SANGITA KURL

Lumbar bone mineral status during infancy and at preschool age in children born preterm

Doctoral dissertation

To be presented by permission of the Faculty of Medicine of the University of Kuopio for public examination in Auditorium L3, Cantia building, University of Kuopio, on Friday 12th September 2003, at 12 noon

Department of Pediatrics
Department of Clinical Physiology and Nuclear Medicine
University of Kuopio, Kuopio University Hospital
ISBN 951-781-348-1
ISSN 1235-0303

Abstract

Metabolic bone disease of prematurity affects in particular extremely low birth weight (ELBW) infants born less than 32 weeks of gestation. The main objective of this study was to identify the factors affecting lumbar spine bone mineral content (BMC) and bone mineral density (BMD) in 98 prematurely-born infants during early infancy; for comparison we similarly measured the lumbar BMC and BMD in 41 term-born reference infants. A further objective was to evaluate the long term effect of prematurity on the bone mineral status in 38 children aged 6-7 years born prematurely. Dual energy x-ray absorptiometry (DXA) was used to measure the lumbar BMC and BMD in all subjects.

Reduced lumbar BMC and BMD were observed in prematurely-born infants when compared to term-born reference infants of similar weight range during early infancy. Postnatal dexamethasone treatment in prematurely-born infants was related to low lumbar BMC while intraterine growth status at birth and the duration of breast-feeding did not associate with lumbar BMC. The prematurely-born appropriately grown infants (AGA) and small for gestational age (SGA) infants had similar lumbar BMC and BMD values.

Infants born less than 32 weeks of gestation developed growth retardation during the initial hospitalization despite following the current dietary recommendations. An important finding was that exclusive breast-feeding after discharge from hospital supported linear catch-up growth and weight gain but was associated with low lumbar BMC. The other factors associated with the risk of having low BMC values later in infancy were low serum phosphate levels at 6 weeks and male gender.

At the age of 6-7 years the lumbar BMD values of the prematurely-born children did not differ from those of the Finnish age- and sex- specific reference values. These children were within the normal range for height and weight. The duration of exclusive breast-feeding after discharge from hospital did not relate to the lumbar BMD. Low body weight at 1 year predicted high BMC at 6-7 years of age.

In future, the growth during the initial hospital stay needs to be more closely followed up in order to identify and treat early nutritional disturbances. Since growth patterns during the first year of life have long-term effects on bone mineral status, it is necessary to improve the post-discharge nutrition. More information is needed about nutrient requirements of very low birth weight (VLBW) infants during the post-discharge catch-up growth period. In particular, the amount and duration of human milk fortification should be studied further.

National Library of Medicine Classification: WS 410, WS 420, WE 200

Medical Subject Headings (MeSH): infant, premature/ growth and development; infant, very low birth weight; infant, small for gestational age; densitometry, x-ray; calcification, physiologic; bone density; dexamethasone; breast-feeding; child.
Tiivistelmä

Hyvin ennenaikaisesti, ennen 32 sikiöviikon ikää syntyneillä keskosilla on syntyvän jälkeen alhainen luoston mineraalipitoisuus ja -tiheys. Tämän tutkimuksen pääasiallisena tarkoituksena oli löytää ne tekijät, jotka vaikuttivat lannerangan mineraalipitoisuuden ja mineraalitiheyden myöhempään kehitykseen 98 imeväiskäisellä keskosella; verrokkiryhmänä oli 41 täysiaikaisena syntynyttä lasta. Tutkimuksessa arvioitiin myös keskosuuden pitkäaikaisvaikutuksia luun mineraalitilaan tutkimalla 38 keskosena syntynyttä lasta 6-7 vuoden iässä. Luun mineraalipitoisuus ja -tiheys mitattiin lannerikamista kaksenergiaisella röntgenabsorptiometrilla (DXA).


Ennen 32 sikiöviikkoa syntyneiden lasten pituuden ja painon kasvu hidastui sairaalassaolon alkuaikoina huolimatta ravitsemusvuositusten mukaisesta ruokinnasta. Tärkeä löydös oli, että pitkäkestoinen yksinomainen rintaruokinta tuki suotuisasti keskoslasten pituuden ja painon kehitystä, mutta oli luun pienen mineraalipitoisuuden riskitekijä. Muita luun pienien mineraalipitoisuuden riskitekijöitä imeväiskäisillä pikkukeskosilla olivat vastasynteineysyskuudella todetut matalat seerumin fosfaatipitoisuudet sekä miessukupuoli.

Luun mineraalitiheyden arvit 6-7-vuotiailla keskoslapsilla eivät eronneet suomalaisista ikä- ja sukupuolivakioiduista viitearvoista. Nämä keskos-lapset olivat myös pituuskasvutaan ja painoitaan normaaleja.

Keskoslasten kasvua tukeva, riittävä ravitsemus jo syntyvän jälkeisenä tehonheitovaikenteessa on edellytykseni luuston mineraalipitoisuuden ja -tiheyden suotuisalle kehitykselle imeväisiässä. Lisää tietoa tarvitaan hyvin pienipainoisten keskosten ravitsemustarpeista myöhemmässä imeväisiässä, johon pituuden ja painon nopein saavutusastia vaihe ajottuu.
I dedicate this work to my mother and in memory of my father
Acknowledgements

This study was carried out at the Department of Pediatrics, in collaboration with the Department of Clinical Physiology and Nuclear Medicine, Kuopio University Hospital, University of Kuopio, Kuopio, Finland.

This study was financially supported by grants from the Helena Vuoren mies Foundation, Kuopio University Foundation, Kuopio University Hospital EVO grants, Finnish Cultural Foundation of North Savo, Finnish Pediatric Research Foundation, Päiviikki and Sakari Sohberg Foundation, and Jalmari and Rauha Ahokas Foundation, which are all gratefully acknowledged.

I have been very fortunate to have three supervisors, whose broad expanse of knowledge has educated and inspired me greatly. I am most deeply indebted to Professor Kari Launiala, Emeritus, M.D., Ph.D., the former head of the Department of Pediatrics, also my supervisor, for providing me this first opportunity to do scientific work. I also wish to thank Professor Raimo Voutilainen, M.D., Ph.D., the present head of the department for his help during the final stages of this study.

I want to express my deepest respect and gratitude to the principal supervisor of this thesis, Docent Kirsti Heinonen, M.D., Ph.D., without whose guidance and encouragement this thesis would not have been possible. It has been a great privilege to work under her supervision.

I also wish to express my sincere thanks to Professor Esko Länsimies, M.D., Ph.D., also my supervisor, for his tireless encouragement, valuable advice, criticism and support throughout this work.

I want to thank the official referees Docent Harri Sievänen, Sc.D., and Docent Marja Ala-Houhala, M.D., Ph.D., for the constructive criticism and expert advice during the preparation of the final version of this thesis. My warmest thanks to Ms. Eila Koski, Registered Nurse, Department of Clinical Physiology and Nuclear
Medicine, Kuopio University Hospital, for her expert work in the bone density measurements. I wish to thank Docent Jukka Jurvelin, Ph.D., for his collaboration in this study.

I am very grateful to Mrs. Pirjo Halonen, M.Sc., Computing Center, University of Kuopio, for kind and patient advice concerning statistical analysis. I especially wish to thank Mrs. Liisa Korkalainen, for her technical assistance during this work. I also want to thank Mrs. Liisa Markkanen for her help in the initial part of this study. I extend my thanks to the staff of the Kuopio University Library for their help in searching and collection of the vast literature, especially, Mrs. Liisa Salmi, M.Sc. My thanks also go out to many others who have cheerfully answered my frequently asked questions and requests: you know who you are, and consider yourselves thanked!

I am indebted to all the doctors and the nursing staff at the Neonatal Intensive Care Unit, Kuopio University Hospital, who took care of the practical performance of this study. I also send my warmest thanks to the parents of all the infants who participated and made this study possible.

I am grateful to Vivian Paganuzzi M.A. for revising the language used in this manuscript.

I acknowledge the permission granted by the copyright owners to reproduce the original articles of this thesis.

I wish to express my eternal gratitude to my mother, Shakuntia Panhani and father Bansilal Panhani, who gave me the foundation to enter into this academic field. I dedicate this thesis to both of you!

Special thanks go out to my in-laws and friends for their impressive support and encouragement given to me at all times.
Finally, my overwhelming thanks must go to my family especially my loving husband Sudhir for his continued and unfailing patience, support and faith in me and to my dearest daughter Ansha without whom this thesis would not be...................

Sangita Kurl
Kuopio, Finland, August 2003
Abbreviations used in the text

AAP: American Academy for Pediatrics
AGA: appropriate for gestational age
BMC: bone mineral content
BMD: bone mineral density
DPA: dual photon absorptiometry
DXA: dual energy x-ray absorptiometry
ESPGAN: European Society of Pediatric Gastroenterology and Nutrition
FLBW: extremely low birth weight
GA: gestational age
MeSH: medical subject headings
SD: standard deviation
SGA: small for gestational age
SPA: single photon absorptiometry
VLBW: very low birth weight
1,25(OH)\(_2\)D: 1,25-dihydroxyvitamin D, calcitriol
25OHD: 25-hydroxyvitamin D, calcidiol

Conversion

<table>
<thead>
<tr>
<th>Traditional units vs SI units</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,25(OH)(_2)D</td>
</tr>
<tr>
<td>25OHD</td>
</tr>
<tr>
<td>biological activity of vitamin D</td>
</tr>
</tbody>
</table>
LIST OF ORIGINAL PUBLICATIONS

This dissertation is based on the following published original articles. In the text, Study I refers to article I, Study II to article II, Study III to article III and Study IV to article IV.


