# Vitamin D in northern latitudes

## Intake and status in Icelandic children

**Birna Þórisdóttir**

**Thesis for the degree of Doctor of Philosophy**

UNIVERSITY OF ICELAND
SCHOOL OF HEALTH SCIENCES

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*Supervised by*

Professor Ingibjörg Gunnarsdóttir, University of Iceland

Professor Bryndís Eva Birgisdóttir, University of Iceland

*PhD Committee*

Professor Ingibjörg Gunnarsdóttir, University of Iceland

Professor Bryndís Eva Birgisdóttir, University of Iceland

Professor Geir Gunnlaugsson, University of Iceland

Professor Kim F. Michaelsen, University of Copenhagen

*Opponents*

Professor Lene Frost Andersen, University of Oslo

Professor Ásgeir Haraldsson, University of Iceland



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*“Lærdómstími ævin er.”*

Helgi Hálfdánarson

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# Abstract

**Background:** Adequate nutrition in childhood is essential for growth, development and health. Vitamin D supplement use is recommended in Iceland, starting in infancy. Little information exists on the vitamin D status of Icelandic infants and children. Vitamin D has been suggested to affect the development of sensitization to food allergens and food allergy.

**Aim:** To study adherence to dietary guidelines among 6-year-old children (paper I), their vitamin D intake and vitamin D status at 12 months and 6 years (papers II and III) and compare vitamin D and feeding in infancy between 6-year-old children IgE-sensitized to food allergens and non-sensitized children (paper IV).

**Methods:** The study population is a nationally representative Icelandic cohort of infants born in 2005, followed up at 6 years of age. Three-day weighed food records were kept at 9 months (n=196), 12 months (n=170) and 6 years (n=162). Total vitamin D intake was calculated from both diet and supplements. Further infant nutrition data was collected by dietary history from birth to 5 months and by monthly 1-day food records at 5-8 and 10-11 months of age. Serum 25-hydroxyvitamin D (25(OH)D) was measured at 12 months (n=76) and 6 years (n=139) and serum-specific IgE-antibodies against food at 6 years (n=144). Cut-off values for vitamin D deficiency, insufficiency, sufficiency and possibly adversely high levels were set at 25(OH)D <30 nmol/L, 30-50 nmol/L, >50 nmol/L and >125 nmol/L, respectively. The cut-off value for sensitization was set at specific IgE ≥0.35 kUA/L.

**Results:** Adherence to dietary guidelines varied among 6-year-old children but was poor in general. A quarter, or less, of the children followed the guidelines for fruit and vegetables, fish, wholegrain bread and other fiber-rich cereals and vitamin D supplements. The food intake was mirrored in a non-optimal distribution of macronutrients, fiber and salt intake. Vitamin and mineral density of the diet seemed however adequate, except for vitamin D.

Supplements (fish liver oil or liquid infant drops during the first months) were the main sources of vitamin D. Total vitamin D intake was higher at 9-12 months than at 6 years (the median intakes were 8.7 µg/d vs. 4.9 µg/d, respectively, $p<0.01$).

The mean±SD concentration of 25(OH)D at 12 months was 98±32 nmol/L, with 92% of infants defined as having sufficient vitamin D status and none deficient. Vitamin D intake at 9-12 months, either from use of vitamin D supplements or consumption of fortified foods in significant amounts (e.g., 200 ml/d of formula), or both, was the main observed determinant for vitamin D status 12 months. Breastfeeding and season were not associated with vitamin D status at 12 months. Taking vitamin D supplements increased the risk of serum 25(OH)D at possibly adversely high levels, observed in a quarter of 12-month-old infants.

At 6 years, vitamin D status was lower than in infancy (57±18 nmol/L), 30% of children were insufficient and 6% deficient. Higher total vitamin D intake at 6 years, blood samples collected in summer and higher serum 25(OH)D measured at 12 months, were associated with higher likelihood of vitamin D sufficiency at 6 years. The vitamin D status decreased during autumn in children not using vitamin D supplements.

Fourteen 6-year-old children (10%) were sensitized to food allergens. Higher vitamin D intake at 12 months (OR=0.8, 95% CI=0.7-0.99) and vitamin D supplement use at 6 years (OR=0.2, 95% CI=0.1-0.98) were associated with lower risk of sensitization. Introduction of solid foods prior to four months was associated with increased risk of sensitization (OR=4.9, 95% CI=1.4-17). More weight gain and head circumference growth from 0-2 months and higher prevalence of overweight at 6 years was observed among sensitized than non-sensitized children.

**Conclusions:** The results indicate that a public health effort among Icelandic children is needed to increase adherence to dietary guidelines, including vitamin D supplement use. Healthy Icelandic infants and children receiving the recommended 10 µg/d vitamin D are likely to be vitamin D sufficient. Vitamin D insufficiency and deficiency may however be prevalent among 6-year-old children due to insufficient intake. Monitoring of diet and vitamin D status among Icelandic infants and children is important, looking both at low and high vitamin D levels. Further studies are needed on the associations of vitamin D and diet with food sensitization and allergy.

**Keywords:** infant, child, vitamin D, nutrition policy, public health

Ritgerð fyrir doktorsvörn í næringarfræði við Háskóla Íslands 2018

# Ágrip

**Inngangur:** Næring á fyrstu árum ævinnar skiptir miklu máli fyrir vöxt, þroska og heilsu. Á Íslandi eru D-vítamínbætiefni ráðlögð frá ungbarnaskeiði. D-vítamínbúskapur íslenskra barna hefur lítið verið rannsakaður. D-vítamín hefur verið tengt við þróun á næmi fyrir fæðu og fæðuofnæmi.

**Markmiðið:** Að rannsaka fylgni sex ára barna við opinberar ráðleggingar um mataræði (grein I), D-vítamíninntöku og D-vítamínbúskap við 12 mánaða og sex ára aldur (greinar II og III) og tengsl D-vítamíns og mataræðis við næmi fyrir fæðu við sex ára aldur (grein IV).

**Aðferðir:** Þátttakendur voru börn fædd árið 2005 sem tóku þátt í ungbarnarannsókn á fyrsta ári og eftirfylgnirannsókn við sex ára aldur. Allur matur, drykkur og bætiefni voru vigtuð og skráð í matardagbækur í þrjá samfellda daga við 9 mánaða (n=196), 12 mánaða (n=170) og sex ára aldur (n=162). Heildar D-vítamíninntaka var reiknuð úr mat og bætiefnum. Frekari upplýsingar um mataræði fengust með fæðusögu fram til fimm mánaða aldurs og mánaðarlegum eins dags matardagbókum á aldrinum 5-8 og 10-11 mánaða. Kannaður var styrkur 25-hýdroxývítamíns D (25(OH)D) í sermi við 12 mánaða (n=76) og sex ára aldur (n=139) og sértæk IgE mótefni fyrir fæðu voru mæld við sex ára aldur (n=144). D-vítamínbúskapur var skilgreindur út frá 25(OH)D gildum sem skortur (<30 nmól/L), ófullnægjandi (30-50 nmól/L), fullnægjandi (>50 nmól/L) og mögulega óæskilega hár (>125 nmól/L). IgE mótefnaprófið var jákvætt ef svarið var >0,35 kUA/L.

**Niðurstöður:** Mataræði sex ára barna samræmdist almennt séð ekki ráðleggingum. Fjórðungur barna, eða tæplega það, fylgdi ráðleggingum um neyslu á ávöxtum og grænmeti, fiski, heilkorna brauðum og trefjaríkum kornvörum og D-vítamínbætiefnum. Samsetning orkugefandi næringarefna, trefjaneysla og saltneysla var ekki eins og best verður á kosið. Hins vegar veitti mataræðið sem svarar ráðlögðum dagskammti fyrir flest vítamín og steinefni að undanskildu D-vítamíni.

D-vítamínbætiefni (lýsi og AD dropar á fyrsta ári) voru aðal D-vítamíngjafarnir. Miðgildi D-vítamíninntöku var hærra við 9-12 mánaða aldur (8,7 µg/d) en við sex ára aldur (4,9 µg/d, p<0,01).

Meðalstyrkur D-vítamíns var 98±32 nmól/L við 12 mánaða aldur, 92% barna hafði fullnægjandi D-vítamínbúskap og ekkert þeirra D-vítamínskort. D-vítamíninntaka við 9-12 mánaða aldur, annaðhvort með notkun D-vítamínbætiefna eða D-vítamínbættra ungbarnavara (t.d. 200 ml/d af Stoðmjólk), var tengd fullnægjandi D-vítamínbúskap. Brjóstagjöf og árstíð voru ekki tengd D-vítamínbúskap við 12 mánaða aldur. Fjórðungur 12 mánaða barna hafði mögulega óæskilega háan D-vítamínbúskap og notkun D-vítamínbætiefna jók líkur á því.

Meðalstyrkur D-vítamíns var lægri við sex ára aldur (57±18 nmól/, $p<0.01$), 30% barna höfðu ófullnægjandi D-vítamínbúskap og 6% voru með D-vítamínskort. Hærri D-vítamíninntaka við sex ára aldur, D-vítamín mælt að sumri til og hærri D-vítamínbúskapur mældur við 12 mánaða aldur juku líkur á fullnægjandi D-vítamínbúskap. D-vítamínbúskapur sex ára barna lækkaði að hausti til meðal barna sem ekki notuðu D-vítamínbætiefni.

Fjórtán sex ára börn (10%) voru næm fyrir fæðu. Hærri D-vítamíninntaka við 12 mánaða aldur (OR=0,8; 95% CI=0,7-0,99) og notkun D-vítamínbætiefna við sex ára aldur (OR=0,2; 95%CI=0,1-0,98) tengdust minni hættu á næmi. Líkur á næmi jukust ef byrjað var að gefa fasta fæðu fyrir 4 mánaða aldur (OR=4,9; 95% CI=1,4-17). Meðal næmra barna sást meiri þyngdaraukning og höfuðvöxtur frá fæðingu til tveggja mánaða aldurs og hærri tíðni ofþyngdar við sex ára aldur.

**Ályktanir:** Niðurstöðurnar benda til þess að átaks sé þörf svo að íslensk börn fylgi betur ráðleggingum um mataræði, þar á meðal um notkun D-vítamínbætiefna. Þær benda til þess að heilbrigð íslensk börn sem fylgja ráðleggingum, þ.e.a.s. fá 10 µg af D-vítamíni á dag, séu líklega með fullnægjandi D-vítamínbúskap. Ófullnægjandi D-vítamínbúskapur og D-vítamínskortur var hins vegar algengur meðal sex ára barna í rannsókninni vegna lítillar D-vítamíninntöku. Mikilvægt er að fylgjast með mataræði og D-vítamínbúskap íslenskra barna og beina sjónum bæði að lágum og háum blóðgildum. Þörf er á frekari rannsóknum á tengslum D-vítamíns og mataræðis við næmi og ofnæmi.

**Lykilorð:** ungbörn, börn, D-vítamín, næringarstefna, lýðheilsa

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# Contents

# List of abbreviations

| | |
|---|---|
| $1,25(OH)_2D$ | 1,25-dihydroxyvitamin D |
| 25(OH)D | 25-hydroxyvitamin D |
| 3-epi-25(OH)D | C-3 epimers of 25-hydroxyvitamin D |
| BMI | body mass index |
| CI | confidence interval |
| EBF | exclusive breastfeeding |
| EFSA | European Food Safety Authority |
| ESPGHAN | European Society for Paediatric Gastroenterology, Hepatology and Nutrition |
| FBDG | food-based dietary guideline |
| IGE | immunoglobulin E |
| IOM | Institute of Medicine |
| LC-MS/MS | liquid chromatography tandem-mass spectrometry |
| LI | lower intake level |
| NNR | Nordic Nutrition Recommendations |
| OR | odds ratio |
| RI | recommended intake |
| SD | standard deviation |
| UL | upper intake level |
| WFR | weighed food records |
| WHO | World Health Organization |

# List of figures

# List of tables

# List of original papers

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals:

I. Gunnarsdottir I, Helgadottir H, Thorisdottir B, Thorsdottir I. Landskönnun á mataræði sex ára barna 2011-2012 (Diet of six-year-old Icelandic children - National dietary survey 2011-2012). Læknabladid (The Icelandic Medical Journal). 2013 Jan;99(1):17-23.

II. Thorisdottir B, Gunnarsdottir I, Steingrimsdottir L, Palsson GI, Thorsdottir I. Vitamin D intake and status in 12-month-old infants at 63-66° N. Nutrients. 2014 Mar 21;6(3):1182-93.

III. Thorisdottir B, Gunnarsdottir I, Steingrimsdottir L, Palsson GI, Birgisdottir BE, Thorsdottir I. Vitamin D intake and status in 6-year-old Icelandic children followed up from infancy. Nutrients. 2016 Feb 4;8(2):75.

IV. Thorisdottir B, Gunnarsdottir I, Vidarsdottir AG, Sigurdardottir S, Birgisdottir BE, Thorsdottir I. Feeding in infancy, vitamin D and IgE sensitization to food allergens at 6 years: a longitudinal cohort (manuscript submitted for publication).

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# Declaration of contribution

The data in this thesis originates from an Icelandic longitudinal, nationally representative, study on nutrition, growth and health in the first year of life (2005/2007) and a follow-up at 6 years (2011/2012). The doctoral candidate, Birna Þórisdóttir (BÞ), participated in data collection in the follow-up study under the supervision of Professor Ingibjörg Gunnarsdóttir and the principal investigator Professor Inga Þórsdóttir as part of her MSc thesis, from which two scientific papers focusing on protein intake, growth and BMI were published [1, 2]. The data collection involved explaining study procedures to participants and their parents, entering consumption data into the nutrient calculation program and assisting the collaborating pediatrician in the collection of blood samples, to give some examples.

BÞ has been the data manager of the overall cohort study from the start of her Ph.D. studies. She has been responsible for merging data from the infant and follow-up studies and assuring the performance, integrity and security of the resulting database. As such, BÞ has participated in and guided other students' work with the data. BÞ carried out the analyses in cooperation with coworkers, interpreted the data and participated in the drafting of paper I. The identification of vitamin D as a critical nutrient in the diet of Icelandic children, supported by previous studies, set the tone for a focus on vitamin D in her Ph.D. thesis. BÞ developed the research questions and planned the research work for papers II-IV, with guidance and feedback from her supervisors, co-authors and doctoral committee. BÞ performed the statistical analyses, was responsible for data interpretation, drafted papers II-IV and had primary responsibility for the final content. During her studies, BÞ presented the results of the research project at several domestic and international conferences to ensure feedback and discussion and to share knowledge about the results of the study.

During her time as a Ph.D. student, BÞ has actively participated in two other ongoing research projects, through which she has gained further experience in writing study protocols, seeking ethical permission and funding and collecting data. The Icelandic Maternal and Child Health (ICE-MCH) study has access to nationwide infant nutrition data from the Primary Health Care in Iceland and authorizations to merge it with certain data from other registries. The IceAge2 study is a prospective study of the interaction between nutrition, body composition and other determinants of growth and development during infancy. The data collection is in final stages, with 80 out of the planned 100 participants recruited. Furthermore, BÞ was a member of an expert group appointed by the Directorate of Health to revise the Icelandic infant nutrition recommendations in 2016-2017. BÞ initiated and was responsible for updating the national nutrient database on infant foods in 2017. She has given lectures at courses offered by the Faculty of Food Science and Nutrition at the University of Iceland and co-supervised two M.Sc. students' and four B.Sc. students' final research projects. BÞ has participated with nutrition education in the University for the Young and University Knowledge Train (outreach programs at the University of Iceland). She has also given lectures on infant and young child's nutrition for child-care professionals and the public.

# 1 Introduction

Adequate nutrition in infancy (used in this thesis as the first two years of life) and childhood is essential for growth, development and both short- and long-term health. Two studies, with data collection in the first year of life and a follow-up at age 6 in nationally representative cohorts, were carried out in Iceland. The former study was in 1995/1996 with the follow-up in 2001/2002. The latter was in 2005 with the follow-up in 2011/2012. The overall aim of studying the Icelandic infant and child cohorts was to investigate diet and nutrition, growth, development and several biomarkers relevant to health. The former study brought to light critical issues related to iron and protein intake among infants [3-6]. The Icelandic infant nutrition recommendations were revised in 2003 to emphasize longer breastfeeding and the consumption of a new product, Icelandic formula intended for infants from 6 months to 2 years (*Stoðmjólk*), instead of regular cow‘s milk [7]. The latter study showed that dietary intake in infancy had moved towards the revised recommendations, followed by improved iron status and lower prevalence of overweight/obesity in childhood [2, 8]. These results highlight the importance of monitoring diet and nutritional status, as findings can be used in the development of strategies aiming at improving dietary habits and public health. **Paper I** reports the dietary intake of 6-year-old children in the latter study.

The importance of fish liver oil (*lýsi*) during the long Icelandic winter has long been known. Between 1913 and 1940, contributions from research groups in the US, England and Scotland led to the discovery of vitamin D and the findings that either the use of cod liver oil or exposure to sunlight were effective in preventing and treating rickets [9]. Based on this new knowledge, Icelandic children started receiving cod liver oil in schools around 1930, replaced by vitamin D tablets in 1954 [10]. Children who were prone to getting colds, were slim or had general poor health according to school doctors or nurses were additionally administered to light therapy (*ljósböð*) [10]. Both the provision of cod liver oil and administration to light therapy in schools gradually ceased with changes in the Icelandic diet and society. Fish liver oil (or more recently any type of vitamin D supplement) has been a part of the Icelandic dietary guidelines since they were first launched in 1987 [11]. Consistent with the most recent Icelandic dietary guidelines [12] the term “vitamin D supplement” used in this thesis covers both fish liver oil, liquid vitamin D (e.g., vitamin A- and D-drops, recommended for infants until 2008, or vitamin D-drops, currently recommended), and vitamin D tablets.

In the past decade or so, associations found between vitamin D and several non-communicable diseases have stirred great interest in vitamin D among both scientists and currently the public. In Iceland, like many other countries, a common belief about widespread vitamin D deficiency in the population has led to public discussions over the optimal dose of vitamin D supplements and increased frequency of vitamin D measurements in blood. In this social context, recent local data on the population's vitamin D status is important. **Paper II** presents the first assessments of vitamin D status among Icelandic infants. **Paper III** presents the vitamin D status at 6 years of age.

Another current matter of both scientific and public interest is the association of diet, including breastfeeding, age of introduction of solid foods, and vitamin D, and food allergy. **Paper IV** compares feeding in infancy and vitamin D between sensitized and non-sensitized 6-year-old children.

# 2 Background

## 2.1 Importance of childhood nutrition

In a world where both under- and overnutrition continue to challenge health and well-being, there are many good reasons to study childhood nutrition [13, 14]. There is general agreement about the long-term benefits of breastfeeding and avoidance of overweight/obesity during childhood [15-20]. Systematic reviews also report convincing relationships between very high protein intake during complementary feeding and higher body mass index (BMI) and body fatness [21-23] as well as between reduced saturated fatty acid intake in childhood and a more favorable blood lipid profile and blood pressure [24]. Modest relations have been reported between dietary patterns in childhood and adolescence and depression [25], executive function [26] and academic achievements [27]. Furthermore, dietary patterns are already established in childhood and have been shown to track through childhood into adulthood and associate with disease risk [28-30]. The best available scientific evidence on dietary patterns, foods or nutrients that help to maintain good health, is the basis for dietary guidelines [31, 32].

## 2.2 Dietary guidelines

Infants have different nutritional needs than older children due to rapid growth, development and progress from depending fully on liquid feedings to being ready to eat the family diet. Infant nutrition recommendations by the World Health Organization (WHO) [33], European Food Safety Authority (EFSA) [34], European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) [35] and Nordic co-operation, resulting in the Nordic Nutrition Recommendations (NNR) [31, 36], are basically similar although the optimal age for the introduction of solid foods is currently a matter of great debate and research focus [37, 38]. The Icelandic infant nutrition recommendations encourage exclusive breastfeeding (EBF) for the first 6 months of life, if possible [39]. If this is not possible, then only infant formula should replace breast milk in the first 4 months. Nutritionally adequate and safe complementary feeding should preferably start at 6 months, and no earlier than at 4 months. Continued breastfeeding is encouraged as is the use of follow-on formula (such as the Icelandic *Stoðmjólk*, hereafter called formula) from 6 months instead of regular cow's milk up to 1-2 years of age. Vitamin D supplements are recommended from 2-4 weeks of age.

Recommendations for individual nutrients are age- and sex-specific and presented as intake ranges of percentages of total energy intake for protein, carbohydrates and fats, and absolute values in mg/d or µg/d for vitamins and minerals [31]. In the first 6 months of life, requirements for individual nutrients are generally considered equal to the supply from breast milk, with the exception of vitamin D [31]. Breast milk, even from well-nourished mothers using vitamin D supplements, contains little vitamin D [40]. Therefore, vitamin D supplementation of 10 µg/d to infants up to 1 year of age is currently universally accepted and recommended in Europe and North America [31, 41-43].

The Icelandic food-based dietary guidelines (FBDGs) apply to adults and children from 2 years of age [12]. They are based on the NNR [31] and are in general agreement with national guidelines from the other Nordic countries [44-47], recommendations on prevention of cancer [15] and cardiovascular disease [48, 49], to give some examples. The Icelandic FBDGs encourage high consumption of fruit and vegetables, fish, wholegrain and other fiber-rich breads and cereals; modest amounts of meat and low-fat milk and milk products; limitation of salt and sugar; choosing vegetable oils over butter; and daily use of vitamin D supplements [12, 50].

## 2.3 The sunshine vitamin

Iceland is one of few countries officially recommending vitamin D supplements for the whole population at all ages [31, 51]. This emphasis is based on expectations of little vitamin D skin synthesis in the population living at 63-66°N. Vitamin D is a unique vitamin for many reasons, one being that most people in the world do not rely on dietary vitamin D but produce enough vitamin D in their skin to meet their needs [52]. When exposed to ultraviolet B with wavelengths 290-315 nm, the cholesterol derivative 7-dehydrocholesterol in the skin is converted, first to previtamin $D_3$, and then to vitamin $D_3$ (cholecalciferol), which is bound to the vitamin D binding protein before entering the circulation for transport to the liver [53]. Latitude and season are important factors affecting the possibility for cutaneous vitamin D synthesis [53, 54]. In Iceland, sun availability for cutaneous vitamin D synthesis is considered negligible for 7-8 months of the year [55, 56]. Therefore, dietary vitamin D is of uttermost importance.

## 2.4 Dietary vitamin D

Few foods, most of them of animal origin, naturally contain useful sources of vitamin D. Fatty fish is the best source (estimated range 5-15 µg/100 g), but meat and offal (<1 µg/100 g), full-fat dairy products such as butter and cream (1-2 µg/100 g) and egg yolk (6 µg/100 g) can also contain useful amounts [57]. A method called biofortification has the potential to enhance the vitamin D content in natural sources although the bio-accessibility of vitamin D in these biofortified foods warrants further study [58]. For example, the addition of vitamin D to animal feeds can increase the vitamin D content of fish, meat and eggs [58] and radiation of mushrooms and yeast can increase their vitamin D content [58].

Traditional fortification, i.e., the addition of vitamin D to selected, widely consumed foods, is a more established public health strategy to increase the vitamin D intake of populations [32]. Infant and follow-on formulas are mandatorily fortified with 1-3 µg vitamin D per 100 kcal in Iceland and elsewhere [59-61] and cereal-based infant products (e.g., porridge) can be voluntarily fortified [62]. Vitamin D fortification of milk, spreads (butter and margarine) and breakfast cereals are important for the general population in the Nordic countries [31, 63].

Vitamin $D_3$ occurs naturally in foods, while vitamin $D_2$ (ergocalciferol) is a synthesized form used in some fortified foods, supplements and drugs [64]. Vitamin $D_2$ is very rarely used in foods and beverages on the Icelandic market, and its use elsewhere may be decreasing due to studies reporting that it is less effective than $D_3$ in raising and maintaining vitamin D status [65-67]. An additional form, 25-hydroxyvitamin D (25(OH)D) is considered a biologically active vitamin $D_3$ metabolite that is absorbed from animal foods and can raise serum 25(OH)D concentrations [68-70].

Vitamin D from the diet is absorbed together with fat in the small intestine, with levels of absorbed vitamin D peaking around 24 hours after ingestion, and packaged into chylomicrons [66]. Vitamin D absorption is improved with concurrent consumption of fat-containing food and is impaired in conditions involving intestinal fat malabsorption [66]. In the circulation, vitamin D from the diet is transported by chylomicron remnants or bound to the vitamin D binding protein to reach the liver [71].

## 2.5 Activation of vitamin D

Vitamin D from cutaneous synthesis or diet is hydroxylated in the liver to 25(OH)D (calcidiol) [72]. Studies suggest that following oral doses of vitamin D, 25(OH)D levels increase gradually, peaking after approximately 7-14 days [66]. This circulating form has a half-life of 2-3 weeks [73]. In the kidneys, a second hydroxylation, converts 25(OH)D to the biologically active 1,25-

dihydroxyvitamin D (1,25$(OH)_2$D, calcitriol) [72]. The concentration of many factors in the blood, including parathyroid hormone, phosphorus, calcium and 1,25$(OH)_2$D itself affect the renal production of 1,25$(OH)_2$D [71]. This biologically active form has a short half-life (4 hours) and circulates at much lower concentrations than 25(OH)D [73]. The functions of 1,25$(OH)_2$D are that of a steroid hormone. It mediates its biological effects through an intracellular vitamin D receptor [74].

## 2.6 Vitamin D and health

The primary role of vitamin D is to regulate blood calcium and phosphorus levels by enhancing calcium and phosphorus absorption in the small intestine, and together with parathyroid hormone if necessary, assisting the release of calcium from bone into blood and stimulate reabsorption of calcium in the kidney [71]. Therefore, vitamin D is essential for bone health throughout life. Interestingly, the symptoms associated with vitamin D deficiency in children were well described before vitamin D was identified, and exposure to sunlight and intake of cod liver oil were suggested as cures [75]. In children, this disease, nutritional rickets, caused by deficiency of vitamin D, calcium or both, involves defective mineralization of growing bones and cartilage [71]. This can lead to bone pain, skeletal deformities, adverse effects on growth and motor development and increased infection risk [73]. In adults, vitamin D deficiency is associated with osteomalacia [73].

Vitamin D receptors have been found in many cells and tissues beyond those associated with the maintenance of calcium homeostasis [74]. A few hundred systematic reviews and meta-analyses have addressed the relationship between vitamin D and disease, with varying results. It has been suggested that studies focusing on those with low vitamin D status are more likely to find effects [76, 77]. The strongest evidence for benefits of vitamin D in adults are for all-cause mortality [78-82], and various cancer types [83-86]. In children, vitamin D deficiency has been associated with an adverse lipid profile [87] and increased incidence of type 1 diabetes [88, 89].

The theory has been advanced that tracking of biomarkers, i.e., their stability over time, may be more associated with health and disease than single measurements [90]. For example, that constant low vitamin D status in childhood is more predictive of peak bone mass or later disease than low vitamin D status measured once [91, 92]. Studies have found tracking of vitamin D status from birth to 10 years of age [91], from childhood into early adulthood [92] and over a 14-year period among adults [93]. This is however not supported by all studies [94]. It remains unknown whether low vitamin D status might be the cause or effect of ill health [95].

