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Studies on nutrition, body composition and  
bone mineralization in healthy 8-year-olds in  
an urban Swedish community

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*Character cannot be developed in ease and quiet.  
Only through experience of trial and suffering  
can the soul be strengthened, ambition inspired,  
and success achieved.*

*- Helen Keller*

*To Pauline, Pedram, Peiman and Armin*

# Studier över kostvanor, bentäthet och metabola markörer hos friska 8-åringar i en svensk storstadspopulation

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## Sammanfattning

Andelen överviktiga ökar i världen både bland barn och vuxna. I Sverige är ca 20% av barnen överviktiga eller feta och liknande siffror gäller både för barn från områden med låg socioekonomisk status och barn till högutbildade föräldrar. En studie av friska 4-åringar i Göteborg visade könsskillnader i metabola markörer och relation mellan kostintag och kroppsstorlek, vilket motiverade en uppföljningsstudie av barnen.

Syftet med studien var att undersöka 118 friska 8-åringar med avseende på kroppssammansättning, bentäthet, och metabola markörer i relation till kostvanor, fysisk aktivitet och socioekonomiska variabler. Barnens kostintag undersöktes med en 24-timmars kostanmänska och ett frekvensformulär om livsmedelsval. Frågeformulär användes också för frågor om socioekonomiska variabler, fysisk aktivitet och hälsa.

I denna population med högutbildade föräldrar var 17% av barnen överviktiga. Koncentrationen av blodparametrarna glukos, HOMA-index och leptin skiljde mellan pojkar och flickor trots liknande kroppssammansättning. Leptin var den bästa markören för övervikt. Sextiotvå procent av barnen hade låga vitamin D koncentrationer i blodet ( $<75$  nmol/L). Livsmedelsvalet skiljde sig inte påtagligt från det vid 4 års ålder vilket tyder på att kostvanorna är etablerade redan vid en tidig ålder. Barn som åt fet fisk mer än 1 gång/vecka hade högre koncentration av omega-3 fettsyror i blodet och en lägre kvot av omega-6/omega-3 fettsyror. Intaget av mättat fett var negativt korrelerat till vikt och fettmassa. Barn som drack standardmjölk regelbundet hade lägre BMI än de som sällan eller aldrig drack mjölk. Intaget av läsk och juice var associerat till föräldrarnas utbildning och intaget av mjölk till mammas ursprung. Inga andra associationer fanns till socioekonomiska variabler. Pojkar och överviktiga barn hade högre bentäthet i helkropp och höft än flickor och normalviktiga, men flickor hade högre bentäthet i ländryggen när justering gjordes för benets vidd. De flesta barnen (81%) var engagerade i någon typ av fysisk aktivitet på fritiden och resultaten visade att viktberärande fysisk aktivitet var positivt associerat till bentäthet i höften hos både pojkar och flickor. Mönstret av fettsyror i serum fosfolipider skiljde inte mellan pojkar och flickor men var associerat till bentäthet hos friska barn.

*Sammanfattningsvis* visade studien att 17% av barnen var överviktiga i en population med högutbildade föräldrar. BMI och leptin var goda markörer för övervikt och fettmassa. Ett regelbundet intag av standardmjölk var associerat till ett lägre BMI. Intag av fet fisk en gång i veckan eller mer var associerat till en högre koncentration av omega-3 fettsyror i blodet. Serum fettsyror var korrelerade till bentäthet hos friska barn. Studien visar på betydande samband redan hos friska 8-åringar mellan kost, fysisk aktivitet och hälsotillstånd och även visst inflytande av socioekonomiska faktorer.

STUDIES ON NUTRITION, BONE MINERALIZATION AND METABOLIC MARKERS IN  
HEALTHY 8-YEAR-OLDS IN AN URBAN SWEDISH COMMUNITY

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**Background:** The incidence of welfare diseases including overweight in childhood is increasing worldwide. The results from a study of healthy pre-school children showed that in a population with well educated parents 17% of the children were overweight or obese at the age of 4 years. Gender differences in metabolic profiles and correlations between food intake and anthropometry motivated a follow-up study at the age of 8 years.

**Aim:** The aim of the study was to investigate nutritional intake, bone mineralization and metabolic markers in a group of healthy 8-year-olds and relate these parameters to body composition, growth, socio-economic variables, physical activity and health.

**Subjects & Methods:** Ninety-two, previously examined children, accepted participation and an additional 28 children were included. A 24-hour dietary recall was performed. Questionnaires on food choice, health, physical activity and socioeconomic variables were used. Anthropometry was measured and bone mineralization and body composition were assessed by dual energy x-ray absorptiometry. Blood samples were obtained for analysis of metabolic markers.

**Results:** The population was representative of that in Sweden except that more parents held a university degree. Seventeen % of the children were overweight. Glucose, HOMA-index and leptin differed by gender despite no difference in anthropometry. Leptin was the best marker for overweight. Serum concentration of vitamin D was low (<75 nmol/L) in 62% of the children. Food choice was similar to that at 4 years of age suggesting that food habits were established at an early age. Children who consumed fat fish once a week or more had higher concentrations of n-3 serum phospholipid fatty acids and a lower n-6/n-3 ratio. Intake of saturated fat was negatively associated to anthropometry and children who consumed full fat milk regularly had a lower BMI compared to those who seldom or never drank milk. With the exception for the intake of milk and soft drinks no socioeconomic influences were seen on the children's nutritional intake. Bone mass differed by gender and weight and larger bones were found in boys and overweight children. Physical activity was associated with the bone mass in the hip of both boys and girls. Serum phospholipid fatty acid pattern was associated with bone mineralization.

**Conclusions:** BMI correlated strongly to fat mass and leptin was the best marker of overweight and fat mass in 8-year-olds. Food choice was similar to that at 4 years of age. An intake of fat fish once a week was associated with higher serum concentrations of n-3 fatty acids. Saturated fat and intake of full fat milk were inversely associated with BMI. Serum phospholipid fatty acids were associated with bone mineralisation. The results for metabolic markers may provide preliminary reference intervals in healthy children.

*Keywords: body composition, bone mineralization, dual energy x-ray absorptiometry, healthy, lipids, metabolic markers, overweight, physical activity.*

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## List of papers

This thesis is based on the following papers:

- Study I** Eriksson S, Strandvik B. Relation between socio-economic variables, food choice and overweight in healthy 8-year-olds. (Manuscript, submitted)
- Study II** Eriksson S, Palsdottir V, Garemo M, Mellström D, Strandvik B. Fat and glucose metabolic profiles differ by sex in healthy 8-year-olds. (*Acta Paediatr*, in press)
- Study III** Eriksson S, Mellström D, Strandvik B. Volumetric bone mineral density is an important tool when interpreting bone mineralization in healthy children. *Acta Paediatr*. 2009;98:374-9. Epub 2008 Oct 1.
- Study IV** Eriksson S, Mellström D, Strandvik B. Fatty acid pattern is associated with bone mineralization in healthy 8-year-old children. *Br J Nutr*. 2009;102:407-12. Epub 2009 Jan 28.

The papers will be referred to in the text by their Roman numerals.

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## **ABBREVIATIONS**

25(OH)D	25-hydroxyvitamin D
BMAD	bone mineral apparent density
BMC	bone mineral content
BMD	bone mineral density
BMI	body mass index
DXA	dual energy x-ray absorptiometry
FFQ	food frequency questionnaire
FM	fat mass
FMI	fat mass index
FN	femoral neck
HOMA	homeostatic model assessment
IOTF	International Obesity Task Force
LS	lumbar spine
z-score	standard deviation score

## BACKGROUND

### *Obesity and welfare diseases in children*

The incidence of welfare diseases including overweight and obesity in childhood has risen dramatically all over the world in recent decades<sup>1,2</sup>. The rapid increase in overweight and obesity affects people of all ages, socioeconomic classes and ethnic groups. In Europe the number of overweight children 7-11 years old varies from 12-36%, using the cut off values recommended by the International Obesity Task Force (IOTF)<sup>2</sup>. In Sweden a twofold increase in the number of overweight and a fourfold increase in obese 10-year-olds have occurred between 1984 and 2000<sup>3</sup>, confirmed by a more recent study showing that not only the prevalence of obesity but also the severity is increasing<sup>4</sup>. Obesity has been found to be more frequent in Swedish 4-year-old girls than boys in areas with low socioeconomic status<sup>5</sup>, whereas one study has suggested a recent decrease in the prevalence of overweight in 10-11-year old girls<sup>6</sup>.