CONTENTS

1. INTRODUCTION ............................................................................................................. 13

2. REVIEW OF LITERATURE .......................................................................................... 14
   2.1 Collection of the literature ....................................................................................... 14
   2.2 Bone structure ........................................................................................................... 15
       2.2.1 Techniques to measure bone mineral content and density ......................... 15
       2.2.2 Dual energy x-ray absorptiometry (DXA) ..................................................... 18
   2.3 Intrauterine bone mineral accretion ......................................................................... 19
       2.3.1 Placental transport of minerals during pregnancy ....................................... 19
       2.3.2 Effect of intrauterine growth on bone mineral status .................................. 20
   2.4 Bone mineral status from birth to childhood in term infants ................................. 20
   2.5 Bone mineral status in preterm infants and influencing factors ......................... 21
       2.5.1 Metabolic bone disease of prematurity - definition and incidence ............ 21
       2.5.2 Diagnosis of metabolic bone disease ............................................................. 22
       2.5.3 Bone mineral accretion after preterm birth ................................................. 23
       2.5.4 Effects of calcium and phosphorus intake .................................................... 24
       2.5.5 Vitamin D intake ............................................................................................. 25
       2.5.6 Effects of steroids .......................................................................................... 25
       2.5.7 Effect of gender .............................................................................................. 26
       2.5.8 Effect of physical activity .............................................................................. 27
   2.6 Long term effect of prematurity on bone mineral status ........................................ 27

3. AIMS OF THE STUDY ................................................................................................... 29

4. PATIENTS AND METHODS ....................................................................................... 30
   4.1 Patients ..................................................................................................................... 30
   4.2 Study design ............................................................................................................ 34
   4.3 Methods ................................................................................................................... 35
1. INTRODUCTION

Metabolic bone disease of prematurity is defined as decreased bone mineralization. Deficiency of minerals, especially phosphates, is the main etiological factor. Rickets, the severe form of this disease, was described as early as the year 1910 (Ylppö 1919). Since then, the development of new non-invasive densitometric techniques have helped to illustrate the complex clinical picture of metabolic bone disease which ranges from osteopenia to overt rickets and fractures of the ribs and long bones. The frequency of metabolic bone disease varies inversely with the gestational age (GA). The incidence of metabolic bone disease varies from 23% in infants weighing less than 1500 grams and 55% in infants less than 1000 grams at birth (McIntosh et al. 1986, Lyon et al. 1987). Recently, a large proportion of extremely low birth weight (ELBW) infants are surviving due to development in their care and advances in technology (Tommiska et al. 2001). This group is at the greatest risk of developing this disease.

The main objective of this study was to investigate the factors affecting lumbar bone mineral status in prematurely-born infants and term-born reference infants during the first year of life by using a densitometric technique. We also evaluated the long-term effect of prematurity on the bone mineral status of children aged 6-7 years born prematurely.
2. REVIEW OF LITERATURE

2.1 Collection of the literature

The review of literature for this thesis is based on a computer assisted search and retrieval system until early 2003. The citations and abstracts were searched from PubMed, a web-based retrieval system developed by the National Center for Biotechnology and Information (NCBI) at the National Library of Medicine (NLM) located at National Institutes of Health (NIH), Bethesda, MD, USA. PubMed provides access to bibliographic information that includes MEDLINE. MEDLINE contains citations and abstracts from biomedical journals published in the United States of America and 70 other countries indexed since 1966. MEDLINE is searchable via the internet at http://www.ncbi.nlm.nih.gov/entrez/. The coverage of this database is worldwide, but only records from English sources were included in this review. Medical Subject Headings (MeSH) terminology were used to retrieve the required information from the MEDLINE. MeSH is controlled vocabulary used for indexing the articles and provides a consistent way to obtain information that may use different terminology for the same concepts.

The following terminology were used in the search from the database: infant, premature/ growth and development (MeSH); prematurity, preterm infants, term born infants; infant, very low birth weight; infant, small for gestational age (MeSH); intrauterine growth retardation (IUGR), intrauterine growth status, small for gestational age (SGA), appropriate for gestational age (AGA); densitometry, x-ray; calcification, physiologic; densitometry, X-ray; bone density (MeSH); dual energy x-ray absorptiometry (DXA), bone mineral content (BMC), bone mineral density (BMD), bone mineralization; dexamethasone, bronchopulmonary dysplasia (MeSH); steroid use; growth (MeSH) breast-feeding (MeSH); child (MeSH); prematurely born children.
2.2 Bone structure

Bone is a specialized connective tissue, which together with cartilage makes up the skeletal system. It consists of cells and extracellular matrix, 35% of the matrix being composed of organic and 65% of inorganic constituents. The inorganic part is formed of minerals (calcium and phosphorus) and the organic components are collagen and noncollagenous proteins. During the course of bone formation, the extracellular matrix becomes calcified by the deposition of calcium and phosphorus to form hydroxyapatite [Ca_{10}(PO_{4})_6(OH)_2]. The crystals of hydroxyapatite are spindle or plate shaped. Histologically, bone is of two types, cortical (compact) and trabecular (cancellous) bone. Compact bone is found predominantly in the long bone shafts of the appendicular skeleton. On the other hand trabecular bone is the primary component of axial bones (vertebrae [66-90%], flat bones of skull and pelvis). The metabolic activity of the trabecular bone is several times greater than that of cortical bone, leading to functional differences: the cortical bone fulfills mainly mechanical and protective function whereas the trabecular bone has metabolic function. Trabecular bone appears to be more sensitive to mineral changes than cortical bone (Seeman et al. 1982).

2.2.1 Techniques to measure bone mineral content and density

Several non-invasive methods have been used to assess the bone mineral status in infants and children. These techniques involve different methodologies which vary in precision and accuracy.

Conventional radiographic evaluation is relatively insensitive and can detect decreased bone mineralization only after 30-40% loss of minerals has occurred (Ardan 1951, Lachman et al. 1985). In infants and children, single photon absorptiometry (SPA) of the peripheral skeleton has been the widely used technique
for several years (Pohlandt et al. 1989, Pittard et al. 1990, Schanler et al. 1990). Dual photon absorptiometry (DPA) and quantitative computed tomography (QCT) offer several advantages allowing measurements of axial skeleton. Relatively long scanning times and high radiation exposure, however, limit their use in children. Ultrasound measurements of the bone may provide additional information about bone quality, but more studies are needed in children to prove their validity. Quantitative magnetic resonance (QMR) is a promising technique for analysis of trabecular bone, but its very expensive and time consuming. Dual energy x-ray absorptiometry (DXA), at present, is the preferred method for clinical evaluation of the skeletal status in infants and children. Summary of the studies performed using DXA in children have been presented in Table 1.
<table>
<thead>
<tr>
<th>Study &amp; Publication year</th>
<th>Age</th>
<th>Site</th>
<th>Instrument used</th>
<th>Number (n)</th>
<th>Main finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Koo et al. 2000</td>
<td>Newborns, 27-42 wks 3A</td>
<td>L1-L4 BMC, BMD</td>
<td>Hologic QDR 1000</td>
<td>201</td>
<td>Body weight main predictor of BMC, no effect of gender, race or season</td>
</tr>
<tr>
<td>Braillon et al. 1992</td>
<td>Newborns, 33-40 wks 3A</td>
<td>L1-L5 BMC, BMD</td>
<td>Hologic QDR 1000</td>
<td>30</td>
<td>Increased with GA</td>
</tr>
<tr>
<td>Park et al. 1998</td>
<td>Term-born infants at 2-5 months</td>
<td>L2-L4 BMC</td>
<td>Lunar</td>
<td>35</td>
<td>No difference between breast-fed and formula-fed infants</td>
</tr>
<tr>
<td>Salle et al. 1992</td>
<td>Newborns, 31-40 wks 3A</td>
<td>L1-L5 BMC, BMD</td>
<td>Hologic</td>
<td>57</td>
<td>BMC &amp; BMD correlated with birth weight, body area, length &amp; GA</td>
</tr>
<tr>
<td>Salle et al. 1992</td>
<td>1-24 months</td>
<td>L1-L5 BMC, BMD</td>
<td>Hologic</td>
<td>22</td>
<td>BMC &amp; BMD correlated with weight, age, length &amp; body area</td>
</tr>
<tr>
<td>Koo et al. 1998</td>
<td>1-391 days</td>
<td>TB BMC, BMD</td>
<td>Hologic</td>
<td>130</td>
<td>Weight main predictor of TB BMC, no gender or race effect</td>
</tr>
<tr>
<td>Rupch et al. 1996</td>
<td>1-18 months</td>
<td>TB BMC, BMD</td>
<td>Hologic</td>
<td>64</td>
<td>BMC higher in males</td>
</tr>
<tr>
<td>del Rio et al. 1994</td>
<td>3 months-21 years</td>
<td>L2-L4 BMC</td>
<td>Lunar DPX-L</td>
<td>471</td>
<td>No gender differences, puberty related differences</td>
</tr>
<tr>
<td>Glastre et al. 1990</td>
<td>1-15 years</td>
<td>L1-L4, BMD</td>
<td>Hologic, QDR '1000</td>
<td>135</td>
<td>No differences between boys and girls, at 12 years girls had higher BMD values due to puberty</td>
</tr>
<tr>
<td>Kröger et al. 1992</td>
<td>6-19 years</td>
<td>L2-L4 BMC, BMD</td>
<td>Lunar DPX</td>
<td>84</td>
<td>BMD higher in girls after adjusting for age, height and weight</td>
</tr>
<tr>
<td>Lu et al. 1994</td>
<td>4-27 years</td>
<td>L2-L4 BMC, BMD</td>
<td>Lunar DPX</td>
<td>266</td>
<td>BMD increased with age till 17.5 years in boys and 15.8 years in girls</td>
</tr>
</tbody>
</table>