Associations between increasing latitude and food allergy prevalence have been reported, suggesting a role of vitamin D in allergy [96]. Also, although not published until recently, we had knowledge of findings from another Icelandic research group of associations between fish liver oil consumption in infancy and food sensitization and allergy [97]. Our data provided an opportunity to measure sensitization to food allergens in a cohort with well documented vitamin D intake (including fish liver oil intake) in infancy and at age 6.

## 2.7 Vitamin D and food allergen sensitization

The immune response in immunoglobulin E (IgE) mediated food allergy, which is the most common form of food allergy, begins with sensitization by a specific food allergen and leads to upregulation of type 2 helper T cell responses and secretion of allergen-specific IgE antibodies [98]. If a medical history and physical examination give cause to suspect IgE-mediated food allergy, the presence of allergen-specific IgE should be assessed and allergy confirmed by a positive double-blind, placebo-controlled food challenge when necessary [99]. The detection of serum IgE antibody identifies whether a state of sensitization exists (i.e., marks the individual as “sensitized”) but does not in isolation prove the existence of clinical allergy [100]. Allergen-specific IgE can be detected by skin prick tests or by immunoassay of serum allergen-specific IgE [99].

The likelihood of clinical allergy correlates with allergen-specific IgE results [101-105]. It has been suggested that primary prevention of allergy should focus on preventing immunological sensitization [106]. Therefore, studies on sensitization may be important to study determinants of and monitor changes in the prevalence of allergic sensitization. US and European studies, for example, include such research and monitoring [107, 108].

Despite known immune modulation by vitamin D, the role of vitamin D in the development of food allergies is unclear. While a systematic review found weak, but suggestive, evidence of a negative association between vitamin D intake and allergy risk (i.e., the lower the vitamin D intake, the higher the allergy risk) [109], the ESPGHAN Committee on Nutrition found the evidence insufficient to support an association between vitamin D supplementation in infants and children and prevention of allergic disease [41]. Two randomized trials investigating the effects of infant vitamin D supplementation on allergy risk are ongoing [110, 111].

Recent systematic reviews have reported no significant associations between vitamin D status and food allergy in children [112] or between prenatal vitamin D status and offspring allergic sensitization [113]. However, some studies suggest that vitamin D insufficiency increases the risk of IgE-mediated food allergy in infants [114] or food sensitization in infants and children [115, 116]. A longitudinal study with repeated cross-sectional measurements throughout the first 10 years of life suggested the same [91]. However, other studies suggest that prenatal vitamin D excess [117, 118] and high-dose vitamin D supplementation in infancy [119] may increase the risk of food allergy or atopy.

The relationship between 25(OH)D and allergy may not be linear. One study on adults proposes a U-shaped association between 25(OH)D and total serum IgE [120]. In this study, elevated IgE levels were observed in participants with the lowest (<25 nmol/L) and highest (>135 nmol/L) serum 25(OH)D. In line with this, high maternal vitamin D status (>125 nmol/L) has been associated with an increased risk of allergic diseases in offspring [118]. Animal studies support this dual effect of vitamin D [121]. Similarly, U-shaped associations between maternal vitamin D status during pregnancy or cord blood 25(OH)D levels and aeroallergen-specific IgE and asthma have been suggested [122, 123]. Insufficient knowledge regarding the optimal vitamin D status for immune function limits interpretation of studies [112].

## 2.8 Assessment of vitamin D status

The quantitative assessment of circulating 25(OH)D in serum or plasma is considered the best indication of vitamin D status in humans [43]. Beyond general acceptance that 25(OH)D <25-30 nmol/L may increase the risk for rickets in infants and children, there is no global consensus on the terminology and cut-off levels used to describe vitamin D status [41, 43, 82].

The literature review prepared for the NNR 2012, a position paper by the ESPGHAN Committee on Nutrition in 2013 and a review carried out for the Institute of Medicine (IOM) in 2011, however, all found evidence supporting that a 25(OH)D concentration of 50 nmol/L could be a reasonable threshold for bone health and other outcomes [41, 82, 124]. In NNR 2012 and by the IOM 2011, 25(OH)D above 50 nmol/L is used to indicate vitamin D sufficiency, 30-50 nmol/L to indicate an insufficient vitamin D status and below 30 nmol/L to indicate deficiency [31, 124].

While the US Endocrine Society concluded the evidence sufficient to suggest that 25(OH)D levels above 75 nmol/L may have additional benefit for risk reduction of cancer, diabetes, autoimmune disease, cardiovascular disease and infections [125], the IOM did not find 25(OH)D levels above 75 nmol/L consistently associated with increased health benefit [124].

Using hypercalcemia as an indicator of vitamin D toxicity and taking into account evidence linking high vitamin D levels with all-cause mortality, cardiovascular disease, some cancers, falls and fractures, the IOM considered that there may be reason for concern regarding vitamin D excess at 25(OH)D concentrations above 125 nmol/L [124]. EFSA currently uses 150 nmol/L as the upper cut-off concentration before risk increases [126]. In a new, but unpublished, update by EFSA, available data from infants is used to suggest that 25(OH)D levels up to 200 nmol/L may be unlikely to increase risk of adverse health outcomes in healthy infants [127]. While it is considered impossible to get vitamin D toxicity from skin synthesis alone, very high doses of vitamin D supplements pose a risk [128].

## 2.9 Dietary reference values

For infants up to 1 year, the NNR, EFSA and IOM define an adequate intake of 10 µg/d vitamin D [31, 124, 129]. The adequate intake is based on infant supplemental studies where a dose of 10 µg/d is associated with no clinical deficiency, serum 25(OH)D ≥50 nmol/L as well as traditional use of this supplemental dose without reported adverse effects.

The literature review prepared for the NNR 2012 suggested that assuming minimal cutaneous synthesis and the target serum 25(OH)D of 50 nmol/L, an average intake of 10 µg/d vitamin D would be required from 3 years of age (50% of a population may need more, 50% less) [82]. Adding 2 SD would result in an intake of 15 µg/d that would meet the needs of 97.5% of a population. This is in agreement with the estimated average requirement (10 µg/d) and recommended dietary allowance (15 µg/d) from 1 year of age defined by the IOM [124] and the adequate intake of 15 µg/d from 1 year of age defined by EFSA [129]. The IOM and EFSA also used 50 nmol/L as target serum 25(OH)D and performed meta-regression analysis of studies assessing the relationship between total vitamin D intake and serum 25(OH)D [124, 129]. In the NNR 2012 itself some cutaneous vitamin D synthesis was assumed and the average requirement was set at 7.5 µg/d and the recommended intake (RI) at 10 µg/d from 6 months of age [31].

The Icelandic dietary guidelines are based on the NNR but set the RI (*ráðlagður dagskammtur*) of vitamin D on their own. In the two latest revisions, 2006 and 2014, the RI for children from 6 months of age is 10 µg/d [12, 50].

The upper intake level (UL) is the maximum level of long-term vitamin D intake that is unlikely to pose a risk of adverse effects [31]. The NNR 2012 cites EFSA and IOM for UL for vitamin D [124, 126]. Both EFSA and IOM scale children levels down from adult levels, using slightly different methods. EFSA suggests an UL of 25 µg/d up to age 1 and 50 nmol/L for 1-10-year-old children [126]. In the previously mentioned new, but unpublished, update, EFSA suggests increasing the UL for 6-11-month-old infants to 35 µg/d, with other UL remaining unchanged [127]. The IOM suggests using 25 µg/d up to 6 months, 38 µg/d for 6-11-month-old, 63 µg/d for 1-3-year-old and 75 µg/d for 4-8-year-old children [124].

The NNR defines the lower intake level (LI) as a cut-off value below which most individuals are considered at risk of clinical deficiency symptoms. The cut-off LI was set at 2.5 µg/d for adults but data considered insufficient to set LI for infants and children [31].

## 2.10 Determinants of vitamin D status

Determinants of vitamin D status in children vary considerably between and within populations because of the complex interplay of determinants at different levels (e.g., personal, environmental, cultural, and political factors). At birth, the serum 25(OH)D of infant and mother is highly correlated [130]. While the correlation may persist for some months after birth [131], very soon other factors start to affect the infants' vitamin D status. Vitamin D status is often not significantly different between boys and girls [132-135]. Since vitamin D in the body can be stored in fat cells, adiposity (BMI, obesity, body fat mass) has been associated with lower circulating 25(OH)D levels in the blood [135, 136].

Exclusive breastfeeding compared with formula feeding is a risk factor for vitamin D deficiency among infants not using supplements [41] and even in a cohort where all infants used vitamin D supplements, the duration of exclusive and total breastfeeding negatively associated with vitamin D status [137]. However, breastfeeding is frequently associated with higher socioeconomic status and health-conscious behavior [138-140], which in turn associate with higher vitamin D status in some studies [132, 141, 142]. These results show the complexity of the situation.

Young children of (non-Western) immigrant parents were suggested to have 50 times higher risk for rickets when compared with children of parents of Nordic origin in a study on the diet of minority groups in the Nordic countries [143]. This is also evident in other large population-based samples in Europe and North America [132, 141, 144], even despite higher prevalence of reported vitamin D supplement use and higher total vitamin D intake among immigrant than native children in Germany [141]. Vitamin D supplement use was found to be very common among 6- and 12-month-old infants of Somali and Iraqi origin residing in Norway in a recent study [145, 146].

Large epidemiological studies have suggested that genetics may explain some of the variance in 25(OH)D between individuals [147-149]. These same studies, and others, strongly suggest that factors affecting vitamin D skin synthesis are the main determinants of vitamin D status in most populations of the world, including children in northern Europe [132, 135, 137, 141, 150-153]. Among factors other than latitude and season that can affect skin synthesis are the time of day, altitude, thickness of the ozone layer, heavy clouds, pollution, skin color, covering clothing, use of sunscreen, time spent outside and sun/shadow-seeking behavior [53, 154].

Systematic reviews of worldwide vitamin D status have reported higher average 25(OH)D in North America than Europe, and within Europe, higher 25(OH)D in northern than southern populations [155, 156]. The former has been attributed to more widespread vitamin D fortification in North America than Europe [155]. The latter sounds contradictory, based on latitude. However, it has mainly been attributed to vitamin D supplement use in northern Europe [155, 156].

## 2.11 Supplementation and fortification

In the European Nutrition and Health Report 2009, mean vitamin D intake among 4-6-year-olds and 7-9-year-old boys and girls ranged from 1.6 to 6.5 µg/d in 10 European countries, with the highest mean intakes in Norway, Sweden and Finland [157]. The contribution of vitamin D supplements to total vitamin D intake could not be determined from this report but was seen in another review of 8 European nationally representative dietary survey data [158]. Excluding supplements from calculations, mean national vitamin D intake did not exceed 2.3 µg/d in the youngest children (1-3 years) or 3.5 µg/d in older children (4-10 years). When supplements were included in calculations, however, total vitamin D intake increased, especially in countries where supplement use was customary. To give an example from Denmark, the mean total vitamin D intake, including supplements, was 7.8 μg/d among 4-10-year-old children [158].

Finnish studies have shown that a national recommendation of vitamin D fortification of fluid milk and margarine in 2003 had beneficial effects on the vitamin D status of infants and children up to 12 years [159, 160]. The frequency of 0-12-year-old children with 25(OH)D levels below 50 nmol/L was reduced by almost half (37% vs. 70%), and fewer children had low vitamin D status (25(OH)D <25 nmol/L) [160]. Furthermore, the studies found no evidence of either excessive vitamin D intake or alarmingly high 25(OH)D levels because of the fortification.

However, an example from Canada demonstrates the obvious, that vitamin D food fortification is not effective in raising its status if the fortified products are not consumed commonly enough. Following decreased consumption of vitamin D fortified milk from 2007/2009 to 2012/2012, a decrease in the proportion of vitamin D sufficient children was later found [161].

## 2.12 Vitamin D status in neighboring countries

The ESPGHAN Committee on Nutrition suggested, after summarizing published data on vitamin D intake and status in European children, that 25(OH)D levels below 50 nmol/L were commonly observed among healthy European infants, children and adolescents [41].

Studies on healthy infant cohorts from Denmark and Finland, with high prevalence of vitamin D supplement use (>95%), have found the majority of infants (>80%) vitamin D sufficient, with relatively high mean serum 25(OH)D concentrations, e.g., 94 nmol/L at 4 months [131], 77-82 nmol/L at 9 months [131, 137] and 74 nmol/L at 14 months [162].

Around 76% vitamin D sufficiency was reported in 1- and 2-year-old children in Oslo in 2000 [163]. Vitamin D sufficiency was observed among 90% of 4-year-old and 70% of 8-year-old children in Gothenburg in 1999/2000 [164]. Samples considered nationally representative of US and Canadian children (1-11-year-old and 3-12-year-old, respectively) have shown >74% vitamin D sufficiency, with mean levels ranging from 63 to 70 nmol/L [161, 165]. Somewhat lower mean levels have however been observed among 1.5-3-year-old (58 nmol/L) and 4-10-year-old children (50 nmol/L) in a UK national diet and nutrition survey [166].

Children using vitamin D supplements had higher 25(OH)D levels than non-users (estimated 5-10 nmol/L higher, based on the US, Canadian, Swedish and Norwegian studies presented above). Seasons affected vitamin D status in the studies measuring vitamin D during different times of the year, even among 9-month-old infants in Denmark [131, 137]. The Norwegian and Canadian studies also showed that frequent consumption of vitamin D fortified formula or milk was associated with higher 25(OH)D levels (compared with breastfed infants in the study on 1-year-old children in Oslo, compared with children using fortified milk less frequently in the Canadian study). Overweight or obese children and those having non-native mothers had lower 25(OH)D in the US, Canadian and Swedish studies. Also, higher vitamin D levels in younger than older groups were generally observed, likely due to higher vitamin D intake.

This highlights the importance of monitoring vitamin D status of vulnerable groups, including infants and children, in each country, as determinants vary significantly and cannot easily be transferred to other populations. We therefore considered it important to measure vitamin D in Icelandic infants and children.

# 3 Aims

The aim of this thesis was to study food and nutrient intake in a nationally representative sample of Icelandic children, their vitamin D status and food allergen sensitization.

The specific aims were to study:

- adherence to dietary guidelines among 6-year-old children (paper I).
- vitamin D intake in infancy and at 6 years (papers II and III).
- vitamin D status at 12 months and 6 years (papers II and III).
- vitamin D intake, seasons and other possible determinants of vitamin D status (papers II and III).
- vitamin D and feeding in infancy among IgE-sensitized and non-sensitized 6-year-old children (paper IV).

# 4 Methods

## 4.1 Study design

This thesis uses data from an Icelandic cohort of infants born in 2005, prospectively investigated for diet and growth throughout the first year of life and followed-up at 6 years of age. Blood samples were collected with the primary aim of analyzing iron status and blood lipids. Excess serum samples were frozen for the possibility of later analysis. Information on socioeconomic status and other characteristics of children and their parents, as well the children‘s development was gathered from questionnaires answered by parents. The study design, including the data collected at each age and key numbers of participants, is shown in Figure 1.

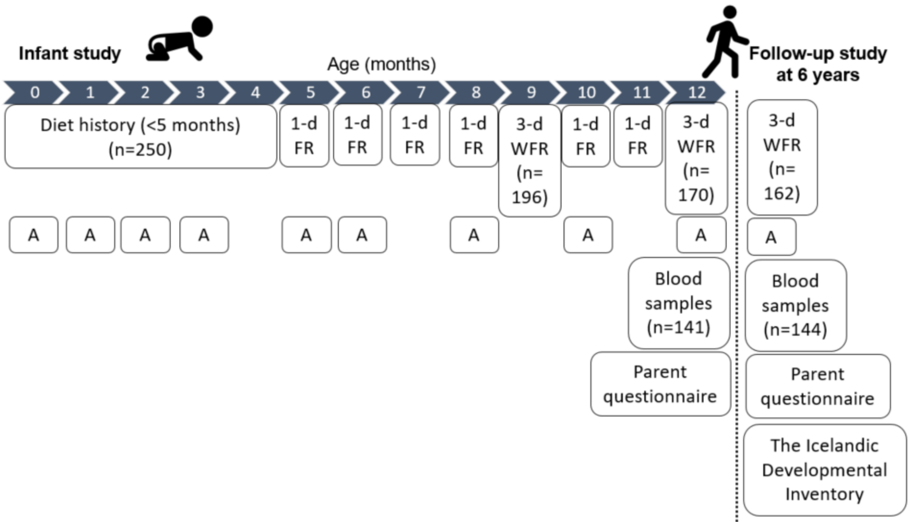


**Figure 1.** A schematic demonstration of the study design, from birth to 12 months in the infant study and at 6 years in the follow-up study.

Abbreviations: A=anthropometrics, d=day, FR=food record, WFR=weighed food record.

## 4.2 Study population

A random nationwide sample of 250 Icelandic infants born January-December 2005 (6% of live-born infants in Iceland in 2005) was selected by Statistics Iceland. The inclusion criteria were Icelandic parents, singleton birth, gestational length of 37-41 weeks, birth weight within the 10$^{th}$ and 90$^{th}$ percentiles, no birth defects or congenital long-term diseases and mothers' regular antenatal care. The sample was considered statistically representative for the infant population of the whole country, with a 50/50 girl/boy ratio, even distribution of births throughout the year, 66% urban living (Reykjavik area or Akureyri), birth weight, length and head circumference of 3747±360 g, 51.9±1.7 cm and 35.9±1.2 cm, respectively. From 5 to 8 months, 54 infants dropped-out of the study. Infants still participating after 9 months (n=196) were invited to a blood sample collection at 12 months, resulting in 141 blood tests. Participants with any available data at 9 months or later were invited to a follow-up at 6 years, resulting in 162 food records (65% of original sample) and 144 blood tests.

Table 1 gives an overview of the main dietary and biochemical data, as well as the number of participants studied in **papers I-IV** and characteristics of participants in the follow-up. The number of participants studied in each paper depends on the main outcome variable. **Paper II** has the smallest number of participants (n=76), restricted by the number of serum samples from 12-month-old infants available for analysis of 25(OH)D.

Neither basic characteristics of the child or parent, growth or breastfeeding in the first months of life differed between children included in the papers/thesis and the original sample. During the years leading to the follow-up study, mothers gained on average 1.6 kg (SD 6.0) and fathers 1.3 kg (SD 5.6). In the infant study vs. follow-up, 48% vs. 62% of mothers had completed university education.

**Table 1.** Overview of the main data used in this thesis and characteristics of the participants in the follow-up at 6 years.

<table>
<tr><th></th><th>Dietary data</th><th>Biochemical data</th><th>Characteristics (n=162)<br>Mean±SD or %</th></tr>
<tr><td>Paper I</td><td>Overall diet at 6 years (n=162)</td><td></td><td rowspan="4">Girl/boy 52/48%<br>Urban living 68%<br>First born 32%<br>Birth weight 3747±362 g<br>Birth length 51.9±1.6 cm<br>Birth head circumf. 35.8±1.2 g<br>Weight at 12 mo. 10.1±1.1 kg<br>Weight at 6 years 22.9±3.5 kg<br>EBF duration 3.6±1.9 months<br>Total BF duration 7.9±3.2 mo.<br>Mother/father smoking 5/17%<br>Mothers vs. fathers age<br>31.2±5.0 vs. 34.0±5.8 years<br>Mothers vs. fathers BMI<br>24.9±5.2 vs. 26.2±3.1 kg/m$^2$</td></tr>
<tr><td>Paper II</td><td>Vitamin D intake at 9-12 months, 0-9 months for details</td><td>Serum 25(OH)D at 12 months (n=76)</td></tr>
<tr><td>Paper III</td><td>Vitamin D intake at 6 years, also infancy for details</td><td>Serum 25(OH)D at 6 years (n=139), tracking from 12 months</td></tr>
<tr><td>Paper IV</td><td>Vitamin D intake in infancy and at 6 years, feeding style 0-6 months</td><td>Serum-specific IgE at 6 years (n=144)</td></tr>
</table>

Abbreviations: 25(OH)D=25-hydroxyvitamin D, EBF=exclusive breastfeeding, BF=breastfeeding, BMI=body mass index, IgE=immunoglobulin E.

## 4.3 Dietary assessment

Detailed information about infant feeding from birth until 4 months (17 weeks), including breastfeeding, introduction of formula and solid foods, and use of vitamin D or other dietary supplements, was gathered with one structured dietary recall facilitated by a trained interviewer (mean infant age at dietary recall was 21 weeks). The exact age when the infant received vitamin D supplement for the first time was registered along with the type. The exact age when the infant received something other than breastmilk and vitamin D supplements was also registered. One-day non-weighed food records, using common household measures, were kept at 5, 6, 7, 8, 10 and 11 months. Information on breastfeeding duration was collected from food records and retrospectively confirmed with parents.

At 9 months, 12 months and 6 years, 3-day weighed food records (WFR) were kept for 72 consecutive hours, preferably over two weekdays and one day during the weekend (i.e., Thursday-Saturday or Sunday-Tuesday). Parents were responsible for the registration, but staff at daycare or schools also registered intake if needed. All food items and fluids were weighed on accurate scales (Philips type HR 2385, Hungary and Austria) with 1-g precision. Parents got detailed directions on recording, use of scales, and how to account for leftovers. In addition, they were instructed to register the type and dose of dietary supplements if they were used during the recording days.

The nutrient calculation program used in the infant and 6-year-old studies, ICEFOOD, calculates energy, nutrients, heavy metals, caffeine and artificial sweeteners from information contained in ISGEM, the Icelandic food composition database [167], and additional recipe databases for products on the Icelandic market for the general population and infants and presents values per 100 g of the edible part, after adjusting for possible loss due to storage and food preparation. The average daily consumption of foods and nutrients was calculated from the 3-d WFR. Total vitamin D intake is the sum of vitamin D from both diet and supplements. More details can be found in **papers I-IV**.

## 4.4 Blood sampling and biochemical analysis

At 12 months and 6 years, venous blood samples were collected in the morning in fasting state at Landspitali by a certified pediatrician and centrifuged within 6 h of collection. Separated serum samples were stored at -80°C at the biobank at Landspitali. They were later analyzed by biomedical scientists at Landspitali for serum 25(OH)D at 12 months (April 2013), serum 25(OH)D at 6 years (December 2013) and serum-specific IgE to food allergens at 6 years (January 2014).

Serum 25(OH)D levels were analyzed by using an electro-chemiluminescence immunoassay (Elecsys Vitamin D total assay, Modular Analytics E170, Roche Diagnostics, Mannheim, Germany) (measuring range 7.5-175.0 nmol/L, precision 0.1 nmol/L). Tests were performed according to the manufacturer's instructions [168]. Cut-off values for vitamin D deficiency, insufficiency, sufficiency and possibly adversely high levels were set, as suggested by the IOM and NNR, at 25(OH)D <30 nmol/L, 30-50 nmol/L, ≥50 nmol/L and >125 nmol/L, respectively [31, 124].

To classify participants according to the expected contribution of cutaneous synthesis to vitamin D status, four categories were constructed for this thesis; blood samples collected in July-August (major contribution, "summer"), September-October (moderate contribution, "autumn“), November-April (minor contribution; "winter/spring"), April-June (moderate contribution; “spring”).

Specific IgE levels were analyzed for ImmunoCAP® allergen Fx5, using ImmunoCAP fluoroenzyme immunoassay (Phadia 250, Thermo Scientific, Uppsala, Sweden) (measuring range 0-100 kUA/L, precision 0.01 kUA/L). This is a food mix test detecting specific IgE to six major food allergens in children (cow's milk, egg white, cod, wheat, soy bean and peanut). If there was a positive Fx5 test, specific IgE was further analyzed for each of the six major food allergens. Tests were performed according to the manufacturer's instructions and classified as a positive test, using the term “IgE sensitized” or simply “sensitized”, when specific IgE was ≥0.35 kUA/L [100, 107, 108].

## 4.5 Parental and child characteristics

Structured questionnaires answered by parents (usually mothers), when participants were 12 months and 6 years old, collected information on the number of siblings, parental smoking after birth, parents' age, parents' self-reported weight and height (from which BMI was calculated) and parental education. Educational level was defined as the highest level of completed education and categorized into: basic (elementary school, 10 years), medium (high school or vocational school, approx. 14 years), and higher education (university, >16 years). At 6 years, the questionnaire included questions on children‘s habitual physical activity and children's regular use of antihistamines and asthma inhalers.

## 4.6 Anthropometrics

Information about weight, length and head circumference at birth was gathered from the maternity wards. The primary healthcare provided measurements of weight, length and head circumference as close to the 2-, 6-, 10- and 12-month birthdays as possible. Weight was recorded with 5-g precision, length measured on length boards and recorded with 0.1-cm precision, and head circumference was recorded with 0.1-cm precision. At follow-up at 6 years (mean age 73.4±3.2 months), weight (Marel M series 110, Iceland) (precision 0.1 kg) and height (Ulmer stadiometer, Professor Heinze, Busse design, Ulm, Germany) (precision 0.1 cm) were measured at Landspitali. Children were classified as being normal weight or overweight/obese according to the International Obesity Task Force cut-off levels for overweight, defined to pass through the BMI of 25 $kg/m^2$ at 18 years of age [169]. Cut-off points of 17.55 $kg/m^2$ and 17.34 $kg/m^2$ were applied for 6-year-old boys and girls, respectively. Gain in weight, length or head circumference were calculated for each participant as the difference in measurements between two time-points.

## 4.7 Statistical analysis

Statistical analysis was performed with SAS (9.2, Enterprise Guide 4.3, 7.1; SAS Institute Inc., Cary, NC, USA). Variables were examined for normality using Quantile Quantile-plots and presented as n (%), mean and standard deviations (SD) or medians and $25^{th}$ to $75^{th}$ percentiles. For comparison between groups, chi-square, two-sided t-test or Mann Whitney U-test was used. Linear and logistic regression analyses were used to examine possible associations with continuous and categorical variables, respectively, and presented as standardized coefficients (β) or the odds ratio (OR) with 95% confidence intervals (CI). The level of significance was set at <0.05. Further information can be seen in **papers I-IV**.

## 4.8 Approvals

Informed written consent from the parents was obtained, and all individual information was processed with strict confidentiality. The study was conducted according to the Declaration of Helsinki. The infant study was approved by the National Bioethics Committee (VSNb2005040019/037) and registered at the Icelandic Data Protection Authority (S2449/2005). The follow-up study was also approved by the National Bioethics Committee (VSNb2011010008/037) and registered at the Icelandic Data Protection Authority (S5099/2011).

# 5 Results

Characteristics of the infant diet, from birth to 12 months of age, have been extensively reported elsewhere [2, 8, 170, 171]. Therefore, only a short summary is provided before moving on to the results of this thesis.

Almost all infants (99%) were breastfed. The proportion of EBF infants declined every month (82% at 1 months, 75% at 2 months, 68% at 3 months, 57% at 4 months, 42% at 5 months) and was only 8% at 6 months of age. More than a quarter of 4-month-old children had received solid food, most commonly infant porridge, and this proportion increased to 50% at 5 months and 92% at 6 months. Most infants (88%) were still breastfed when introduced to solid food and 21% were still breastfed at 12 months of age. Formula consumption was more common than regular cow's milk at 6-12 months. Overall adherence to infant nutrition recommendations in the first year of life was rather good [8].