Several factors have been investigated in relation to the increasing prevalence of overweight. The urbanisation of societies is creating an obesogenic environment by minimizing the space for play and leisure time activities. Children who want to be active are restrained by factors in regard to safety and convenience. Physical activity defined as organised sports and school physical activity is declining in many countries<sup>7</sup>.

The availability of foods has increased and a more frequent intake of energy dense foods and soft drinks has led to changes in dietary habits. During the latest half century recommendations have been given to substitute saturated fat with vegetable oils which has led to an increased intake of omega-6 (n-6) fatty acids<sup>8,9</sup>. An increased ratio of n-6/n-3 has been found to promote both adiposity<sup>10</sup> and the pathogenesis of diseases such as inflammatory and cardiovascular disease and cancers<sup>9</sup>.

A study of healthy 4 year old children recruited from three paediatric health care centres was performed in Göteborg in 1999/2000<sup>11</sup>. The population was representative of that in Sweden except for the fact that more parents held a university degree. At the age of 4 years, 17 % of the children were already

overweight or obese. Twenty five % of the daily energy intake consisted of junk food. Most children had an intake of saturated fatty acids and sucrose exceeding the recommendations whereas the intake of vitamin D, iron and calcium was lower than the recommendations in many children. A higher BMI was associated with lower fat intake and higher sucrose intake. In the girls, serum insulin concentrations and HOMA insulin resistance index (HOMA-IR) were associated with the change in z-score for both height and weight from birth to 4 years but not to the actual height, weight or BMI at the age of 4 years. These findings motivated the interest in performing a follow-up study on the same children at the age of 8 years.

High insulin concentrations is usually associated with obesity, but a study of Pima Indians in Arizona, USA, as well as the results from Swedish 4-year olds have suggested that hyperinsulinemia may precede the development of obesity<sup>12,13</sup>, which has also been implied in animal studies<sup>14</sup>.

Sedentary activities such as watching television has been found to be related to obesity, not only by displacing physical activity but also by increasing energy intake<sup>15</sup>. An increased intake of sugar sweetened drinks is associated with a higher energy intake<sup>16</sup> but not always corresponding to a higher BMI<sup>17</sup>.

Growth rate in utero, overfeeding in early childhood, and a rapid growth rate for both height and weight have been associated with an increased risk for later development of obesity, diabetes, hypertension and coronary heart disease<sup>18-23</sup>.

Efforts have been made to identify markers of the metabolic syndrome and adipocytokines, produced by the adipose tissue, seems to be of importance in this matter. Adiponectin and leptin, has been suggested as such predictors<sup>24-26</sup> but reference values for several biomarkers in children are generally lacking<sup>27</sup>.

### *Definitions of overweight and obesity*

Due to the increasing prevalence of overweight and obesity in childhood it is important to identify overweight/obesity early in life and the matter is of major public health concern. Sweden has a paediatric health care system, where the children are weighed and measured on a regular basis from birth up to the age of 18 years. Thus, the development of Swedish children's height and weight has been followed for decades and resulted in national growth charts; 1976 based on

children born between 1955-1958<sup>28</sup> and 2002 based on children born between 1973-1975<sup>29</sup>. The growth charts in 2002 were based on the same population as the national body mass index (BMI) references presented in 2000<sup>30</sup>.

The definition of normal weight for adults is a BMI between 18.5 and 24.9 according to the World Health Organisation (WHO). In 2000, Cole et al. published international age- and gender-specific reference values for BMI in children and adolescents, corresponding to the cut off points used for adults<sup>31</sup>. Overweight in children can also be defined as a z-score  $>2$  for BMI ( $\text{kg}/\text{m}^2$ ) or body weight. The use of BMI cut-off values has been recommended by IOTF for comparing the prevalence of overweight and obesity between different countries.

### *Body composition*

Body composition is defined as the percentage of fat, muscle and bone in the body and is influenced by energy intake, food choice, physical activity and health.

There are different methods of assessing body composition, for instance bio-impedance analysis, dual energy x-ray absorptiometry (DXA), magnetic resonance imaging and computed tomography (CT); the last two however, being expensive and exposing the subject to higher radiation. DXA provides information on both body composition and bone mineralization in terms of bone mineral content, fat free soft tissue and fat mass. The former two components can be combined to represent fat free mass. The previously mentioned methods are mainly used for research purposes. In clinical settings, waist circumference and BMI are feasible tools and used together suggested as an adequate measure in assessing adiposity in children<sup>32</sup>.

### *Bone mass*

The main function of the skeleton is to protect the internal organs, provide support and enable the body to move. The skeleton also provides storage for the body's calcium reservoir.

DXA and quantitative computed tomography (QCT) are two methods of measuring bone composition. In children DXA is the preferred method due to its high accuracy, low radiation and availability of paediatric reference values.

The technique has limitations since it only measures the cross sectional area of the bone and therefore the influence of bone size can lead to an underestimation of bone mineral density in short people and an overestimation in tall people. Correction is made for bone size by calculating volumetric bone density. Quantitative CT is a more expensive tool but measures the three dimensional bone and provides measures of volumetric density, but due to the higher radiation exposure and as a consequence lack of reference values in children, this method is hitherto less commonly used.

There are two types of bones; the trabecular, spongy bone in the interior region of the bones, and the cortical bone, which form the hard outer layer of the bones. The trabecular bone provides only about 20% of the total bone mass but is the most metabolically active. Therefore the bones with the highest content of trabecular bone are most studied, like the hip and the lumbar spine.

The bone is under constant renewal, remodelling, but starts in childhood with the formation process called modelling until the peak bone mass is reached in early adulthood (Figure 1).

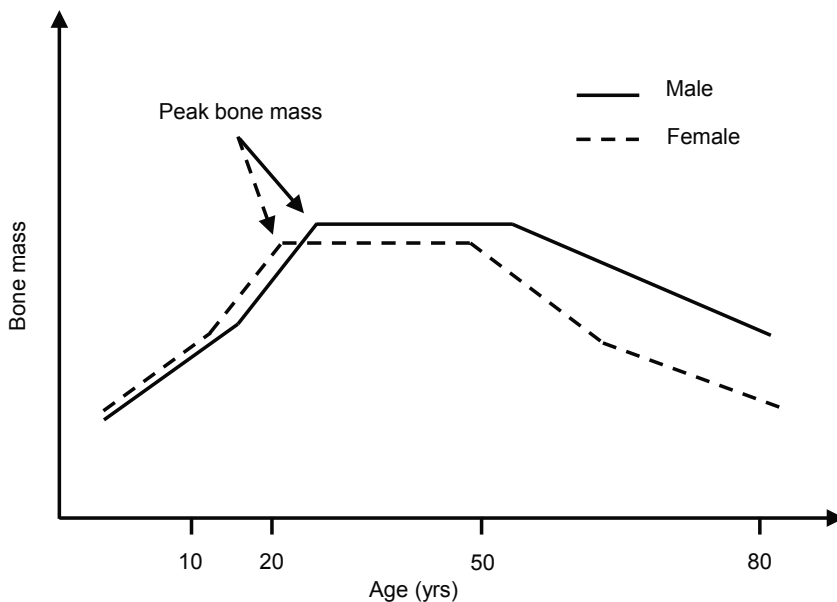


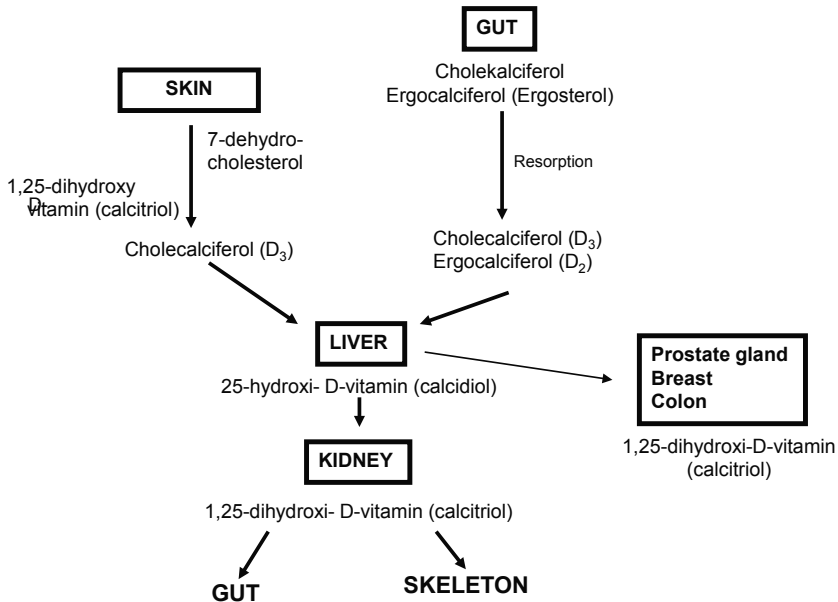
Figure 1. Brief description of the course of bone mass acquisition and loss during the life span.