Abbreviations: GA = gestational age, BMC = bone mineral content, BMD = bone mineral density, TB = total body
2.2.2 Dual energy x-ray absorptiometry (DXA)

DXA is based on the method of x-ray spectrophotometry that was developed in the 1970s, and was introduced commercially as the direct successor to DPA. DXA uses the same principles as DPA, but in DXA the radionuclide source is replaced by an x-ray tube. Depending on the manufacturer, beams of two distinct energy levels are produced by the x-ray generator or selectively filtered from the x-ray spectrum. The main advantages of an x-ray system over the DPA radionuclide system are shortened examination time due to increased influx of the x-ray tube and greater accuracy and precision due to higher resolution and removal of errors due to source decay corrections. DXA uses two different x-ray energy levels (high and low photon energies). The photons produced by the x-ray tube after exiting the bone of interest are detected and measured by a computer which converts them to BMC and BMD. BMC is expressed in grams (g), this is a bulk parameter highly dependent on bone size. BMD does not represent a volumetric density but an areal density expressing the BMC in a projected area of bone (g/cm²). The duration of DXA scans range from 3 to 5 minutes for the lumbar spine. The preferred anatomic sites for DXA measurements are lumbar spine, proximal femur, whole body and some peripheral sites such as radius and calcaneus. At all sites, DXA reflects a composite of trabecular and cortical bone. The lumbar spine is the most frequently studied skeletal site (Glastre et al. 1990, Braillon et al. 1992, Kröger et al. 1992, Salle et al. 1992, del Rio et al. 1994, Lu et al. 1994, Park et al. 1998, Koo et al. 2000). The region of interest studied is usually either L1-L5 or L2-L4 which serves as a sampling site for bone mineral estimation in the vertebral column. Portable BMD instruments have been used to measure the sick preterm infants requiring intensive care (Williams et al. 1994, Truscott et al. 1996).
2.3 Intrauterine bone mineral accretion

2.3.1 Placental transport of minerals during pregnancy

Mineralization of the fetal skeleton is provided by the active transport of minerals from mother to fetus across the placenta for skeletal growth. Fetal bone mineralization begins at about 8 weeks of gestation (Salder 1995). Analysis of fetal bone mineral content has given insight into the process of fetal skeletal formation. The average daily calcium need raises from 2-3 mg in the first trimester to 250-300 mg in the last trimester (Kumar 1980). Mineral accretion of calcium and phosphorus increases during the pregnancy and reaches a maximum by the third trimester (Ziegler et al. 1976). During the third trimester, 70-80 % of fetal ossification occurs. Calcium transport through the placenta to the fetus occurs via an active mechanism. Phosphate transport is reduced by parathyroid hormone (Brunette et al. 1989), and is modulated by pH, sodium and amino acid concentrations (Brunette et al. 1985, Lajeunesse et al. 1988). The fetal parathyroid glands are essential for maintaining elevated plasma calcium levels for stimulation of fetal osteoblasts and mineralization of cartilage and osteoid (MacIsaac et al. 1991).

Maternal calcium and vitamin D concentrations also influence the placental calcium transport. Maternal dietary deficiency of calcium and vitamin D has been found to result in the birth of infants with rickets (Park et al. 1987). Poorly treated maternal hypoparathyroidism has been reported to result in maternal hypocalcemia and led to fetal transient congenital hyperparathyroidism (Landing et al. 1970). Under these conditions decreased placental calcium transfer has been found to cause fetal hypocalcemia, leading to fetal hyperparathyroidism (Jacobson et al. 1978).
2.3.2 Effect of intrauterine growth on bone mineral status

Reduced utero-placental blood flow in small for gestational age (SGA) infants results in reduced transplacental mineral supply, reduced fetal bone formation (Namgung et al. 1996, Namgung et al. 2000), reduced fetal placental production of 1,25-dihydroxyvitamin D, calcitriol [1,25(OH)₂D], low BMC and low serum osteocalcin valuee (Namgung et al. 1993). Term SGA infants when compared with appropriate for gestational age (AGA) infants had lower BMC of the lumbar spine soon after birth (Namgung et al. 1993, Chen et al. 1995, Chunga Vega et al. 1996, Lapillonne et al. 1997). Decreased transplacental mineral transfer is related to low BMC in infants of diabetic mothers (Namgung et al. 2000). Infants of diabetic mothers had low BMC of the radius when compared with control infants at birth using SPA (Mimouni et al. 1988). However, Lapillonne et al. (1997) reported that infants of diabetic mothers had higher whole body mineral content when compared with control infants of same weights using DXA.

Large for gestational age term infants have higher BMC than AGA infants of similar weights (Kurl et al. Abstract 2001, Hammani et al. 2001). A seasonal effect can be seen: summer-born infants had lower BMC and higher serum osteocalcin and 1,25-(OH)₂D than winter-born infants born at term (Namgung et al. 1994).

2.4 Bone mineral status from birth to childhood in term infants.

Smoking during pregnancy, maternal thinness, a faster walking pace and vigorous activity during late pregnancy have been shown to associate with lower whole body BMC and BMD in term infants (Godfrey et al. 2001). In healthy term infants, body weight is the best determinant of lumbar spine BMC at birth (Koo et al. 2000) and of total body BMC during infancy (Koo et al. 1998).

There was no effect of gender on bone mineral status of term infants during the first year of life (Glastre et al. 1990, Koo et al. 1998, Koo et al. 2000, Avila-Diaz et al.
2001), although another study (Rupich et al. 1996) has reported gender-related
differences in BMC and BMD values during infancy.

There are several studies on lumbar bone mineral status during childhood
presented either in relation to age or body weight, but the use of different
absorptiometric instruments prevents the comparison of the results. Reference data
on BMC and BMD values measured by DXA in term infants (Braillon et al. 1992,
1998, Koo et al. 2000) have been reported. In children the available data suggest
that BMC and BMD values increase with age in both boys and girls. Earlier studies
have shown high annual increments in BMD values during the first 3 years of life (del
Rio et al. 1994) and they increase until puberty. Due to earlier onset of puberty girls
have higher BMD values than boys at 12 years (Glastre et al. 1990), at 12-13 years
(Kroger et al. 1992), at 14-15 years (del Rio et al. 1994) and at 15 years (Lu et al.
1994) of age. It has been found that BMD values increased with age in both boys and
girls until 20-21 years before they flattened off (del Rio et al. 1994, Lu et al. 1994).

2.5 Bone mineral status in preterm infants and influencing factors
Postnatal skeletal mineralization is affected by various factors in preterm infants.

2.5.1 Metabolic bone disease of prematurity – definition and incidence
Metabolic bone disease of prematurity is defined as decreased bone mineralization
(as assessed by absorptiometric techniques) ranging from osteopenia to overt rickets
(frontal bossing, cranialubes, swelling of the costochondral junctions and cupping
and fraying of the wrist joints) and fractures of the ribs and long bones. It’s incidence
varies from 55% in infants with birth weight less than 1000 g (McIntosh et al. 1986)
to 23% in infants with birth weight less than 1500 g (Koo et al. 1989). Lucas et al.
(1989) found that 66% of human-milk fed infants under 1200 g at birth had high
alkaline phosphatase levels, but fewer than 2 % of these infants had overt clinical manifestations. The clinical onset is usually between the sixth and twelfth postnatal week.

2.5.2 Diagnosis of metabolic bone disease

Clinical diagnosis of metabolic bone disease in premature infants has been based on varying criteria including physical signs, radiological observations and biochemical markers. Recently, several non-invasive methods, especially bone densitometry, have been developed to assess bone mineral density and content in infants and children.

Physical signs

The physical signs frontal bossing, craniotubes, swelling of the costochondral junctions, cupping and fraying of the wrist joints and fractures of the ribs and long bones usually appear between the sixth and twelfth postnatal week (Lyon et al. 1987). The physical signs appear at the established stages of the disease, which limits their use for early diagnosis.