## 5.1 Diet at 6 years (paper I)

Table 2 shows the adherence of 6-year-old children to the Icelandic FBDGs. Less than 20% of the children consumed dietary fiber in line with recommendations. Fruit and vegetable consumption was less than recommended (mean±SD 187±119 g/d when excluding juice from calculations), fiber-rich bread (≥6 g dietary fiber per 100 g) was 20% of total bread consumed and oatmeal porridge was consumed by 25% of participants. Consumption of biscuits and cake was almost three times that of fiber-rich bread. Cereal-based foods, such as bread and breakfast cereals, contributed much of the fiber intake but also much of the salt intake.

The highest adherence (87%) was to the recommendation of at least two portions per day of milk and milk products. However, 10% of children consumed over 600 g/d of milk/milk products. This food group was the largest dietary source of saturated fat, followed by oils/fats, and meat/meat products. The proportional distribution of fatty acid intake was not optimal (see paper I, Table III). The contribution of saturated plus trans fatty acids to total energy intake was 14.1%. Foods with low nutrient density, i.e., biscuits, cakes, candy, ice cream and sugar sweetened beverages, provided up to 25% of total energy, over 25% of saturated fatty acids and 60% of added sugar in the diet.

**Table 2.** Adherence to the Icelandic food-based dietary guidelines at 6 years (n=162).

| Food-based dietary guidelines[1] | Criteria<br>Adjusted for 6-year-old children | n (%) |
|---|---|---|
| Fruits and vegetables | ≥400 g of fruits, vegetables and pure juice per day | 30 (19) |
| Fish at least two times per week or more | ≥34 g per day, which equals a fish meal two times per week | 37 (23) |
| Whole grain bread and other fiber-rich cereals | ≥2.5 g dietary fiber/MJ/d[2] | 28 (17) |
| Milk products containing less fat | 2 portions of milk or milk products daily (1 portion 200 g of milk products or ≥20 g of cheese) | 141 (87) |
| Oils or soft fat instead of saturated fat | <10% of the energy from saturated fat | 10 (6) |
| Moderation in salt intake | <3200 mg of salt per day[3] | 7 (4) |
| Cod liver oil or other vitamin D source | ≥5 ml per day, which equals daily recommendations for vitamin D | 44 (27) |
| Water is the best drink | <10% of the energy from added sugar | 70 (43) |

[1]Dietary guidelines and nutrition recommendations for adults and children from two years of age, 2006 edition [50].

[2]The Nordic Nutrition Recommendation on intake of dietary fiber is used to assess adherence to this recommendation (≥2.5 g/MJ/d) [36].

[3]The Nordic Nutrition Recommendation on salt intake ≤0.5 g/1000 kJ/d [36] corresponds to <3.2 g salt per day according to energy intake in the present study.

In general, the vitamin and mineral density of the diet seemed adequate (see paper I, Table IV). However, the distribution of fat-soluble vitamins was large, especially for vitamin A, where the highest intakes were above the UL set for adults (3000 µg/d). The results also suggest that some children may not satisfy their needs for iodine. Only a quarter of children consumed fish in line with recommendations. Vitamin D intake was low, with more than 25% of children below the LI set for adults (2.5 µg/d), and only a quarter of children were above the RI (10 µg/d). These children were the ones following the official recommendations of vitamin D supplement use during all three registration days.

These results, establishing vitamin D once more as a critical nutrient in the diet of Icelandic children, warranted a more thorough look at vitamin D intake and status in the cohort.

## 5.2 Vitamin D intake (papers II and III)

The proportion of infants receiving vitamin D supplements increased during the first months (50% at 1 month of age, 75% at 2 months, 85% at 3 and 4 months). In the first half year, vitamin D supplement use did not differ between EBF infants and infants receiving formula and/or solid food. Almost all infants (95%) received vitamin D supplements at some point.

Table 3 shows the total vitamin D intake and quantities from different sources, at 9 months, 12 months and 6 years. Total vitamin D intake did not differ significantly between 9 and 12 months, but it was significantly lower at 6 years than at both 9 and 12 months. The distribution of vitamin D intake was large, ranging from <0.5 µg/d to 25.1 µg/d at 9 months (one infant above 25 µg/d), 34.2 µg/d at 12 months (two infants above 25 µg/d) and 26.1 µg/d at 6 years. Very low vitamin D intake (<2.5 µg/d) was more common at 6 years (28%) than 9 and 12 months (13% and 15%, respectively). At 9 months, 12 months and 6 years, 45%, 42% and 23%, respectively, reached the RI of 10 µg/d. 40% of 6-year-old children reached the average intake of 7.5 µg/d.

**Table 3.** Vitamin D intake (µg/d) at 9 months, 12 months and 6 years.

| | Mean±SD | 5% | 10% | 25% | 50% | 75% | 90% | 95% |
|---|---|---|---|---|---|---|---|---|
| **9 months (n=196)** | | | | | | | | |
| Total vitamin D | 9.6±6.0 | 0.4 | 1.5 | 4.7 | 9.2 | 14.3 | 18.0 | 19.6 |
| From supplements | 5.3±4.9 | 0.0 | 0.0 | 0.0 | 3.8 | 10.0 | 11.3 | 15.1 |
| fish liver oil | 2.6±3.6 | 0.0 | 0.0 | 0.0 | 0.0 | 4.0 | 10.0 | 10.0 |
| other e.g., drops | 2.7±5.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.8 | 11.3 | 15.1 |
| From food | 4.3±3.4 | 0.2 | 0.4 | 1.4 | 3.5 | 6.6 | 9.0 | 9.8 |
| formulas | 2.4±2.4 | 0.0 | 0.0 | 0.0 | 1.5 | 4.7 | 5.9 | 6.5 |
| infant porridges | 1.6±2.2 | 0.0 | 0.0 | 0.0 | 0.7 | 2.7 | 4.3 | 5.3 |
| **12 months (n=170)** | | | | | | | | |
| Total vitamin D | 8.6±5.9 | 0.6 | 1.6 | 4.1 | 7.7 | 12.3 | 15.8 | 17.9 |
| From supplements | 5.1±5.0 | 0.0 | 0.0 | 0.0 | 5.0 | 10.0 | 10.0 | 11.4 |
| fish liver oil | 4.0±4.4 | 0.0 | 0.0 | 0.0 | 2.3 | 7.0 | 10.0 | 10.0 |
| other e.g., drops | 1.1±3.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.8 | 11.2 |
| From food | 3.5±2.9 | 0.2 | 0.3 | 0.9 | 3.0 | 5.1 | 7.3 | 8.3 |
| formulas | 2.3±2.4 | 0.0 | 0.0 | 0.0 | 1.8 | 4.0 | 5.5 | 6.8 |
| infant porridges | 0.6±1.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.7 | 2.6 | 3.6 |
| **6 years (n=162)** | | | | | | | | |
| Total vitamin D | 7.5±6.4 | 1.1 | 1.4 | 2.2 | 4.9 | 11.9 | 17.8 | 19.6 |
| From supplements | 4.9±6.1 | 0.0 | 0.0 | 0.0 | 1.6 | 10.0 | 16.0 | 16.0 |
| fish liver oil | 4.5±5.8 | 0.0 | 0.0 | 0.0 | 0.0 | 10.0 | 13.3 | 16.0 |
| other e.g., tablets | 0.4±1.7 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 1.5 |
| From food | 2.5±1.5 | 0.8 | 1.0 | 1.6 | 2.2 | 3.3 | 4.1 | 5.2 |
| breakfast cereals | 1.1±1.2 | 0.0 | 0.0 | 0.4 | 0.8 | 1.5 | 2.5 | 3.7 |
| fish | 0.4±0.9 | 0.0 | 0.0 | 0.0 | 0.1 | 0.3 | 0.7 | 1.2 |
| butter | 0.3±0.4 | 0.0 | 0.0 | 0.1 | 0.2 | 0.5 | 0.8 | 1.0 |

Supplements were the largest vitamin D sources at all three time-points, used by 72% of 9-month-old, 70% of 12-month-old and 57% of 6-year-old children during at least one of the three recording days. Fish liver oil was the main vitamin D supplement, although at 9 months, vitamin drops and fish liver oil provided similar amounts of vitamin D. Vitamin D intake was 3-4 times higher among those using supplements vs. those not using supplements (mean±SD: 11.7±5.3 µg/d vs. 4.0±3.6 µg/d at 9 months, 10.8±5.6 µg/d vs. 3.5±2.8 µg/d at 12 months, 11.9±5.7 µg/d vs. 2.5±1.5 µg/d at 6 years, respectively).

Formulas and infant porridges were additional important sources of vitamin D at 9 and 12 months. Vitamin D intake from food was higher at 9 than 12 months ($p=0.04$). This was mainly attributable to more vitamin D intake from infant porridge. At 6 years, breakfast cereals, fish and butter provided some vitamin D. At none of the three time-points (9 months, 12 months, and 6 years) did vitamin D intake from food differ between children using and not using vitamin D supplements.

Vitamin D supplement use at an earlier time-point increased the likelihood of later vitamin D supplement use. The correlation between vitamin D intake at 9 and 12 months was modest ($r=0.4$, $p<0.01$) and weaker but still significant between vitamin D intake at 9-12 months and 6 years ($r=0.2$, $p=0.02$). Calculated per kg of bodyweight, vitamin D intake decreased from 1.1±0.7 µg/kg/d at 9 months, to 0.9±0.6 µg/kg/d at 12 months and was 0.3±0.3 µg/kg/d at 6 years.

We have previously reported that socioeconomic factors were not associated with vitamin D supplement use in infancy [172]. At 6 years, higher BMI of the children (OR=0.8, 95 CI=0.6-0.96) and their mothers (OR=0.9, 95% CI=0.9-0.99) was associated with lower odds of the child's vitamin D supplement use. Neither gender, father's BMI nor parental age, parental education or parental smoking was associated with supplement use at 6 years of age. There were no significant differences in supplement use according to the month of record keeping, neither at 12 months or 6 years (Figure 2).

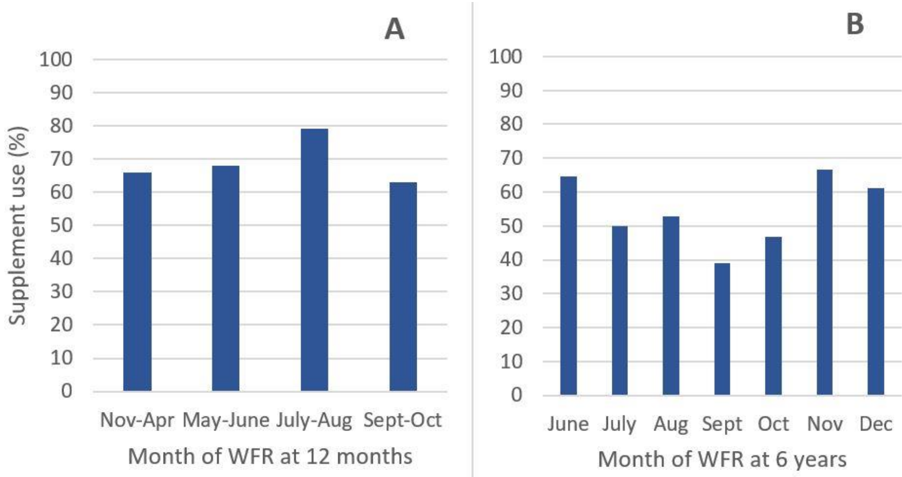


**Figure 2.** Vitamin D supplement use by recording month, at: **A)** 12 months; **B)** 6 years.

## 5.3 Vitamin D status (papers II and III)

Table 4 presents the distribution of serum 25(OH)D. Mean serum 25(OH)D levels were higher at 12 months than at 6 years (98±32 nmol/L vs. 57±18 nmol/L, $p<0.01$). At 12 months vs. 6 years, 92% vs. 64% of participants, respectively, were vitamin D sufficient (>50 nmol/L), 8% vs. 30%, respectively, vitamin D insufficient, and 0% vs. 6% at risk of deficiency (<30 nmol/L). At 12 months, 24% had serum 25(OH)D >125 nmol/L, but none exceeded 200 nmol/L. One 6-year-old child had 25(OH)D above 125 nmol/L.

**Table 4** Distribution of serum 25(OH)D levels (nmol/L) at 12 months (n=76) and 6 years (n=139).

| | min | 5% | 10% | 25% | 50% | 75% | 90% | 95% | max |
|---|---|---|---|---|---|---|---|---|---|
| 12months | 39 | 44 | 56 | 71 | 98 | 124 | 142 | 150 | 166 |
| 6 years | 14 | 24 | 35 | 46 | 56 | 66 | 77 | 87 | 135 |

Seventy-four children had serum 25(OH)D measured at both 12 months and 6 years, with a correlation of 0.3 ($p<0.01$). Almost half of the children stayed within the same serum 25(OH)D tertile from 12 months to 6 years (Table 5). Nine children (12%) moved either from the lowest or highest 25(OH)D tertile at 12 months to the other extreme at 6 years.

**Table 5.** Serum 25(OH)D tertiles at 12 months and 6 years for children measured at both time-points (n=74).

| 6 years / 12 months | Tertile 1, n | Tertile 2, n | Tertile 3, n |
|---|---|---|---|
| Tertile 1, n (% of total 74) | 12 (16) | 9 (12) | 4 (5) |
| Tertile 2, n (% of total 74) | 8 (11) | 10 (14) | 7 (9) |
| Tertile 3, n (% of total 74) | 5 (7) | 6 (8) | 13 (18) |

Figure 3 shows a strong inverse association between 25(OH)D at 12 months and the change in 25(OH)D (delta 25(OH)D) from 12 months to 6 years. This means that there is a regression towards the mean, i.e., those with very high serum 25(OH)D at 12 months had a greater decrease in serum 25(OH)D compared to those with lower serum 25(OH)D at 12 months. Thereby, the variability in serum 25(OH)D decreases from 12 months to 6 years. Apart from serum 25(OH)D at 12 months, the only baseline variable associated with the delta 25(OH)D was vitamin D intake in infancy.

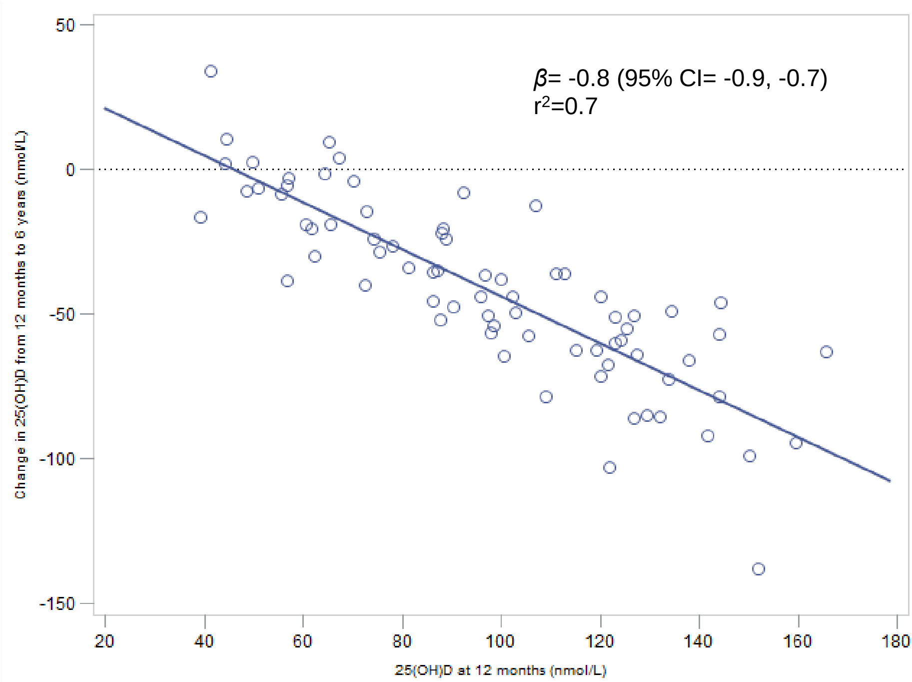


**Figure 3**. Relationship between serum 25(OH)D level at 12 months and change in serum 25(OH)D from 12 months to 6 years (n=74).

## 5.4 Determinants of vitamin D status at 12 months (paper II)

In a univariate linear regression model, vitamin D intake at 9-12 months (median 8.7 µg/d) was associated with serum 25(OH)D at 12 months ($\beta=2.9$, $p<0.01$). Other variables, such as duration of exclusive and total breastfeeding and socioeconomic status, were not associated with serum 25(OH)D at 12 months. No significant differences in 25(OH)D by season were observed, neither when splitting into four seasons (Figure 4) nor when combining summer/autumn months (103.5±32.3 nmol/L) and winter/spring months (94.6±34.7 nmol/L) ($p=0.2$).

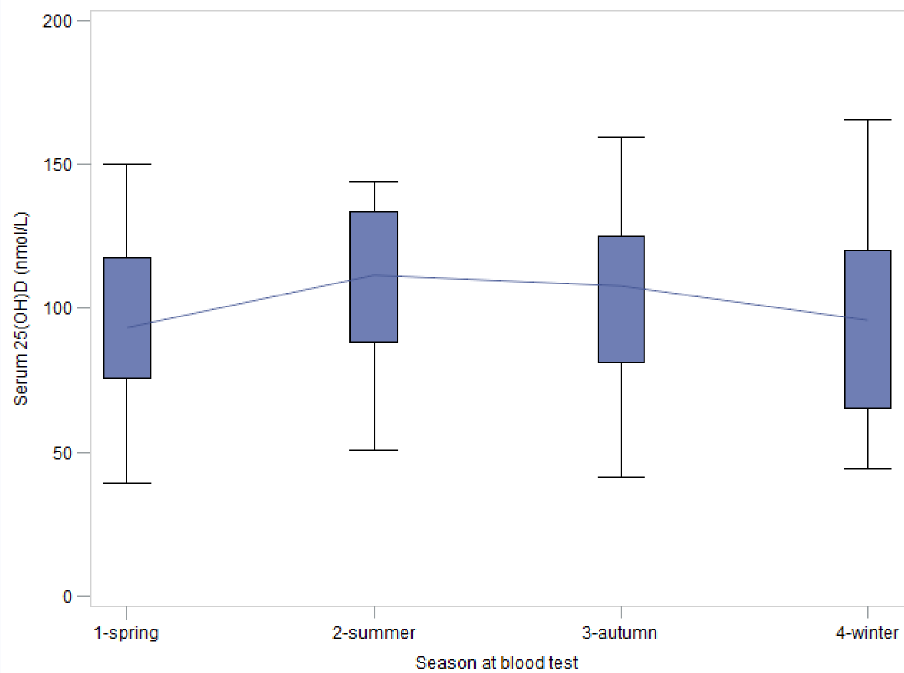


**Figure 4.** Serum 25(OH)D levels at 12 months by season at blood test.

Infants not using vitamin D supplements or fortified products in significant amounts at 9-12 months (i.e., "no or irregular" group) had significantly lower 25(OH)D levels than children consuming either supplements, or fortified products, or both (Table 6). Five out of six infants with 25(OH)D levels below 50 nmol/L belonged to this group. Moreover, those five children did not use vitamin D supplements, formula or infant porridge at all in infancy, according to the dietary data. The use of vitamin D supplements, increased the odds of having 25(OH)D levels above 125 nmol/L, OR=4.6 (95% CI=1.4-15.8). However, the risk of 25(OH)D levels above 125 nmol/L was not significantly higher in the “combined” than the “supplement” group.

**Table 6.** Vitamin D intake and status in infants categorized into groups, based on vitamin D intake at 9-12 months.

| Vitamin D intake groups | N (%) | Vitamin D intake (µg/d) | 25(OH)D (nmol/L) |
|---|---|---|---|
| "No or irregular" (neither fortified products nor supplements in significant amounts) | 20 (26) | 2.5±1.9 | 76.8±27.1 |
| "Fortified" (≥2.4 µg/d vitamin D from fortified products, e.g., ≥200 mL/d formula) | 17 (22) | 6.5±2.2 | 100.0±31.4 |
| "Supplement" (≥5.0 µg/d vitamin D from supplements, i.e., recommended dose on half of registration days) | 14 (18) | 8.8±2.7 | 104.6±37.0 |
| "Combined" (both fortified products and supplements in significant amounts) | 25 (33) | 14.3±3.0 | 110.3±26.6 |

## 5.5 Determinants of vitamin D sufficiency at 6 years (paper III)

Almost half of the blood tests (n=67) were performed in months categorized as summer (July-August), one-quarter in autumn (September-October, n=34) and fewer in winter (November-December, n=21) and spring (June, n=17). Vitamin D levels at 6 years, by month of blood sample collection, are shown in Figure 5. After seeing that both vitamin D supplement use (see Figure 2) and serum 25(OH)D levels in June had much more in common with the winter than the summer months, November-June were combined as "winter/spring.

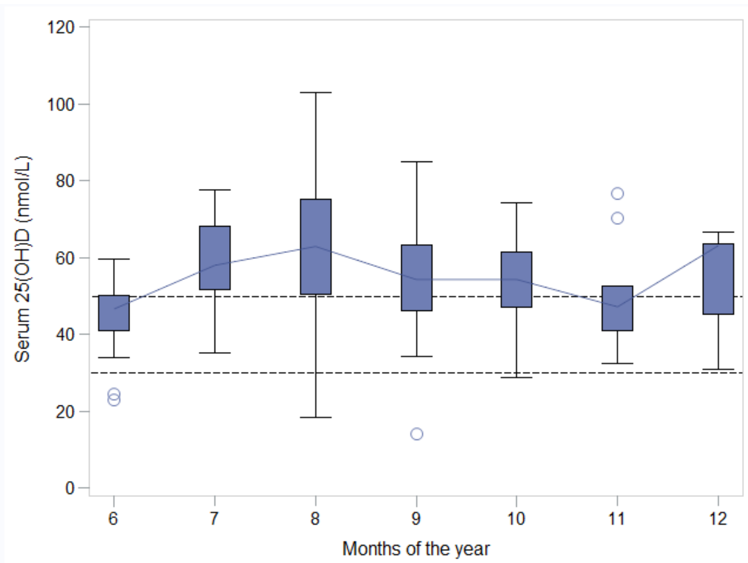


**Figure 5.** Serum 25(OH)D levels at 6 years, by month of blood test (6=June, 7=July, 8=August, 9=September, 10=October, 11=November, 12=December).

Compared with insufficient or deficient children, vitamin D sufficient 6-year-old children had been breastfed longer (median 9 vs. 7 months, p=0.02), had a higher total vitamin D intake (median 7.5 vs. 3.2 µg/d, p<0.01), and their blood samples were significantly more likely to have been obtained during summer and significantly less likely during winter/spring (see paper III, Table 1). Higher vitamin D intake, summer season and higher vitamin D status at 12 months were associated with vitamin D sufficiency at 6 years in a multivariate logistic regression analysis model (Table 7).

**Table 7.** Multivariate model of factors associated with vitamin D sufficiency at 6 years.

| | Model 1 (n=139)<br>OR (95% CI) | Model 2 (n=74)<br>OR (95% CI) |
|---|---|---|
| Breastfeeding duration, month | 1.1 (0.9-1.2) | 1.1 (0.9-1.3) |
| Vitamin D intake at 6 years, 10 µg/d | 3.2 (1.5-6.8) | 7.3 (1.8-29) |
| Blood test at 6 years during summer | 3.2 (1.4-7.1) | 3.3 (1.0-11) |
| Serum 25(OH)D at 12 mo., 10 nmol/L | | 1.2 (1.0-1.4) |

Eighty-three percent of 6-year-old children using vitamin D supplements in recommended amounts (average ≥10 µg/d from the 3-d WFR) were vitamin D sufficient, with no significant differences in serum 25(OH)D between seasons (Figure 6A). Among children using vitamin D supplements below recommended amounts (average <10 µg/d from the 3-d WFR), 94% of children with blood tests in summer were vitamin D sufficient compared with 75% in autumn and 33% in winter/spring (Figure 6B). If only looking at children not using vitamin D supplements at all in the 3-d WFR, 68% of those children with blood tests in summer were vitamin D sufficient compared with 41% in autumn and 17% in winter/spring (Figure 6C).

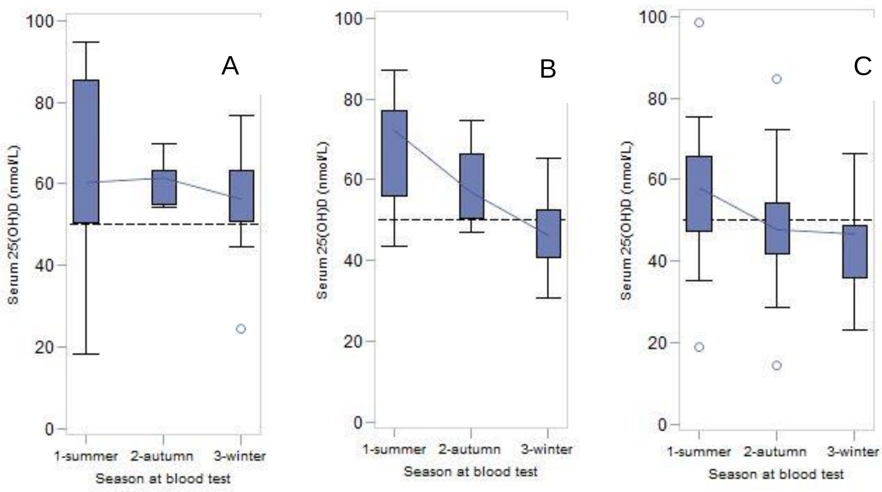


**Figure 6.** Serum 25(OH)D levels at 6 years by season of blood test and vitamin D supplement use: **A)** ≥10 µg/d; **B)** <10 µg/d; **C)** no vitamin D supplement use.

## 5.6 Sensitization to food allergens (paper IV)

Fourteen 6-year-old children (10%) were sensitized to one or more food allergens. Birth measurements and socioeconomic factors did not differ between sensitized and non-sensitized children, apart from maternal smoking during infancy (15% among sensitized vs. 3% among non-sensitized children, p=0.05). From birth to 2 months of age, sensitized vs. non-sensitized children had greater increase in weight (mean±SD; 2.2±0.4 kg vs. 1.8±0.5 kg, p=0.04) and head circumference (4.9±1.2 cm vs. 4.2±1.0 cm, p=0.02). At 6 years, sensitized children were more likely to be overweight/obese than their non-sensitized peers (29% vs. 10%, p=0.04). Breastfeeding was not significantly associated with sensitization. Introduction of solid foods prior to 4 months increased the odds for sensitization, OR=4.9 (95% CI=1.4-17), adjusted for maternal smoking.

Figure 7 shows the proportion of participants using vitamin D supplements in infancy and at 6 years, by sensitization. No significant differences in infant vitamin D supplement use by sensitization were observed, either when looking at any type of vitamin D supplement, or when specifically considering fish liver oil use. However, vitamin D supplement use at 6 years was significantly less common among sensitized (23%) than non-sensitized (56%) participants (p=0.03). When specifically considering fish liver oil use, the difference was still significant (23% vs. 52%, p=0.04). Vitamin D supplement use at 6 years decreased the odds for sensitization, OR=0.2 (95% CI=0.1-0.98), adjusted for maternal smoking.