To reach the full potential for peak bone mass and thereby decreasing the risk of later osteoporosis related fractures, satisfactory physical activity, nutrition, hormonal balance and body weight are important.

Different dietary and metabolic factors have been investigated in association with bone health, particularly calcium and vitamin D<sup>33</sup>. Associations between bone growth and fatty acids has mainly been investigated in animal studies<sup>34-37</sup> and there has been a lack of studies in humans until recently<sup>38-40</sup>.

- Vitamin D

The main source of vitamin D in humans is the exposure to the sun which initiates a conversion of 7-dehydrocholesterol in the skin to vitamin D<sub>3</sub>. Vitamin D in the skin or ingested in the diet is metabolized in the liver to 25-hydroxyvitamin D (25(OH)D, calcidiol), the circulating form used to determine vitamin D status. Calcidiol is further converted into the bioactive form 1,25 dihydroxy vitamin D (calcitriol) in the kidneys. Calcitriol enhances the absorption of calcium in the small intestine and the absorption of calcium from the bone (Figure 2).



With permission from Gronowitz E

Figure 2. Vitamin D synthesis.

Vitamin D deficiency can cause osteomalacia, osteopenia and osteoporosis, increasing the risk of future fractures. Rickets, caused by insufficient sun exposure, was a common disease a century ago and was thought to be eradicated. New figures have shown a resurrection of the disease<sup>41,42</sup>, in which also a low intake of dietary calcium has been debated<sup>43</sup>.

The optimal concentrations of 25(OH)D and the definition of deficiency has been discussed over the years and today, based on available results, concentrations <50 nmol/L (20 ng/ml) are proposed as indicative of deficiency and concentrations >75 nmol/L (30 ng/ml) are considered to be sufficient<sup>44,45</sup>. No optimal concentrations have been defined for children although assumed to be the same as in adults.

Few foods contain vitamin D naturally. The main dietary sources are fish, fortified - mainly dairy - products, and supplements. Several studies have shown that the dietary intake of vitamin D do not provide sufficient amounts<sup>46-48</sup> and therefore new dietary recommendations for vitamin D are urgently asked for<sup>49</sup>.

#### - Fatty acids

Fatty acids are components of the fat structure in the body. Triglycerides are made up of a glycerol molecule and three fatty acids and compose the major dietary intake of fat and the body's energy reserve. The phospholipids consist of glycerol, two fatty acids and a phosphate group with a polar head and are incorporated in the cell membranes of every cell in the body in the form of a double layer with the hydrophilic phosphate head outwards and the hydrophobic tails of fatty acids towards the middle. The phospholipids have specific properties depending on the structure.

The fatty acids are grouped into saturated, monounsaturated or polyunsaturated depending on the number of double bonds in the carbon chain. The body can synthesize all fatty acids but two, linoleic acid (LA, 18:2 n-6) and alpha-linoleic acid (ALA, 18:3 n-3), which must be obtained by diet. These essential fatty acids are the mother substances of the other important fatty acids of the n-6 and n-3 families.

Omega-6 and omega-3 fatty acids are found in vegetable oils but long chain n-3 fatty acids are mainly found in fish and sea food. During the conversion of LA and ALA into the long chain fatty acids; arachidonic acid (AA, 20:4 n-6), eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3), LA and ALA competes for the same enzymes and therefore the balance between the fatty acids is important (Figure 3).

During recent decades, an increased intake of the n-6 fatty acids has occurred when recommendations to replace saturated fat with polyunsaturated fat has been given. This has lead to a change in the ratio of n-6/n-3 fatty acids<sup>8,9,50</sup>. It is not known what the optimal ratio should be but it is believed that humans evolved on a diet with a ratio of n-6/n-3 of 1:1<sup>51</sup>. In Sweden the recommendation is a ratio of 5:1 but the ratio in Western diet today is estimated to be 10:1 - 15:1<sup>9</sup>. A high intake of n-6 fatty acids and a high n-6/n3 ratio has been suggested to promote the development of several diseases including cardiovascular disease, cancer, autoimmune and inflammatory diseases<sup>9</sup>.

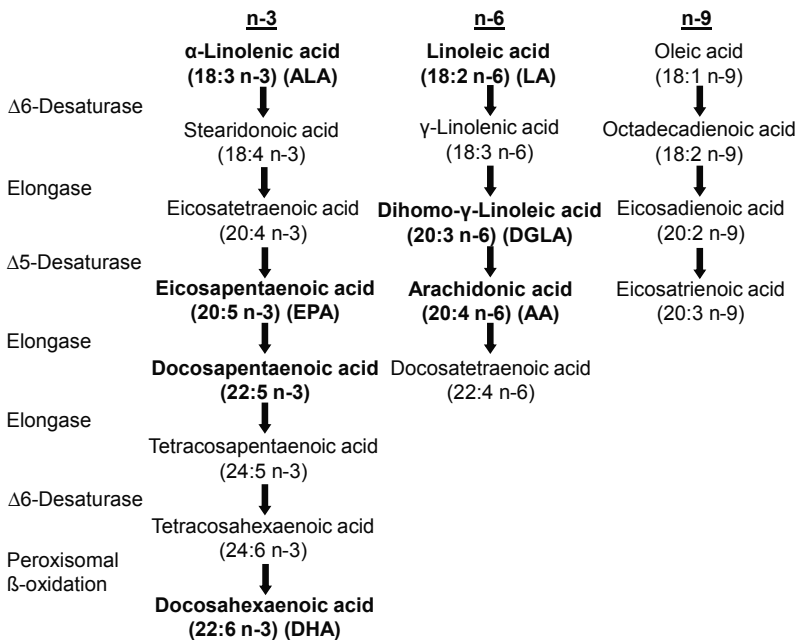


Figure 3. Major pathways of the fatty acid metabolism; elongation and desaturation.

It is well established that the long chain n-6 and especially n-3 fatty acids are important for mental development during infancy. In children with developmental and behavioural difficulties deficiency in n-3 and n-6 fatty acids was found but supplementation with fatty acids showed significant effects on behaviour and learning<sup>52</sup>. Deficient concentrations of n-3 fatty acids have been associated with aggressive and depressive disorders<sup>53</sup>.

Many systems in the body, including immune system and blood pressure regulation are dependent on the essential fatty acids since they are further synthesized into hormone like substances, such as eicosanoids. There are different kinds of eicosanoids such as prostaglandins (PG), prostacyclins, thromboxanes and leukotrienes, which have important roles for instance causing constriction or dilatation in the blood vessels, stimulate bone metabolism and regulating the inflammatory response of the body. Prostaglandin E<sub>2</sub>, synthesized from AA, has mainly pro-inflammatory effects and is the primary PG affecting bone metabolism by dose-dependently regulating bone modelling<sup>54</sup>. Prostaglandins E<sub>1</sub> and E<sub>3</sub> derived from dihomo- $\gamma$ -linolenic acid (DGLA) and EPA, respectively, have primarily anti-inflammatory properties. Also EPA and DHA are transformed into anti-inflammatory products. Increased intake of n-3 fatty acids altering the n-6/n-3 ratio provides positive conditions for bone modelling by lowering the concentrations of AA and thereby decreasing the PGE<sub>2</sub> production<sup>36</sup>. Animals fed long-chain n-3 fatty acids have shown an increased rate of bone formation, proposing a beneficial effect on osteoblastic activity<sup>55</sup>.

