Linderborg K, Niinikoski H, Yang B, Lagström H.** Breast milk fatty acid composition differs between overweight and normal weight women: the STEPS Study. *Eur J Nutr* 2013a Mar;52(2):727-735.

**Mäkelä J, Vaarno J, Kaljonen A, Niinikoski H, Lagström H.** Maternal overweight impacts infant feeding patterns-the STEPS Study. *Eur J Clin Nutr* 2013b Nov 13.

**Männistö S, Laatikainen T, Vartiainen E.** Suomalaisten lihavuus ennen ja nyt. Tutkimuksesta tiiviisti 4, marraskuu 2012. Terveysten ja hyvinvointilaitos, Helsinki.

**Mäyränpää MK, Tamminen IS, Kroger H, Mäkitie O.** Bone biopsy findings and correlation with clinical, radiological, and biochemical parameters in children with fractures. *J Bone Miner Res* 2011 Aug;26(8):1748-1758.

**Nwaru BI, Takkinen HM, Niemelä O, Kaila M, Erkkola M, Kaila M, Erkkola M, Ahonen, Haapala A-M, Kenward MG, Pekkanen J, Laheesmaa R, Kere J, Simell O, Veijola R, Ilonen J, Hyöty H, Knip M, Virtanen S.** Timing of infant feeding in relation to childhood asthma and allergic diseases. *J Allergy Clin Immunol* 2013 Jan;131(1):78-86.

**Oddy WH, Kendall GE, Li J, Jacoby P, Robinson M, de Klerk NH, Silburn SR, Zubirck SR, Landau LI, Stanley FJ.** The long-term effects of breastfeeding on child and adolescent mental health: a pregnancy cohort study followed for 14 years. *J Pediatr* 2010 Apr;156(4):568-574.

**Oddy WH, Robinson M, Kendall GE, Li J, Zubrick SR, Stanley FJ.** Breastfeeding and early child development: a prospective cohort study. *Acta Paediatr* 2011 Jul;100(7):992-999.

**Official statistics of Sweden,** Statistics – Health and Diseases. Breastfeeding, children born 2005. 12th ed. Stockholm: Socialstyrelsen; 2007.

**Olsson C, Hernell O, Hornell A, Lonnberg G, Ivarsson A.** Difference in celiac disease risk between Swedish birth cohorts suggests an opportunity for primary prevention. *Pediatrics* 2008 Sep;122(3):528-534.

**Olufadi R, Byrne CD.** Clinical and laboratory diagnosis of the metabolic syndrome. *J Clin Pathol* 2008 Jun;61(6):697-706.

**Oranta O, Pakkala K, Ruottinen S, Niinikoski H, Lagström H, Viikari JS, Jula A, Loo BM, Simell O, Rönnemaa T, Raitakari OT.** Infancy-onset dietary counseling of low-saturated-fat diet improves insulin sensitivity in healthy adolescents 15-20 years of age: the Special Turku Coronary Risk Factor Intervention Project (STRIP) study. *Diabetes Care* 2013 Oct;36(10):2952-2959.

**O'Tierney PF, Barker DJ, Osmond C, Kajantie E, Eriksson JG.** Duration of breast-feeding and adiposity in adult life. *J Nutr* 2009 Feb;139(2):422S-5S.

**Owen CG, Martin RM, Whincup PH, Davey-Smith G, Gillman MW, Cook DG.** The effect of breastfeeding on mean body mass index throughout life: a quantitative review of published and unpublished observational evidence. *Am J Clin Nutr* 2005a Dec;82(6):1298-1307.

**Owen CG, Martin RM, Whincup PH, Smith GD, Cook DG.** Effect of infant feeding on the risk of obesity across the life course: a quantitative review of published evidence. *Pediatrics* 2005b May;115(5):1367-1377.

**Owen CG, Martin RM, Whincup PH, Smith GD, Cook DG.** Does breastfeeding influence risk of type 2 diabetes in later life? A quantitative analysis of published evidence. *Am J Clin Nutr* 2006 Nov;84(5):1043-1054.