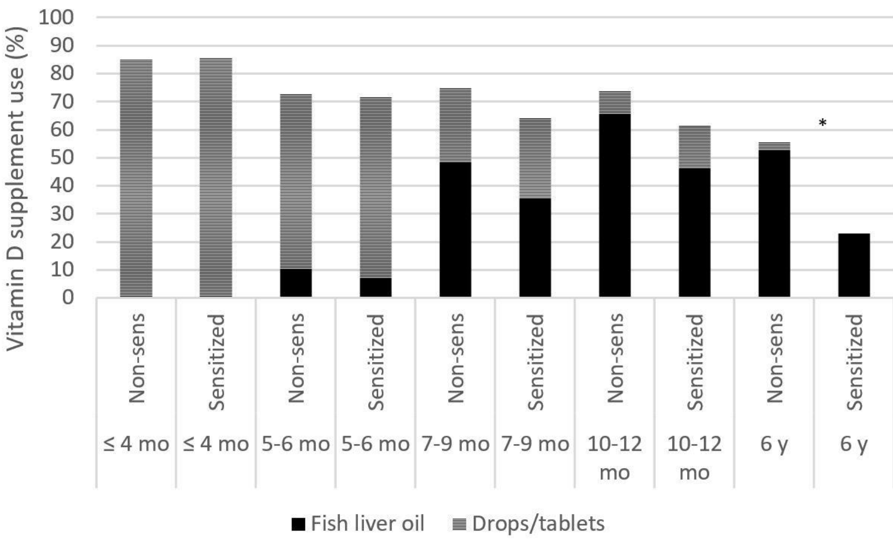


**Figure 7.** Vitamin D supplement use in infancy and 6 years among sensitized and non-sensitized 6-year-old children.

Because we considered fish liver oil of special interest for sensitization, Table 8 shows the proportions of non-sensitized and sensitized children categorized by age when they first received fish liver oil. No infant received fish liver oil prior to 5 months. There was no significant difference in age of fish liver oil introduction in infancy by sensitization. 28% of non-sensitized vs. 43% of sensitized participants received no fish liver oil at all in infancy (non-significant, p=0.2).

**Table 8.** Initiation of fish liver oil in infancy according to sensitization.

| Subgroup | Non-sensitized, n (%) | Sensitized, n (%) |
|---|---|---|
| Begin fish liver oil age 5-6 mo. | 13 (10) | 1 (7) |
| Begin fish liver oil age 7-9 mo. | 55 (42) | 5 (36) |
| Begin fish liver oil age 10-12 mo. | 26 (20) | 2 (14) |
| No fish liver oil use in the first year | 36 (28) | 6 (43) |

Vitamin D intake at 12 months was lower among sensitized than non-sensitized 6-year-old children (Table 9). This could partly be explained by significantly less frequent consumption of formula among sensitized compared to non-sensitized children. Also at 9 months and 6 years, the medians for vitamin D intake were lower among sensitized children, but no significant differences were observed. Higher vitamin D intake at 12 months decreased the odds to sensitization, OR=0.8 (95% CI=0.7-0.99), adjusted for maternal smoking.

**Table 9.** Vitamin D intake according to sensitization.

| | Non-sensitized, median ($25^{th}$,$75^{th}$perc) | Sensitized median ($25^{th}$, $75^{th}$ perc) |
|---|---|---|
| Vitamin D intake 9 mo., µg/d | 9.8 (5.6, 13.7) | 7.2 (3.2, 10.5) |
| Vitamin D intake 12 mo., µg/d | 8.1 (4.4, 12.3) | 3.9 (3.2, 7.2)[1] |
| Vitamin D intake 6 y, µg/d | 5.2 (2.3, 12.3) | 3.1 (2.1, 3.6) |

[1]p=0.03

Mean serum 25(OH)D did not differ between sensitized and non-sensitized children, at either 12 months (96.8±33.6 vs. 99.3±32.2 nmol/L, respectively) or 6 years (59.3±15.9 vs. 56.0±16.7 nmol/L, respectively). Sensitized children were not more likely than others to be in the lowest or highest quartiles for vitamin D status.

# 6 Discussion

## 6.1 Paper I: Diet at 6 years

In agreement with previous Icelandic studies on 6- and 7-year-old children [173, 174], the diet of 6-year-old children was not in line with dietary guidelines. From a public health perspective, this is concerning as childhood diet may affect short-term health and well-being [24, 25] and track into adulthood [28-30], affecting disease risk [14].

The results indicate a need for actions aiming to increase Icelandic children's consumption of fruit, vegetables, whole grain bread and other fiber-rich grain products, fish and vitamin D supplements while limiting salt and high-energy foods with low nutrient density (such as candy, ice-cream, biscuits, cakes and sugar-sweetened beverages). Despite suggestions of lower intake of saturated fatty acids and added sugar among 6-year-old children in this study (2011/2012) than in the Icelandic cohort studied 10 years prior (2001/2002) [175], continued efforts are needed to restrict high intakes of these macronutrients among Icelandic children.

As many individual and environmental factors affect the dietary habits and diet quality of children, modifications in the dietary environment of children (at the governmental level, at home, during school/day-care or in locations where leisure time is spent) are suggested to be effective for reaching most of the population and promoting better diets [176, 177]. Using the Health in All Policies framework [178], many municipalities in Iceland (including the most populated ones) have committed to being "health promoting communities", and many preschools, primary and secondary schools have similarly committed to health promotion [179]. Promoting healthy food choices is one of the key factors in these approaches. However, a recent observational study in a sports club located in one of the first "health promoting communities" in Iceland found the majority of foods and beverages sold at the sports club to be energy dense and nutrient poor [180]. A school-based intervention targeting children's knowledge and attitudes, their parents and teachers was effective in increasing fruit/vegetable consumption and fiber intake among 7-9-year-old children [181].

Iceland participates in the *Nordic monitoring of diet, physical activity and overweight* surveillance system and the Icelandic Directorate of Health also uses public health indicators (*Lýðheilsuvísar*) to surveil many indicators of public health, such as consumption of fruit, vegetables and sugar-sweetened beverages [182, 183]. National dietary surveys of both adults and children are a crucial addition to these measures but have not been carried out since 2010/2011.

In addition to vitamin D, our results suggest that some 6-year-old children may not satisfy their needs for iodine. This is in line with findings from the national dietary studies on adults in 2002 and 2010/2011 and adolescents in 2002/2003 [63, 184, 185]. Iodine deficiency may have negative effects on children's mental development and cognitive function [186, 187]. The iodine status of Icelandic adolescent girls and pregnant women in 2007-2009, which are groups with average low fish and milk intake, was however within the optimal range defined by WHO [188, 189], as was the iodine status of 6-month-old Icelandic infants in 2014-2017 [190]. Our results further suggest that very high vitamin A intake among some 6-year-old children may require attention.

## 6.2 Papers II and III: Vitamin D

Our findings of higher mean serum 25(OH)D levels (97.5 vs. 56.5 nmol/L) and higher rates of vitamin D sufficiency at 12 months than 6 years (92% vs. 64%) are in line with other population-based studies reporting higher serum 25(OH)D in younger than older children [141, 160, 163-166, 191]. This has mainly been explained by higher vitamin D intake in the younger age groups, in accordance with our results. Studies from Denmark, Norway and Finland have previously reported over 75% vitamin D sufficiency among healthy infants with high prevalence of vitamin D supplement use [137, 162, 163]. As very limited data is available about vitamin D status in nationally-representative infant samples, this study is an important contribution to the literature and has been cited in review articles [43, 192]. Transferring the 6% vitamin D deficiency among 6-year-old participants to the entire children population in Iceland would suggest that over 200 children in the general population would be deficient (25(OH)D <30 nmol/L).

Vitamin D intake in our study was somewhat higher than in the Icelandic cohort studied 10 years prior (median intakes 2 µg higher at 9 and 12 months and 1 µg higher at 6 years) [193]. This may partly be explained by higher prevalence of vitamin D supplement use, and for the infants, also by the replacement of regular cow's milk with formula (fortified with 1.2 µg vitamin D per 100 g) as the main milk type consumed between 6 and 12 months of age [8]. Our results suggest that fortified infant products (formula or infant porridge) contribute significantly not only to vitamin D intake but also to serum 25(OH)D levels at 12 months among regular consumers.

Other Icelandic studies have given rise to suggestions of more widespread vitamin D fortification in Iceland as a part of the solution to tackle low vitamin D status [55, 194]. In our study, breakfast cereals were the largest food source of vitamin D among 6-year-old children. Shortly after the data-collection finished, vitamin D fortified full-fat and semi-skimmed milk (both containing 1 µg vitamin D per 100 g) for the general population was put on the market. Therefore, vitamin D intake among 6-year-old Icelandic children today may be somewhat higher than in our study. European randomized controlled trials have shown significant improvements in vitamin D status in 1- to 6-year-old children randomized to vitamin D fortified milk compared with control groups drinking unfortified milk [195, 196]. The products used in the trials were fortified with higher amounts of vitamin D (1.7 or 2.85 µg/100 ml) than the Icelandic milk. Adherence to the FBDG of two portions of milk/dairy per day would increase vitamin D intake by approximately 4 µg/d for those using the fortified milk. The consumption of vitamin D fortified milk among Icelandic children today is however unknown.

### 6.2.1 Too little?

Protocols of the Primary Health Care in Iceland call for addressing the infant's vitamin D supplement use at every visit to the infant care, by asking about supplement use and informing about or encouraging use if needed [197]. Infant care is offered free of charge every 1-2 months in the first year of the infant's life and is very well attended [198]. Or results suggest that despite the emphasis on vitamin D in official guidelines and infant care, some infants do not use vitamin D supplements in the first year of life. Infants without regular use of vitamin D supplements or fortified products were identified as a risk group for lower serum 25(OH)D levels in our study, and the few insufficient infants in our study did not use vitamin D supplements or fortified products at all from birth. This knowledge could be used in infant care to underpin encouragement to parents who are not adhering to vitamin D recommendations. Despite being largely preventable, there are still cases of nutritional rickets in Iceland [199].

It has been suggested that part of the reason that some infants do not receive vitamin D supplements is infants' upset stomach when receiving vitamin D drops [200]. Vitamin $D_3$ drops are recommended until complementary feeding starts [39], after which, 1 teaspoon fish liver oil (10 µg vitamin $D_3$) is recommended. This is based on the assumption that no other nutrients than vitamin D are needed in the first 6 months of life given adequate breast- or formula-feeding [33]. In Norway, however, the use of fish liver oil is encouraged from the first month of life, with a dose gradually increasing from a half teaspoon at 4 weeks up to the recommended intake of 1 teaspoon by 6 months for the EBF infant [201]. Fish liver oil might be considered an alternative in the first 6 months of life in Iceland as well, if resulting in higher rates of vitamin D supplement use. Other forms of encouragement might be considered, such as the Swedish model of offering vitamin D supplements free of charge via the infant care [43], also done in Norway for infants with immigrant background [201, 202].

In our study, only 50-65% of 6-year-old children used vitamin D supplements, even during winter months. This is similar to other Icelandic studies reporting low vitamin D supplement use among second grade children, adolescents and adults [63, 174, 185] and low vitamin D status among those not using supplements [55, 150, 194]. In many preschools in Iceland, fish liver oil is included in food budgets and offered every day. This is usually not the case in primary schools, but could be a way of increasing vitamin D supplement use among children if implemented successfully.

### 6.2.2 Too much?

There are safety concerns with both vitamin D supplement use and food fortification, not least for infants and young children since the UL is only 2.5- to 5-times higher than the adequate intake or RI, depending of age [124, 126, 127]. In our study, only three infants had an intake above 25 µg/d at 9 or 12 months and none above 35 µg/d. However, serum 25(OH)D levels defined as possibly adversely high by the IOM (>125 nmol/L) [124] were observed in almost a quarter of 12-month-old infants, more so in infants using supplements than others. However, no infant had 25(OH)D above 200 nmol/L, which the unpublished EFSA update suggests using as cut-off for adversely high vitamin D status among infants [127].

The Norwegian infant nutrition recommendations suggest that smaller doses of vitamin D supplements can be given if the infant receives formula and/or vitamin D fortified infant porridge [201]. This is similar to recommendations by the UK National Health Service and American Academy of Pediatrics, both of which recommend vitamin D supplements only for infants not drinking sufficient amounts of vitamin D fortified formula or milk (>500 mL NHS, >1000 mL AAP) [203, 204]. Our results give cause to discuss the possibility of applying approaches as applied in Norway in the next update of the Icelandic infant nutrition guidelines.

According to a 2014 review, cases of vitamin D intoxication in infants, related to errors in manufacturing, formulation or prescription of vitamin D supplements, typically involved total intakes above approximately 6000 µg or 1000 µg/kg that resulted in serum levels above 600 nmol/L, leading to severe hypercalcemia and life-threatening symptoms [128]. Infants and children in our study were far from such levels. In 2016, dozens of intoxication cases among Danish infants were reported due to vitamin D supplement drops on the market containing 75 times the level indicated (750 µg in the recommended five drops) [205]. These cases duly attracted attention in Iceland as elsewhere, possibly causing some parents to worry about giving their infant vitamin D drops. Cases like these further underpin the importance of local monitoring and having updated information about the vitamin D status of each individual country, to be able to soothe possible worry.

### 6.2.3 Breastfeeding and seasons

Among infants without supplement use, breastfeeding compared with formula feeding has been associated with lower vitamin D status, due to the low vitamin D content in breast milk compared to formula [133, 206]. Even in a population having a high prevalence of supplement use (97%), a longer duration of exclusive breastfeeding, and breastfeeding at 9 months of age were associated with lower vitamin D status at 9 months [137]. We did however not find associations between the duration of exclusive or total breastfeeding and vitamin D status at 12 months, or a difference in vitamin D status between breastfed and non-breastfed 12-month-old infants. It can be speculated whether we would have seen differences if measuring vitamin D status earlier in infancy, in periods when breastmilk contributes more to infant diet than at 12 months.

Seasons were found to be associated with vitamin D status at 6 years, but not at 12 months. The relatively high vitamin D intake in infancy may contribute to the absence of association, supported by our results of no seasonal change in vitamin D status among 6-year-old children using the recommended amount of vitamin D supplements. The results may also partly be explained by less outdoor play, more covering clothing and sunscreen use among infants than children. Other studies have found seasonal differences in vitamin D status among infants [137] and children [132, 135, 150, 151, 153, 163]. In a study on Icelandic 7-year-old children, median vitamin D levels dropped from 60 nmol/L in September to <40 nmol/L in November [150]. We similarly see mean vitamin D levels dropping in autumn and winter among 6-year-old children not using vitamin D supplements as recommended. Mean vitamin D status was low in June and only three-quarters of 6-year-old children were vitamin D sufficient in July-August. The results therefore suggest that to maintain vitamin D sufficiency, it may be important to use vitamin D supplements throughout the year in Iceland.

### 6.2.4 Tracking of vitamin D status

We found modest tracking of vitamin D status during the five-year period from 12 months to 6 years, with a clear regression towards the mean. The observed correlation was similar in strength as what is observed for some other biomarkers in children, for example, tracking of serum lipid and apolipoprotein levels from infancy to age 4 in Swedish children [207]. Despite vastly different serum 25(OH)D levels at 12 months, participants in our study grow closer together at 6 years, around 50 nmol/L. Similar findings in the age-period 6-12 months have been reported in Polish infants [208]. Our findings may be partly explained by lower vitamin D intake at 6 years, especially when calculated per kg bodyweight. Although age and body weight have been associated with variation in serum 25(OH)D concentrations [209, 210], the same vitamin D supplement dose is recommended from 2 weeks to 10 years of age in Iceland [12, 197]. It is also possible that C-3 epimers may have contributed to overestimations of serum 25(OH)D levels at 12 months. We discuss this in detail in the chapter on methodology, 8.2.3.

## 6.3 Paper IV: Sensitization to food allergens

Compared to other European countries, food allergy prevalence among Icelandic infants is suggested to be relatively low (approximately 2% in the first year of life) [211]. A higher prevalence of atopic disease among 10-11-year-old Icelandic children has been found (24% of children sensitized to any allergen, e.g., grass, cats, trees) [212]. In the latter study, the high prevalence of atopic disease was reported in conjunction with low allergenic load, similar to another study suggesting that IgE sensitization and asthma prevalence rise at low levels of allergen exposure [213].

A recent Icelandic observational study found postnatal fish liver oil consumption associated with decreased food sensitization and clinical food allergies in infants and young children up to 2.5 years of age [97]. Furthermore, children starting to receive fish liver oil in their first half-year of life were significantly more protected than those whose introduction came later [97]. In our study, the timing of introduction did not associate with sensitization. IgE sensitization is not the same as clinical allergy. It only indicates that a state of sensitization exists and not necessarily that it is associated with clinical outcomes.

Fish liver oil consumption was very rare in the first six months of life in the present study and did not become more common than AD drops until the age 9 months. In addition to providing vitamin D, fish liver oil is a valuable source of omega-3 long-chain polyunsaturated fatty acids (omega-3). Omega-3 can influence cell membrane structure, cell signaling and antigen presentation and thereby exert anti-inflammatory effects [214]. Its role in primary prevention of sensitization and allergy has however been challenged in a recent systematic review and meta-analysis [215]. Another systematic review suggested that infant fish intake per se, not specifically omega-3, may have protective effects against allergy [216]. This warrants further study.

Our findings of sensitized children being less likely to use vitamin D supplements (fish liver oil) at 6 years of age are interesting, especially in light of findings from the ProMeal Study. There, 10-11-year-old Icelandic children with reported food allergies or intolerance were less likely to use vitamin D supplements, according to questionnaires [217]. We did not see a difference in vitamin D status by sensitization, but the small sample size may have weighed very heavily there, especially if the relationship between vitamin D and sensitization/allergy is U-shaped [120]. A large sample size would be needed to get extreme groups. Not using vitamin D supplements at 6 years was however associated with increased likelihood of vitamin D insufficiency/deficiency in our study.

Although very rare, maternal smoking during infancy was more common among sensitized than non-sensitized children in our study. This aligns with the literature on associations between passive smoking and increased risk of food allergy [218].

The optimal age to introduce solid foods, as primary prevention to reduce allergy, is an area currently receiving much research and debate [106, 219]. Some studies, but not all, suggest that introduction of solid food from 3-6 months of age may be effective in the prevention of IgE-mediated food allergy [106, 220-223]. However, the introduction of solid foods prior to 4 months is frequently associated with faster infant growth and childhood obesity [224-228]. Our results suggest greater weight gain and head circumference growth in the first two months following birth and more frequent introduction of solids prior to four months for sensitized than non-sensitized children. Other studies have found associations between rapid intrauterine or postnatal weight gain and large head circumference at birth and atopic disorders [229-235]. If prevention of sensitization is considered important for prevention of food allergy [106], our findings may have implications with regard to primary and secondary prevention and support current recommendations of vitamin D intake as recommended and avoidance of solid foods prior to the age of 4 months.

# 7 Strengths and limitations

General strengths of the study are the population-based sample, the longitudinal design and the careful collection of dietary data, anthropometrics, blood samples and parental and child characteristics in infancy and at 6 years. Therefore it was possible to both study diet on the group level, e.g., adherence to recommendations and identification of critical nutrients in the study population, and test hypotheses regarding the relations between diet and special outcomes, e.g., motor and verbal development [236], growth [171], overweight/obesity [1, 2], and, in this thesis, vitamin D status and sensitization to food allergens. The main limitations were the relatively small sample size and incomplete datasets for some participants.

Assessment of the dietary intake at 6 years of age (**paper I**) was one of the primary aims of the study. Later, we decided to assess the vitamin D status of Icelandic infants and children (**papers II and III**). We saw it as highly ethical to make good use of the available data and decided to use already available blood samples from this study instead of initiating new data collection, including intrusive blood withdrawal from the children. The measurement of specific IgE to common foods (**paper IV**) was similarly a secondary outcome. This entailed working around certain limitations. The next chapter will present a methodological discussion on selection bias, information bias, confounding and reproducibility, with a focus on data and methods used in this thesis.

# 8 Methodological discussion

## 8.1 Selection bias

In longitudinal studies, selection bias (i.e., when the study population does not represent the target population) can arise at inclusion or during the study period [32]. The sampling method, random selection by the independent institution Statistics Iceland, helps minimize selection bias during inclusion. The mean birth weight in this cohort is close to the national mean of the study year and similar to other cohorts of healthy Icelandic children [6, 237-239]. Acceptable distribution of birth months throughout the year and areas of residence throughout the country were met [172].

In the literature, participation in birth cohorts is commonly associated with high socioeconomic status and healthy behaviors [240-242]. This cohort was no exception as parents had high levels of education and were unlikely to smoke, compared to the overall population [243, 244]. This may affect the distribution of dietary factors [32], e.g., result in higher adherence to nutrition recommendations and supplement use. However, breastfeeding duration, vitamin D supplement use and the proportion of infants introduced to formula, porridge and other solid foods in the first year of life compares well with results from the registrations in the Primary Health Care's electronic records from the general infant population in Iceland born in 2004-2008 [198]. Also, comparison of the infants born 1995/1996 in the former Icelandic study with a control group only participating with WFR at 9 months of age, showed that participation in the infant study did not affect the infant's food or nutrient intake [238].

As previously mentioned, basic characteristics, breastfeeding and growth in the first months of life did not differ between the children participating in the follow-up at 6 years of age (n=162) and the original sample (n=250). The characteristics of the 76 infants (30% of the original sample) in **paper II** were not different from the original sample or other infants with blood tests at 12 months of age. Given the above, the study cohort is considered representative of Icelandic children. The inclusion criteria, such as born at term, Icelandic parents and absence of disease, must however be kept in mind when interpreting the results.

## 8.2 Information bias

### 8.2.1 Dietary assessment

Weighed food records, kept for enough days, are the best estimate for energy intake, especially for young children [245], and are commonly used to validate other methods. WFR measure actual intake, directly measured amounts, are open-ended and do not rely on memory [32]. Their main limitations are the high participant burden, risk of underreporting and the possibility that the process of writing down the foods leads to changes in eating behavior, resulting in the record not being representative of what the participant would normally have eaten [32].

The longitudinal design and study protocol is believed to reduce this risk as unmotivated parents were likely to have dropped-out in the first months of the study. Prior to each registration, parents received face-to-face training in the methods of keeping complete and accurate WFR and written directions and examples were provided on the front page of the records. Parents were encouraged to maintain a normal eating environment for their child during the recording period. This was not without challenges. At 6 years of age, children consumed many meals outside their home, and sometimes caregivers other than parents were required to keep the WFR. When necessary, members of the research team visited participants at school during lunch to weigh and record the meal (10 participants). WFR were screened for missing meals or obvious underreporting, and this was also studied during the calculation of the nutrient intake.

For infants, 2 days are considered sufficient to assess micronutrient intake with an acceptable degree of accuracy and 3-5 days for energy and macronutrients [246, 247]. For 6-year-old children, more recording days are preferred, especially to acceptably assess foods that are infrequently eaten (e.g., fatty fish) and some nutrients that largely depend on foods infrequently eaten [246]. However, calculations including supplements that are used by some, but not all, participants (e.g., fish liver oil in Iceland) leads to fewer recording days needed for adequate intake estimates of the nutrient provided by supplements (e.g., vitamin D and omega-3) [246]. For estimates of the group's means of overall diet in **paper I,** we consider the 3-day WFR a good method [32, 246, 247]. Another method very often chosen to measure dietary intake in epidemiologic studies is a food frequency questionnaire.

A 3-day WFR would not be our method of choice for the primary purpose of assessing the use of vitamin D from supplements on the individual level.

On one hand, it is easy to forget to record supplements and, on the other hand, it is possible that the process of keeping a WFR reminds participants to use supplements. Also, fatty fish, a good vitamin D source, is commonly consumed once a week or more infrequently. Therefore, more recording days or another assessment method would be needed to reliably assess its intake. To address this issue in **paper II**, we decided to calculate average daily consumption of vitamin D from the 3-d WFR kept at 9 and 12 months. To categorize infants by vitamin D intake, we added the 1-d non-weighed food records kept at 10 and 11 months (from which calculations of vitamin D intake were not possible), resulting in a total of 8 recording days.

### 8.2.2 Food databases and food composition values

Food databases can be a source of error and need regular updating. The Icelandic food composition database, ISGEM, is kept as up-to-date as possible at Matis and contains data on 45 nutrients in around 1250 foods, from chemical analyses conducted in Iceland, information from food labels or food producers, food importers and food composition tables from other countries [167]. There is a lack of recent Icelandic chemical analyses of vitamins, including vitamin D.

The recipe database for commercial infant products in Iceland was constructed in 2005 for the current study. The values come from food labels, food producers or importers. Information on vitamin content is scarce except for fortified formulas and porridges. Basing values in databases on the content of food labels should be done cautiously. A recent pilot study from the Netherlands showed that compared to values on the labels, analytically determined vitamin D values ranged from 50% to 153% for fortified foods intended for infants (n=29 products) and 8% to 177% for dietary supplements (n=15 products) [248]. Such bias can contribute to systemic over- or underestimation of vitamin D intake, with the potential to affect the perceived need for action on the population level. Therefore, chemical analyses of food contents are important. This also emphasizes that it is not enough to study critical nutrients in vulnerable populations by dietary assessment alone. Biomarkers should also be measured when possible.

### 8.2.3 Assessment of vitamin D status

The standard method to assess vitamin D status in the clinical laboratories at Landspitali was used for samples in this thesis. The Roche chemiluminescence immunoassay employs a vitamin D binding protein to bind 25(OH)D [168]. In a multicenter comparison study of different methods to measure serum 25(OH)D, using isotope dilution liquid chromatography-tandem mass spectrometry (LC-MS/MS) as the gold standard, the Roche method showed little systemic bias in measured 25(OH)D but increasing random bias at increasing 25(OH)D concentrations [249]. The inability of this method to distinguish between 25(OH)$D_3$ and 25(OH)$D_2$ is not considered of significance in this study, since vitamin $D_2$ is unlikely to contribute much to vitamin D status of Icelandic infants and children and we were interested in the total 25(OH)D. However, a limitation of the method is that it does not separately measure the C-3 epimers of 25(OH)D (3-epi-25(OH)D).

Little is known about what leads to C3 epimerization, and to what extent separate reporting of 3-epi-25(OH)D might be of clinical relevance [250-253]. 3-epi-25(OH)D appear to suppress parathyroid hormone secretion and have been shown to have some calcemic and non-calcemic regulatory effects, but at lower levels than non-epimeric vitamin D forms [250]. Studies suggest that the 3-epi-25(OH)D can contribute significantly to total 25(OH)D concentration, particularly in early infancy (frequently reported to be around 10%, less frequency as high as 40-60% of total 25(OH)D) and to a smaller degree also in later infancy and among children and adults [249, 250, 253-261]. Recent studies have found 3-epi-25(OH)D in almost all studied infants. They however suggest that the epimers may only be of clinical significance in the first months of life and not around 12 months of age [254-256].

The implication for our findings is that there is a possibility that the 25(OH)D levels in some participants, especially at 12 months of age rather than 6 years, may be overestimated due to the presence of 3-epi-25(OH)D. If we would have had sufficient leftover blood available from participants at 12 months, we would have liked to measure 25(OH)D again in the samples, using the recommended LC-MS/MS [43, 262]. However, this was not possible. A Danish study using LC-MS/MS found high mean 25(OH)D levels at 9 months (82 nmol/L) but did not mention 3-epi-25(OH)D in the paper [131]. Even though 3-epi-25(OH)D might be present in the 12-month-old infants in our study, we consider it unlikely that vitamin D insufficient/deficient infants are being misclassified as being vitamin D sufficient. Therefore, the clinical significance of this limitation may be low.