## **AIM OF THE STUDY**

The principal aim of the study was to investigate nutritional intake, metabolic markers and bone mineralization in a group of healthy 8-year-olds and relate these parameters to socio-economic variables, growth, physical activity and health. Since a large group of the children had been examined previously at the age of 4 years, a second aim was to follow-up these children prospectively, to try to identify early markers for overweight/obesity.

The specific aims of the thesis have been:

- to examine socioeconomic variables such as parental education and ethnicity in association to the children's nutritional intake and food choice.
- to examine the association between metabolic markers and body composition.
- to examine bone mineralization in association to physical activity and overweight.
- to examine the association between bone mineralization and serum phospholipid fatty acids.
- to investigate the development of metabolic markers in a follow-up study from 4 to 8 years of age.

The study was approved by The Ethical Committee of the University of Gothenburg and informed consent was obtained from children and parents.

## SUBJECTS AND METHODS

### *Subjects*

When initiating the follow-up study, the parents of those children who had given blood and/or participated in the DXA scanning at the age of 4 years were contacted, a total of 120 children. Seventy-seven % of the families accepted to participate in the follow-up but since the drop-out rate among the 4-year-olds were higher in the area with low socio-economic status, 45 additional children were invited from such an area. Seventeen children were excluded due to unsatisfactory language skills among these parents or unwillingness to participate resulting in a total of 118 children, including three pair of twins (Figure 4).

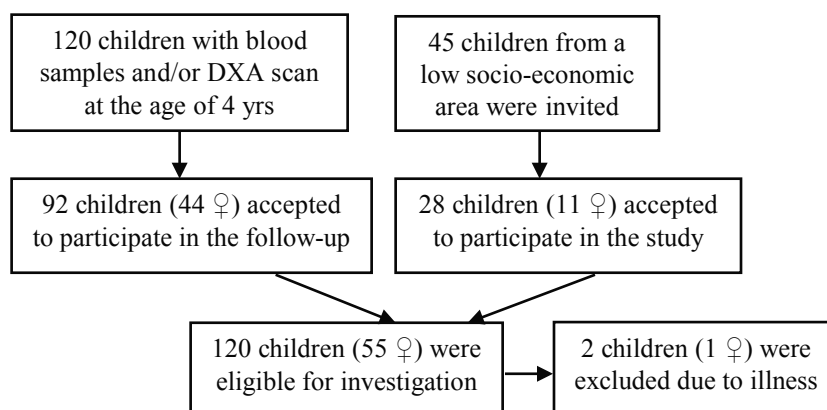


Figure 4. Schematic picture of the inclusion procedure resulting in a total of 118 participating children.

The same methods as was used in the initial study were intended to be used also at 8 years, but a large group of parents rejected to participate in a 7-day food registration. Therefore a combined diet history, 24-hour recall and an extended food frequency questionnaire (FFQ) was used to get a description of the children's habitual dietary intake and food habits. The questionnaires on health,

socio-economic variables and physical activity (PA) were the same as used in the original study with minor age related adjustments. The birth length and weight of the children who participated at the age of 4 years had been stated previously<sup>13</sup> and for the new participants these data were supplied by the school nurse with permission from the parents.

The children were defined as healthy since they had no known diseases or conditions affecting growth, bone metabolism or the metabolic markers intended for investigation.

#### *Nutritional intake (paper I)*

A 24-hour dietary recall was performed together with the child and with the support of the parents. The children described their food intake on the previous day and in order to recall the portion sizes, the booklet “Matmallen” (National Food Administration, Sweden 1999) was used. This booklet provides pictures of different portion sizes of common foods such as pasta, rice, fruit and bread. Daily intake was then summarized and approved by both the child and the parent as being representative for the intake during the previous day. The 24-hour dietary recall was then calculated into daily energy and nutrient intake using the computer software for nutrient calculation MATs (Rudans Lättdata 6<sup>th</sup> ed. 1999).

#### *Questionnaires (paper I & III)*

An extended version of the FFQ used at the age of 4 years was given to the parents to fill out at home. The parents were instructed to also consider foods eaten by the child away from home to get a representative picture of the habitual intake of different foods.

In the questionnaire on socio-economy the parents were asked about country of birth, educational level, income, housing and family size.

A health questionnaire was used for identifying food allergies, intolerances and associated symptoms. Questions regarding present or past diseases and intake of vitamins or other food supplements were also included.

To assess self-reported physical activity, a fourth questionnaire was used and this was filled out by the child and one/both parents. The questionnaire included

questions on leisure time activities and sedentary activities, such as watching TV or playing computer.

A validation of the physical activity questionnaire was intended, using an actigraph (ActiReg®, PreMed AS, Oslo, Norway), but could not be fulfilled since only four of 16 participating children were able to complete the recommended minimum of 4 days of registration.

#### *Blood sampling (paper I, II & IV)*

The parents were given local anaesthetic plasters (EMLA®, AstraZeneca AB, Södertälje, Sweden) to apply on the skin of the child one hour before blood sampling, which was performed after an overnight fast.

Concentrations of plasma glucose, and serum cholesterol and triglycerides, calcium (Ca), parathyroid hormone (PTH), vitamin D (25(OH)D) and IgE were analysed according to routine (Sahlgrenska University Hospital, Göteborg, Sweden).

Serum insulin concentrations were measured by Insulin Ultrasensitive ELISA kit (Mercodia AB, Uppsala, Sweden). HOMA insulin resistance and HOMA  $\beta$ -cell index were calculated according to Matthews et al.<sup>56</sup>.

Serum concentrations of adiponectin and leptin were measured using Quantikine human ELISA kits (R&D Systems, Minneapolis, USA). All samples were analyzed in duplicates.

#### *Bone measurements and body composition (paper II-IV)*

The children were weighed and measured using standardized equipment and body mass index (BMI, kg/m<sup>2</sup>), z-scores for height, weight and BMI were calculated<sup>28,30</sup>. The z-score indicates how many standard deviations an observation is from the mean value, calculated as (measured value - mean of the population)/standard deviation of the population. The IOTF cut-off values for BMI was used to define overweight/obesity<sup>31</sup>.

Bone measurements and body composition were analysed using DXA (Lunar DPX-IQ, GE Lunar Corp, Madison, WI, USA) and results were given as bone mineral content and bone mineral density for total body, lumbar spine, total femur and femoral neck. Bone mineral apparent density of lumbar spine (volumetric BMD, BMAD) was calculated using the formula:  $BMAD_{LS} =$

$BMD_{LS} * [4 / (\pi * width)]^{57}$ . The reference values obtained from the manufacturer were expressed in age- and sex- matched z-scores except for femur, for which no data were available. Results for fat mass and lean body mass were also given. Fat mass index (FMI,  $kg/m^2$ ) was calculated.

### *Statistical analysis*

Statistical analyses were performed using the software program Statistical Package for the Social Sciences (SPSS®) 14.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Statistical tests were chosen as appropriate after checking distributional assumptions.

Student's t-test and Pearson correlation coefficients were used for symmetric, continuous observations and Mann-Whitney's U-test and Spearman rank correlation test for data with non-normal distribution. For normally distributed variables, mean value and standard deviation (SD) are given. Percentile values were also given for the bone measurements (paper III).

The distribution of leptin, triglycerides and insulin were negatively skewed and were logarithmically transformed in regression analysis. For skewed variables, averages were given in terms of median and interquartile range (IQR) (paper II).

The  $\chi^2$ -test was used to assess associations between categorical variables and the Breslow-Day test to assess for sex differences, and if not present, the Mantel-Haentzel method was used to obtain a common odds ratio (OR) given with 95% confidence interval (CI) (paper II).

Paired sample t-test and Wilcoxon signed rank test were used for analysing changes in variables in the children followed longitudinally (paper II).

Multivariate linear regression analyses were used to analyse the relation between dependent variables and influencing co-variables. Statistical significance was set at  $p < 0.05$  (2-sided test).

## RESULTS AND DISCUSSION

### *Food choice in healthy 8-year-olds (Paper I)*

In this cross-sectional study 114 healthy children were included, out of which 97 (85%) participated in the blood sampling. Seventeen % of the children were overweight or obese according to the IOTF definition. The population was representative for Sweden except for the fact that more parents held a university degree than the population in general. The food habits at the age of 8, were similar to those at the age of 4 years, thus it seemed like food habits had been established early. The intake of chips, chocolate, sugar sweetened drinks was restricted to once or twice a week for the majority of children as was seen also at the age of 4 years<sup>46</sup>. Mothers with university education were more likely to restrict the intake of soft drinks with artificial sweetener whereas a trend was seen for a higher intake of sugar sweetened drinks in children of mothers with compulsory school. There was also a trend towards lower intake of fruit juice for children of fathers with compulsory school in comparison with fathers with upper secondary school.