**Owen CG, Whincup PH, Gilg JA, Cook DG.** Effect of breast feeding in infancy on blood pressure in later life: systematic review and meta-analysis. *BMJ* 2003 Nov 22;327(7425):1189-1195.

**Owen CG, Whincup PH, Kaye SJ, Martin RM, Davey Smith G, Cook DG, Bregström E, Black S, Wadsworth ME, Fall CH, Freudenheim JL, Nie J, Huxley RR, Kolacek S, Leeson CP, Pearce MS, Raitakari OT, Lisinen I, Viikari JS, Ravelli AC, Rudnicka AR, Strachan**

- DP, Williams SM.** Does initial breastfeeding lead to lower blood cholesterol in adult life? A quantitative review of the evidence. *Am J Clin Nutr* 2008 Aug;88(2):305-314.
- Parsons TJ, Power C, Manor O.** Infant feeding and obesity through the lifecourse. *Arch Dis Child* 2003 Sep;88(9):793-794.
- Pearce MS, Birrell FN, Francis RM, Rawlings DJ, Tuck SP, Parker L.** Lifecourse study of bone health at age 49-51 years: the Newcastle thousand families cohort study. *J Epidemiol Community Health* 2005 Jun;59(6):475-480.
- Pearce MS, Relton CL, Groom A, Peaston RT, Francis RM.** A lifecourse study of bone resorption in men ages 49-51years: the Newcastle Thousand Families cohort study. *Bone* 2010 Apr;46(4):952-956.
- Peltonen M, Harald K, Männistö S, Saarikoski L, Peltomäki P, Lund L, Sundvall J, Juolevi A, Laatikainen T, Aldén-Nieminen H, Luoto R, Jousilahti P, Salomaa V, Taimi M, Vartiainen E.** Kansallinen FINRISKI 2007-terveysutkimus. Tutkimuksen toteutus ja tulokset. Kansanterveyslaitoksen julkaisu B:B34/ 2008 2008;B34.
- Pesonen J, Sirola J, Tuppurainen M, Jurvelin J, Alhava E, Honkanen R, Kröger H.** High bone mineral density among perimenopausal women. *Osteoporos Int* 2005 Dec;16(12):1899-1906.
- Pettitt DJ, Forman MR, Hanson RL, Knowler WC, Bennett PH.** Breastfeeding and incidence of non-insulin-dependent diabetes mellitus in Pima Indians. *Lancet* 1997 Jul 19;350(9072):166-168.
- Polgreen LE, Jacobs DR, Nathan BM, Steinberger J, Moran A, Sinaiko AR.** Association of Osteocalcin With Obesity, Insulin Resistance, and Cardiovascular Risk Factors in Young Adults. *Obesity* (Silver Spring) 2012 Apr 30.
- Pollock NK, Bernard PJ, Gutin B, Davis CL, Zhu H, Dong Y.** Adolescent obesity, bone mass, and cardiometabolic risk factors. *J Pediatr* 2011 May;158(5):727-734.
- Quigley MA, Kelly YJ, Sacker A.** Breastfeeding and hospitalization for diarrheal and respiratory infection in the United Kingdom Millennium Cohort Study. *Pediatrics* 2007 Apr;119(4):e837-42.
- Ravelli AC, van der Meulen JH, Osmond C, Barker DJ, Bleker OP.** Infant feeding and adult glucose tolerance, lipid profile, blood pressure, and obesity. *Arch Dis Child* 2000 Mar;82(3):248-252.
- Reilly JJ, Ashworth S, Wells JC.** Metabolisable energy consumption in the exclusively breast-fed infant aged 3--6 months from the developed world: a systematic review. *Br J Nutr* 2005a Jul;94(1):56-63.
- Reilly JJ, Wells JC.** Duration of exclusive breast-feeding: introduction of complementary feeding may be necessary before 6 months of age. *Br J Nutr* 2005b Dec;94(6):869-872.
- Rich-Edwards JW, Stampfer MJ, Manson JE, Rosner B, Hu FB, Michels KB, Willet WC.** Breastfeeding during infancy and the risk of cardiovascular disease in adulthood. *Epidemiology* 2004 Sep;15(5):550-556.
- Robinson S, Ntani G, Simmonds S, Syddall H, Dennison E, Sayer AA, Barker D, Cooper C, The Hertfordshire Cohort Study Group.** Type of milk feeding in infancy and health behaviours in adult life: findings from the Hertfordshire Cohort Study. *Br J Nutr* 2012 Jul 5:1-9.
- Saarinen UM.** Need for iron supplementation in infants on prolonged breast feeding. *J Pediatr* 1978 Aug;93(2):177-180.
- Saarinen UM.** Prolonged breast feeding as prophylaxis for recurrent otitis media. *Acta Paediatr Scand* 1982 Jul;71(4):567-571.
- Saarinen UM, Bjorksten F, Knekt P, Siimes MA.** Serum IgE in healthy infants fed breast milk or cow's milk-based formulas. *Clin Allergy* 1979 Jul;9(4):339-335.