### 8.2.4 Season

According to the literature, the availability of sun for cutaneous vitamin D synthesis in Iceland is believed to be high in June and July, moderate in May and August (perhaps into September) and negligible in September/October to April [55, 56]. In **paper II**, we used this literature to classify by season as summer/autumn (May-October) and winter/spring (November-April). To confirm our findings of non-significant differences in 25(OH)D by season at 12 months of age, we further looked at the darkest winter months (November-March, 98±32 nmol/L) vs. summer months (June-August, 105±30 nmol/L, or July-August, 105±31 nmol/L) and did not find significant differences by season. The number of participants in these analyses is however low, limiting the power to detect significant differences, but even if numbers were very large, a difference of 7 nmol/L, at a concentration of around 100 nmol/L, cannot be considered of any clinical significance.

Our categorization of months into seasons in **paper III**, especially categorizing June as "winter/spring", is unorthodox but does not influence our results. Excluding June did not change any of our findings.

### 8.2.5 Assessment of sensitization

The ImmunoCAP method to assess sensitization is one of the top verified IgE assays [101]. Based on the WHO IgE standard, the top verified IgE assays (others being HYTEC-288 by Hycor-Agilent and Immulite by Siemens) report comparable analytic sensitivity, with the coefficients of variation of the precision, reproducibility and linearity being <15%, which is considered excellent for a clinical assay [101]. Still, the ImmunoCAP method, as other methods for assessing sensitization, has the potential for false-negative and false-positive results and should preferably be interpreted in the context of the patient's history and confirmed by the double-blind, placebo-controlled oral food challenge [101], which we did not do. A false-negative situation arises if an individual with objective allergic symptoms or a definite history of a severe allergic reaction is not measured as sensitized, i.e., has undetectable or negative serum IgE. A false-positive situation arises if an asymptomatic individual who can tolerate the allergen exposure without objective symptoms is measured as sensitized, i.e., has above cut-off levels of serum IgE. When measured with a verified assay and in a regulated clinical laboratory, like the one at Landspitali, the false-positive situation is in most cases analytically true and some suggest that it should rather be identified as clinically irrelevant rather than false-positive [100].

In our study, because of the time difference between blood sample collection and measurement of sensitization, we could not invite sensitized participants to a double-blind, placebo-controlled oral food challenge. We also did not consider it ethical to contact parents of sensitized participants to ask questions related to the child's allergic history, potentially causing them to worry in hindsight about their child. However, four sensitized (29%) vs. one non-sensitized child (<1%) were reported regular users of asthma inhalers or antihistamine at 6 years.

## 8.3 Confounding and multiple testing

Our findings cannot determine causality, and the possibility of confounding by unmeasured factors cannot be excluded. Due to the small sample size, adjustments were a challenge. The study still gives very valuable indications of associations in a field where it would be unethical to perform randomized trials. Such examples are randomizing to the introduction of solid foods prior to 4 months and not using vitamin D supplements in a country like Iceland with official, evidence-based recommendations on exclusive breastfeeding up to 6 months and daily use of vitamin D supplements.

More information to determine skin vitamin D synthesis (skin type, outdoor play, travel to sunny countries) would have been valuable as would information about clinical food allergy and family history of allergy.

In this thesis we examined several outcomes. The more inferences are made, the more false inferences are likely to occur (i.e., type I error) [263]. However, because our results are align with literature e.g., regarding associations between vitamin D intake and status, solids prior to 4 months and sensitization, we consider them unlikely to be false findings.

## 8.4 Reproducibility

The papers were drafted in accordance with the STROBE guidelines for cohort studies. It is important to note that participants in the cohort are 13 years old this year. Nutrition recommendations have not changed drastically since the time of the study, so even though some factors of the dietary environment may have changed, we do not expect great differences between the study diet and the diet today. It is however important to monitor diet regularly.

# 9 Conclusions

Adherence to the Icelandic food-based dietary guidelines varied among 6-year-old children but was poor in general. The food intake was mirrored in a non-optimal distribution of macronutrients and fiber intake. Vitamin and mineral density of the diet seemed however adequate for most nutrients, except for vitamin D. The results indicate that a public health effort is needed to improve the diet of Icelandic children starting school-age, for example by finding ways to increase their consumption of fruit and vegetables, wholegrain bread and cereals, fish and vitamin D supplements and decrease their consumption of unhealthy foods.

Serum 25(OH)D levels were higher at 12 months (mean 98 nmol/L) than at 6 years (mean 57 nmol/L), reflecting higher total vitamin D intake and more use of vitamin D supplements in infancy compared with 6 years. Healthy Icelandic infants and children receiving the recommended 10 µg/d vitamin D are likely to be vitamin D sufficient. Vitamin D insufficiency and deficiency may however be prevalent among 6-year-old children due to insufficient intake.

The results indicate that either regular use of vitamin D supplements or consumption of vitamin D fortified infant products at 9-12 months would be enough to reach vitamin D sufficiency (>50 nmol/L) at 12 months. The results suggest that dietary vitamin D is important throughout the year for Icelandic infants.

Among 6-year-old children not using vitamin D supplements, vitamin D sufficiency decreased during autumn and winter. It is possible that the relatively high proportion of vitamin D measurements carried out during summer months among 6-year-old children in the study might have caused some underestimation of vitamin D insufficiency and deficiency in the population studied. While the results suggest a contribution from cutaneous vitamin D synthesis during high summer (July and August) among 6-year-old children, they do not suggest that vitamin D supplement use (10 µg/d) during summer cause adversely high levels among Icelandic 6-year-old children.

Vitamin D supplement use at 6 years was associated with decreased risk of sensitization to food allergens at 6 years of age, while introduction of solid foods prior to 4 months was associated with increased risk of sensitization. These findings support current recommendations regarding vitamin D supplements and avoidance of solid foods before the age of 4 months.

# 10 Future perspectives

Infancy and childhood are important windows of opportunity to implement dietary change with a potential to track into adolescence and adulthood. Research should focus on how to effectively implement health promoting strategies among Icelandic school-children. A new national dietary study among Icelandic infants and children should ideally be prepared sooner than later as the dietary environment and food habits may change and health promotion and evidence-based advice to parents need to keep up with that.

In a country where vitamin D supplements are officially recommended, monitoring the vitamin D status of vulnerable groups may be seen as not only desirable but a moral duty of relevant official bodies. Special risk groups for both low and high intake and status warrant special attention. Children with non-Western background have been identified as risk-groups for vitamin D deficiency in our neighboring countries but were not included in our study. It is also important to monitor the effects of voluntary vitamin D fortification in milk, breakfast cereals and other products on children's status.

Keeping food databases updated, preferably with locally analyzed food composition data when possible, is important. This is not least important for nutrients with few significant dietary sources, such as vitamin D, where changes in the nutrient content of individual foods or supplements can have a large impact and variation from values on food labels can matter significantly. Chemical analysis on vitamin D content of breast milk, infant foods and supplements in Iceland would be ideal.

The dataset used in the present study and the former Icelandic infant and child study still offer opportunities for further research, e.g. on determinants of diet quality at 6 years and associations between diet (e.g. saturated fat, vitamin D, fruit and vegetable consumption, dietary pattern) and lipid profile at 12 months and 6 years.

Unique national registries on breastfeeding, formula feeding and introduction of solid food (timing and type) as well as vitamin D supplement use among the whole Icelandic infant population, was established in 2005. The data from the ICE-MCH study (cohort based on this registry) provides a good opportunity for indirectly following up findings from paper IV, e.g., studying the association between vitamin D supplementation and age of introduction of solid food and several health outcomes and drug use. Validation on the dataset is ongoing and will be ready for analysis in the next few months.

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# Papers I-IV

# Paper I

# Landskönnun á mataræði sex ára barna 2011-2012

**Ingibjörg Gunnarsdóttir** næringarfræðingur, **Hafdís Helgadóttir** nemi í næringarfræði, **Birna Þórisdóttir** nemi í næringarfræði, **Inga Þórsdóttir** næringarfræðingur


## ÁGRIP

**Tilgangur:** Þekking á mataræði er grundvöllur stefnumótunar stjórnvalda í manneldismálum. Tilgangur rannsóknarinnar var að kanna mataræði 6 ára barna.

**Efniviður og aðferðir:** Þátttakendur voru 6 ára börn (n=162) valin með slembiúrtaki úr þjóðskrá. Matur og drykkur sem börnin neyttu var vigtaður og skráður í þrjá daga. Fæðuval og neysla næringarefna voru borin saman við fæðutengdar ráðleggingar og ráðlagða dagsskammta (RDS) fyrir viðkomandi næringarefni.

**Niðurstöður:** Samanlögð meðalneysla ávaxta og grænmetis var 275±164 grömm á dag (g/dag), en innan við 20% þátttakenda neytti ≥400 g/dag. Fisk- og lýsisneysla um fjórðungs þátttakenda var í samræmi við ráðleggingar. Meirihluti (87%) neytti ≥2 skammta af mjólk og mjólkurvörum daglega. Fæða með lága næringarþéttni (kex, kökur, gos- og svaladrykkir, sælgæti, snakk og ís) veitti að meðaltali nær 25% af heildarorku. Einungis um 5% barna nær viðmiðum um hlutfall harðrar fitu í fæðu og neyslu matarsalts og innan við 20% barna nær viðmiðum um neyslu fæðutrefja. Meðalneysla vítamína og steinefna var almennt hærri en RDS fyrir viðkomandi næringarefni. Undantekning var D-vítamín þar sem einungis fjórðungur barnanna neytti RDS eða meira af vítamíninu.

**Ályktun:** Matarvenjur 6 ára barna veita sem svarar RDS fyrir flest vítamín og steinefni að undanteknu D-vítamíni. Mataræðið samræmist ekki ráðleggingum hvað varðar grænmeti, ávexti, fisk og lýsi. Samsetning orkugefandi efna og trefjaefnainnihald er ekki eins og best verður á kosið enda veita vörur með lága næringarþéttni stóran hluta orkunnar. Mikilvægt er að leita leiða til að bæta mataræði íslenskra barna.


Rannsóknastofu í næringarfræði, við matvæla- og næringarfræðideild, Háskóla Íslands og Landspítala.

Fyrirspurnir: Ingibjörg Gunnarsdóttir *ingigun@hi.is*

Greinin barst 19. september 2012, samþykkt til birtingar 14. desember 2012.

Engin hagsmunatengsl gefin upp.

## Inngangur

Fyrstu ár ævinnar eru einstaklega mikilvæg með tilliti til næringar og benda rannsóknir til þess að næringarástand og fæðuval fyrstu ár ævinnar geti haft langtímaáhrif á vöxt, þroska og heilsu.[1-8]

Þekking á mataræði mismunandi hópa er grundvöllur stefnumótunar stjórnvalda í manneldismálum og á því sinn þátt í að efla lýðheilsu í landinu. Rannsóknastofa í næringarfræði hefur undanfarin 15 ár rannsakað mataræði barna og hafa niðurstöðurnar meðal annars nýst við endurskoðun opinberra ráðlegginga.[3, 9-13]

Margar þjóðir, þar á meðal Íslendingar, hafa mótað fæðutengdar ráðleggingar samhliða hefðbundnum ráðleggingum um lífsnauðsynleg næringarefni, vítamín og steinefni, orku og hlutfallslega skiptingu orkuefnanna.[14] Þannig eru gefnar leiðbeiningar um ráðlagða neyslutíðni og/eða magn matvæla úr ákveðnum fæðuflokkum. Markmiðið er að auðvelda fólki að velja sér fjölbreytta og góða fæðu, sem rannsóknir hafa sýnt að stuðla að bættri heilsu og minnkar líkur á ýmsum sjúkdómum, svo sem hjarta- og æðasjúkdómum, offitu, sykursýki og ákveðnum tegundum krabbameina.[15]

Markmið þessarar rannsóknar var að afla upplýsinga um neyslu matvæla og næringarefna 6 ára Íslendinga. Rannsóknin á mataræði 6 ára barna 2011-2012 er framskyggn rannsókn á handahófsvöldu landsúrtaki ungbarna sem fylgt var til 6 ára aldurs. Opinberar ráðleggingar um fæðuval og næringarefni[16] eru notaðar til að meta niðurstöðurnar um neyslu matvæla og næringarefni í fæðu 6 ára barna og eru niðurstöðurnar kynntar hér.

## Efniviður og aðferðir

Þátttakendur voru 6 ára börn sem áður höfðu tekið þátt í langtímarannsókn á mataræði og heilsu íslenskra ungbarna.[2] Ungbörn fædd árið 2005 voru valin af Hagstofunni með slembiúrtaki úr þjóðskrá fjórum sinnum það ár. Við hvert slembiúrtak voru börnin innan við fjögurra mánaða gömul. Skilyrðum fyrir þátttöku mættu 250 börn, en skilyrði voru íslenskir foreldrar, einburi, meðgöngulengd 37-41 vika, fæðingarþyngd milli 10-90 hundraðshluta, engir fæðingargallar eða meðfæddir sjúkdómar og að móðir hefði notið mæðraverndar. Alls luku 219 börn að minnsta kosti einum þætti rannsóknarinnar við 12 mánaða aldur og var þeim boðið að taka þátt í eftirfylgni við 6 ára aldur. Fullnægjandi matardagbókum var skilað inn fyrir 162 sex ára börn (74%). Hlutfall háskólamenntaðra foreldra í rannsókninni var 58% meðal mæðra og 42% meðal feðra. Rannsóknin á næringu ungbarna var fyrst samþykkt af Vísindasiðanefnd (VSNb2005040019/037) og skráð hjá Persónuvernd (S2449/2005) og síðan áður en rannsóknin á 6 ára hófst af Vísindasiðanefnd (VSNb2011010008/37) og Persónuvernd (2010111049AMK).

Allur matur og drykkur sem barnið neytti nálægt sjötta afmælisdegi sínum var vigtaður með ± 1 g nákvæmni (PHILIPS HR 2385, Ungverjaland) og neyslan skráð í matardagbók í þrjá daga samfellt, tvo virka daga og einn helgardag. Skráningin var fyrst og fremst í höndum foreldra og forráðamanna, en samstarf var einnig við leik- og grunnskóla um vigtun og skráningu þeirra máltíða sem börnin neyttu á skólatíma. Foreldrar, forráðamenn og aðrir sem sáu um skráningu mataræðis fengu skriflegar og munnlegar leiðbeiningar um notkun á vogunum og nákvæmni við skráningu (til dæmis um mikilvægi þess að skrá nákvæma tegund og jafnvel vöruheiti þeirra matvæla sem neytt var). Niðurstöður voru færðar inn í næringarútreikningaforritið ICEFOOD sem hannað var fyrir landskönnun á mataræði

**Tafla I.** *Neysla algengra matvæla og matvælaflokka í grömmum á dag (meðaltal ± staðalfrávik (SF) og dreifing neyslunnar). n=162.*

| | | Meðaltal ± SF | 5% | 10% | 25% | 50% | 75% | 90% | 95% |
|---|---|---|---|---|---|---|---|---|---|
| **Grænmeti, ávextir og hreinir safar** | Grænmeti | 52 ± 47 | 1 | 5 | 14 | 39 | 77 | 124 | 134 |
| | Ávextir | 136 ± 102 | 3 | 24 | 57 | 114 | 201 | 292 | 318 |
| | Hreinir safar | 87 ± 107 | 0 | 0 | 0 | 45 | 155 | 242 | 287 |
| | Ávextir, grænmeti og safar alls | 275 ± 164 | 46 | 89 | 155 | 257 | 369 | 533 | 583 |
| **Kartöflur og kornmeti** | Kartöflur, nýjar | 21 ± 28 | 0 | 0 | 1 | 16 | 29 | 49 | 56 |
| | Franskar kartöflur | 6 ± 12 | 0 | 0 | 0 | 0 | 0 | 24 | 37 |
| | Brauð | 80 ± 39 | 30 | 38 | 52 | 71 | 104 | 127 | 151 |
| | Gróf brauð, trefjaefni ≥6% | 16 ± 21 | 0 | 0 | 0 | 8 | 23 | 48 | 59 |
| | Morgunkorn | 43 ± 38 | 0 | 6 | 19 | 33 | 54 | 93 | 126 |
| | Hafragrautur | 16 ± 36 | 0 | 0 | 0 | 0 | 0 | 67 | 107 |
| | Pasta og núðlur | 19 ± 29 | 0 | 0 | 0 | 3 | 28 | 61 | 81 |
| | Pizza | 12 ± 28 | 0 | 0 | 0 | 0 | 0 | 49 | 75 |
| **Fiskur og kjöt** | Fiskur og fiskafurðir | 21 ± 24 | 0 | 0 | 0 | 16 | 30 | 53 | 62 |
| | Kjöt og kjötafurðir | 65 ± 35 | 15 | 21 | 39 | 63 | 84 | 112 | 128 |
| **Mjólk og mjólkurvörur** | Nýmjólk | 78 ± 117 | 0 | 0 | 0 | 0 | 123 | 239 | 290 |
| | Léttmjólk | 102 ± 144 | 0 | 0 | 0 | 60 | 137 | 301 | 385 |
| | Undanrenna/Fjörmjólk | 17 ± 49 | 0 | 0 | 0 | 0 | 0 | 67 | 103 |
| | Kókómjólk/Kakó | 32 ± 53 | 0 | 0 | 0 | 0 | 67 | 83 | 133 |
| | Mjólkurvörur* | 22 ± 29 | 0 | 1 | 4 | 14 | 36 | 51 | 58 |
| | Ostar | 17 ± 14 | 0 | 0 | 6 | 14 | 27 | 35 | 39 |
| | Mjólk og mjólkurvörur alls | 357 ± 193 | 97 | 143 | 205 | 345 | 458 | 597 | 720 |
| **Feitmeti** | Smjör og smjörlíki | 9 ± 8 | 1 | 2 | 4 | 7 | 12 | 16 | 21 |
| | Sósur og ídýfur | 13 ± 11 | 0 | 0 | 4 | 10 | 18 | 26 | 33 |
| | Lýsi | 2 ± 3 | 0 | 0 | 0 | 0 | 5 | 8 | 8 |
| **Drykkir aðrir en mjólk** | Gosdrykkir og svaladrykkir | 117 ± 129 | 0 | 0 | 23 | 83 | 165 | 266 | 417 |
| | Svaladrykkir | 71 ± 99 | 0 | 0 | 0 | 31 | 89 | 203 | 274 |
| | Gosdrykkir | 46 ± 76 | 0 | 0 | 0 | 0 | 67 | 151 | 220 |
| | Vatn og sódavatn | 285 ± 235 | 10 | 49 | 123 | 221 | 404 | 545 | 737 |
| **Kex, kökur, sætindi og snakk** | Kökur | 32 ± 34 | 0 | 0 | 4 | 27 | 42 | 73 | 93 |
| | Kex | 13 ± 18 | 0 | 0 | 0 | 7 | 18 | 34 | 49 |
| | Sælgæti og ís | 32 ± 33 | 0 | 0 | 0 | 25 | 53 | 81 | 96 |
| | Popp, snakk, hnetur | 8 ± 14 | 0 | 0 | 0 | 1 | 10 | 24 | 32 |

* Mjólkurvörur aðrar en drykkjarmjólk og ostar

2002[16] og endurbætt fyrir landskönnun á mataræði 2010-2011.[17] Við útreikninga á næringargildi fæðu var annars vegar stuðst við íslenska gagnagrunninn um efnainnihald matvæla, ÍSGEM,[18] og hins vegar gagnagrunn fyrrum Lýðheilsustöðvar um samsetningu algengra rétta og skyndibita á íslenskum markaði. Tekið var tillit til rýrnunar næringarefna við eldun.

Birtar eru niðurstöður um neyslu valinna fæðutegunda (í grömmum á dag (g/dag)), orku (sem kkal/dag), orkugefandi næringarefna (í g/dag og sem hlutfall af heildarorkuneyslu) og neyslu vítamína og steinefna. Eins var gerð greining á framlagi fæðuflokka til heildarorkuneyslu og valinna næringarefna (harðrar fitu, viðbætts sykurs, salts og fæðutrefja). Við flokkun fæðutegunda í skilgreinda fæðuflokka er stuðst við sama kerfi og lýst er í skýrslu er geymir helstu niðurstöður landskönnunar á mataræði fullorðinna Íslendinga 2010-2011.[17] Niðurstöðurnar voru bornar saman við ráðleggingar um fæðuval og ráðlagða dagsskammta (RDS) næringarefna fyrir viðkomandi aldurshóp.[14,19] Skammtastærðir sem miðað er við í ráðleggingum um fæðuval voru aðlagaðar miðað við orkuþörf 6 ára barna, sem nemur um það bil 60% af áætlaðri orkuþörf fullorðinna.[20] RDS fyrir vítamín og steinefni er skilgreindur sem það magn næringarefnis sem fullnægir þörfum alls þorra fólks, eða um 98% þýðis. Þegar lagt er mat á vítamín- og steinefnaneyslu hópa er venjan að styðjast við fleiri hugtök en einungis ráðlagðan dagsskammt, það er að segja meðalþörf, lægri mörk æskilegrar neyslu og efri mörk hættulausrar neyslu. Meðalþörf vítamína og steinefna hefur ekki verið skilgreind fyrir börn, en fyrir fullorðna samsvarar hún yfirleitt 60-80% af RDS.[19] Í þessari grein er stuðst við 2/3 hluta RDS sem mælikvarða á áætlaða meðalþörf þýðisins, en slík nálgun er algeng og viðurkennd þegar niðurstöður neyslukannana eru metnar og meðalþörf fyrir efni er ekki þekkt.[19] Ef neysla fer undir áætlaða meðalþörf þýðis er talið að hætta sé á að þörf fyrir viðkomandi næringarefni sé ekki mætt.

Forritið SAS (9.2) var notað við tölfræðigreiningar. T-próf var notað til að kanna hugsanlegan mun á neyslu næringarefna milli kynja. Marktækni var skilgreind sem $p<0,05$.

### Niðurstöður

Heildarorka fæðunnar var meðal stúlkna 1529±314 kkal/dag og drengja 1558±336 kkal/dag ($p>0,05$). Neysla fæðutegunda, hlutfallsleg skipting heildarorku á orkuefnin og neysla vítamína og steinefna reyndust ekki vera mismunandi milli kynja ($p>0,05$). Niðurstöður eru þar af leiðandi birtar sem meðaltöl og staðalfrávik fyrir bæði kyn saman, ásamt dreifingu neyslunnar.

Tafla I sýnir neyslu matvæla úr völdum fæðuflokkum. Um helmingur 6 ára barna neytti minna en 40 gramma af grænmeti daglega, en til samanburðar má geta þess að einn tómatur vegur um það bil 80 grömm. Ávaxtaneysla var að jafnaði ríflegri en grænmetisneyslan. Neysla á mjólk og mjólkurvörum nam rúmlega 350 grömmum á dag að jafnaði. Meðalneysla gos- og svaladrykkja samsvaraði rúmum 800 ml á viku og þau 10% barna sem mest neyttu af gos- og svaladrykkjum drukku að jafnaði nálægt tveimur lítrum á viku. Neysla á kexi og kökum var næstum þrisvar sinnum meiri en neysla á trefjaríku brauði (skilgreint sem ≥6 grömm tefjaefni í 100 grömmum af brauði) og meðalneysla á sælgæti og öðrum sætindum var einnig rífleg. Þó ber að taka fram að helmingur barnanna neytti hvorki sætinda né gosdrykkja þá daga sem skráning fór fram.

Í töflu II má sjá ráðleggingar um fæðuval,[14] auk fjölda og hlutfalls barna með neyslu í samræmi við þau viðmið sem gefin eru. Töluvert er í land með að 6 ára börn nái almennt ráðleggingum um grænmetis- og ávaxtaneyslu. Um fjórðungur barna neytti fisks og lýsis skráningardagana. Eins er mjög langt í land með að neysla á harðri fitu (mettuðum fitusýrum og trans-fjölómettuðum fitusýrum), fæðutrefjum og salti sé í samræmi við ráðleggingar. Meirihluti barna (87%) neytti í það minnsta tveggja skammta af mjólk og mjólkurvörum daglega. Þess má geta að tæplega 10% barnanna neytti sem svarar fjórum skömmtum af mjólk og mjólkurvörum eða meira daglega.

**Tafla II.** *Fjöldi og hlutfall 6 ára barna (n=162) með mataræði í samræmi við opinberar ráðleggingar.*

| Ráðleggingar um fæðuval* | Viðmið aðlöguð fyrir 6 ára börn | n | % |
|---|---|---|---|
| Grænmeti og ávextir | ≥400 g af grænmeti og ávöxtum á dag | 30 | 19 |
| Fiskur tvisvar í viku eða oftar | ≥34 g á dag, sem samsvarar fiskmáltíð tvisvar í viku | 37 | 23 |
| Gróf brauð og annar trefjaríkur kornmatur | ≥2,5 g fæðutrefjar/MJ/dag ** | 28 | 17 |
| Fituminni mjólkurvörur | 2 skammtar af mjólk eða mjólkurvörum daglega (1 sk 200 g af mjólkurvörum eða ≥20 g af osti) | 141 | 87 |
| Olía eða mjúk fita í stað harðrar fitu | <10% orkunnar úr harðri fitu | 10 | 6 |
| Salt í hófi | <3,2 g af salti á dag*** | 7 | 4 |
| Þorskalýsi eða annar D-vítamíngjafi | ≥5 ml á dag, sem samsvarar ráðlögðum dagskammti af D-vítamíni | 44 | 27 |
| Vatn er besti svaladrykkurinn | <10% orkunnar úr viðbættum sykri | 70 | 43 |

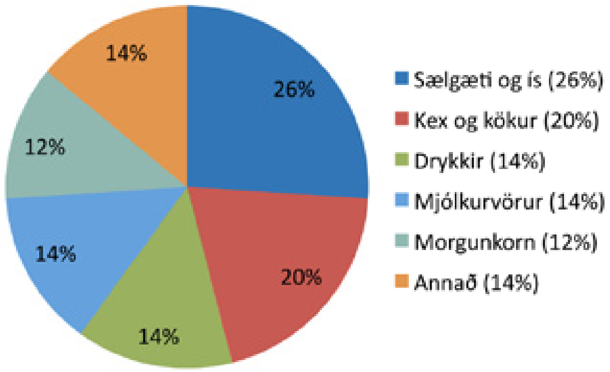
*Ráðleggingar um mataræði og næringarefni fyrir fullorðna og börn frá tveggja ára aldri.[14]
**Notuð eru viðmið um ráðlagða neyslu fæðutrefja samkvæmt norrænum ráðleggingum um næringarefni ≥2,5 g/MJ/dag.[19] Ástæðan er sú að flokkunarkerfi næringarútreikningsforritsins, ICEFOOD, gefur ekki tilefni til nákvæmrar skoðunar á trefjaríkum kornmat nema fyrir brauð.
***Norrænar ráðleggingar um næringarefni:[19] ≤0,5 g salt/1000 kJ (0,5 g salt/239 kcal) sem samsvarar <3,2 g af salti daglega miðað við orkuneyslu barnanna í þessari rannsókn.