The children who consumed fat fish once a week or more had significantly higher concentrations of n-3 fatty acids and a lower ratio of n-6/n-3, but no associations were found to anthropometric measures in the healthy children. An increased ratio of n-6/n-3 fatty acids have in both animal and human studies been shown to promote adiposity<sup>10</sup>. Low concentrations of n-3 fatty acids as well as high concentrations of n-6 fatty acids have been found in overweight children<sup>58,59</sup>. An intake of fish once a week or more has an impact on cognitive functions in healthy adolescents<sup>60</sup>, a parameter not measured in this study but suggesting the importance of the fatty acids in several health aspects.

Garemo et al. found a negative association between fat intake and BMI in healthy 4-year olds<sup>13</sup>, a finding which was also seen in the 8-year olds where a high intake of saturated fatty acids was negatively associated to BMI, height, weight and fat mass.

For many years the recommendations have emphasized on a reduction of fat intake, especially saturated fatty acids in order to prevent obesity, some types of cancers and cardiovascular diseases, but this opinion has been debated. In adult

men no significant association was seen between the intake of total fat or saturated fat and the risk of cardiovascular disease<sup>61</sup>. Another study in men showed a positive association between serum concentrations of saturated fatty acids and cardiovascular mortality, while linoleic acid (18:2 n-6) was inversely related to cardiovascular and total mortality<sup>62</sup>. In women, a positive association was seen between trans fatty acids and the risk of heart disease and a negative association between heart disease and polyunsaturated fatty acids<sup>63</sup>.

An inverse association between consumption of full fat milk and BMI was seen in our population (Figure 5). No similar association was seen for the intake of medium or low fat milk.

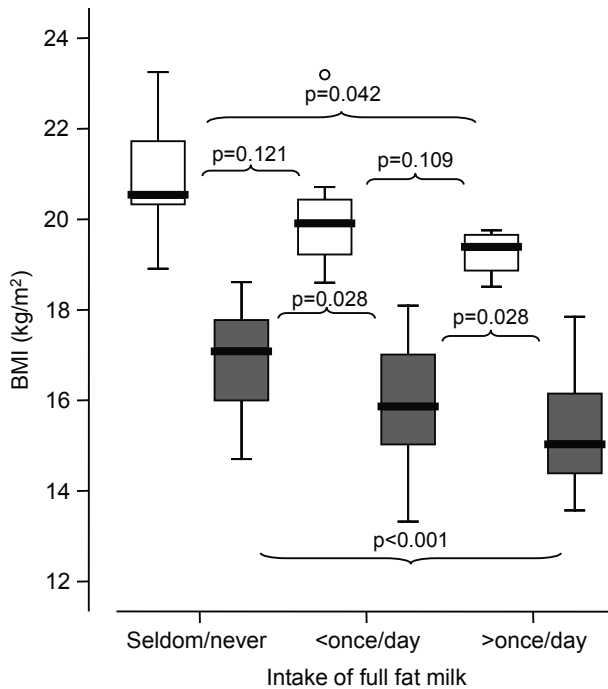


Figure 5. Children who consumed full fat milk on a regular basis (more than once a day) had a lower BMI than the children who seldom or never drank full fat milk. The difference was significant in both normal weight children as well as in the group of overweight children.

The intake of dairy products has been found to influence the regulation of body weight in both children and adults<sup>64-66</sup>, but the mechanisms behind the findings are not clear. Almost all the children (96%) in the study population drank milk on a regular basis, on average 4 dl/day. The serum concentration of the phospholipid fatty acid pentadecanoic acid (15:0), which is a marker of intake of milk fat<sup>66</sup>, was positively correlated to the amount of milk consumed ( $r=0.225$ ,  $p=0.03$ ). The amount of full fat and medium fat milk were similarly consumed but a difference was seen in regard ethnic origin; children of mothers born abroad drank more full fat milk.

The nutritional intake of vitamin D was reflected in the serum concentrations of 25(OH)D, which was below the suggested lower reference interval of 75 nmol/L for 62% of the children. The serum concentrations of vitamin D were lower in children of mothers born abroad, than children of mothers born in Sweden (Figure 6).

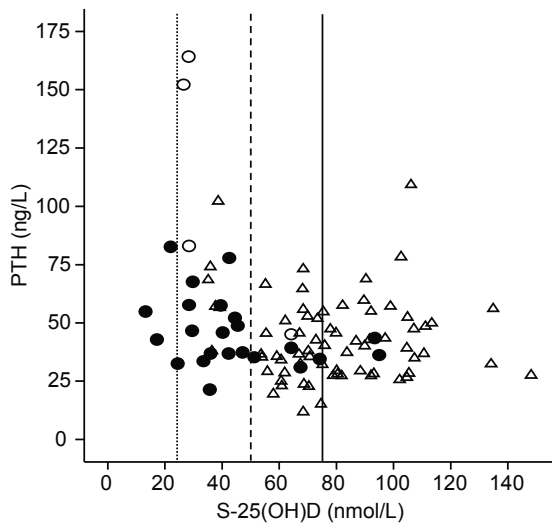


Figure 6. Serum concentrations of S-25(OH)D in correlation to parathyroid hormone in 97 children. Children of mothers born in Sweden ( $\Delta$ ), children of mothers born abroad ( $\bullet$ ) including four children with dark skin ( $\circ$ ). Lines indicate deficiency:  $<25$  nmol/L, insufficiency:  $<50$  nmol/L and recommended sufficient levels  $>75$  nmol/L.

Four of the children with mothers born abroad were children who, also wore concealing clothing and/or had dark pigmented skin, further obstructing the synthesis of vitamin D in the skin. No correlation was seen between PTH and 25(OH)D when the two outliers were excluded.

Fulfilling the dietary recommendation of vitamin D only by foods is difficult. This was found in the national investigation of children (Riksmaten 2003)<sup>67</sup> initiated by the National Food Administration (SLV) as well as in the investigation of the healthy 4 year olds in 1999/2000<sup>46</sup> and in a previous study of healthy adolescents<sup>68</sup>. Therefore it seems contradictory that the recommendation to supplement infants and young children with vitamin D has changed to only be given up to the age of 2 years instead of the previous recommended 5 years, except for specific risk groups; children who does not eat vitamin D enriched dairy products and those with dark pigmented skin and/or concealing clothing. A compulsory enrichment of vitamin D in dairy products does not appear to be enough and a re-evaluation of the recommendations is proposed.

Since the sun is the main provider of vitamin D for synthesis in the skin, seasonal variation and the latitude is of major importance. In countries, positioned on a high latitude such as Sweden (Göteborg N 57°), it is difficult to fulfil the needs during wintertime.

Reports from Scandinavia and northern Europe have shown that vitamin D deficiency is common in both the native population as well as in immigrant populations<sup>47,69,70</sup>. To the best of my knowledge this is the first time results for serum concentrations in healthy Swedish children has been presented.

**Summary paper I:**

- **Food pattern was similar to that at 4 years of age suggesting that food habits were established at an early age.**
- **Intake of beverage, chocolate and sweets were influenced by parental education. The ethnicity of the mother influenced the choice of milk.**
- **High saturated fat intake was negatively associated to anthropometry.**
- **Intake of fish, milk and vitamin D was reflected by serum markers.**

*Metabolic markers in healthy 8-year-olds (Paper II)*

Ninety-seven children participated in the blood sampling for analysis of metabolic markers in relation to anthropometry and body composition. The boys in the study were longer and heavier than the girls at birth and had higher LBM than girls at the age of 8 years. No other differences were seen between boys and girls in regard to anthropometric measures.

Sixteen % of the children were overweight or obese according to the IOTF cut off values for BMI. BMI is widely used for measuring adiposity, but has been questioned since it is rather a measure of excess weight in relation to height than excess body fat and therefore a poor marker for cardiovascular risk factors<sup>71</sup>. However, in the 8-year-olds, BMI correlated strongly with fat mass in both boys and girls, confirming the results seen at the age of 4 years<sup>11</sup>. Thus BMI seems to reflect body fat in young children. There was a parallel opposite shift in boys and girls in the correlation between a given BMI to FM and LBM representing a higher FM for girls and higher LBM for boys, respectively, (Figure 7).