- Saarinen UM, Juntunen K, Kajosaari M, Bjorksten F.** Serum immunoglobulin E in atopic and non-atopic children aged 6 months to 5 years. A follow-up study. *Acta Paediatr Scand* 1982 May;71(3):489-494.
- Saarinen UM, Kajosaari M.** Breastfeeding as prophylaxis against atopic disease: prospective follow-up study until 17 years old. *Lancet* 1995 Oct 21;346(8982):1065-1069.
- Saarinen UM, Kajosaari M, Backman A.** Birch pollen allergy in children. Role of milk feeding during the first birch season of life. *Allergy* 1982 Jul;37(5):345-350.
- Saarinen UM, Kajosaari M, Backman A, Siimes MA.** Prolonged breast-feeding as prophylaxis for atopic disease. *Lancet* 1979 Jul 28;2(8135):163-166.
- Saarinen UM, Siimes MA.** Developmental changes in serum iron, total iron-binding capacity, and transferrin saturation in infancy. *J Pediatr* 1977a Dec;91(6):875-877.
- Saarinen UM, Siimes MA.** Iron absorption from infant milk formula and the optimal level of iron supplementation. *Acta Paediatr Scand* 1977b Nov;66(6):719-722.
- Saarinen UM, Siimes MA.** Developmental changes in red blood cell counts and indices of infants after exclusion of iron deficiency by laboratory criteria and continuous iron supplementation. *J Pediatr* 1978a Mar;92(3):412-416.
- Saarinen UM, Siimes MA.** Serum ferritin in assessment of iron nutrition in healthy infants. *Acta Paediatr Scand* 1978b Nov;67(6):745-751.
- Saarinen UM, Siimes MA.** Iron absorption from breast milk, cow's milk, and iron-supplemented formula: an opportunistic use of changes in total body iron determined by hemoglobin, ferritin, and body weight in 132 infants. *Pediatr Res* 1979a Mar;13(3):143-147.
- Saarinen UM, Siimes MA.** Role of prolonged breast feeding in infant growth. *Acta Paediatr Scand* 1979b Mar;68(2):245-250.
- Saarinen UM, Siimes MA, Dallman PR.** Iron absorption in infants: high bioavailability of breast milk iron as indicated by the extrinsic tag method of iron absorption and by the concentration of serum ferritin. *J Pediatr* 1977 Jul;91(1):36-39.
- Sadeharju K, Knip M, Virtanen SM, Savilahti E, Tauriainen S, Koskela P, Åkerblom H, Hyöty H, Finnish TRIGR Study Group.** Maternal antibodies in breast milk protect the child from enterovirus infections. *Pediatrics* 2007 May;119(5):941-946.
- Salmenperä L, Perheentupa J, Siimes MA.** Exclusively breast-fed healthy infants grow slower than reference infants. *Pediatr Res* 1985 Mar;19(3):307-312.
- Savage SA, Reilly JJ, Edwards CA, Durnin JV.** Weaning practice in the Glasgow Longitudinal Infant Growth Study. *Arch Dis Child* 1998 Aug;79(2):153-156.
- Savilahti E, Saarinen KM.** Early infant feeding and type 1 diabetes. *Eur J Nutr* 2009 Jun;48(4):243-249.
- Savino F, Liguori SA.** Update on breast milk hormones: leptin, ghrelin and adiponectin. *Clin Nutr* 2008a Feb;27(1):42-47.
- Savino F, Liguori SA, Fissore MF, Palumeri E, Calabrese R, Oggero R, Silvestro L, Miniero R.** Looking for a relation between serum leptin concentration and body composition parameters in healthy term infants in the first 6 months of life. *J Pediatr Gastroenterol Nutr* 2008b Mar;46(3):348-351.
- Scholes D, Ichikawa L, LaCroix A, Spangler L, Beasley J, Reed S, Ott S.** Oral contraceptive use and bone density in adolescent and young adult women. *Contraception* 2010;81(1):35-40.
- Scholten S, Brunekreef B, Smit HA, Gast GC, Hoekstra MO, de Jongste JC, Postma DS, Gerritsen J, Seiden JC, Wijga AH.** Do differences in childhood diet explain the reduced overweight risk in breastfed children? *Obesity* (Silver Spring) 2008 Nov;16(11):2498-2503.