Tafla III sýnir heildarorkuneyslu (kkal/dag) og hlutfallslega skiptingu orkugefandi næringarefna. Að jafnaði gáfu prótein um 15,4±2,9% af heildarorku. Niðurstöðurnar sýna að gæði fitu í fæði er ábótavant, en hörð fita (það er mettaðar fitusýrur og transómettaðar fitusýrur) veitti 14,1±2,9% af heildarorku. Lítil gæði kolvetna í fæði barnanna endurspeglast í lítilli neyslu fæðutrefja og að jafnaði hærra hlutfalli viðbætts sykurs af heildarorku en mælt er með. Ef skoðað er framlag mismunandi fæðuflokka til heildarorku í fæði má sjá að fæða með lága næringarþéttni (sem inniheldur lítið sem ekkert af vítamínum, steinefnum og fæðutrefjum), veitir allt að 25% af heildarorku. Þetta eru fæðuflokkar á borð við snakk,

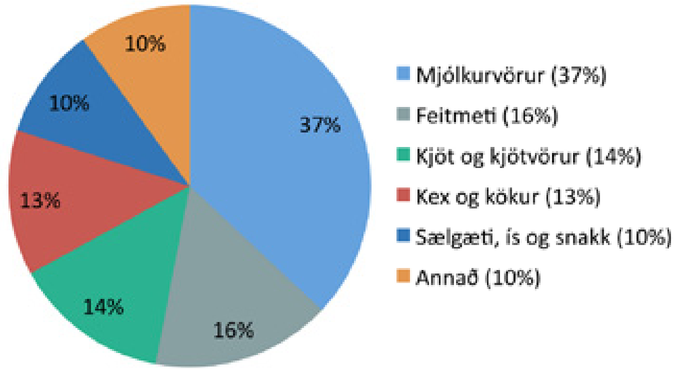
**Tafla III.** *Heildarorkuneysla og hlutfallsleg skipting orkugefandi næringarefna (E%) ásamt neyslu fæðutrefja (g/MJ)*. n=162.*

| | Viðmið* | Meðaltal ± SF | 5% | 10% | 25% | 50% | 75% | 90% | 95% |
|---|---|---|---|---|---|---|---|---|---|
| Orka (kkal) | 1600 | 1543 ± 324 | 1039 | 1161 | 1323 | 1550 | 1742 | 1906 | 1994 |
| Prótein | 10-20 | 15,4 ± 2,9 | 10,7 | 11,7 | 13,5 | 15,0 | 16,9 | 19,6 | 20,5 |
| Fita alls | 25-35 | 32,2 ± 4,9 | 23,9 | 26,0 | 29,2 | 32,0 | 35,8 | 38,5 | 40,1 |
| Mettaðar fitusýrur | ≤10** | 13,3 ± 2,7 | 9,3 | 10,0 | 11,5 | 13,2 | 15,4 | 16,5 | 17,6 |
| Cis-einómettaðar fitusýrur | 10-15 | 10,1 ± 1,8 | 7,2 | 7,7 | 9,0 | 10,0 | 11,2 | 12,5 | 13,2 |
| Cis-fjölómettaðar fitusýrur | 5-10 | 4,7 ± 1,5 | 2,6 | 2,9 | 3,5 | 4,5 | 5,5 | 6,9 | 7,9 |
| Transfitusýrur | ** | 0,8 ± 0,3 | 0,4 | 0,5 | 0,6 | 0,7 | 0,9 | 1,1 | 1,3 |
| Hörð fita | ≤10** | 14,1 ± 2,9 | 9,8 | 10,5 | 12,2 | 14,0 | 16,2 | 17,6 | 19,0 |
| Kolvetni alls | 50-60 | 50,3 ± 5,5 | 41,8 | 44,0 | 46,8 | 50,2 | 54,1 | 56,9 | 58,7 |
| Viðbættur sykur | ≤10 | 11,2 ± 4,5 | 4,7 | 5,7 | 7,7 | 11,0 | 14,5 | 17,1 | 18,8 |
| Trefjar* (g/MJ) | ≥2,5 | 2,1 ± 0,5 | 1,4 | 1,5 | 1,7 | 2,0 | 2,3 | 2,7 | 2,9 |

*Ráðleggingar um mataræði og næringarefni fyrir fullorðna og börn frá tveggja ára aldri,[14] og norrænar ráðleggingar um næringarefni.[19] Orkuþörf barna er mjög breytileg.
** Í ráðleggingum er mælt með að neysla harðrar fitu (mettaðra fitusýra auk transfitusýra) sé að hámarki 10% af heildarorku. Mælt er með því að neysla transfitusýra sé eins lág og mögulegt er.




**Mynd 1**. *Viðbættur sykur í fæði 6 ára barna. Framlag fæðuflokka.*




**Mynd 2**. *Mettuð fita í fæði 6 ára barna. Framlag fæðuflokka.*

sælgæti, ís, kex, kökur og sykraða svaladrykki. Mynd 1 sýnir framlag valinna fæðuflokka til heildarneyslu á viðbættum sykri. Sælgæti, ís, kex, kökur og drykkir aðrir en mjólk veita samtals um 60% af viðbættum sykri í fæðu sex ára barna. Um 37% af mettaðri fitu í fæði 6 ára barna kemur úr mjólk og öðrum mjólkurvörum ásamt ostum (mynd 2). Brauð og aðrar kornvörur eru helstu trefjagjafar fæðunnar (55%) en um leið stærsta uppspretta natríums (matarsalts) í fæði, en 34% af natríum í fæði barnanna kemur úr þessum fæðuflokki, þar af tæp 17% úr brauðum.

Í töflu IV má sjá neyslu vítamína- og steinefna og ráðlagða dagsskammta (RDS) fyrir sex ára börn.[14,19] Meðalneysla vítamína og steinefna var almennt meiri en RDS fyrir viðkomandi næringarefni. Þetta á þó ekki við um D-vítamín þar sem einungis fjórðungur barnanna neytti RDS eða meira af efninu (sá fjórðungur barna sem tók lýsi). Lægri mörk neyslu hafa verið áætluð 2,5 µg/dag og var D-vítamínneysla fjórðungs barna undir þeim mörkum. Athygli vekur mikil dreifing í neyslu á A-vítamíni, allt frá mjög lágri neyslu upp í neyslu sem er yfir þeim mörkum sem skilgreind hafa verið sem efri mörk hættulausrar neyslu fyrir fullorðna (3000 µg/dag). Um fjórðungur barna neytti minna en 2/3 hluta RDS fyrir E-vítamín, sem gæti bent til þess að sá hópur væri í hættu á að fullnægja ekki þörf sinni fyrir efnið. Neysla á B-vítamínum og C-vítamíni var rífleg og ólíklegt að í hópnum séu börn sem ekki fullnægja þörf sinni fyrir þau efni. Um það bil 10% barna neyttu minna en sem nemur 2/3 af RDS af kalki. Miðað við þá skilgreiningu sem stuðst er við í þessari grein er um fjórðungur barnanna í hættu á að fullnægja ekki þörf sinni fyrir joð.

## Umræða

Nýleg landskönnun á mataræði fullorðinna bendir til þess að ýmsar jákvæðar breytingar hafi orðið á mataræði þjóðarinnar á síðastliðnum árum.[17,21] Niðurstöður þessarar rannsóknar benda til þess að almennt veiti mataræði barna sem eru að hefja skólagöngu ríflegt magn vítamína og steinefna, að D-vítamíni undanskildu. Niðurstöðurnar benda til þess að ákveðinn hópur barna neyti ef til vill ekki ákjósanlegs magns næringarefna eins og joðs, E-vítamíns og A-vítamíns. Langt er í land með að neysla grænmetis, ávaxta og heilkornaafurða sé í takt við ráðleggingar og neysla á gos- og svaladrykkjum, sælgæti og öðrum sætindum er rífleg og meiri en æskilegt getur talist. Áskoranir sem við stöndum frammi fyrir er kemur að því að bæta fæðuval og þar með næringargildi fæðunnar eru svipaðar og í nágrannaríkjum okkar.[22]

Ýmislegt bendir til þess að ávaxta- og grænmetisneysla Íslendinga hafi almennt aukist á undanförnum árum.[17,21] Þó er neyslan enn langt frá þeim viðmiðum sem tengd hafa verið minni líkum á ýmsum sjúkdómum.[14,17,19,23] Innan við fimmtungur barna í þessari rannsókn neytti ávaxta og grænmetis í samræmi við ráðleggingar. Innlendar rannsóknir benda til þess að hlutfall óhollustu í fæði barna aukist með hækkandi aldri.[24,25] Um 20-25% af heildarorku í fæði barnanna í þessari rannsókn kom úr fæðuflokkum með takmarkaða næringarþéttni. Heildarorkuneysla var þó í samræmi við áætlaða orkuþörf.[19] Rannsókn á mataræði 15 ára unglinga árið 2003 sýndi að um þriðjungur af heildarorku kom úr kökum, kexi, sælgæti og ís, gosdrykkjum og öðrum sætindum.[25] Þessir fæðuflokkar eru einmitt þeir sem veita hvað mest af viðbættum sykri í fæði íslenskra barna, bæði í þessari rannsókn sem og eldri rannsóknum.[25] Neysla 6 ára barna í þessari rannsókn á kexi, kökum, sælgæti og ís (í grömmum á dag) er sambærileg og meðal fullorðinna einstaklinga (18-80 ára) í landskönnun á mataræði 2010-2011[17] þrátt fyrir að orkuþörf sex ára barna sé einungis um 60% af orkuþörf fullorðinna. Niðurstöður rannsóknarinnar sýna að gæði fitu og kolvetna er ábótavant í fæði íslenskra barna, en rannsóknir benda til þess að báðir þættir geti haft umtalsverð áhrif á þróun sjúkdóma.[4,26,27] Þörf er á markvissum íhlutunum meðal barna í landinu sem miða að því að bæta mataræði barna og unglinga. Rannsóknir hafa sýnt að íhlutun í formi fræðslu og verkefna í samstarfi við heimili og skóla er áhrifarík leið til þess að auka ávaxta- og grænmetisneyslu barna, eða koma í veg fyrir minnkandi neyslu með hækkandi aldri.[20]

Ísland er eitt fárra ríkja þar sem D-vítamíngjafi er nefndur sem hluti af fæðutengdum ráðleggingum[14] og skýrist áherslan meðal annars af legu landsins og þar með takmarkaðri D-vítamínframleiðslu í húð stóran hluta ársins. Skilaboðin virðast þó ekki hafa náð til almennings þar sem rannsóknir hafa endurtekið sýnt fram á litla neyslu vítamínsins[17,20,25,28] og sama má segja um þá rannsókn sem hér er kynnt. Helsti D-vítamíngjafi í fæði Íslendinga er lýsi. Eftir að gagnasöfnun þessarar rannsóknar lauk kom á markað D-vítamínbætt léttmjólk. Óljóst er á þessari stundu hver markaðshlutdeild hennar kemur til með að verða og þar með hver áhrifin verða

**Tafla IV.** *Dagleg neysla vítamína, steinefna auk þungmálma. Meðaltal ± staðalfrávik (SF) og dreifing neyslunnar. n=162.*

| | RDS* (2/3 af RDS) | Meðaltal ± SF | 5% | 10% | 25% | 50% | 75% | 90% | 95% |
|---|---|---|---|---|---|---|---|---|---|
| A-vítamín jafngildi (µg/dag) | 400 (267) | 963 ± 1051 | 223 | 282 | 421 | 630 | 939 | 1963 | 3635 |
| Retínól (µg/dag) | - | 850 ± 1032 | 177 | 241 | 308 | 508 | 778 | 1855 | 3582 |
| β-karótín (µg/dag) | - | 1262 ± 1396 | 153 | 196 | 306 | 608 | 1794 | 3084 | 3798 |
| D-vítamín (µg/dag) | 10 (6,5) | 7,5 ± 6,4 | 1,1 | 1,4 | 2,2 | 4,9 | 11,9 | 17,8 | 19,6 |
| E-vítamín jafngildi (mg/dag) | 6 (4) | 8,1 ± 4,9 | 3,1 | 3,4 | 4,3 | 6,3 | 10,8 | 14,5 | 16,5 |
| $B_1$-vítamín þíamín (mg/dag) | 0,9 (0,6) | 1,2 ± 0,5 | 0,6 | 0,7 | 0,9 | 1,1 | 1,4 | 2,0 | 2,4 |
| $B_2$-vítamín ríbóflavín (mg/dag) | 1,1 (0,7) | 1,7 ± 0,7 | 0,9 | 1,0 | 1,3 | 1,6 | 2,1 | 2,7 | 3,1 |
| Níasín jafngildi /dag | 12 (8) | 25 ± 8 | 15 | 16 | 19 | 23 | 29 | 36 | 39 |
| Níasín (mg/dag) | - | 15 ± 7 | 7 | 8 | 10 | 13 | 18 | 25 | 27 |
| $B_6$-vítamín þíamín (mg/dag) | 1,0 (0,7) | 1,6 ± 0,8 | 0,7 | 0,8 | 1,1 | 1,4 | 1,9 | 2,8 | 2,9 |
| Fólasín (µg/dag) | 130 (87) | 309 ± 158 | 133 | 150 | 194 | 275 | 368 | 513 | 623 |
| $B_{12}$-vítamín þíamín (µg/dag) | 1,3 (0,9) | 5,3 ± 3,3 | 1,9 | 2,4 | 3,4 | 4,4 | 5,9 | 9,0 | 14,1 |
| C-vítamín (mg/dag) | 40 (27) | 99 ± 61 | 20 | 33 | 57 | 91 | 125 | 176 | 213 |
| Kalsíum (mg/dag) | 800 (533) | 812 ± 246 | 428 | 513 | 628 | 790 | 996 | 1087 | 1174 |
| Fosfór (mg/dag) | 600 (400) | 1134 ± 277 | 723 | 786 | 958 | 1126 | 1309 | 1459 | 1543 |
| Magnesíum (mg/dag) | 200 (133) | 206 ± 51 | 137 | 147 | 170 | 204 | 233 | 270 | 293 |
| Natríum (mg/dag) | - | 2032 ± 563 | 1343 | 1395 | 1634 | 2004 | 2325 | 2649 | 2886 |
| Kalíum (mg/dag) | 2000 (1334) | 2034 ± 518 | 1254 | 1385 | 1710 | 2025 | 2334 | 2720 | 2836 |
| Járn (mg/dag) | 9 (6) | 11,1 ± 5,1 | 5,1 | 5,5 | 7,5 | 10,0 | 13,7 | 17,1 | 19,9 |
| Sink (mg/dag) | 7 (4,7) | 9,5 ± 4,8 | 4,5 | 5,4 | 6,6 | 8,3 | 10,4 | 14,5 | 20,6 |
| Kopar (mg/dag) | 0,5 (0,33) | 1,0 ± 0,6 | 0,5 | 0,6 | 0,7 | 0,9 | 1,1 | 1,5 | 2,4 |
| Joð (µg/dag) | 120 (80) | 123 ± 73 | 43 | 47 | 74 | 103 | 158 | 216 | 276 |
| Selen (µg/dag) | 30 (20) | 49 ± 21 | 26 | 28 | 35 | 45 | 54 | 77 | 92 |
| Kadmín (µg/dag) | - | 6,4 ± 1,9 | 3,7 | 4,2 | 5,4 | 6,3 | 7,7 | 8,9 | 9,6 |
| Blý (µg/dag) | - | 16,1 ± 13,2 | 3,6 | 5,7 | 7,5 | 11,6 | 20,1 | 30,7 | 46,3 |
| Kvikasilfur (µg/dag) | - | 2,3 ± 1,9 | 0,2 | 0,3 | 0,8 | 1,8 | 3,4 | 4,5 | 5,0 |

* Ráðlagðir dagsskammtar vítamína og steinefna fyrir 6-9 ára börn.[14]

til að auka D-vítamínneyslu barna. Ólíklegt er að D-vítamínbætt mjólk muni leysa af hólmi lýsið sem helsti D-vítamíngjafi íslenskra barna þar sem 100 ml af D-vítamínbættri mjólk veitir aðeins 1 µg af D-vítamíni (RDS 10 µg/dag). Þó gæti neysla hennar orðið góð viðbót við aðra mikilvæga D-vítamíngjafa eins og feitan fisk og lýsi.[18]

Eftirtekt vakti mikil neysla á salti. Einungis 4% barna í rannsókninni neyttu minna en 3,2 gramma af salti á dag, sem þykir hæfilegt magn ef tekið er mið af orkuneyslu sex ára barna.[19] Mikil saltneysla tengist auknum líkum á háþrýstingi, jafnvel meðal barna og unglinga.[6] Saltið leynist víða en þeir fæðuflokkar sem gáfu mest af salti í þessari rannsókn voru kornvörur (þar með talið brauð og morgunkorn), borðsalt og önnur krydd, súpur og sósur, kjöt og kjötvörur. Þar sem kornvörur voru einnig helsti trefjagjafi í fæði barnanna (55%) þá er æskilegt að leita leiða til að minnka saltmagn í brauðum á íslenskum markaði og velja að öðru leyti saltlitlar kornvörur. Fitugæði í fæði barna, sem og fullorðinna Íslendinga[17], er annað verkefni sem halda þarf áfram að minna á, en hlutfall harðrar fitu af heildarfitu var hærra en æskilegt getur talist í þessari rannsókn.[7]

Niðurstöðurnar benda til þess að eitt af þeim efnum sem hugsanlega gæti verið af skornum skammti í fæði ákveðins hóps barna sé joð. Eru niðurstöðurnar í samræmi við áhyggjur sem áður hafði verið lýst eftir birtingu niðurstaðna kannana á mataræði fullorðinna Íslendinga 2002[16] og barna og unglinga 2002-2003[25] sem bentu til minnkaðrar fiskneyslu samhliða minnkaðri neyslu á mjólkurvörum meðal Íslendinga. Þessir tveir fæðuflokkar eru helstu uppsprettur joðs í fæðu. Joðbætt salt er almennt ekki notað á Íslandi. Hins vegar benda niðurstöður nýrra rannsókna á joðhag annars vegar unglingsstúlkna,[29] og hins vegar þungaðra kvenna,[30] til þess að ekki sé þörf á því að ráðleggja notkun joðbætts salts á Íslandi. Gæti ráðlegging um almenna notkun á joðbættu salti á Íslandi jafnvel verið óráðleg, þar sem slíkt gæti leitt til þess að ákveðinn hópur myndi ef til vill fá of mikið af efninu.[19,30] Hins vegar gæti verið þörf á sérstökum ráðleggingum til þeirra hópa sem hvorki neyta fisks né mjólkurvöru eða neyta þessa í mjög takmörkuðu magni.[30]

Þrátt fyrir að mjólkurneysla Íslendinga hafi dregist saman undanfarna áratugi[17,29-31] telst mjólkurneysla íslenskra 6 ára barna rífleg. Yfir 85% barna í rannsókninni neytir mjólkurvara í samræmi við ráðleggingar og kalkneysla er rífleg í samræmi við það. Hins vegar er ástæða til þess að benda sérstaklega á að tæplega 10% barnanna í rannsókninni neyttu sem svarar fjórum skömmtum eða meira af mjólk og mjólkurvörum daglega. Þessi fæðuflokkur veitti um það bil 37% af mettaðri fitu í fæði, en hlutfall mettaðrar fitu var hærra en æskilegt getur talist. Neysla mjólkurvara umfram ráðleggingar (tveir skammtar á dag) gæti talist óæskileg og ef hún er langt umfram stuðlar hún að of einhæfu fæðuvali. Eins eru faraldsfræðilegar vísbendingar um að of mikil mjólkurneysla gæti verið skaðleg til lengri tíma litið.[4] Mjólk og mjólkurvörur teljast þó mikilvægur hluti af hollu mataræði, enda uppspretta mikilvægra næringarefna, svo sem kalks og joðs.[14]

Mataræði er einn af lífsstílstengdum áhrifaþáttum heilsu og er það á ábyrgð samfélagsins í heild að stuðla að heilsusamlegu mataræði landsmanna. Þátttakendur í rannsókninni voru fjölskyldur barna sem áður höfðu tekið þátt í rannsókn á mataræði ungbarna. Einstaklingar sem eru áhugasamir um mikilvægi næringar eru ef til vill líklegri til að taka þátt í rannsókn sem þessari og má því álykta að þær niðurstöður sem hér eru birtar gefi betri mynd af mataræði íslenskra 6 ára barna heldur en það er í raun og veru. Matarvenjur byrja að mótast í æsku[32-34] og þess vegna er mikilvægt að börn læri í skólum um hollar venjur, þeim standi til boða að velja hollan mat og hreyfa sig. Aðgengi er nauðsynlegt til að hollt val geti átt sér stað, eins og til dæmis það að ávextir og grænmeti séu til á heimilum og séu hluti af skólamáltíðum.[30] Þrátt fyrir það að einstaklingurinn sjálfur beri ábyrgð á eigin heilsu er mikilvægt að matvælaframleiðendur axli sína ábyrgð, ekki síst þegar kemur að markaðssetningu fæðu sem ætluð er börnum. Heilbrigðiskerfið þarf að vera í stakk búið til að sinna forvörnum, meðal annars með ráðleggingum til þeirra sem þurfa á leiðbeiningum að halda um val á hollum mat fyrir börn sín. Eins er mikilvægt að fylgst sé reglulega með þróun mataræðis meðal barna.

## Þakkir

Við þökkum Hrafnhildi Evu Stephensen MS í næringarfræði og Jónu Halldórsdóttur BS-nema í næringarfræði fyrir aðstoð við gagnasöfnun, Hólmfríði Þorgeirsdóttur matvæla- og næringarfræðingi hjá Embætti landlæknis og Ólafi Reykdal matvælafræðingi hjá MATÍS ohf fyrir samstarf um gagnagrunna og forrit, Ívari Guðmundssyni fyrir aðstoð við næringarefnaútreikninga og Gesti I. Pálssyni barnalækni fyrir samstarf við gagnasöfnun.

Rannsóknasjóður Háskóla Íslands og Vísindasjóður Landspítala styrktu rannsóknina og Embætti landlæknis styrkti úrvinnslu gagna fyrir þessa grein um Landskönnun á mataræði 6 ára Íslendinga. Rannsóknarnámssjóður RANNÍS styrkir MS-nám Birnu Þórisdóttur.

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# Diet of six-year-old Icelandic children – National dietary survey 2011-2012


## Abstract

**Aim:** Knowledge of dietary habits makes the basis for public nutrition policy. The aim of this study was to assess dietary intake of Icelandic six-year-olds.

**Material and methods:** Subjects were randomly selected six-year-old children (n=162). Dietary intake was assessed by three-day-weighed food records. Food and nutrient intake was compared with the Icelandic food based dietary guidelines (FBDG) and recommended intake of vitamins and minerals.

**Results:** Fruit and vegetable intake was on average 275±164 g/d, and less than 20% of the subjects consumed ≥400 g/d. Fish and cod liver oil intake was in line with the FBDG among approximately 25% of subjects. Most subjects (87%) consumed at least two portions of dairy products daily. Food with relatively low nutrient density (cakes, cookies, sugar sweetened drinks, sweets and ice-cream) provided up to 25% of total energy intake. The contribution of saturated fatty acids to total energy intake was 14.1%. Less than 20% of the children consumed dietary fibers in line with recommendations, and for saturated fat and salt only 5% consumed less than the recommended upper limits. Average intake of most vitamins and minerals, apart from vitamin D, was higher than the recommended intake.

**Conclusion:** Although the vitamin and mineral density of the diet seems adequate, with the exception of vitamin D, the contribution of low energy density food to total energy intake is high. Intake of vegetables, fruits, fish and cod liver oil is not in line with public recommendations. Strategies aiming at improving diet of young children are needed.



**Key words:** Child nutrition sciences, food habits, nutrition policy, child, dietary surveys, nutritive value.

# Introduction

The first years of life are particularly important with respect to nutrition. Research indicates that nutritional circumstances and food selection the first years of life can have long-term effects on growth, development and health.[1-8]

Information on the diets of different groups is the basis of the Government's formulation of policy on human nutrition. Knowledge therefore plays a part in strengthening the country's public health. The last 15 years, the Unit for Nutrition Research has studied children's diets. The findings have been utilised, for example, in revising official recommendations.[3, 9-13]

Many countries, including Iceland, have formulated recommendations on foods, in parallel with traditional recommendations on essential nutrients, vitamins and minerals, energy, and proportions of energy nutrients.[14] Thus, recommendations are published on the advised frequency of the consumption of and/or quantity of foods from certain food groups. The goal is to make it easier for people to choose varied and good foods that studies have shown to promote improved health and reduce the risk of various diseases, such as cardiovascular disease, obesity, diabetes and certain types of cancer.[15]

The goal of this study was to collect information on the food consumption and nutrients of six-year-old Icelanders. The research on the diet of six-year-old children in 2011-2012 is a prospective study of a randomly selected national sample of infants that were followed through the age of six. Official recommendations on food selection and nutrients[16] are used to evaluate the findings on the consumption of food and nutrients in the diet of six-year-old children, and the findings are presented here.

# Study population and methods

The participants were six-year-old children who had previously participated in long-term research on the diet and health of Icelandic infants.[2] The infants were born in 2005. Statistics Iceland randomly selected the infants at four times that year from the National Registry. At each random selection, the children were younger than four months. 250 children met the conditions for participation. These conditions were Icelandic parents, single birth, gestation period of 37-41 weeks, birth weight between the $10^{th}$ and $90^{th}$ percentile, no birth defects or diseases at birth, and the mother had received maternal care. Altogether, 219 children had completed at least one part of the study at the age of 12 months. These were invited to participate in the follow-up at age six. Satisfactory food diaries were submitted for 162 six-year-old children (74%). The proportion of university-educated parents in the study was 58% for mothers and 42% for fathers. The Bioethics Committee first approved the research on the nutrition of infants (VSNb2005040019/037), and the study was registered at the Data Protection Authority (S2449/2005). Then, before the research on the six-year-olds started, the Bioethics Committee and the Data Protection Authority registered that, respectively (VSNb2011010008/37 and 2010111049AMK).

All food and beverages that the children consumed near their sixth birthday was weighed to an accuracy of ± 1 g (PHILIPS HR PHILIPS HR 2385, Hungary), and the consumption was recorded in a food diary for three days in a row, on two working days and one weekend day. Recording was primarily the responsibility of parents and guardians, but preschools and compulsory schools also collaborated on the weighing and recording of the meals that the children ate at school. Parents, guardians, and others who saw to the recording of diet received written and oral instructions on the use of the scales and the preciseness of recording (for example, the importance of recording the exact type and even the trademark of the foods eaten). The results were entered into the nutrition calculation programme ICEFOOD that was designed for the National Dietary Survey 2002[16] and improved for the National Dietary Survey 2010-2011.[17] The references for calculating the nutritional value of foods were, on one hand, the Icelandic Database on the Contents of Foods, ISGEM,[18] and, on the other, the database of the former Public Health Centre on the composition of common meals and snacks on the Icelandic market. The loss of nutrients during cooking was taken into consideration.