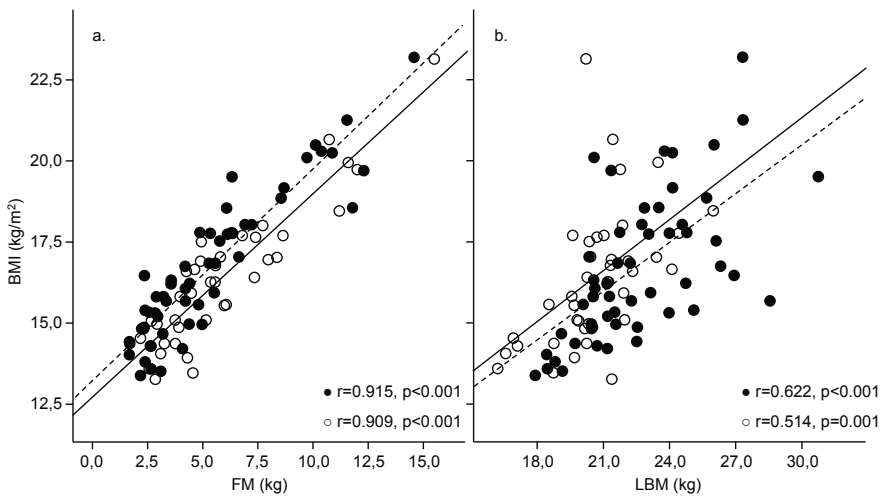


Figure 7. BMI was strongly correlated to both fat mass (FM) and lean body mass (LBM) in both boys (n=52) and girls (n=40). Boys indicated with filled figures and broken line, girls with open figures and solid line.

The use of different reference materials and definitions of overweight makes comparisons between studies difficult<sup>1</sup>. Depending on the reference values used, significant discrepancies in prevalence of overweight can be seen in a population sample. This issue became evident during the prospective study of healthy 4 to 8 year olds in Göteborg. When the children initially were examined at the age of 4<sup>13</sup>, the reference values for BMI by Cole et al.<sup>31</sup> were used and 17% of the children were overweight. Defining overweight as z-score for weight >2, identified 16% of the 4 year olds as being overweight using the references from 1976<sup>28</sup> but in comparison with the latest reference material from 2002<sup>29</sup> only 9% were defined as overweight<sup>11</sup>. Similar divergences were seen at the age of 8 years (Table 1). Therefore the decision was made to use the older reference values for height and weight<sup>28</sup> and the IOTF cut off values for definition of overweight and obesity in this study to be able to compare the children longitudinally. Adjusting the reference values to the changes in the population indicates an acceptance and tolerance of increasing body weight, disguising the true prevalence of overweight. This issue has previously been stressed by Jebb and Prentice<sup>72</sup>, urging health professionals and researchers to adopt a standard definition of overweight/obesity, suggesting the IOTF definition to be used.

Table 1. Prevalence of overweight/obesity in a 116 healthy 8 year old children using different definitions and references.

	Total (n=116)		Boys (n=63)		Girls (n=53)	
	%	n	%	n	%	n
BMI (kg/m <sup>2</sup> ) IOTF cut-off <sup>31</sup>	16	19	19	12	13	7
BMI z-score >2 <sup>30</sup>	12	14	16	10	7	4
Weight z-score>2SD <sup>28</sup>	15	17	19	12	9	5
Weight z-score>2SD <sup>29</sup>	8	9	10	6	6	3

During the latest decades, height, weight and BMI have increased in Swedish children<sup>3</sup> and this might reflect changes in the society, but not genetic changes. The purpose of identifying overweight and obesity is to treat and prevent the negative effects of the condition. In order to be able to start an early prevention

for later welfare diseases and decrease the risk of overlooking early signs of bad health the cut off values should be stringent. For comparisons between different countries and cohorts the IOTF definition should be used. National age and gender specific reference values may no longer be a useful tool since the populations in many countries is getting increasingly multicultural.

The concentration of plasma glucose was higher in the boys, and the HOMA  $\beta$ -cell function was higher in the girls. The girls also had higher leptin concentrations than the boys. Leptin has, in girls, been found to be positively associated to age, BMI and body fat<sup>27</sup>. Since there was no difference in age or BMI between gender but a trend to higher body fat in the girls this might be a probable explanation to the findings. Adipocytokines has been proposed as predictors of metabolic syndrome in children<sup>24</sup>. Apart from a weak correlation between adiponectin and BMI in girls, adiponectin was not associated to any anthropometric or metabolic measures in healthy 8-year old children and its role as a risk marker in healthy children has also previously been questioned<sup>73</sup>. In boys, none of the metabolic markers generally used to indicate metabolic disturbances were associated to the anthropometric measures. However, leptin and the leptin/adiponectin (L/A) ratio were positively correlated to weight, BMI and FMI in both boys and girls suggesting leptin to be the best marker of overweight and fat mass.

An increased z-score of height and weight from birth to 4 years of age in healthy children was associated with high fasting insulin concentrations in children, who were not overweight or obese<sup>13</sup>. At the age of 8 the more expected finding of an association between serum insulin concentrations and the actual body weight was seen.

The serum concentrations of cholesterol and triglycerides were similar or lower than those found in 7-9 year old children in the Finnish STRIP-study, where dietary advice on lowering the fat intake had been given from birth to 14 years of age<sup>74</sup>. Boys in that study had lower total cholesterol concentrations than the girls throughout childhood proposing that the intervention effect was larger in boys. However, a similar decrease was seen in the 8-year old boys, who had been examined at both 4 and 8 years of age, without any dietary intervention. Interestingly, the 8-year old boys in the highest quartile of body fat had lower serum cholesterol concentrations than the boys in the lowest quartile. In the

girls the serum cholesterol concentrations were stable during 4 and 8 years of age. No associations were seen between concentrations of triglycerides and anthropometric measures or body fat.

In both boys and girls followed from 4 to 8 years of age an increase was seen in the concentrations of leptin and the L/A ratio, whereas the adiponectin levels had decreased. This may be an age related adjustment but also a reflection of increasing fat mass during the four years.

Preliminary reference intervals for several metabolic markers in normal weight children at the age of 4 and 8 years are presented in table 2. Even though this population is rather small, these reference values would serve as guidelines since reference intervals for metabolic markers in healthy children are generally lacking.

Table 2. Preliminary reference intervals for metabolic markers in healthy, normal weight<sup>#</sup> 4 and 8 year old children. All blood samples were taken after an overnight fast. (Methods: see page 20)

	4 years		8 years	
	Boys (n=43) <sup>a-c</sup>	Girls (n=43) <sup>d-f</sup>	Boys (n=54)	Girls (n=43)
fS-Calcium (mmol/L)				
Mean (SD)	2.39 (0.38)	2.48 (0.08)	2.45 (0.06)	2.49 (0.07)**
Median	2.44	2.47	2.45	2.49
5 <sup>th</sup> -95 <sup>th</sup> percentiles	2.31-2.56	2.34-2.62	2.37-2.57	2.35-2.58
fS-Phosphate (mmol/L) <sup>a</sup>				
Mean (SD)	1.51 (0.11)	1.54 (0.12)	1.59 (0.14)	1.58 (0.14)
Median	1.50	1.60	1.60	1.60
5 <sup>th</sup> -95 <sup>th</sup> percentiles	1.39-1.71	1.32-1.70	1.40-1.88	1.40-1.91
fS-Cholesterol (mmol/L) <sup>c</sup>				
Mean (SD)	4.49 (0.77)	4.57 (0.63)	4.45 (0.83)	4.43 (0.72)
Median	4.40	4.50	4.40	4.40
5 <sup>th</sup> -95 <sup>th</sup> percentiles	3.50-5.97	3.62-5.80	2.94-6.28	2.79-5.64
fS-Triglycerides (mmol/L) <sup>c</sup>				
Mean (SD)	0.64 (0.15)	0.73 (0.21)*	0.62 (0.20)	0.66 (0.21)
Median	0.60	0.69	0.58	0.60
5 <sup>th</sup> -95 <sup>th</sup> percentiles	0.44-0.94	0.42-1.20	0.38-1.08	0.41-1.21
fS-Adiponectin <sup>§</sup> (mg/L) <sup>b,e</sup>				
Mean (SD)	18.09 (6.68)	17.77 (6.76)	12.28 (5.57)	12.09 (5.03)
Median	16.77	16.58	11.51	11.66
5 <sup>th</sup> -95 <sup>th</sup> percentiles	9.09-31.10	8.98-36.09	4.53-22.18	5.43-23.13
fS-Leptin <sup>§</sup> (ng/ml) <sup>b,e</sup>				
Mean (SD)	2.04 (1.10)	2.89 (1.55)**	3.93 (5.62)	5.32 (3.70)
Median	1.80	2.45	2.61	4.24
5 <sup>th</sup> -95 <sup>th</sup> percentiles	0.60-4.09	1.38-6.69	0.85-8.52	1.56-13.97
fS-25(OH)D (nmol/L) <sup>c,f</sup>				
Mean (SD)	78.58 (19.82)	77.62 (15.41)	65.70 (26.39)	77.20 (30.01)
Median	74.75	77.50	63.50	74.25
5 <sup>th</sup> -95 <sup>th</sup> percentiles	46.29-112.34	54.85-111.83	24.75-109.35	27.41-134.91