Plain radiography

The radiological characteristics of metabolic bone disease in preterm infants are dependent on the severity and duration of the impaired bone mineralization. As radiological examinations can detect decreased bone mineralization only after 30-40 % loss of minerals has occurred (Ardan 1951, Lachman et al. 1985), radiographs are not useful for early diagnosis. Koo and co-workers (1982) have, however, proposed a simple grading of radiological changes observed on single view radiographs of wrists and ankles taken 5 to 10 weeks postnataally. Plain x-ray of the wrist is useful in the diagnosis of rickets (Lyon et al. 1987).
Biochemical markers

Serum calcium and phosphate concentrations, and activities of alkaline phosphatase (Backström et al. 2000) and serum osteocalcin have been used for screening of metabolic bone disease in preterm infants. Serum calcium is typically normal but may be elevated when there is phosphate depletion (Lyon et al. 1984). BMC was not associated with serum phosphate values measured at term (Faerk et al. 2002). Hypophosphatemia is common at 3 months of post-term age in preterm infants (Backström et al. 2000). Alkaline phosphatase, an important enzyme derived from the bones and liver and to a lesser extent from the intestines, placenta and kidneys, is a commonly used marker for bone formation. Preterm infants with radiological evidence of rickets have elevated activity of alkaline phosphatase (Lyon et al. 1987). However, poor correlation has been found between alkaline phosphatase and BMC measured by SPA (Pittard et al. 1992, Ryan et al. 1993) and DXA (Faerk et al. 2002). In another study, a combination of alkaline phosphatase along with serum phosphate levels has improved the detection of low BMD in preterm infants (Backström et al. 2000). Osteocalcin is a vitamin K-dependent non-collagenous bone protein synthesized by osteoblasts. A significant positive correlation has been reported between serum osteocalcin levels and alkaline phosphatase activity, but both values were poor indicators of the radiological status (Petitfor et al. 1986). As yet no ideal biochemical marker has been found for diagnosis of metabolic bone disease in preterm infants.

2.5.3 Bone mineral accretion after preterm birth

In preterm neonates a large skeletal mineral deficit builds up between birth and 40 weeks postconception (Horsman et al. 1989, Rigo et al. 1998, Tsukahara et al. 1993). Catch-up in bone mineralization occurs during the first 20 weeks, though most of it is in the first 12 weeks post term. and is completed by 25-50 weeks post term
(Horsman et al. 1989, Congdon et al. 1990, Pittard et al. 1990, Lapillonne et al. 1994), and is possibly influenced by increased mineral intake (Bishop et al. 1993).

2.5.4 Effects of calcium and phosphorus intake

Although, mother’s milk is the gold standard for feeding of term infants at least for the first six months of postnatal life (Lawrence et al. 1991), its role in the feeding of preterm infants is controversial. Human milk contains inadequate amounts of calcium and phosphorus for such infants, and therefore does not meet the mineral requirements for intrauterine mineral accretion rate. Evidence supporting the inadequacy of mineral intake from human milk includes elevated serum alkaline phosphatase activity, elevated 1,25(OH)2D and low BMC (Steichen et al. 1980, Steichen et al. 1981, Greer et al. 1981, Koo et al. 1989). Mineral supplementation of human milk used for feeding of preterm infants is recommended during the initial hospital stay, but the current recommendations vary in Europe and in the United States; the European Society for Pediatric Gastroenterology and Nutrition (ESPGAN 1987) recommends calcium 70-140 mg/100 kcal and phosphate 50-90 mg/100 kcal whereas the American Academy of Pediatrics (AAP 1985) recommends calcium 140-160 mg/100 kcal and phosphate 95-108 mg/100 kcal.

Duration of calcium and phosphorus supplementation is also under debate. The mineral supplementation should at least be continued till term, or until the infant has reached 3.5 kg as recommended by Koo and Tsang (1991). Other studies recommend even longer duration of supplementation. Increasing the mineral supplementation and extending it into the post hospitalization period up to the first year of life has improved short term bone mineral accretion and growth in preterm infants (Bishop et al. 1993, Chan 1993, Cooke et al. 1999, Craver et al. 2001). After discharge from hospital, preterm infants fed unsupplemented breast milk, which is a low mineral, low phosphate diet, gained less weight and had lower BMC when
compared with formula-fed infants (Chan 1993, Wauben et al. 1998, Lucas et al. 2001). The impairment in skeletal mineralization in preterm infants while on unsupplemented breast milk feeds persists through the first postnatal year (Abrams et al. 1980, Schanler et al. 1992) and tends to disappear by the second year (Schanler et al. 1992). More data are needed on the amount and duration of the mineral supplementation of very low birth weight (VLBW) infants after initial hospital discharge.

2.5.5 Vitamin D Intake

Vitamin D intake is important for all infants during the first few years of life. The current recommendations for term infants is 10 ug of vitamin D (AAP 1985). Both breast-fed and formula-fed infants need vitamin D supplementation. The vitamin D content is 0.01 ug/100 ml of breast milk (Fewtrell et al. 1999). Since infant formulae are currently fortified with vitamin D, formula-fed infants need smaller doses of vitamin D. For preterm infants, the European Society for Pediatric Gastroenterology and Nutrition recommends 20-40 ug/day (ESPGAN 1987), whereas the American Academy of Pediatrics recommends 10 ug/day (AAP 1985). Ten micro grams of vitamin D has been shown to be adequate for maintaining the serum levels of 25-hydroxyvitamin D, calcidiol [25OHD] and 1,25(OH)₂D in preterm infants (Cooke et al. 1990, Koo et al. 1995, Backström et al. 1999). In Finland, the recommended dose of vitamin D is 10 ug/day until two years of age (Ministry of Social Affairs and Health 1994).

2.5.6 Effect of steroids

Dexamethasone treatment in preterm infants with chronic lung disease improves pulmonary compliance and facilitates weaning from the ventilator (Avery et al. 1985, Sinkin et al. 2000, Halliday et al. 2001) Unfortunately, corticosteroids are known to
cause growth impairment and a delay in bone formation and skeletal maturation (Chesney et al. 1978, Rickers et al. 1982). One study shows that preterm infants treated with dexamethasone for a considerably long mean period of 37 days had lower radial bone mineral accretion than controls at six months post term age (Weiler et al. 1997), while other studies (Ryan et al. 1987, Greer et al. 1987) fail to show differences in forearm BMC values between dexamethasone-treated preterm infants with bronchopulmonary dysplasia and controls. Dexamethasone has been associated with a significant fall in growth velocity without changes in the BMC of the radius measured by DXA (Shrivastava et al. 2000).

The mechanism of steroid action on bone is complex. Glucocorticoids are known to induce disturbances in mineral and vitamin D metabolism (Chesney et al. 1978, Kimberg et al. 1971). In vivo, they stimulate bone resorption by decreasing intestinal calcium absorption (Kimberg et al. 1971) and increase the calcium and phosphate excretion, possibly mediated through a decrease in production of 1,25(OH)₂D (Avery et al. 1985). They also have a direct effect on bone metabolism, decreasing the replication of bone cells, which leads to both reduced bone formation and bone resorption (Canalis 1996, Canalis et al. 2002).

The dexamethasone effect may also be related to overall caloric intake. A recent study (Bolt et al. 2002) shows that those preterm infants who were treated with dexamethasone for chronic lung disease and who received extra caloric intake until term had similar body composition at 3 months of age as preterm infants without chronic lung disease.

2.5.7 Effect of gender

Preterm male but not female infants had better growth and higher BMC when fed a nutrient-enriched post-discharge formula between nine and eighteen months of post-term age (Cooke et al. 1999, Cooke et al. 2001, Carver et al. 2001, Lucas et al.
2001). This has been attributed to gender-related differences in nutritional needs and nature of growth.

2.5.8 Effect of physical activity

2.6 Long term effect of prematurity on bone mineral status
BMC of VLBW infants reaches 80 % of that of full term infants by one year of age (Salle et al. Abstract 1992), whereas other studies have shown that bone mineral status of the preterm infants is normal after the first year of life (Rubinacci et al. 1993) or by the age of 2 years (Ichiba et al. 2002). Other cross-sectional studies report catch up by three years of age (Fewtrell et al. 1999, Hori et al. 1995). The effect of mineral supplementation on BMC is no longer evident at the age of 2 or 5 years (Schlaner et al. 1992, Bishop et al. 1996).

However, contrasting data have been reported. Amstrong and colleagues (Abstract 1997) reported lower BMD in former preterm VLBW infants at the age of 7 years when compared to term infants of similar age. Lower BMC at the lumbar spine when compared to controls has been reported in prematurely born children at the age of 8 years, but this did not remain so when adjusted for height and weight (Bowden et al. 1999). There are also long-term effects of gender. Lower BMC in comparison with
age matched controls has been found in preterm boys but not in girls at the age of 4-16 years (Helin et al. 1985). In contrast, girls aged 7-9 years born preterm had low femoral neck BMD when compared with age-matched term controls (Zamora et al. 2001). In both the reports (Bowdon et al. 1990, Helin et al. 1985), the children with lower BMC values, were shorter and lighter than the controls. Weiler et al. (2002) reported that young adults around 17 years of age born with VLBW were shorter and had lower whole body BMC than those born at term.