Findings are published on the consumption of selected types of foods (in grams per day (g/day)), energy (in kcal/day), energy nutrients (in g/day and as total energy consumption) and consumption of vitamins and minerals. The study likewise analysed the contribution of food groups to total energy consumption and selected nutrients (hard fat, added sugar, salt and dietary fiber). In classifying food types into defined food groups, we referred to the system described in a report on the main findings of the National Dietary Survey of Adult Icelanders 2010-2011.[17] The findings were compared with the recommended food selection and recommended daily allowances of nutrients for the relevant age group.[14,19] The portion sizes of the food selection were adapted to the energy requirement of six-year-old children. This is about 60% of adults' estimated energy requirement.[20] RDAs for vitamins and minerals are defined as the amount of nutrients fulfilling the needs of most people, i.e., about 98% of the population. When assessing the vitamin and mineral consumption of groups, the custom is to refer to additional references besides RDA, i.e., average requirement, the lower limit of desirable consumption and the upper limit of safe consumption. The average requirement of vitamins and minerals has not been defined for children, but for adults it generally corresponds 60-80% of RDA.[19] This article uses 2/3 of RDA as the estimated average requirement of the population, for this is the common recognised approach when findings of consumption surveys are assessed, and the average requirement for the subjects is not known.[19] If consumption falls below the estimated average requirement of the population, it may be inferred that there is a risk that the requirement for the relevant nutrients is not being met. The programme SAS (9.2) was used for statistical analysis. The T-test was used to check for differences in the consumption of nutrients between the sexes. The level of significance was defined as $p<0.05$.

# Findings

The total energy of the diet for girls was 1529±314 kcal/day, and for boys it was 1558±336 kcal/day ($p>0.05$). The intake of foods and composition of the total energy nutrients and intake of vitamins and minerals proved not to differ between the sexes ($p>0.05$). The findings are consequently published as average figures and standard deviations for both sexes together, along with the distribution of consumption.

**Table I** shows food intake from selected food groups. About half of the six-year-old children ate less than 40 g of vegetables daily. For comparison, a tomato weighs about 80 g. Fruit intake was usually more ample than vegetable intake. The consumption of milk and milk products was usually more than 350 grams per day. The average consumption of soda and soft drinks was more than 800 ml per week, and the 10% of children consuming the most soda and soft drinks usually drank close to 2 litres per week. The consumption of cookies and cakes was nearly 3 times more than the consumption of high-fiber bread (defined as ≥6 grams of fiber per 100 grams of bread), and the average consumption of candy and other sweets was also ample. However, it bears stating that half of the children consumed neither sweets nor soda on the days of recording.

**Table II** shows the recommended food selection,[14] in addition to the number and percentage of children whose consumption was in accordance with the given criteria. Six-year-old children are generally quite a ways from reaching the recommended intake of vegetables and fruits. About one quarter of the children consumed fish and fish oil on the recording days. Likewise, the children are a very long ways from consuming hard fat (saturated and trans-polyunsaturated fatty acids), dietary fiber and salt in accordance with recommendations. Most participants (87%) daily consumed at least two portions of milk and dairy products. We can say that nearly 10% of children daily consumed four portions of milk and dairy products or more.

**Table III** shows total energy consumption (kcal/day) and the percentage distribution of energy nutrients. Protein usually provided about 15.4±2.9% of total energy. The findings show that the quality of fat in food is deficient, for hard fat (that is saturated fatty acids and trans-polyunsaturated fatty acids) provides 14.1±2.9% of total energy. Low quality of carbohydrates in children’s diet is reflected in low consumption of dietary fiber and usually a higher than recommended proportion of total energy that is added sugar. Examination of the contribution of different food groups to total energy in the diet shows that food with low nutritional density (containing next to no vitamins, minerals and dietary fiber), provides up to 25% of total energy. These are food groups like snacks, sweets, ice cream, cookies, cakes and sweetened soft drinks. **Figure 1** shows the contribution of added sugar in selected food groups to total energy consumption. Sweets, ice cream, cookies, cakes, and drinks other than milk provide about 60% of added sugar in the diet of six-year-old children. About 37% of saturated fat in six-year-olds' diet comes from milk and other dairy products along with cheese (**Figure 2**). Bread and other cereal products are the main sources of dietary fiber (55%). At the same time, they are the biggest source of sodium (dietary salt) in the diet–34% of dietary salt in the children’s diet comes from this food group (17% from bread).

**Table IV** shows the intake of vitamins, minerals and RDAs for six-year-old children.[14,19] The average intake of vitamins and minerals generally exceeded the RDAs for nutrients. However, this does not apply to vitamin D since only one quarter of the children reached or exceeded the RDA for vitamin D (the quarter of the children who took fish oil). The lower intake limit was estimated at 2.5 µg/day, and the vitamin D intake of one quarter of the children was below the limit. The broad distribution for the vitamin A intake is striking. It ranges from very low intake to intake exceeding the upper limit defined as safe consumption for adults (3000 µg/day). About one quarter of children got less than 2/3 of the RDA for vitamin E. This could indicate that this group was at risk of not fulfilling their requirement for the nutrient. The intake of vitamins B and C was ample, and it is unlikely that there are children in the group that did not meet their requirement for these vitamins. About 10% of the children got less than 2/3 of the RDA for calcium. Based on the definition this article uses as a guide, about a quarter of the children are at risk of not meeting their requirement for iodine.

# Discussion

The recent National Dietary Survey of Adults’ Diet indicates that positive changes have occurred in the nation's diet over the last several years.[17,21] The findings of this study indicate that the diet of children starting school generally provides ample quantities of vitamins and minerals, with the exception of vitamin D. The findings indicate that a certain group of children may not consume ideal quantities of nutrients like iodine, vitamin E and vitamin K. Iceland has a long way to go before the intake of vegetables, fruits and whole grain products is in accordance with recommendations, and the consumption of soda and soft drinks, candy and other sweets is ample and more than is deemed desirable. The challenges facing us when it comes to improving food selection, and thereby the nutritional value of foods, are similar to those in our neighbouring countries.[22]

Several factors indicate that Icelanders' fruit and vegetable consumption has generally increased in recent years.[17, 21] However, the consumption is far from the criteria connected with lower risks of various diseases.[14,17,19,23] Less than one fifth of the children in this study consumed fruits and vegetables in accordance with recommendations. Icelandic studies indicate that the proportion of unhealthy foods in children's diet will increase as they grow up.[24,25] About 20-25% of total energy in the diet of the children in this study came from food groups having limited nutritional density. However, total energy consumption was in accordance with the estimated energy need.[19] A study in 2003 of the diet of 15-year-old youths showed that one third of total energy came from cakes, cookies, candy and ice cream, soda, and other sweets.[25]It is precisely these food groups that provide the most added sugar in the diet of Icelandic children, in this as well as previous studies.[25] In this study, six-year-old children's consumption (grams per day) of cookies, cakes, candy and ice cream is comparable to that of adult individuals (aged 18-80) in the National Dietary Survey 2010-2011[17] despite the energy requirement of the six-year-old children being only about 60% of the energy requirement of adults. The study's findings show that the quality of fat and carbohydrates is deficient in the diet of Icelandic children. However, research indicates that both factors can have considerable effect on the development diseases.[4,26,27] Iceland needs purposive interventions for its children, aimed at improving the diets of children and youths. Research has shown that intervention in the form of education and health promotion, in collaboration with homes and schools, is an effective way to increase children's consumption of fruits and vegetables, or to prevent decrease in consumption with increasing age.[20]

Iceland is one of the few countries where vitamin D sources are mentioned as part of recommendations on foods[14]. This emphasis is due partially to the country's geographical position, thereby resulting in limited vitamin D production in the skin for a big part of the year. However, the message appears not to have reached the public since studies have repeatedly proved low vitamin D intake[17,20,25,28], and the same can be said for the study presented here. The main source of vitamin D in Icelanders' diet is fish oil. After the data collection for this research was finished, vitamin D-enriched low-fat milk came on the market. It is unclear at this time what its market share will be, and thereby whether it will increase children's vitamin D intake. It is unlikely that vitamin D-enriched milk will replace fish oil as Icelandic children's main source of vitamin D since 100 ml of vitamin D-enriched milk provides only 1 µg of vitamin D (RDA 10 µg/day). However, consumption of the milk could be a good addition to other important sources of vitamin D, like fatty fish and fish oil.[18]

The great consumption of dietary salt really stirred up attention. Only 4% of children in the study consumed less than 3.2 g of dietary salt per day. This seems a suitable quantity, considering the energy consumption of six-year-old children.[19] Heavy salt consumption is linked to increased risk of high blood pressure, even amongst children and youths.[6] Salt lurks in various foods. However, those giving the highest amounts in this study were cereal products (including bread and breakfast cereal), table salt and spices, soups and gravies, meat and meat products. Since cereal products were also one of the main sources of dietary fiber in the children's diet (55%), it is desirable to seek ways to reduce the quantity of salt in breads on the Icelandic market and otherwise choose low-salt cereal products. The fat quality in the food of Icelandic children, as well as adults[17], is another project that we must continually remind people of, for in this study, hard fat as a proportion of total energy was higher than desirable.[7]

The findings indicate that iodine may be an element that could possibly be lacking in the diet of a certain group of children. The findings are in accordance with concerns previously expressed after publication in 2002 of survey findings on the diet of Icelandic adults[16] and children and youths in 2002-2003[25]. They indicated Icelanders' reduced consumption of fish in parallel with reduced consumption of dairy products. These two food groups are the main sources of iodine in food. Iodised salt is generally not used in Iceland. On the other hand, findings in later studies on the iodine status of, on one hand, young girls,[29] and, on the other, pregnant women,[30] indicated that there is not a need to recommend the use of iodised salt in Iceland. A recommendation on general use of iodised salt in Iceland could even be inadvisable since this could lead to a certain group perhaps getting too much of the substance.[19,30] On the other hand, there could be a need for special recommendations to groups that consume neither fish nor dairy products or consume them in very limited quantities.[30]

Despite a decrease in recent decades of Icelanders' consumption of dairy products,[17,29-31] six-year-olds' consumption of dairy products is deemed ample. Over 85% of children in the study consume dairy products in accordance with recommendations, and the consumption of calcium is accordingly ample. On the other hand, there is reason to point out specifically that less than 10% of children in the study consumed the equivalent of four or more helpings of milk and dairy products daily. This food group provided about 37% of saturated fat in the diet, which was a higher percentage of saturated fat than researchers deem desirable. More consumption of dairy products than recommended (two helpings per day) can be deemed undesirable, and if it is far beyond this, it promotes food selection with too little variety. There are also epidemiological indications that too much consumption of dairy products could be harmful over the longer term.[4] However, milk and milk products are deemed part of a healthy diet since they are a source of important nutrients, such as calcium and iodine.[14]

Diet is one of the life-style factors important to health, and it is the responsibility of society as a whole to promote a healthy diet of its citizens. Participants in the study were families of children who had previously participated in a study on the diet of infants. Individuals interested in the importance of nutrition are perhaps more likely to participate in a study like this one. One may therefore infer that the results published here provide a better picture of the diet of Icelandic six-year-olds than is actually the case. Dietary habits begin to form early in life,[32-34] and it is for this reason important for children to learn healthy habits in school, and that they have the option to choose healthy food and be active. Accessibility is necessary to make healthy choice possible, for example, having fruits and vegetables available in households and as part of school meals.[30] Despite individuals being responsible for their own health, it is important for food manufacturers to shoulder their responsibility, not least when it comes to marketing food intended for children. The healthcare system must be prepared to take preventive measures, for example, with recommendations to those needing instructions on choosing healthy food for their children. Likewise, it is important to monitor the trends in children's diet regularly.

# Thanks

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**Table I**. Consumption (g/day) of selected food from different food groups (Mean±standard deviation (SD) and distribution of the consumption). N=162.

| | Mean±SD | 5% | 10% | 25% | 50% | 75% | 90% | 95% |
|---|---|---|---|---|---|---|---|---|
| **Vegetables, fruits and pure juice** | | | | | | | | |
| Vegetables | 52±47 | 1 | 5 | 14 | 39 | 77 | 124 | 134 |
| Fruits | 136±102 | 3 | 24 | 57 | 114 | 201 | 292 | 318 |
| Pure juice | 87±107 | 0 | 0 | 0 | 45 | 155 | 242 | 287 |
| Total (vegetables, fruits and pure juice) | 275±164 | 46 | 89 | 155 | 257 | 369 | 533 | 583 |
| **Potatoes and cereals** | | | | | | | | |
| Potatoes, new | 21±28 | 0 | 0 | 1 | 16 | 29 | 49 | 56 |
| French fries | 6±12 | 0 | 0 | 0 | 0 | 0 | 24 | 37 |
| Bread, total | 80±39 | 30 | 38 | 52 | 71 | 104 | 127 | 151 |
| Whole grain bread, fiber ≥6% | 16±21 | 0 | 0 | 0 | 8 | 23 | 48 | 59 |
| Breakfast cereals | 43±38 | 0 | 6 | 19 | 33 | 54 | 93 | 126 |
| Oatmeal | 16±36 | 0 | 0 | 0 | 0 | 0 | 67 | 107 |
| Pasta og noodles | 19±29 | 0 | 0 | 0 | 3 | 28 | 61 | 81 |
| Pizza | 12±28 | 0 | 0 | 0 | 0 | 0 | 49 | 75 |
| **Fish and meat** | | | | | | | | |
| Fish and fish products | 21±24 | 0 | 0 | 0 | 16 | 30 | 53 | 62 |
| Meat and meat products | 65±35 | 15 | 21 | 39 | 63 | 84 | 112 | 128 |
| **Milk and milk products** | | | | | | | | |
| Whole milk | 78±117 | 0 | 0 | 0 | 0 | 123 | 239 | 290 |
| Low fat milk | 102±144 | 0 | 0 | 0 | 60 | 137 | 301 | 385 |
| Skimmed milk | 17±49 | 0 | 0 | 0 | 0 | 0 | 67 | 103 |
| Chocolate milk/cocoa | 32±53 | 0 | 0 | 0 | 0 | 67 | 83 | 133 |
| Milk products* | 22±29 | 0 | 1 | 4 | 14 | 36 | 51 | 58 |
| Cheeses | 17±14 | 0 | 0 | 6 | 14 | 27 | 35 | 39 |
| Total dairy intake | 357±193 | 97 | 143 | 205 | 345 | 458 | 597 | 720 |
| **Fats** | | | | | | | | |
| Butter and margarine | 9±8 | 1 | 2 | 4 | 7 | 12 | 16 | 21 |
| Sauces and dips | 13±11 | 0 | 0 | 4 | 10 | 18 | 26 | 33 |
| Cod liver oil | 2±3 | 0 | 0 | 0 | 0 | 5 | 8 | 8 |
| **Beverages** | | | | | | | | |
| Soda and soft drinks | 117±129 | 0 | 0 | 23 | 83 | 165 | 266 | 417 |
| Soft drinks | 71±99 | 0 | 0 | 0 | 31 | 89 | 203 | 274 |
| Soda | 46±76 | 0 | 0 | 0 | 0 | 67 | 151 | 220 |
| Water and soda water | 285±235 | 10 | 49 | 123 | 221 | 404 | 545 | 737 |
| **Cakes, cookies, sweets and snacks** | | | | | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Cakes | 32±34 | 0 | 0 | 4 | 27 | 42 | 73 | 93 |
| Cookies | 13±18 | 0 | 0 | 0 | 7 | 18 | 34 | 49 |
| Sweets and ice-cream | 32±33 | 0 | 0 | 0 | 25 | 53 | 81 | 96 |
| Popcorn, snacks, nuts | 8±14 | 0 | 0 | 0 | 1 | 10 | 24 | 32 |

* Milk products other than drinking milk and cheeses

**Table II**. Number and the proportion of six-year-olds (n=162) with dietary intake in line with the Icelandic food based dietary guidelines.

| Food based dietary guidelines* | Criteria<br>Adjusted for six-year-olds | Number<br>n | Proportion<br>% |
|---|---|---|---|
| Fruits and vegetables | ≥400 g of fruits, vegetables and pure juice per day | 30 | 19 |
| Fish at least two times per week or more | ≥34 g per day, which equals a fish meal two times per week | 37 | 23 |
| Whole grain bread and other fiber rich cereals | ≥2.5 g dietary fiber/MJ/day ** | 28 | 17 |
| Milk products that contain less fat | 2 portions of milk or milkproducts daily (1 portion 200 g of milk products or ≥20 g of cheese) | 141 | 87 |
| Oils or soft fat instead of hard fat | <10% of the energy from hard fat | 10 | 6 |
| Moderation in salt intake | <3200 mg of salt per day*** | 7 | 4 |
| Cod liver oil or other vitamin D source | ≥5 ml per day, which equals daily recommendations for vitamin D | 44 | 27 |
| Water is the best soft drink | <10% of the energy from added sugar | 70 | 43 |

*Recommendations on diet and nutrition for adults and children from two years of age. [10]

**The Nordic Nutrition Recommendation on intake of dietary fibre is used to assess adherance to this recommendation (≥2.5 g/MJ/day).[15]The reason is that the nutrient calculating program used for analysis, ICEFOOD, does not allow this categorisation except for bread.

*** The Nordic Nutrition Recommendation on salt intake: [15]≤0.5 g salt/1000 kJ/day (0.5 g salt/239 kcal/day) corresponding to <3.2 g salt/day according to the energy intake in the present study.

**Table III**. Total energy intake, contribution of energy giving nutrients to total energy intake (E%) and intake of dietary fiber (g/MJ). N=162.

| | Mean±SD | 5% | 10% | 25% | 50% | 75% | 90% | 95% |
|---|---|---|---|---|---|---|---|---|
| Energy (kcal) | 1543±324 | 1039 | 1161 | 1323 | 1550 | 1742 | 1906 | 1994 |
| Protein | 15.4±2.9 | 10.7 | 11.7 | 13.5 | 15.0 | 16.9 | 19.6 | 20.5 |
| Total fat | 32.2±4.9 | 23.9 | 26.0 | 29.2 | 32.0 | 35.8 | 38.5 | 40.1 |
| Saturated fatty acids | 13.3±2.7 | 9.3 | 10.0 | 11.5 | 13.2 | 15.4 | 16.5 | 17.6 |
| Cis-monounsaturated fatty acids | 10.1±1.8 | 7.2 | 7.7 | 9.0 | 10.0 | 11.2 | 12.5 | 13.2 |
| Cis-polyunsaturated fatty acids | 4.7±1.5 | 2.6 | 2.9 | 3.5 | 4.5 | 5.5 | 6.9 | 7.9 |
| Trans fatty acids | 0.8±0.3 | 0.4 | 0.5 | 0.6 | 0.7 | 0.9 | 1.1 | 1.3 |
| Carbohydrates total | 50.3±5.5 | 41.8 | 44.0 | 46.8 | 50.2 | 54.1 | 56.9 | 58.7 |
| Added sugar | 11.2±4.5 | 4.7 | 5.7 | 7.7 | 11.0 | 14.5 | 17.1 | 18.8 |
| Fiber (g/MJ) | 2.1±0.5 | 1.4 | 1.5 | 1.7 | 2.0 | 2.3 | 2.7 | 2.9 |

**Table IV**. Daily intake of vitamins, minerals and heavy metals. Mean±standard deviation (SD) and distribution of the consumption. N=162.

| | RDI* (2/3 af RDI) | Mean±SD | 5% | 10% | 25% | 50% | 75% | 90% | 95% |
|---|---|---|---|---|---|---|---|---|---|
| Vitamin A equalent (µg/day) | 400 (267) | 963±1051 | 223 | 282 | 421 | 630 | 939 | 1963 | 3635 |
| Retinol(µg/ day) | - | 850±1032 | 177 | 241 | 308 | 508 | 778 | 1855 | 3582 |
| β-carotin (µg/day) | - | 1262±1396 | 153 | 196 | 306 | 608 | 1794 | 3084 | 3798 |
| Vitamin D (µg/day) | 10 (6.5) | 7.5±6.4 | 1.1 | 1.4 | 2.2 | 4.9 | 11.9 | 17.8 | 19.6 |
| Vitamin E equalent (mg/day) | 6 (4) | 8.1±4.9 | 3.1 | 3.4 | 4.3 | 6.3 | 10.8 | 14.5 | 16.5 |
| Vitamin $B_1$ thiamin (mg/day) | 0.9 (0.6) | 1.2±0.5 | 0.6 | 0.7 | 0.9 | 1.1 | 1.4 | 2.0 | 2.4 |
| Vitamin $B_2$ riboflavin (mg/day) | 1.1 (0.7) | 1.7±0.7 | 0.9 | 1.0 | 1.3 | 1.6 | 2.1 | 2.7 | 3.1 |
| Niacin equalent (NJ /day) | 12 (8) | 25±8 | 15 | 16 | 19 | 23 | 29 | 36 | 39 |
| Niacin (mg/day) | - | 15±7 | 7 | 8 | 10 | 13 | 18 | 25 | 27 |
| Vitamin $B_6$ thiamin (mg/day) | 1.0 (0.7) | 1.6±0.8 | 0.7 | 0.8 | 1.1 | 1.4 | 1.9 | 2.8 | 2.9 |
| Folate (µg/day) | 130 (87) | 309±158 | 133 | 150 | 194 | 275 | 368 | 513 | 623 |
| Vitamin $B_{12}$ (µg/day) | 1.3 (0.9) | 5.3±3.3 | 1.9 | 2.4 | 3.4 | 4.4 | 5.9 | 9.0 | 14.1 |
| Vitamin C (mg/day) | 40 (27) | 99±61 | 20 | 33 | 57 | 91 | 125 | 176 | 213 |
| Calcium (mg/day) | 800 (533) | 812±246 | 428 | 513 | 628 | 790 | 996 | 1087 | 1174 |
| Phosphate (mg/day) | 600 (400) | 1134±277 | 723 | 786 | 958 | 1126 | 1309 | 1459 | 1543 |
| Magnesium (mg/day) | 200 (133) | 206±51 | 137 | 147 | 170 | 204 | 233 | 270 | 293 |
| Sodium (mg/day) | - | 2032±563 | 1343 | 1395 | 1634 | 2004 | 2325 | 2649 | 2886 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Potassium (mg/day) | 2000 (1334) | 2034±518 | 1254 | 1385 | 1710 | 2025 | 2334 | 2720 | 2836 |
| Iron (mg/day) | 9 (6) | 11.1±5.1 | 5.1 | 5.5 | 7.5 | 10.0 | 13.7 | 17.1 | 19.9 |
| Zink (mg/day) | 7 (4.7) | 9.5±4.8 | 4.5 | 5.4 | 6.6 | 8.3 | 10.4 | 14.5 | 20.6 |
| Copper (mg/day) | 0.5 (0.33) | 1.0±0.6 | 0.5 | 0.6 | 0.7 | 0.9 | 1.1 | 1.5 | 2.4 |
| Iodine (µg/day) | 120 (80) | 123±73 | 43 | 47 | 74 | 103 | 158 | 216 | 276 |
| Selenium (µg/day) | 30 (20) | 49±21 | 26 | 28 | 35 | 45 | 54 | 77 | 92 |
| Cadmium (µg/day) | - | 6.4±1.9 | 3.7 | 4.2 | 5.4 | 6.3 | 7.7 | 8.9 | 9.6 |
| Lead (µg/day) | - | 16.1±13.2 | 3.6 | 5.7 | 7.5 | 11.6 | 20.1 | 30.7 | 46.3 |
| Mercury (µg/day) | - | 2.3±1.9 | 0.2 | 0.3 | 0.8 | 1.8 | 3.4 | 4.5 | 5.0 |

* Recommended daily intake of vitamins and minerals for 6-9-year-old children.[10]

**Figure 1**. Added sugar in the diet of six-year-olds. Contribution of food groups.

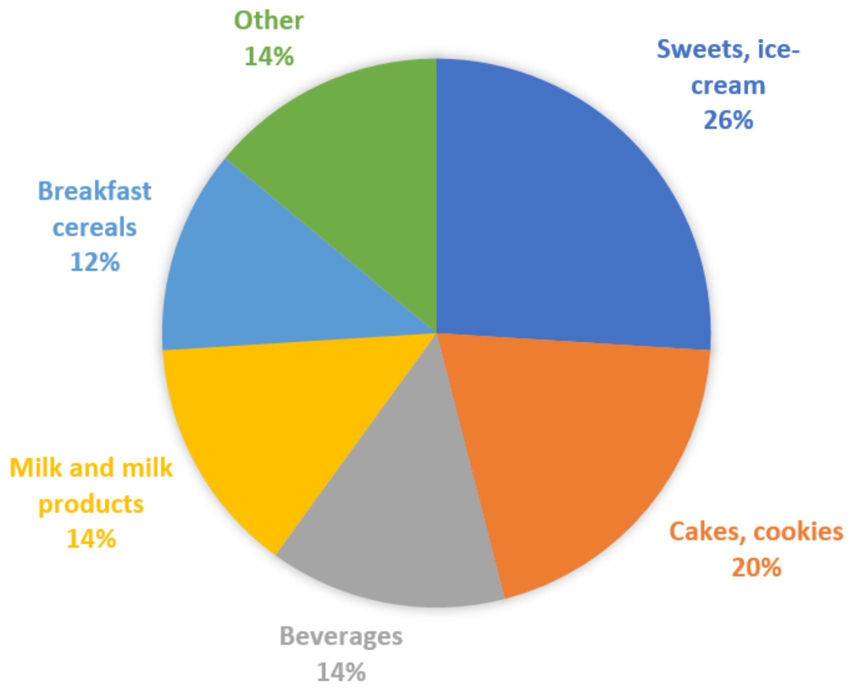
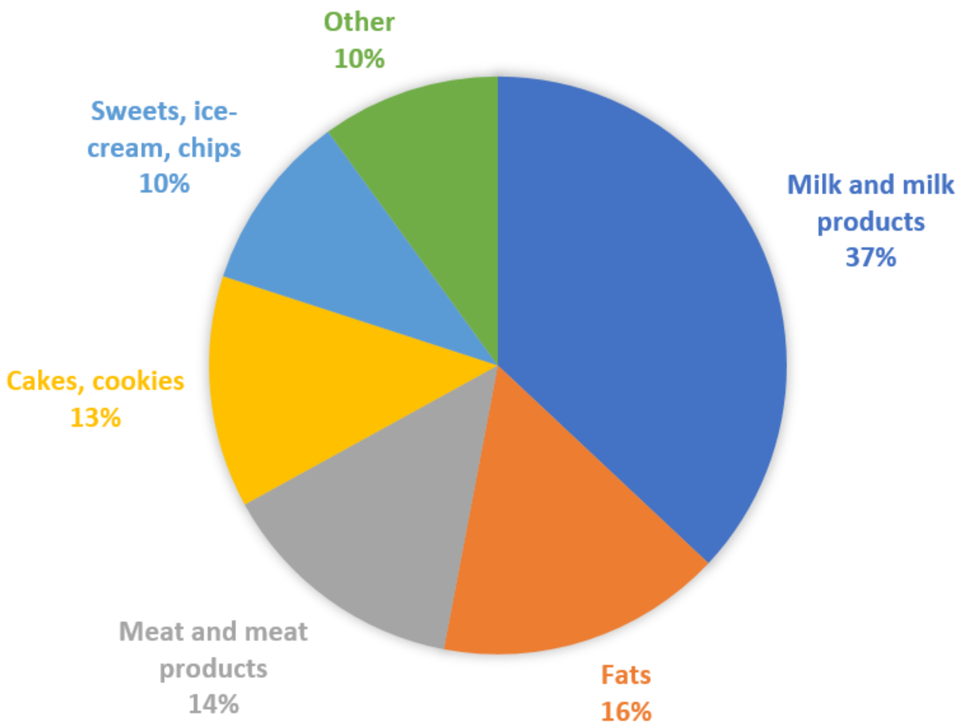



**Figure 2**. Saturated fat in the diet of six-year-olds. Contribution of food groups.



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*Article*

# Vitamin D Intake and Status in 12-Month-Old Infants at 63–66° N

**Birna Thorisdottir [1,*], Ingibjorg Gunnarsdottir [1], Laufey Steingrimsdottir [1], Gestur I. Palsson [2] and Inga Thorsdottir [1]**

[1] Unit for Nutrition Research, Landspitali University Hospital & Faculty of Food Science and Nutrition, School of Health Sciences, University of Iceland, Eiriksgata 29, IS-101 Reykjavik, Iceland; E-Mails: ingigun@landspitali.is (I.G.); laufey@hi.is (L.S.); ingathor@hi.is (I.T.)