fS-PTH (ng/ml) <sup>f</sup>				
Mean (SD)	27.51 (11.99)	31.46 (12.72)	46.03 (17.06)	49.11 (31.65)
Median	25.90	29.20	43.60	41.50
5 <sup>th</sup> -95 <sup>th</sup> percentiles	11.90-54.98	14.51-59.30	24.12-78.06	15.83-153.60
fP-Glucose (mmol/L) <sup>f</sup>				
Mean (SD)	4.50 (0.35)	4.47 (0.43)	4.57 (0.27)	4.37 (0.27)***
Median	4.50	4.40	4.60	4.40
5 <sup>th</sup> -95 <sup>th</sup> percentiles	3.84-5.08	3.80-5.20	4.10-4.98	4.00-4.91
fS-Insulin <sup>s</sup> (mU/L) <sup>b,e</sup>				
Mean (SD)	3.88 (2.18)	4.95 (2.76)	4.15 (2.66)	4.07 (1.72)
Median	3.60	4.10	3.40	3.75
5 <sup>th</sup> -95 <sup>th</sup> percentiles	1.20-8.41	1.70-11.00	0.92-9.48	2.08-7.85
HOMA-IR <sup>b,d</sup>				
Mean (SD)	0.79 (0.47)	1.02 (0.61)	0.84 (0.51)	0.79 (0.34)
Median	0.71	0.80	0.71	0.70
5 <sup>th</sup> -95 <sup>th</sup> percentiles	0.23-1.83	0.32-2.21	0.17-1.89	0.36-1.52
HOMA β-cell function (%) <sup>b,d</sup>				
Mean (SD)	74.24 (59.77)	101.84 (80.28)	76.30 (44.00)	100.46 (52.53)*
Median	67.27	91.11	64.29	94.17
5 <sup>th</sup> -95 <sup>th</sup> percentiles	22.64-179.14	8.60-247.33	30.44-173.53	42.96-199.26

# according to IOTF definition. <sup>s</sup>Not analysed by accredited laboratory.

Number of 4 year olds missing: a:5 boys, b:2 boys, c:1 boy, d:6 girls, e:4 girls, f:2 girls

\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  for differences between gender at the same age

#### Summary paper II:

- The metabolic profile of plasma glucose, serum leptin and HOMA β-cell function differed by sex, despite no difference in BMI or FM.
- Boys in the highest quartile of body fat had lower serum concentrations of cholesterol than the boys in the lowest quartile.
- Leptin was the best marker of overweight.
- Preliminary reference intervals for metabolic markers are provided.

*Bone in healthy 8-year-olds (Paper III)*

Bone mineralization in relation to anthropometry and physical activity was examined in 96 Caucasian children. Bone mass differed between boys and girls at the age of 8 years except for the measurement of  $BMD_{LS}$ . The boys had a wider bone than the girls, whereas the calculated bone mineral density, BMAD, of the lumbar spine was higher in the girls. This finding probably reflected the larger bone in the boys. The boys had higher values for all other measurements, and all measures except BMAD were correlated to anthropometry.

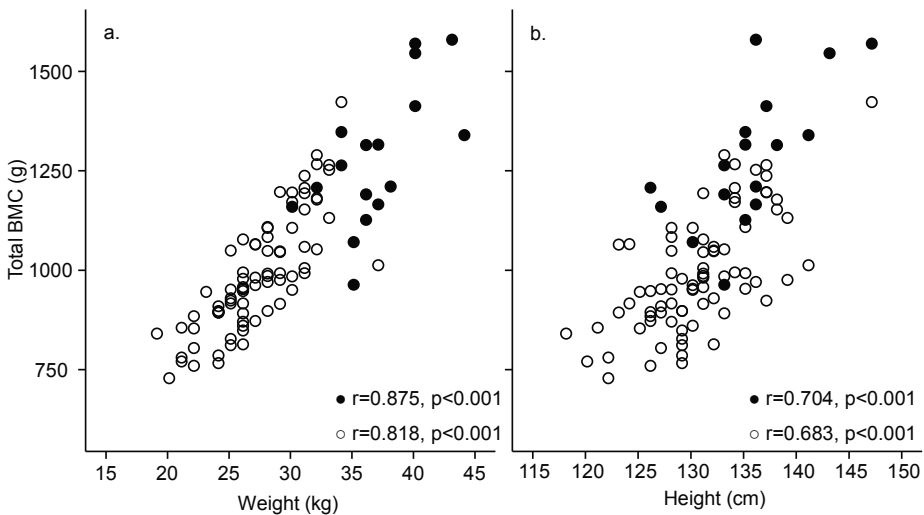


Figure 8. A correlation of BMC in total body to weight (a) and height (b) respectively was seen both for children with normal weight (○) and overweight children (●). Overweight children were taller than children of normal weight and therefore also height is important when interpreting the results of bone measurements.

Overweight children had higher BMC and BMD than children of normal weight, but no difference was seen for BMAD. The results suggest that the higher value of  $BMD_{LS}$  was due to bone size, rather than a higher mineralization and therefore not necessarily indicating a stronger skeleton. If overweight children have higher or lower bone mass compared to children of normal weight has been investigated previously with ambiguous conclusions<sup>75,76</sup>, depending on

whether height or weight was used as reference (Figure 8). Due to the relatively narrow age and weight range in this specific population conclusion can not be drawn, but the results point to the importance of considering the bone size, when interpreting results from bone measurements in growing subjects. Boys have been found to be at risk of being misdiagnosed for osteopenia when non-sex specific references are used and this is especially important in early adolescence since girls reach puberty earlier than boys and this is associated with an increased bone accretion<sup>77</sup>.

Physical activity is known to be beneficial for skeletal growth, in particular weight-bearing physical activity, which was confirmed in this study. School based intervention studies in young children have also shown that moderate physical activity, without specific osteogenic programs were positively influencing bone mineral acquisition and bone width in pre-pubertal boys<sup>78</sup> and girls<sup>79</sup>. Exercise during the pre-pubertal years is especially beneficial to increase bone density<sup>80,81</sup>.

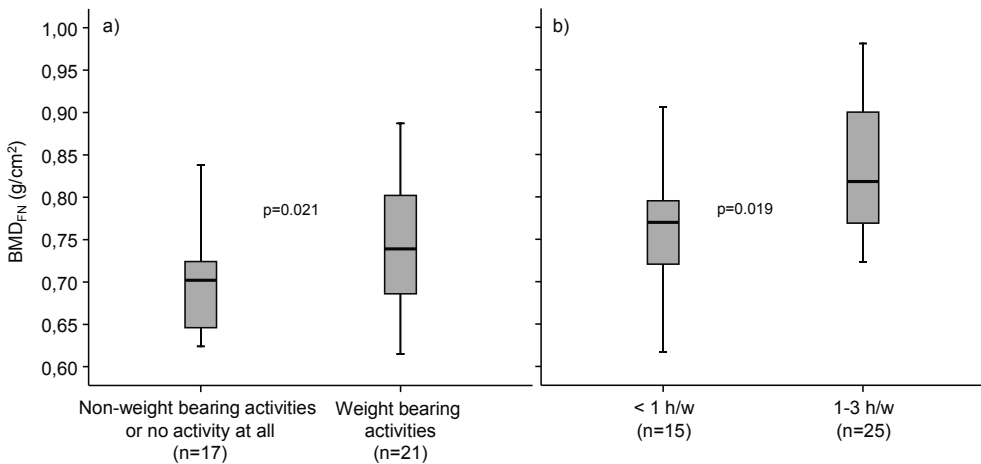


Figure 9. Girls performing weight bearing activities had higher bone mineral density in the femoral neck (FN) than girls performing non-weight bearing activities or no activities at all (a). Boys spending 1-3 hours/week on physical activities had higher bone mineral density in FN than boys spending less than 1 hour/week on physical activity (b).