Although breast milk feeding is associated with low BMC during the first two years of life (Abrams et al. 1989, Schanler et al. 1992), data on long-term effects are limited. One study reported that increased human milk intake early in life was associated with greater BMC later in life. The greater the proportion of mother’s milk consumed, the higher was the BMC at the age of 5 years in children born prematurely (Bishop et al. 1996). This effect was no longer seen at the age of 8-12 years in children born prematurely (Fewtrell et al. 1999).
3. AIMS OF THE STUDY

The main objective of this study was to investigate the factors affecting lumbar bone mineral status in prematurely-born infants and term-born reference infants during the first year of life by using a densitometric technique. We also evaluated the long term effect of prematurity on the bone mineral status of children aged 6-7 years born prematurely.

The specific aims of the study

1. To assess the association between intrauterine growth status, prematurity, neonatal morbidity and the lumbar BMC and BMD of prematurely-born infants during early infancy (Study I).

2. To measure the lumbar bone mineral status of term-born infants during early infancy in order to get reference values for lumbar BMC and BMD (Study II).

3. To assess the effect of growth and nutrition on lumbar BMC of very prematurely-born infants during early infancy (Study III).

4. To evaluate the long term effect of prematurity and early growth on lumbar BMC and BMD of prematurely-born children at the age of 6-7 years (Study IV).
4. PATIENTS AND METHODS

4.1 Patients

Three different groups were studied; 1) prematurely-born infants during the first year of life, 2) term born reference infants during the first year of life and 3) prematurely-born children at the age of 6-7 years. Neonatal and growth data of the study infants are presented in Tables 2 and 3 respectively.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Study I (n=58)</th>
<th>Study II (n=41)</th>
<th>Study III (n=64)</th>
<th>Study IV (n=33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boys/girls</td>
<td>26/32</td>
<td>19/22</td>
<td>29/35</td>
<td>17/21</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>31(3)</td>
<td>&lt;0(1)</td>
<td>29(2)</td>
<td>31(3)</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>1.5(0.7)</td>
<td>3.7(0.8)</td>
<td>1.1(0.3)</td>
<td>1.7(0.6)</td>
</tr>
<tr>
<td>Birth length (cm)</td>
<td>40.0(4.5)</td>
<td>&lt;9.4(3.1)</td>
<td>36.8(3.4)</td>
<td>41.5(4.3)</td>
</tr>
<tr>
<td>Dexamethasone treated (n)</td>
<td>18</td>
<td>NA</td>
<td>33</td>
<td>-</td>
</tr>
<tr>
<td>Duration of dexamethasone (days)</td>
<td>13(6)</td>
<td>NA</td>
<td>12(4)</td>
<td>-</td>
</tr>
<tr>
<td>Respirator treated (n)</td>
<td>39</td>
<td>NA</td>
<td>58</td>
<td>22</td>
</tr>
<tr>
<td>Respirator (days)</td>
<td>14(10)</td>
<td>NA</td>
<td>12(14)</td>
<td>15(13)</td>
</tr>
<tr>
<td>Extra oxygen (n)</td>
<td>38</td>
<td>NA</td>
<td>58</td>
<td>31</td>
</tr>
<tr>
<td>Extra oxygen (days)</td>
<td>21(17)</td>
<td>NA</td>
<td>30(19)</td>
<td>21(23)</td>
</tr>
</tbody>
</table>

Abbreviation: SD = standard deviation, NA = not applicable
**Table 3.** Standardized growth of the study infants. Data presented as mean (SD).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Study I (n=58)</th>
<th>Study II (n=41)</th>
<th>Study III (n=64)</th>
<th>Study IV (n=38)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>At birth</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>1.5(0.7)</td>
<td>3.7(0.8)</td>
<td>1.1(0.3)</td>
<td>1.7(0.6)</td>
</tr>
<tr>
<td>Weight SD score</td>
<td>-1.0(1.5)</td>
<td>-0.5(1.4)</td>
<td>-0.9(1.4)</td>
<td>-</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>40.5(4.5)</td>
<td>49.4(3.1)</td>
<td>36.8 (3.4)</td>
<td>41.5(4.3)</td>
</tr>
<tr>
<td>Length SD score</td>
<td>-0.8(1.8)</td>
<td>-0.5(1.7)</td>
<td>-0.9(2.0)</td>
<td>-</td>
</tr>
<tr>
<td><strong>At BMC examination</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>5.2(0.3)</td>
<td>6.8(1.2)</td>
<td>5.5(0.7)</td>
<td>23.6(4.7)</td>
</tr>
<tr>
<td>Percent deviation from mean weight for length</td>
<td>1.1(13.5)</td>
<td>3.2(12.4)</td>
<td>-4.2(13)</td>
<td>3.8(13.5)</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>56.2(2.3)</td>
<td>63.0(5.0)</td>
<td>58.5(4.4)</td>
<td>121.1(6.8)</td>
</tr>
<tr>
<td>Length SD score</td>
<td>-0.8(1.8)</td>
<td>-0.7(1.7)</td>
<td>-1.2(1.7)</td>
<td>-0.1(1.0)</td>
</tr>
<tr>
<td>Post-term age (yr)</td>
<td>0.3(0.2)</td>
<td>NA</td>
<td>0.3(0.1)</td>
<td>NA</td>
</tr>
<tr>
<td>Chronological age (yr)</td>
<td>NA</td>
<td>0.4(0.2)</td>
<td>NA</td>
<td>3.8(0.7)</td>
</tr>
</tbody>
</table>

Abbreviation: SD = standard deviation, NA = not applicable
Prematurely-born infants (Study I and III)

In all, 117 prematurely-born infants with GA ranging from 22 to 36 weeks born at Kuopio University Hospital, Kuopio, Finland, between April 1995 to December 1998, were recruited during the first week of life from the Neonatal Intensive Care Unit, Kuopio University Hospital, Kuopio, Finland. The GA was confirmed by antenatal ultrasound before 20 weeks of gestation. Nineteen infants were excluded due to missing biochemical data or transfer to other hospitals. Hence, this group finally consisted of 98 prematurely-born infants. The patients for Study I were 58 prematurely-born infants, 43 AGA and 15 SGA infants with GA less than 37 weeks. The patients for Study III were 64 infants with GA less than 32 weeks. Twenty-four infants with GA less than 32 weeks were included both in Study I and Study III.

Term-born reference infants (Study II)

Forty-one healthy infants born between February 1996 and November 1998 at Kuopio University Hospital, Kuopio, Finland, were recruited from the well baby clinic at the city of Kuopio, Finland, formed the reference group (Study II). The inclusion criteria were GA ranging from 37 to 42 weeks, absence of chronic diseases and milk or food allergies. These infants formed the reference group for the prematurely-born group (Study III). Seventeen term-born infants formed the reference group in Study I.

Six to seven year old children born prematurely (Study IV)

Thirty-eight children who had been prematurely-born at the Kuopio University Hospital, Kuopio, Finland, between 1987 and 1992 were invited to the study when they were 6-7 years old. The criteria for inclusion were GA less than 37 weeks, no history of milk or food allergies and no use of steroids. These children lived in the district of Kuopio. Previously published data was available for age-and sex-specific
lumbar BMC and BMD values measured by DXA using the same instrument and software, hence we did not recruit any controls.

4.2 Study design

Prematurely-born infants

The anthropometric and biochemical parameters of the study infants were followed up from birth until discharge in the framework of a prospective design. Weight and length were measured at birth, at discharge and on the day of the BMC measurement. Serum calcium, phospho, alkaline phosphatase, albumin, prealbumin and protein were measured at specified time points: 2 and 6 weeks and before discharge. Serum 1,25(OH)_{2}D, 25OHD and serum osteocalcin were measured before discharge. Morbidity data were gathered from the hospital records of the infants at the end of the treatment period in data collection sheets. Lumbar spine BMC and BMD were measured when the weight of the infants ranged between 5 to 8 kg.

Term-born reference infants

Lumbar spine BMC and BMD were measured when the weight of the infants ranged between 5 to 8 kg. Weight and length were measured on the same day. No biochemical data were collected from these infants for ethical reasons. A questionnaire on the food and vitamin D intake of the infant based on 24-hour recall was completed by the accompanying parent(s). Data on gestational age and birth measures of the infants were collected from the records of the mothers.

Six to seven year old children born prematurely

The lumbar spine BMC and BMD were measured in children at the age of 6-7 years. Weight and height were measured on the same day. A questionnaire about the
duration of breast-feeding, vitamin D intake and heights of the parents was completed by the accompanying parent(s). Data on neonatal morbidity were collected from the records of the children. Yearly data on weight and height were collected from the children’s health cards.

4.3 Methods

4.3.1 Anthropometric measurements

The infants’ weights (Study I, II and III) were measured using a digital scale (accuracy 5 g, Seca model 727, Hamburg, Germany) and the crown-to-heel lengths to the nearest 0.1 cm measured horizontally, using an infant board (Pedihealth OY, Oulu, Finland). The children’s weights (Study IV) were measured with minimal clothing on with an electronic scale and height was measured using a wall-mounted stadiometer.