- Sears MR, Greene JM, Willan AR, Taylor DR, Flannery EM, Cowan JO, Herbison GP, Poulton R.** Long-term relation between breastfeeding and development of atopy and asthma in children and young adults: a longitudinal study. *Lancet* 2002 Sep 21;360(9337):901-907.
- Shields L, Mamun AA, O'Callaghan M, Williams GM, Najman JM.** Breastfeeding and obesity at 21 years: a cohort study. *J Clin Nurs* 2010 Jun;19(11-12):1612-1617.
- Shu A, Yin MT, Stein E, Cremers S, Dworakowski E, Ives R, Rubin MR.** Bone structure and turnover in type 2 diabetes mellitus. *Osteoporos Int* 2012 Feb;23(2):635-641.
- Simell O, Nünikoski H, Rönnemaa T, Raitakari OT, Lagström H, Laurinen M, Aromaa M, Hakala P, Jula A, Jokinen E, Välimäki I, Viikari J, SRTIP Study Group.** Cohort Profile: the STRIP Study (Special Turku Coronary Risk Factor Intervention Project), an Infancy-onset Dietary and Life-style Intervention Trial. *Int J Epidemiol* 2009 Jun;38(3):650-655.
- Strand TA, Sharma PR, Gjessing HK, Ulak M, Chandyo RK, Adhikari RK, Sommerfelt H.** Risk factors for extended duration of acute diarrhea in young children. *PLoS One* 2012;7(5):e36436.
- Suomen lastenlääkäriyhdistys.** 2001 <http://www.suomenlastenlaakariyhdistys.fi>.
- Sääkslahti A, Numminen P, Varstala V, Helenius H, Tammi A, Viikari J, Välimäki I.** Physical activity as a preventive measure for coronary heart disease risk factors in early childhood. *Scand J Med Sci Sports* 2004 Jun;14(3):143-149.
- Tanaka PA, Yeung DL, Anderson GH.** Infant feeding practices: 1984-85 versus 1977-78. *CMAJ* 1987 May 1;136(9):940-944.
- Tanko LB, Christiansen C, Cox DA, Geiger MJ, McNabb MA, Cummings SR.** Relationship between osteoporosis and cardiovascular disease in postmenopausal women. *J Bone Miner Res* 2005 Nov;20(11):1912-1920.
- Tharner A, Luijk MP, Raat H, Ijzendoorn MH, Bakermans-Kranenburg MJ, Moll HA, Jaddoe VW, Hofman A, Verhulst FC, Tiemeier H.** Breastfeeding and its relation to maternal sensitivity and infant attachment. *J Dev Behav Pediatr* 2012 Jun;33(5):396-404.
- Toschke AM, Martin RM, von Kries R, Wells J, Smith GD, Ness AR.** Infant feeding method and obesity: body mass index and dual-energy X-ray absorptiometry measurements at 9-10 y of age from the Avon Longitudinal Study of Parents and Children (ALSPAC). *Am J Clin Nutr* 2007 Jun;85(6):1578-1585.
- UNICEF.** Baby Friendly Initiative 2007 <http://www.babyfriendly.org.uk>
- Uusitalo L, Nyberg H, Pelkonen M, Sarlio-Lähteenkorva S, Hakulinen-Viitanen T, Virtanen S.** Imeväisikäisten ruokinta Suomessa vuonna 2010. 2012.
- Vaarala O.** Human intestinal microbiota and type 1 diabetes. *Curr Diab Rep* 2013 Oct;13(5):601-607.
- Vahlquist B.** Evolution of breast feeding in Europe. *J Trop Pediatr Environ Child Health* 1975 Feb;21(1):11-18.
- van Odijk J, Kull I, Borres MP, Brandtzaeg P, Edberg U, Hanson LA, Host A, Kuitunen M, Olsen SF, Skerfving S, Sundell J, Wille S.** Breastfeeding and allergic disease: a multidisciplinary review of the literature (1966-2001) on the mode of early feeding in infancy and its impact on later atopic manifestations. *Allergy* 2003 Sep;58(9):833-843.
- Vartiainen E, Borodulin K, Sundvall J, Laatikainen T, Peltonen M, Harald K, Salomaa V, Puska P.** FINRISKI-tutkimus: Väestön kolesterolitaso on vuosikymmenien laskun jälkeen kääntynyt nousuun. *Suomen Lääkärilehti* 2012;67(35):2364-2368.
- Vestman NR, Timby N, Holgerson PL, Kressirer CA, Claesson R, Domellof M, Ohman C, Tanner AC, Hernell O, Johansson I.** Characterization and in vitro properties of oral lactobacilli in breastfed infants. *BMC Microbiol* 2013 Aug 15;13:193-2180-13-193.