Most of the 8-year olds reported some kind of physical activity during leisure time and a difference was seen in  $BMD_{FN}$  between the girls who performed WBA vs. those who performed non-WBA or no physical activity at all (Figure 9a). In the boys, an association was seen between time spent on physical activity and  $BMD_{FN}$  (Figure 9b). These differences might be related to a higher spontaneous activity in the boys which has been shown by others<sup>82</sup>. A limitation of the study was that the proposed activity measurement with an actigraph was not successfully completed and therefore the spontaneous activity could not be evaluated. The results that self-reported physical activity, although a crude measure, correlates to BMD has also been found by others<sup>83</sup>.

The study showed that the BMC and BMD measurements agreed well with the reference values provided by the manufacturer. The reference values are based on 6 studies of a total of 2000 children, aged 5-19 years from Europe, Australia and USA<sup>84-89</sup>. The good agreement between the 8-year-olds investigated and the reference values given by the manufacturer suggest that large control groups may not be needed when performing bone measurements in children.

**Summary paper III:**

- **Bone mineral content and bone mineral density differed by gender.**
- **Overweight children had higher bone mineral density than children with normal weight but this was related to a larger bone size.**
- **Self-reported physical activity correlated to femoral bone mineral density.**
- **Good agreement was found between bone measurements in healthy 8-year-olds and the reference values provided by the manufacturer.**

*Metabolic markers and BMD in 8-year olds. (Paper IV)*

Blood samples for analysis of calcium, vitamin D and serum phospholipid fatty acids were provided by 85 (89%) of the 96 Caucasian children performing the bone measurements.

Serum concentration of calcium and vitamin D was higher in girls than boys. The only association between these metabolic markers and bone mineralisation was to BMAD in the lumbar spine, but the association disappeared when adjusted for anthropometry, gender and fatty acids. The lack of associations between BMD, calcium and vitamin D confirmed previous studies of healthy school children and adolescent girls<sup>90,91</sup>. A negative association was seen between serum concentrations of vitamin D and the total concentration of n-6 fatty acids ( $r = -0.284$ ,  $p = 0.008$ ). Polyunsaturated fatty acids such as AA and LA have been found to compete with 25(OH)D and 1,25(OH)<sub>2</sub>D<sub>3</sub> for binding to the vitamin D-binding protein<sup>92</sup>. Essential fatty acids enhance calcium absorption from the gut by improving the effects of vitamin D, to reduce urinary excretion of calcium, to increase calcium deposition in bone and improve bone strength<sup>93</sup>. This suggests a complex interaction between phospholipid fatty acids, calcium and vitamin D and further longitudinal studies are desirable.

There were no difference in the serum phospholipid fatty acid concentrations between boys and girls. An association was found between serum concentrations of saturated fatty acids and BMD confirming the findings in children with cystic fibrosis<sup>40</sup>. A positive association has also been found between dietary saturated fatty acids and BMD in healthy children and adolescents<sup>94</sup>.

Linoleic acid (LA; 18:2n-6), as well as the total n-6 concentration, and the ratio of n-6/n-3 FA were negatively associated with BMD in the lumbar spine, rich in trabecular bone. Arachidonic acid (AA; 20:4n-6) was positively correlated to both BMC and BMD of the total body, where cortical bone is more apparent. The ratio of AA/LA was positively correlated to the BMD of the total body and the corresponding z-score. The inverse influence of LA and AA on bone mineralization might indicate that different fatty acids have diverse impact on cortical and trabecular bone. Watkins et al. have suggested that balanced levels of AA are of importance since an overload of AA may lead to an

overproduction of PGE<sub>2</sub>, which affects bone formation in a dose dependent way, stimulatory at low levels and inhibitory at high levels<sup>54</sup>. This might be of special importance during bone growth in childhood as also suggested by Gronowitz et al., who only found correlations between serum phospholipid fatty acids and bone mineralisation in children and not in adults with cystic fibrosis<sup>40</sup>.

Omega-3 fatty acids have been positively associated with bone mineral accrual in healthy adolescent males<sup>38</sup>, as previously shown in animal studies<sup>55</sup>. The only association to n-3 fatty acids in the 8-year-olds was a positive correlation between DHA and BMD of the lumbar spine in the children with a body weight >32 kg, the children representing the third tertile for body weight. In adolescents with cystic fibrosis, DHA was associated with the endosteal circumference of the long bones<sup>39</sup>. Since the attainment of peak bone mass is region specific<sup>95,96</sup>, and the long bones are the last to attain peak bone mass, these findings may suggest that n-3 fatty acids acts as regulators in the later stage of bone mineralisation.

Genetic factors vouch for a large part of the variability in bone mass but changes in fatty acid composition of the food may influence the formation of bone in children. Furthermore, optimal nutritional conditions with sufficient intake of vitamin D and calcium during childhood and adolescence will have a positive impact on peak bone mass and thereby decrease the risk of future osteoporosis related fractures.

**Summary paper IV:**

- **Both saturated and polyunsaturated serum phospholipid FA were associated with BMC and BMD in healthy children.**
- **Linoleic acid and arachidonic acid influenced BMD in an opposite pattern suggesting different impact on BMD in total body and lumbar spine.**
- **The interaction between vitamin D and fatty acids implies the need for further investigations.**

## **METHODOLOGICAL CONSIDERATIONS**

The population of the study was representative of that in Sweden, except for the fact that more parents held a university degree. This was also the case at the age of 4 years. Many parents hesitated to participate in the study if a 7-day food registration was to be used as a measure of dietary intake. Therefore the decision was made to use a combination of the 24-hour dietary recall and the FFQ. The 24-hour dietary recall has limitations due to the lack of day to day variation but strong relationships have been found between FFQ and 24-hour recalls suggesting that the methods supplement each other when estimating dietary intake<sup>97</sup>.

Even though the population was relatively small, the results from the bone measurements and blood samples would present preliminary reference intervals, which are mainly lacking at this age. All bone measurements were assessed by the same scanner operator and the same equipment. All blood samples were taken by the same two nurses at both 4 and 8 years of age and analysed with the same methods at the same internationally accredited laboratory (the Sahlgrenska University Hospital).

## CONCLUSIONS

- The population was representative for Sweden except for the fact that more parents held a university degree.
- Seventeen percent of the children were overweight with no difference by gender.
- Food choice was similar to that at the age of 4 years suggesting that food habits are established at an early age.
- Metabolic markers differed between boys and girls although no gender difference was seen in anthropometric measures.
- Between 4 and 8 years of age serum leptin increased and adiponectin decreased in both boys and girls. The boys also showed a decrease in serum cholesterol and the girls in insulin concentrations.
- Most children performed some kind of physical activity and weight bearing activity was reflected in the bone mineral density of the hip for both boys and girls.
- Higher bone mineral density in boys and overweight children seemed to be due to larger bone size.
- Serum phospholipid fatty acids were associated with bone mineralization in healthy children.

## **CLINICAL RELEVANCE**

- BMI was a good marker for overweight and fat mass in healthy 8-year-olds and is easy to use in clinical settings.
- Leptin was the best metabolic marker for overweight in this age group.
- Although a small population study, the results of the metabolic markers may fill a need as preliminary reference intervals for healthy children
- Bone mineralization in healthy children is associated to serum phospholipid fatty acids, previously only shown in animal studies. There seems to be a complex interaction between the fatty acids and vitamin D and calcium.
- The bone measurements agreed with the reference values provided by the manufacturer, thus, suggesting that large control groups may not be needed when performing DXA in children.

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