4.3.2 Biochemical measurements

Serum levels of calcium (Study III), phosphate, alkaline phosphatase, albumin, protein and prealbumin were measured by standard methods using Konelab 60i Clinical Chemistry Analyser (Thermo Clinical Labsystems, Helsinki, Finland). The intra- and interassay coefficients of variation, for serum calcium were less than 0.8 % and 4.1 %, for serum phosphate were less than 1.2 % and 1.6 %, for alkaline phosphatase were less than 2.7 % and 8.8 %, for serum albumin were less than 2.2 % and 2.3 %, for serum protein were less than 4.8 % and 2.5 % and for serum prealbumin were less than 1.6 % and 3.6 %, respectively. Serum concentrations of 1,25 (OH)_{2} D were estimated using the IDS gamma-B 1,25 D kit (Diasorin Corp., Minnesota, USA) and concentrations of 25(OH)D using a radio-immunoassay kit (Diasorin Corp., Minnesota, USA). The intra- and interassay coefficients of variation, for serum 1,25(OH)_{2}D were less than 13.4 % and 18.3 %, and for serum 25(OH)D
were less than 16% and 19.2%, respectively. Serum concentrations of osteocalcin were measured using a radio-immunoassay kit (Diasorin Corp., Minnesota, USA). The intra- and interassay coefficients of variation, for serum osteocalcin were less than 4.2% and 6.2%, respectively.

4.3.3 Dual energy x-ray absorptiometry measurements

All the bone scans (Studies I, II, III, IV) were carried out at the Department of Clinical Physiology and Nuclear Medicine, Kuopio University Hospital, Kuopio, Finland. BMC (g) and BMD (g/cm²) of the lumbar spine were measured by DXA using the Lunar DPX densitometer (Lunar Radiation Corporation, Madison, WI, USA). In this study all BMD values refer to areal BMD values. All measurements were carried out by trained personnel. The lumbar vertebrae L2-L4 of the spine were the site for measurement, since the trabecular bone is more sensitive to mineral changes than the cortical bone. The Pediatric AP spine mode (Version 3.8E) scan was used, the procedure lasted for 3-5 minutes, and the effective dose for radiation exposure was about 0.01 mSv.

Scans on the lumbar vertebrae L2 to L4 were performed by a trained nurse (Eila Koski, Registered Nurse), with the child in a supine position and without sedation. The child was directly observed at all times by the person performing the scan. The scanning procedure was interrupted if any movement artifact was noted and the scan was repeated after the child was pacified.

The scan was typically analyzed using automatic localization of the bone edges. However, if the automatic identification of the bone edges was visually incorrect the edges were adjusted manually. Finally, the recalculate edges function of the software was still used for the fine control of the bone edges. Analyses were carried out using the Lunar DPX software version 3.8E. Quality assurance test using a standard spine phantom were conducted daily according to the manufacturers’ guidelines. The short-
term precision of the scanner (coefficient of variation on repeated scan analysis) using the spine phantom was 0.9%. For ethical reasons, we did not perform repeat scans on the same child after repositioning. To estimate the precision of the analysis, scans of 10 infants were re-analyzed using the same protocol. The coefficient of variation on repeated scan analysis (Gluer et al. 1995) was 0.05% for the BMC values.

4.4 Nutrition

Prematurely-born infants

The preterm infants were fed according to the currently recommended guidelines (ESPGAN 1987), but no milk intake data were collected during the hospital stay. As the nutritional requirements of preterm infants vary according to the degree of maturity, infants with gestational age ≤ 33 weeks and those > 33 weeks had different feeding regimens. Enteral nutrition was started gradually as tolerated with banked human milk or the child’s own mother’s milk. When infants with gestational age ≤ 33 weeks had reached a minimum milk intake of 100 ml/kg/day, milk fortification with a special formula containing hydrolyzed whey protein was started. The full amount of fortified human milk (140 to 160 ml/kg/day) provided protein 2.8 to 3.2 g/kg/day and energy 140-150 kcal/kg/day. Then, a multivitamin preparation (vitamin A 1200 IU/day, vitamin B_{12} 0.8 ug/day, and vitamin E 8 IU/day), vitamin C (2 mg/day), vitamin D (10 ug/day), and iron (4-6 mg/kg/day) were gradually added to the diet. On the basis of the range of calcium and phosphorus concentrations (178-333 mg/l, 99-150 mg/l, respectively), in the banked milk pools during the time of the study, we calculated that non-fortified milk provided calcium 27 to 50 mg/100 kcal and phosphorus 15 to 22 mg/100 kcal; the corresponding intakes from the fortified milk were 48 to 71 mg/100 kcal and 25 to 32 mg/100 kcal. In order to meet the ESPGAN recommendations for daily mineral intake (ESPGAN 1987), additional calcium and
phosphorus were provided as calcium carbonate, 45-50 mg/100 kcal, and as sodium diphosphate, 40-45 mg/100 kcal, divided into three doses per day. The total daily intakes of calcium and phosphorus were 93 to 121 mg/100 kcal and 65 to 77 mg/100 kcal respectively. Fortified milk, mineral supplements, multivitamin preparation and vitamin C were continued at least until the weight was 2.5 kg; thereafter only vitamin D and iron were continued. The infants with gestational age > 33 weeks received non-fortified banked human milk or their own mother’s milk 160-180 ml/kg/day, vitamin D 10 μg/day and iron, if needed, but no other vitamin or mineral supplements.

At home, solids were introduced into their diets as recommended between three to six months of age (ESPGAN 1981, Ministry of Social Affairs and Health 1994). In Finland, vitamin D administration is recommended at a dose of 10 μg daily until two years of age (Ministry of Social Affairs and Health 1994). All the study infants were receiving vitamin D by the time of the BMC examination.

**Term-born reference infants**

All the infants started to breast-feed from birth and 75 % (13 boys and 17 girls) were still exclusively or partially breast-fed at the time of the BMC examination. None of the reference infants were exclusively formula fed. The reference group’s feeding habits demonstrate the prevailing breast-feeding patterns in Finland, where practically all infants begin to breast-feed at birth and 38 % of them are still at least partially breast-fed at the age of 6-8 months (Sairanen et al. 1997). Introduction of solids and administration of vitamin D in the reference group was similar to that in the preterm group.

**4.5 Statistical methods**

Associations between variables were analyzed using Pearson’s correlation coefficients when the variables were normally distributed (Study IV), partial
correlations (Study I) and Spearman’s rank correlation coefficients were used when the variables were apparently skewed (Studies II and III) tests. The nonparametric Mann-Whitney-U test and chi-square test were used to compare the differences between groups (Studies I, II and III). The within group differences were examined by repeated measures analysis of variance (Study III). One sample t-test was used to compare the distribution of the growth data with those of Finnish age- and sex-specific values (Sorva et al. 1984) and BMD of the children aged 6-7 years with that of reference data (Kröger et al. 1992) (Study IV). The parent-specific mean height standard deviation score was calculated based on parental heights of prematurely born children aged 6-7 years (Sorva et al. 1989) (Study IV).

To describe intrauterine growth status adjusted for gestational age and gender, standard deviation (SD) scores of birth weight and length were determined using the means and SD values from an ethnic reference population of 75,061 neonates (Pihkala et al. 1989) (Studies I, II and III). At birth, the infants were classified as SGA based on birth weight SD scores. The normal range for birth weight SD scores was -2 to +2 SD, and the infants with birth weight SD scores below -2 SD formed the SGA group. The SD scores of weight and length at the end of the initial hospitalization were calculated to examine differences between growth status at discharge and normal intrauterine growth of the reference fetus at the same postconceptional age. The post-term age of each infant was calculated using the expected date of delivery [post-term age = (date of the DXA measurement) - (expected date of delivery)] (Studies I and III); then the growth data obtained at the time of the BMC examination was standardized using the gender-specific ethnic growth charts to transform the weights to the percentage deviations from the mean weight for length and the length to the SD scores (Sorva et al. 1984) (Studies I, II, III and IV).

In Study III, the predicted BMC values for the preterm infants were calculated based on the reference infants’ lumbar BMC values and the concurrent weights
(Study II) using the following regression equation: predicted BMC values = [-0.174] + (0.333) \times \text{(weight of the infant)}. The BMC values of preterm infants were expressed as percentages of the predicted values of the healthy term infants of the same weight (Study II).

Regression analyses were used to determine the important factors explaining the variance of the lumbar BMC values (Studies I, II and IV). Continuous variables were converted into natural logarithms to linearize their relation with the BMC, the logarithmic transformation of BMC was used as the dependent variable (Studies I and IV). Logistic regression analysis was used for estimating the associations between various variables having an independent effect on the risk of having low BMC values later in infancy (Study III).

For Study I statistical analyses were performed using SPSS for Windows (SPSS Inc., Chicago, Illinois, USA) version 6.0, for Studies II and III SPSS for Windows version 9.0.1 and for Study IV SPSS for Windows version 6.1.3 were used. All statistical tests were two-tailed and a p-value less than 0.05 was considered as significant.

4.6 Ethics

The informed parental consent was obtained for all the study subjects and the study was approved by the ethical committee of Kuopio University Hospital.
5. RESULTS

This section reviews the main findings of this study. Further details are presented in the original publications (I - IV).

The effect of intrauterine growth status, prematurity and neonatal morbidity on the lumbar BMC and BMD of prematurely-born infants during early infancy (Study I)

The prematurely-born infants had significantly lower lumbar BMC and BMD values than the term-born reference infants (n=17). BMC correlated with gestational age, bone area, duration of respirator treatment and duration of dexamethasone treatment, but not with gestational- age- specific birth weight standard deviation scores or the duration of breast-feeding. There were no significant differences in lumbar BMC and BMD between preterm SGA (n=15) and AGA (n=43) groups. When dexamethasone treated infants were excluded, lumbar BMC and BMD did not differ between the prematurely-born SGA and AGA infants. Bone area, the duration of dexamethasone treatment and weight at examination were the best variables, explaining 54 % of the variance of the BMC values.