- Virtanen SM, Knip M.** Nutritional risk predictors of beta cell autoimmunity and type 1 diabetes at a young age. *Am J Clin Nutr* 2003 Dec;78(6):1053-1067.
- Walfisch A, Sermer C, Cressman A, Koren G.** Breast milk and cognitive development--the role of confounders: a systematic review. *BMJ Open* 2013 Aug 23;3(8):e003259-2013-003259.
- Weiser TM, Lin M, Garikapaty V, Feyerharm RW, Bensyl DM, Zhu BP.** Association of maternal smoking status with breastfeeding practices: Missouri, 2005. *Pediatrics* 2009 Dec;124(6):1603-1610.
- Wells JC, Chomtho S, Fewtrell MS.** Programming of body composition by early growth and nutrition. *Proc Nutr Soc* 2007 Aug;66(3):423-434.
- Weyermann M, Brenner H, Rothenbacher D.** Adipokines in human milk and risk of overweight in early childhood: a prospective cohort study. *Epidemiology* 2007 Nov;18(6):722-729.
- WHO.** The optimal duration of exclusive breastfeeding. Report of an Expert Consultation. Geneva, Switzerland: World Health Organization.; 2001.
- WHO Multicentre Growth Reference Study Group.** Assessment of differences in linear growth among populations in the WHO Multicentre Growth Reference Study. *Acta Paediatr Suppl* 2006 Apr;450:56-65.
- Wickes IG.** A history of infant feeding. V. Nineteenth century concluded and twentieth century. *Arch Dis Child* 1953 Dec;28(142):495-502; concl.
- Wiedmeier JE, Joss-Moore LA, Lane RH, Neu J.** Early postnatal nutrition and programming of the preterm neonate. *Nutr Rev* 2011 Feb;69(2):76-82.
- Williams DM, Martin RM, Davey Smith G, Alberti KG, Ben-Shlomo Y, McCarthy A.** Associations of infant nutrition with insulin resistance measures in early adulthood: evidence from the Barry-Caerphilly Growth (BCG) study. *PLoS One* 2012;7(3):e34161.
- Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB.** Prediction of coronary heart disease using risk factor categories. *Circulation* 1998 May 12;97(18):1837-1847.
- Wolf JH.** Low breastfeeding rates and public health in the United States. *Am J Public Health* 2003 Dec;93(12):2000-2010.
- Young RJ, Antonson DL, Ferguson PW, Murray ND, Merkel K, Moore TE.** Neonatal and infant feeding: effect on bone density at 4 years. *J Pediatr Gastroenterol Nutr* 2005 Jul;41(1):88-93.