The lumbar BMC and BMD of term-born infants during early infancy, in order to get reference values (Study II)

Lumbar BMC correlated with the present weight, length, standardized length, age and with the bone area in term infants. Exclusive breast-feeding did not correlate with the lumbar BMC values. No differences were found in lumbar spine BMC, BMD, bone area and anthropometric data between boys and girls. Present weight and bone area were the major determinants of the lumbar spine BMC and explained 68 % of the variance of the lumbar BMC values.
The effect of growth and nutrition on lumbar BMC of very prematurely-born infants during early infancy (Study III)

Growth

The extremely prematurely born group (gestational age ≤ 28 weeks) had better intrauterine growth, shown by standardized growth measures, than the more mature infants. During the initial hospital stay, standardized weights and lengths of the extremely prematurely-born infants declined more steeply than those of the more mature infants. Still by the time of discharge, 63% of the extremely prematurely-born infants and 44% of the more mature infants were growth retarded. After discharge, significant catch-up growth of length and weight occurred in both gestational age groups. When the lumbar BMC was examined, the extremely prematurely-born infants had significantly smaller standardized lengths than the more mature infants although there were no differences in the post-term age. At the time of the BMC examination, the infants in both gestational age groups had appropriate weight for length.

Growth of breast-fed subgroup

After discharge from hospital to the time of the BMC examination, 13 (20%) prematurely-born infants were exclusively and successfully breast-fed. At the time of the BMC examination, the standardized lengths of the breast-fed infants [mean (SD) -0.8 (1.0) SD] were not different from those of formula-fed infants [-1.4 (1.8) SD]. The breast fed subgroup had relatively larger weight for length [6 (10) %] than the formula fed infants [-7 (13) %].

Biochemical values

The values of serum phosphate increased from week two to week six, no between-
group differences were found. Serum alkaline phosphatase activity also increased during the same time, but the extremely prematurely-born infants had higher values at 6 weeks and at the time of discharge than the more mature infants. No differences in serum calcium values were seen between the gestational age groups. Serum protein and prealbumin values decreased from week two to the time of discharge, but no between-group differences were observed.

Vitamin D status and subsequent bone mineral status

At discharge, the values of 25OHD (storage form) were within the reference range of our laboratory. Thirty-seven percent of the values of 1,25(OH)2D (active form) were above, the rest within the reference range. Serum 1,25(OH)2D (r=0.321; p=0.015) but not 25OHD (r = -0.107; p=0.431) correlated with subsequently measured BMC. Serum 1,25(OH)2D values correlated with serum osteocalcin (r=0.448; p=0.001). No differences between the gestational age groups were found either in vitamin D values or in serum osteocalcin values.

Factors associated with the risk of having BMC below the predicted value

Of the independent variables entered into the logistic regression, the following ones were associated with the risk (95 % CI) of having BMC < the predicted value: (1) low serum phosphate value at 6 weeks, with a 7.8 - fold risk; (2) exclusive breast-feeding after discharge from hospital, with a 7.0 - fold risk; (3) male gender, with a 4.3 - fold risk.

The long term effect of prematurity and early growth on lumbar BMC and BMD of prematurely-born children at the age of 6-7 years (Study IV)

The 6-7 year old children born prematurely had similar BMD values when compared to those of the Finnish age- and sex- specific reference values. There was no
correlation between duration of breast-feeding after discharge from hospital and the lumbar BMC values. At the time of the BMC examination, all children were within the normal range for standardized height and weight percentiles. The parental specific mean height standard deviation scores were within the normal limits. Bone area, current weight, gestational age and weight at 1 year were the significant variables explaining the variance of the lumbar BMC values. After adjusting for the other independent variables the prematurely born children who were thinner at 1 year of age subsequently had higher BMC values at the age of 6-7 years.
6. DISCUSSION

Subjects

For this study, 117 prematurely-born infants were recruited from the Neonatal Intensive Care Unit during the years 1995 to 1998. Sixteen percent (n=19) of these infants were excluded due to transfer to other hospitals or missing biochemical data. The GA and the respiratory morbidity of the excluded infants did not differ from those of the study infants. There were 64 infants born less than 32 weeks of GA, representing a majority (91%) of the survivors of this birth cohort. During the study time period the use of postnatal steroids was common: 52% of infants with GA less than 32 weeks required steroid treatment for chronic lung disease. This should be taken into consideration while interpreting results, since steroids may affect bone mineral status (Greer et al. 1987, Ryan et al. 1987, Weiler et al. 1997, Ward et al. 1999). Reference lumbar BMC and BMD values were obtained from forty-one term-born infants during the first year of life. The feeding habits of this group were representative of the common feeding habits of Finnish infants (Sairanen et al. 1997).

We also measured the lumbar BMC and BMD values in 38 prematurely-born children aged 6-7 years. When these children stayed in the Neonatal Intensive Care Unit between 1987-1992, early steroid treatment for chronic lung disease was unusual. The lumbar BMC and BMD values of these children were compared with reference data of healthy Finnish children (Kröger et al. 1992).

Methods

Dual energy absorptiometry measurements

In the present study, the BMC and BMD values of the lumbar spine L2-L4 were measured by the standard clinical method, DXA and a dedicated pediatric software
was used for the analysis of the lumbar spine measurements. The BMC and BMD values of the prematurely-born infants were compared with those of term-born reference infants of the same weight range using the same densitometer and software. The lumbar BMC and BMD values of the children born prematurely aged 6-7 years were compared to those of healthy Finnish children (Kröger et al 1992) measured using the same DXA instrument as used by us in this study (Lunar DPX). All the measurements were performed by a single person to minimize the source of variability. Quality assurance tests using the standard spine phantom were conducted daily. The precision (coefficient of variation on repeated scan analysis) using the spine phantom was 0.9 %.

We decided to use the lumbar spine scans as the area of interest for the following reasons: (1) it is fast, (2) the radiation dose is low, (3) the infants do not need to be sedated and (4) the risks of movement artifacts are reduced when compared to whole body scans which have a longer scanning time (Zia-Ullah et al. 2002). In addition, the lumbar spine consists mainly of cancellous bone, which is quick in reflecting the metabolic changes and the effects of drugs and nutrition (Ichiba et al. 2001).

In infancy it is important to interpret BMC and BMD values measured by DXA in relation to weight since BMC correlates best with weight rather than with length or gestational age or with age at examination (Salle et al. 1992, Lapillonne et al. 1994). Body weight is the main predictor of BMC values in infants (Rigo et al. 1998). Hence we decided to use body weight but not age as a basis for the measurement of lumbar spine BMC and BMD.
Results

The effect of intrauterine growth status, prematurity and neonatal morbidity on the lumbar BMC and BMD of prematurely-born infants during early infancy

The studies reporting the effect of intrauterine growth retardation on bone mineral status are controversial. We found no differences in lumbar BMC and BMD values between prematurely-born SGA and AGA groups during the first year of life. At birth, Minton et al. (1983) reported similar BMC in preterm SGA and AGA infants while in another study (Lapillonne et al. 1997), decreased BMC was observed in SGA infants when compared with AGA infants of similar gestational age. At term-adjusted age, preterm SGA infants had lower BMCs than the preterm AGA infants (Atkinson et al. 2000). Although we could not confirm intrauterine growth status-related differences in lumbar BMC or BMD values, we did, however, document that prematurely-born infants had lower BMC and BMD values than the term-born reference infants during the first year of life. These results are in agreement with findings of previous studies (Tsukahara et al. 1993, Avila-Diaz et al. 2001).

According to our data, preterm infants receiving dexamethasone treatment for chronic lung disease were particularly at risk of decreased bone mineralisation. Earlier, dexamethasone was commonly used for the treatment of chronic lung disease of prematurity, but nowadays there are serious concerns about possible deleterious effects not only on growth and bone (Greer et al. 1987, Ryan et al. 1987, Weiler et al. 1997, Ward et al. 1999, Shrivastava et al. 2000), but, even more importantly, on neurological outcome (Grier et al. 2003). Corticosteroids inhibit bone growth mainly by decreasing bone formation (Ng et al. 2002). These steroids inhibit bone collagen synthesis (Advani et al. 1997), which results in interruption of osteoblastic function and decreases cell replication (Gronowicz et al. 1991). They also reduce the calcification of newly synthesized matrix (Gronowicz et al. 1991). The steroid effects on growth were also seen in our study. The dexamethasone treated
infants had less weight and height gain per week and lower weight at examination than non-dexamethasone treated prematures. These results imply that low BMC values in dexamethasone treated infants may be related to altered growth patterns of the infants. Infants treated with dexamethasone had more respiratory morbidity than the non-treated infants. This may have compromised their nutrition and by these means led to insufficient mineral intake and decreased bone mineral status. Unfortunately, we did not have a gestational age matched group of infants without chronic lung disease.

There are plenty of reference data on BMC values during infancy presented in relation to age or body weight but these data are difficult to interpret as they have been obtained using different absorptiometric instruments but also due to different skeletal sites and bone variables (BMC, BMD, BMAD). It has been previously reported that in term infants BMC correlates better with weight than with length, GA or age (Salle et al. 1992, Lapillonne et al. 1994). Body weight but not age at examination accounts for the variance of the BMC values in healthy infants during early infancy (Salle et al. 1992, Koo et al. 1998). We decided to measure lumbar BMC and BMD when the prematurely-born infants and the reference infants had attained similar body weight range of 5 to 8 kg. Our results showed that weight at examination was one of the factors explaining the variance of the BMC values in preterm infants, which is in agreement with earlier reports (Avila-Diaz et al. 2001, Koo et al. 2000).

**The effect of growth and nutrition on lumbar BMC of very prematurely-born infants during early infancy**

The main results from this study show that infants born less than 32 weeks of gestation developed postnatal growth retardation during initial hospital stay. Low serum phosphate value at 6 weeks. exclusive breast-feeding after discharge from
hospital and male gender were the factors associated with the risk of having BMC below the predicted value during the first year of life.

In our study, the prematurely-born infants developed growth retardation as exhibited by the declining standardized growth values from birth to the time of discharge from the hospital. By the time of discharge from the Neonatal Intensive Care Unit, 53% of the study group members had weights less than -2 SD. Similarly, growth retardation has been previously reported in VLBW infants during initial hospitalization (Gill et al. 1986, Berry et al. 1997, Ehrenkranz et al. 1999, Embleton et al. 2001, Steward et al. 2001). One study (Steward et al. 2001) reported that 89% of their study infants born with birth weight below 1000 grams were growth retarded by the time of discharge. The growth rate of our study infants was far below the intrauterine growth rate, which resulted in significant growth deficit by the time of discharge close to term age. Factors such as feeding difficulties, prematurity-associated diseases and their treatments may have accounted for this. After discharge from hospital, significant catch-up growth of length and weight occurred in both gestational age groups as evidenced by increases in their standardised values. Even at the time of the BMC examination, 31% of the infants in our study had standardized length below -2 SD and 16% had standardized weight below -20 percent deviation from the mean close to term age. This pattern of postnatal growth retardation should be taken into account while interpreting the bone mineral status.

Low phosphate intake after birth (indicated by hypophosphatemia at 6 weeks of age), and a low phosphate diet (exclusive breast-feeding) after discharge from hospital were independently related to low BMCs of prematurely-born infants during the first year of life in our study. Low phosphate intake early in life has been shown to be the main etiological factor for the development of metabolic bone disease of prematurity. Exclusive breast-feeding after discharge from hospital has been
previously shown to associate with low lumbar BMC values in prematurely-born infants during early infancy (Abrams et al. 1988, Chan et al. 1988, Schanler et al. 1988, Chan 1993, Wauben et al. 1998, Butte et al. 2002). The effect of post discharge breast feeding on growth are debatable. We observed that, post discharge exclusive breast-feeding supported linear catch-up growth and weight gain but not bone mineralization. Some earlier studies (Chan 1993, Chan et al. 1994, Wheeler et al. 1996, Butte et al. 2000) have reported that after initial hospital discharge breast-fed infants were lighter than formula-fed infants. Other studies (Abrams et al. 1988, Chan et al. 1985, Wauben et al. 1998) have reported that growth did not differ between breast-fed and formula-fed infants after discharge from hospital. We do not object to discharging very preterm infants on exclusive breast-feeding, but suggest that breast milk should be fortified using multi-component supplementation. The amount and duration of fortification need further study.

Male gender was also associated with an increased risk of having BMC values below the predicted value. We did not find any differences in respiratory morbidity between boys and girls. We speculate that there may be gender-related differences in nutritional needs, especially in mineral requirements, as previously suggested by Cooke et al. (1998), Cooke et al. (1999), Carver et al. (2001).

A daily vitamin D dose of 10 ug maintained an appropriate vitamin D status even in the extremely preterm infants in our study and in previous reports (Cooke et al. 1990, Backström et al. 1999). There were no differences between the gestational age groups for serum levels of 25OHD and 1,25(OH)₂D values. In fact, more than a third of 1,25(OH)₂D values were above the reference range. We speculate that the high 1,25(OH)₂D values may represent an adaptive process to assure absorption of calcium and phosphorus during bone mineralization. This compensatory mechanism may be needed to support bone mineralization during the subsequent period of rapid growth in infancy.
The long term effect of prematurity and early growth on BMC and BMD of prematurely-born children at the age of 6-7 years

The present study showed that at the age of 6-7 years the lumbar BMD values of the prematurely-born children did not differ from those of the reference population (Kröger et al. 1992). In contrast, lower BMC at the lumbar spine has been reported in prematurely-born children at the age of 8 years when compared with controls, but did not remain so when adjusted for height and weight (Bowden et al. 1999). In another study, girls aged 7-9 years born preterm had low femoral neck BMD when compared with age-matched term controls (Zamora et al. 2001). Lower BMC was found in preterm boys but not in girls at the age of 4-16 years in comparison with age-matched controls (Helin et al. 1985).

We did not find any correlation between the duration of breast-feeding and subsequent lumbar BMD in infancy. Similar results have been reported by Bowden et al. (1999) and Jones et al. (2000). In contrast, earlier studies have reported beneficial long-term effects of breast-feeding on the bone mineral status in prematurely born children (Bishop et al. 1996).

At the age of 6-7 years our prematurely-born children were within the normal range for the standardized height and as well for the weight percentiles. Earlier studies have reported prematurely-born children being shorter and lighter than their peers (Bowden et al. 1999, Helin et al. 1985). Early growth had an impact on the lumbar BMC in the prematurely-born children at the age of 6-7 years, as shown by our results; a low body weight at 1 year predicted high BMC at 6-7 years of age. In contrast, significant positive association was found between weight at 1 year and BMC at the age of 16-19 years in young adults who weighed less than 1500 grams at birth (Waaler et al. 2002) and in women aged 22-23 years (Cooper et al. 1995). This association remained significant even after adjusting for current weight (Cooper et al.)
1995). This indicates that growth patterns during the first year of life have long-term effects on bone mineral status.
7. SUMMARY AND CONCLUSIONS

Our study was undertaken to investigate the lumbar bone mineral status during the first year of life in prematurely-born infants and in term-born reference infants, and to evaluate the long term effect of prematurity on the bone mineral status at 6-7 years of age. The conclusions of this study are as follows:

1. Reduced lumbar BMC and BMD were observed in prematurely-born infants when compared with term-born reference infants of similar weight range during early infancy. Postnatal dexamethasone treatment was related to low lumbar BMC while intrauterine growth status at birth did not associate with lumbar BMC in prematurely-born infants during early infancy. The prematurely-born AGA infants and SGA infants had similar lumbar BMC and BMD.

2. Term-born boys and girls had similar values of lumbar BMC and BMD during early infancy. Infants who were short had low BMC, indicating that patterns of linear growth are related to lumbar BMC values during early infancy. Exclusive breast-feeding did not affect the bone mineral status.

3. Prematurely-born infants with GA less than 32 weeks developed growth retardation during the initial hospital stay despite following the current dietary recommendations. Vitamin D in a dose of 10 µg/daily was sufficient to maintain adequate vitamin D status. An important finding was that exclusive breast-feeding after discharge from hospital supported linear catch-up growth and weight gain but was associated with low lumbar BMC. Our current post-discharge nutritional scheme for preterm infants did not meet their mineral requirements after discharge from hospital. Male gender was another factor associated with low lumbar BMC.

4. The children aged 6-7 years who were prematurely-born had similar lumbar BMD values when compared with the Finnish reference values. The children were within the normal range for height and weight. The duration of exclusive breast-
feeding did not effect the lumbar bone mineral status. Children who were thin at one year of age later had higher BMC at the age of 6-7 years. This indicates that growth patterns during the first year of life have long-term effects on bone mineral status.

In conclusion, very premature infants (born less than 32 weeks of gestation) developed growth retardation during initial hospital stay while on the current early nutritional supplementation. The growth of these infants during the initial hospital stay needs to be more closely followed up in order to identify and treat early nutritional disturbances which threaten these infants. Prematurely-born infants also had lower BMC and BMD values than the term-born reference infants during early infancy. More information on the nutritional requirements of the very premature infants during the post-discharge catch-up growth period is needed.
8. REFERENCES


del Rio L, Carrascosa A, Pons F, Gusinye M, Yeste D, Domenech FM. Bone mineral
density of the lumbar spine in white Mediterranean Spanish children and
adolescents: changes related to age, sex, and puberty. Pediatr Res 1994;35:362-
366.

osteopenia and vitamin D metabolism. Effect of vitamin D2, calcium phosphate and

Rigo J, Nyamugabo K, Picaud JC, Gerard P, Pieltain C, De Curtis M. Reference
values of body composition obtained by dual energy X-ray absorptiometry in preterm

weight and early life nutrition on bone mineral content in preterm born infants and

Rupich RC, Specker BL, Lieuw-A-Fa M, Ho M. Gender and race differences in bone


Ryan SW, Truscott J, Simpson M, James J. Phosphate, alkaline phosphatase and


9. ORIGINAL PUBLICATIONS I-IV

These original articles have been reprinted by permission from the copyright holders.