[2] Children's Hospital, Landspitali University Hospital, Hringbraut, IS-101 Reykjavik, Iceland; E-Mail: gesturip@landspitali.is

* Author to whom correspondence should be addressed; E-Mail: bth50@hi.is; Tel.: +354-5438-410; Fax: +354-5521-331.

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**Abstract:** The objective was to assess the vitamin D status in healthy 12-month-old infants in relation to quantity and sources of dietary vitamin D, breastfeeding and seasons. Subjects were 76 12-month-old infants. Serum levels of 25-hydroxyvitamin D (25(OH)D) ≥ 50 nmol/L were considered indicative of vitamin D sufficiency and 25(OH)D < 27.5 nmol/L as being indicative of increased risk for rickets. Additionally, 25(OH)D > 125 nmol/L was considered possibly adversely high. Total vitamin D at 9–12 months (eight data collection days) included intake from diet and supplements. The mean ± SD of vitamin D intake was 8.8 ± 5.2 µg/day and serum 25(OH)D 98.1 ± 32.2 nmol/L (range 39.3–165.5). Ninety-two percent of infants were vitamin D sufficient and none at increased risk for rickets. The 26% infants using fortified products and supplements never/irregularly or in small amounts had lower 25(OH)D (76.8 ± 27.1 nmol/L) than the 22% using fortified products (100.0 ± 31.4 nmol/L), 18% using supplements (104.6 ± 37.0 nmol/L) and 33% using both (110.3 ± 26.6 nmol/L). Five of six infants with 25(OH)D < 50 nmol/L had no intake of supplements or fortified products from 0 to 12 months. Supplement use increased the odds of 25(OH)D > 125 nmol/L. Breastfeeding and season did not affect vitamin D status. The majority of infants were vitamin D sufficient. Our findings highlight the need for vitamin D supplements or fortified products all year round, regardless of breastfeeding.

**Keywords:** 25-hydroxyvitamin D; vitamin D; infant; dietary supplements; fortified foods

## 1. Introduction

Vitamin D is a key nutrient for children's well-being and growth, is essential for bone health [1] and may contribute to other health benefits [2]. Infant need for vitamin D can be met by synthesis in the skin when exposed to appropriate ultraviolet B wavelengths and by sufficient vitamin D intake, either from breast milk or other dietary sources [3]. At latitudes higher than ~50° N, little or no cutaneous vitamin D synthesis is possible during winter months [4]. Vitamin D from breast milk alone is unlikely to meet the needs of infants during complementary feeding [5]. Few common foods are naturally rich in vitamin D [6]. Vitamin D intake from supplements or fortified foods or beverages is therefore important in northern latitudes [3].

The recommended intake (RI) of vitamin D is 10 μg (400 IU) for infants and children from six months of age according to Nordic nutrition recommendations [7]. This is in accordance with the average intake specified by the Institute of Medicine (IOM) for infants from birth to 12 months of age [8]. To ensure that the RI is met, parents are advised to give their infants a daily supplement of 10 μg $D_3$ from the age of 1–2 weeks. Fortification schemes differ between countries [3]. In Iceland, population-based studies on infants [9,10] and pre-school children [11] have shown that less than two-thirds of young children use vitamin D supplements regularly. Several cases of rickets in the past years have given cause for concern on the vitamin D status of Icelandic infants [12]. In 2003, a follow-up formula intended for infants from 6 to 24 months, fortified with 1.2 μg $D_3$ per 100 mL, was introduced [13], and infant porridges and breakfast cereals fortified with vitamin D are available [14]. Population-based studies on both vitamin D intake and status during the complementary feeding period in the Nordic countries are lacking [3]. The vitamin D status of Icelandic infants is unknown and has never been studied.

The objectives of this study were to assess vitamin D status measured as serum levels of 25-hydroxyvitamin D (25(OH)D) in healthy 12-month-old infants and to consider it in relation to quantity and sources of dietary vitamin D, breastfeeding and seasons.

## 2. Experimental Section

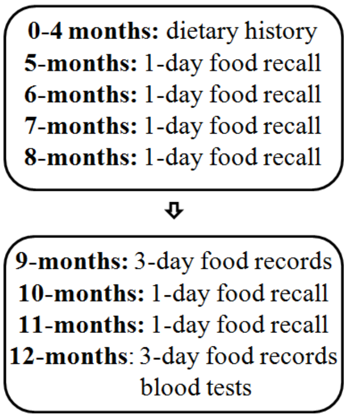
### *2.1. Subjects*

Study subjects were 76 infants with data on dietary intake in infancy and quantitative analysis of serum 25(OH)D levels at 12 months. They were a subsample of participants in a longitudinal cohort study on diet, growth and health outcomes of infants born in the year 2005. In the original study, 250 healthy Icelandic infants born at term were randomly selected from the whole country (63–66° N). Blood samples were obtained with the primary aim of analyzing the iron status and blood lipids of the infants [13]. Analysis of serum 25(OH)D was only possible for those subjects with sufficient amounts of blood available, resulting in the subsample of 76. Anthropometrical variables and dietary intake in infancy, e.g., duration of breastfeeding, intake of vitamin D, formula and cod liver oil, sociodemographic factors (parents' age and education) and parental BMI of the children included in the current analysis did not differ from those of the children in the original study. More detailed information on the original cohort is published elsewhere [13,15]. Informed written consent from the parents was obtained, and all individual information was processed with strict confidentiality. The study was approved by the Icelandic Bioethics Committee, the Icelandic Data Protection Authority and the Local Ethical Committee at Landspitali University Hospital.

### *2.2. Dietary Assessment*

A flowchart of the process of the study, relevant to the present analysis, is presented in Figure 1.

**Figure 1.** Flowchart on the progress of the study.




Dietary data from 0 to 4 months of age were collected by dietary history, including questions on breastfeeding, infant formula-feeding, other food items and supplements. At 5–8 and 10–11 months, 24-h recalls using common household measures, such as cups and spoons, were made. At 9 and 12 months, weighed food records were kept for 3 consecutive days (72-h). All food and fluids were weighed on accurate scales (Philips type HR 2385, Szekesfehervar, Hungary) with 1-g precision. The amount of breast milk consumed was estimated by weighing the breastfed infants in the same clothes before and after each breastfeeding session on baby scales (Tanita model 1583, Tokyo, Japan, or Sega model 336, Hamburg, Germany) with 10-g precision. An average consumption of food and nutrients was calculated using the Icelandic food composition database [14]. The total intake of vitamin D included intake from the diet, breast milk and supplements. For the analysis presented here, the main emphasis was on dietary intake at 9–12 months, because we believe that it may influence serum 25(OH)D concentration at 12 months [3,16]. We divided infants into four groups based on regular intake of significant amounts of the main vitamin D sources at 9–12 months. The "fortified" group included infants getting on average ≥2.4 μg of vitamin D per day from fortified products; the "supplement" group included those getting on average ≥5.0 μg of vitamin D per day from supplements; the "combined" group included those fulfilling both conditions; and the "no or irregular" group included infants fulfilling neither conditions. Fortified products included infant formula, infant porridges and breakfast cereals, and the cut-off at 2.4 μg of vitamin D was applied, because it corresponds to consumption of ≥200 mL of fortified formula, the most commonly consumed product in this category. Supplements included cod liver oil and liquid vitamin A and D supplements (vitamin

AD drops), and the cut-off at 5 μg of vitamin D was applied, because it corresponds to the recommended dose on at least half of the data collection days. We also considered whether or not infants were still partially breastfed at 12 months of age.

### *2.3. Blood Sampling and Biochemical Analyses*

At 12 months of age, blood samples were collected in the morning in the fasting state. The samples were centrifuged within 6 h of data collection. Separated serum samples were then stored at −80 °C until being analyzed. The quantitative analyses of serum 25(OH)D levels were conducted by the Roche Diagnostics Vitamin D total assay (Roche Diagnostics, Mannheim, Germany), with a measuring range of 7.5–175 nmol/L and a precision of 0.1 nmol/L. In accordance with a recent Nordic systematic literature review (SLR), serum 25(OH)D ≥ 50 nmol/L (20 ng/mL) was considered indicative of a sufficient vitamin D status, and serum 25(OH)D < 27.5 nmol/L (11 ng/mL) indicates increased risk for rickets [3]. Additionally, serum 25(OH)D > 125 nmol/L (50 ng/mL) was considered as possibly adversely high, as suggested by the IOM [8]. Infants were classified according to season when blood samples were collected; winter/spring (January 2006–April 2006 and November 2006–December 2006) and summer/autumn (May 2006–October 2006).

### *2.4. Statistical Analyses*

Statistical analyses were performed with SAS (Enterprise Guide 4.3; SAS Institute Inc., Cary, NC, USA). Linear regression analysis was used to examine the relation between vitamin D intake and serum 25(OH)D. Descriptive statistics were used to describe vitamin D intake and serum 25(OH)D concentrations, presented as the means ± SD. For comparison between groups, an independent, two-sample *t*-test with equal variances and a one-way ANOVA with equal variance were used. Logistic regression was used to examine the risk of having serum 25(OH)D above 125 nmol/L among infants using supplements or not. The results were presented as odds ratios (OR), with its 95% CI. Spearman's correlation analysis was used to assess correlations between 25(OH)D and breastfeeding, presented as the correlation coefficient (ρ) and the *p*-value for correlation. A two-sided test with a $p$-value < 0.05 was considered statistically significant.

## 3. Results

At the age of 12 months, the mean ± SD serum 25(OH)D concentration was 98.1 ± 32.2 nmol/L (39.3 ± 12.9 ng/mL) and ranged from 39.3 to 165.5 nmol/L (15.7 to 66.3 ng/mL). Seventy infants (92%) were considered vitamin D sufficient and none at increased risk for rickets. Eighteen infants (24%) were considered to have a possibly adversely high 25(OH)D concentration.

Vitamin D intake at 9–12 months predicted 25(OH)D levels at 12 months (Figure 2). The mean ± SD intake of vitamin D was 8.8 ± 5.2 μg, and 57% of the infants were below the RI of 10 μg. Those infants had significantly lower mean ± SD 25(OH)D than infants above the RI (87.1 ± 31.1 *vs.* 111.8 ± 29.0 nmol/L, $p = 0.001$).

Supplements provided 56% of total vitamin D at 9–12 months. Another 38% came from fortified products; thereof, 24% from formulas, 13% from infant porridges and 1% from breakfast cereals.

Among natural sources of vitamin D were meat and fish (3%) and cow's milk (1%). Breast milk provided <1% of vitamin D. As presented in Table 1, infants in the "combined" group had a higher vitamin D intake than infants in the "supplement" group ($p < 0.001$), who, in turn, had a higher vitamin D intake than infants in the "fortified" group ($p = 0.013$). Mean serum 25(OH)D in these three groups was, however, not significantly different ($p > 0.05$). Infants not using fortified products or supplements regularly in significant amounts at 9–12 months ("no or irregular" group) had significantly lower vitamin D intake than all the other groups ($p < 0.001$) and lower serum 25(OH)D ($p < 0.001$).

**Figure 2.** The linear regression line for serum 25-hydroxyvitamin D (25(OH)D) at 12 months in relation to vitamin D intake from diet and supplements at 9–12 months. The dashed horizontal line at 50 nmol/L is the cut-off line applied for a sufficient vitamin D status, and the dashed vertical line at 10 µg indicates the Nordic recommended intake (RI).

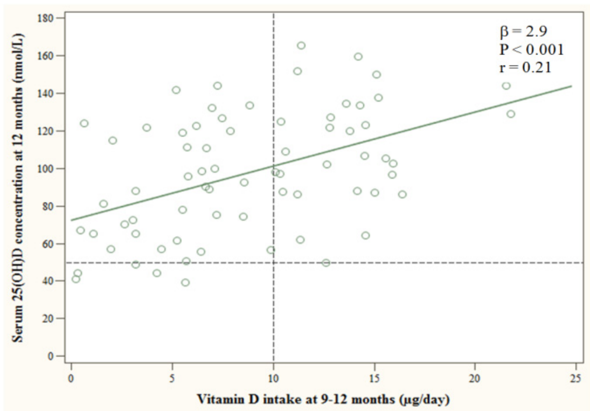



**Table 1.** Variables potentially associated with vitamin D intake at 9–12 months and serum 25(OH)D at 12 months.

| Variables | *n* (%) | Vitamin D Intake (µg/day) | 25(OH)D (nmol/L) |
|---|---|---|---|
| All | 76 (100) | 8.8 ± 5.2 | 98.1 ± 32.2 |
| Boys | 39 (51) | 8.6 ± 5.7 | 96.6 ± 34.3 |
| Girls | 37 (49) | 8.9 ± 4.6 | 99.7 ± 30.3 |
| *Vitamin D sources at 9–12 months* [a] | | | |
| "No or irregular" | 20 (26) | 2.5 ± 1.9 | 76.8 ± 27.1 |
| "Fortified" | 17 (22) | 6.5 ± 2.2 | 100.0 ± 31.4 |
| "Supplement" | 14 (18) | 8.8 ± 2.7 | 104.6 ± 37.0 |
| "Combined" | 25 (33) | 14.3 ± 3.0 | 110.3 ± 26.6 |
| *Partially breastfed at 12 months* | | | |
| No | 62 (82) | 8.7 ± 5.0 | 97.7 ± 32.7 |
| Yes | 14 (18) | 9.1 ± 6.0 | 101.9 ± 31.5 |
| *Season of blood sample collection* | | | |
| Winter/Spring | 33 (43) | 8.1 ± 4.9 | 94.4 ± 31.6 |
| Summer/Autumn | 43 (57) | 9.2 ± 5.4 | 101.0 ± 32.8 |

Abbreviation: 25(OH)D, 25-hydroxyvitamin D. Mean ± SD. [a] Infants were divided into groups based on the regular intake of significant amounts of the main vitamin D sources at 9–12 months. "No or irregular": neither fortified products nor supplements; "Fortified": fortified products; "Supplement": supplements; "Combined": both fortified products and supplements.

Five out of six infants with serum 25(OH)D below 50 nmol/L belonged to the "no or irregular" group, *i.e.*, they did not use fortified products or supplements on a regular basis or in significant amounts at 9–12 months. Their intake of vitamin D was below 5 µg at 9–12 months. Further, they did not use fortified products or supplements at all from birth to nine months of age. The sixth infant with 25(OH)D below 50 nmol/L was categorized in the "supplement" group, but only got half of the recommended amount of supplements daily. Of the 18 infants with 25(OH)D levels above 125 nmol/L, one belonged to the "no or irregular" group (5% of infants in that group), three to the "fortified" group (18%), six to the "supplement" group (43%) and eight to the "combined" group (32%). Infants using supplements (*i.e.*, classified in the "supplement" or "combined" groups) were 4.6 times more likely (95% CI = 1.4, 15.8) to have 25(OH)D above 125 nmol/L than infants not using supplements (*i.e.*, classified in the "no or irregular" or "fortified" groups). Infants in the "combined" group were not more likely to have 25(OH)D above 125 mol/L than infants in the "supplement" group (OR (95% CI) = 0.6 (0.2, 2.4)).

The duration of exclusive breastfeeding ranged from 0 to 6 months, with a median (25th, 75th percentiles) of four (1, 5) months. The total duration of breastfeeding ranged from 0 to 12 months, with a median (25th, 75th percentiles) of eight (6, 10) months. There was no correlation between the duration of exclusive breastfeeding and 25(OH)D ($\rho = -0.02$, $p = 0.895$) or the total duration of breastfeeding and 25(OH)D ($\rho = 0.08$, $p = 0.502$). Among children partially breastfed at 12 months of age, breast milk intake in the age period of 9–12 months ranged from 10 mL to 750 mL per day. No difference was observed in vitamin D intake or 25(OH)D according to breastfeeding at 12 months ($p = 0.923$ and 0.674, respectively), season of blood sample collection ($p = 0.385$ and $p = 0.379$, respectively) or sex ($p = 0.859$ and $p = 0.678$, respectively).

## 4. Discussion

This study provides the first information on vitamin D status in Icelandic infants. Based on thresholds proposed in a recent Nordic SLR [3], 92% of the infants were considered vitamin D sufficient and none at increased risk for rickets. Consensus has not been reached on the optimal 25(OH)D concentration in infants, and uniformity is lacking in the description of sufficient and deficient ranges for 25(OH)D levels. Using cut-off values proposed by IOM [8] or the Pediatric Endocrine Society [16] does not change our results of 92% of infants being classified as vitamin D sufficient, and according to those cut-offs, no infants are classified as vitamin D deficient. According to a European consensus statement, vitamin D deficiency occurs commonly among healthy European infants not adhering to recommendations for vitamin D supplementation [17]. However, studies on healthy infants from Denmark [18], Norway [19] and Finland [20] have previously reported a high proportion of vitamin D sufficiency amongst nine-month-olds, 12-month-olds and 14-month-olds, respectively. Those studies were not population-based, and in the Danish and Finnish studies, selection bias resulted in an unusually high frequency of infants using vitamin D supplements (97% and 100%, respectively). The Nordic countries have a well-established newborn and infant healthcare. According to protocols for the newborn and infant healthcare in Iceland [21], mothers are asked about their infants' vitamin D supplement use and encouraged to follow the recommendations on vitamin D supplements at every visit, which are scheduled at least nine times during the first year of the infant.

This may explain the relatively low proportion of vitamin D deficiency among infants in Iceland and, more broadly, the Nordic countries. To our knowledge, this is the first study on infant vitamin D status in the Nordic countries in a sample that is representative of the general infant population. Therefore, we believe it is an important contribution to the literature on the vitamin D status of healthy infants during complementary feeding in northern latitudes.

The relatively high 25(OH)D levels may, at least partly, be explained by 75% of the infants regularly using vitamin D supplements and/or fortified foods or drinks in significant amounts. The commonly used follow-up formula, fortified with vitamin D, was introduced in 2003. Before that, it was common that regular cow's milk gradually replaced breast milk in the age range of 5–12 months [9]. The main vitamin D source for Icelandic infants has, therefore, historically, been vitamin D drops or cod liver oil, and even though studies on infants and children have shown a little less than two-thirds of children complying with supplement use, the remaining one-third has been seen as a reason for concern. Studies on Icelandic infants and young children have never before assessed how frequently vitamin D fortified products are consumed or how they contribute to vitamin D status. We do not have any data on the vitamin D status of infants and young children previous to the introduction of the fortified follow-up formula.

The wide range of serum 25(OH)D concentrations observed in the study should be considered when interpreting the results. Transferring the 8% of infants in our sample with serum 25(OH)D below 50 nmol/L to the whole infant population in Iceland (around 4600 12-month-olds annually from 2005 to 2012) [22] suggests that about 275 infants every year would be vitamin D insufficient, with the possibility of some being at risk for vitamin D deficiency. Our study, showing that infants with an insufficient vitamin D status did not use fortified products or supplements at all from birth to nine months of age, in addition to a very low vitamin D intake from nine to 12 months of age, could be considered in newborn and infant healthcare in Iceland to identify, at an early age, children with undesirable diet habits that may increase the risk of vitamin D insufficiency or deficiency. Infants using supplements with or without concurrent use of fortified foods or drinks were more likely than infants not using supplements to have 25(OH)D concentrations that may be considered as possibly adversely high [8]. Correct dosing of supplements is important, as well as caution when combining the use of supplements and fortified foods or drinks. However, no infant exceeded the 25 μg vitamin D intake, which is considered the tolerable upper intake level by the European Food Safety Authority [6], and other estimations of the high end for safe concentration levels of 25(OH)D are higher than the 125 nmol/L estimated by IOM [16,23].

Since the time of this study, parents have been encouraged to give their infants vitamin D drops instead of vitamin AD drops. The vitamin D content in the two products is the same, and other infant guidelines remain unchanged. Therefore, we believe that the findings of this study are transferable to Icelandic infants born today. Iceland is among the few countries that includes cod liver oil intake or other vitamin D supplements in the population-based dietary guidelines for children and adults of all ages [24]. A recent study from Denmark showed that parents' perceived relevance of nutritional guidelines declined from the early to later phases of complementary feeding [25], and a Finnish study showed decreased use of supplements as children grew older [26]. Icelandic studies have also shown low vitamin D intakes among children [27], adolescents [28] and adults [29], and results from a follow-up of the infants participating in the current analysis reveal that only 27% used supplements at six-years of age [30]. While the vitamin D status in our study is considered sufficient for the majority of infants, studies on Icelandic children and adults have shown lower 25(OH)D concentrations than presented here [31–33]. This study, showing the importance of supplements and/or fortified products on vitamin D status, is therefore of importance for public health policy.

We did not find differences in 25(OH)D levels between months when cutaneous synthesis is expected to be very low or totally absent at northern latitudes (November to April) and months when the quantity and quality of UV radiation might be sufficient for cutaneous synthesis (May to October). Icelandic parents are advised to keep their infants out of direct sunlight, and summer temperatures in Iceland usually require long sleeves and a hat for infants. In case infants get in contact with sun, the use of sunscreen is advised [21]. We propose that cutaneous synthesis of vitamin D does not contribute significantly to 25(OH)D in Icelandic infants and that the use of supplements and/or fortified foods and drinks is therefore essential all year round in order to maintain a sufficient vitamin D status. Seasons have, however, been shown to affect 25(OH)D in older children and adults [31,33]. No difference was seen in 25(OH)D levels between infants breastfed or not breastfed at 12 months, which may be explained by the emphasis put on supplement use regardless of feeding mode.

The main strengths of our study lie in the assessment of both vitamin D intake and status in a population-based infant sample and the longitudinal design of the study. Although we are aware of the possibility of altered dietary behavior on data collection days, the use of eight data collection days from 9 to 12 months of age in the current analysis strengthens our confidence that we have reliably estimated food and nutrient intake that may affect vitamin D status at 12 months. The dietary information from 0 to 8 months of age gives practical information. The study also has some limitations. Blood samples were obtained with the primary aim of analyzing the iron status and blood lipids of the infants [13]. Analysis of serum 25(OH)D was only possible for those subjects with sufficient amounts of blood available, resulting in a small sample size. Analyses on parameters that have been used to complement 25(OH)D levels and/or used as blood safety measurements in other studies [34,35], such as parathyroid hormone, serum calcium, alkaline phosphatase and C-reactive protein, were not performed. There is a possibility that the method used for quantitative analyses of serum 25(OH)D may overestimate the 25(OH)D concentration in infants, due to the possible presence of *C*-3 epimers [36–38]. As all subjects were of Icelandic origin and healthy, transferring the results to high-risk groups of vitamin D deficiency, such as infants of non-western immigrants residing in northern latitudes and infants with chronic illnesses, should not be advised [7,39,40].

## 5. Conclusions

In conclusion, the majority of infants were vitamin D sufficient. Our findings highlight the need for vitamin D supplements or fortified products all year round, regardless of breastfeeding in infant populations with little or no sun exposure.

## Acknowledgments

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## Author Contributions

B.T. participated in data collection, analyzed the data and drafted the paper. I.G. and L.S. conceived of and designed the study. G.P. performed the blood sampling. I.T. supervised data collection and conceived of and designed the study. All authors contributed in writing and editing the manuscript and approved the final version of the paper as submitted.

## Conflicts of Interest

The authors declare no conflict of interest.

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# Paper III

nutrients

Article

# Vitamin D Intake and Status in 6-Year-Old Icelandic Children Followed up from Infancy

**Birna Thorisdottir [1,*], Ingibjorg Gunnarsdottir [1], Laufey Steingrimsdottir [1], Gestur I. Palsson [2], Bryndis E. Birgisdottir [1] and Inga Thorsdottir [1]**

1 Unit for Nutrition Research, Faculty of Food Science and Nutrition, School of Health Sciences, University of Iceland and Landspitali University Hospital, Reykjavik 101, Iceland; ingigun@hi.is (I.G.); laufey@hi.is (L.S.); beb@hi.is (B.E.B.); ingathor@hi.is (I.T.)

2 Children's Hospital, Landspitali University Hospital, Reykjavik 101, Iceland; gesturip@landspitali.is

* Correspondence: bth50@hi.is; Tel.: +354-5438410

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**Abstract:** High serum 25-hydroxyvitamin D (25(OH)D) levels have been observed in infants in Nordic countries, likely due to vitamin D supplement use. Internationally, little is known about tracking vitamin D status from infancy to childhood. Following up 1-year-old infants in our national longitudinal cohort, our aims were to study vitamin D intake and status in healthy 6-year-old Icelandic children ($n$ = 139) and to track vitamin D status from one year of age. At six years, the mean 25(OH)D level was 56.5 nmol/L (SD 17.9) and 64% of children were vitamin D sufficient (25(OH)D $\geqslant$ 50 nmol/L). A logistic regression model adjusted for gender and breastfeeding showed that higher total vitamin D intake (Odds ratio (OR) = 1.27, 95% confidence interval (CI) = 1.08–1.49), blood samples collected in summer (OR = 8.88, 95% CI = 1.83–43.23) or autumn (OR = 5.64, 95% CI = 1.16–27.32) compared to winter/spring, and 25(OH)D at age one (OR = 1.02, 95% CI = 1.002–1.04) were independently associated with vitamin D sufficiency at age six. The correlation between 25(OH)D at age one and six was 0.34 ($p$ = 0.003). Our findings suggest that vitamin D status in infancy, current vitamin D intake and season are predictors of vitamin D status in early school age children. Our finding of vitamin D status tracking from infancy to childhood provides motivation for further studies on tracking and its clinical significance.

**Keywords:** 25-hydroxyvitamin D; child; dietary supplements; seasons; tracking; vitamin D

## 1. Introduction

Vitamin D sufficiency in childhood is associated with improved bone health [1] and may possibly lower the risk for type 1 diabetes [2], cardiovascular disease [3] and other diseases later in life [4]. It may also have short-term benefits beyond bone metabolism. Some, but not all, studies have found cross-sectional associations between vitamin D status and lipid profile or other cardiometabolic risk factors in children [5–7]. Serum 25-hydroxyvitamin D (25(OH)D) is generally considered the best measure of vitamin D status and is believed to reflect the combined contributions from cutaneous vitamin D synthesis and vitamin D intake from the diet, *i.e.*, food naturally rich in vitamin D, fortified food and supplements [8,9].

Iceland has a small and relatively genetically homogenous population [10]. It is geographically located at 63–66°N, resulting in little or no cutaneous vitamin D synthesis during large parts of the year [11,12]. Similar to other northern regions, e.g., Norway, Sweden, Denmark, Finland [13], the USA [8,14] and Canada [15], the recommended vitamin D intake for children aged 2 weeks to 10 years is 10 μg/day. Daily use of cod liver oil or another form of vitamin D supplement is part of the Icelandic food based recommendations for all age groups [16].

In our previous publication from a cohort designed to examine diet, growth and health in infancy and childhood we reported high serum 25(OH)D levels among 1 year old infants [17]. We suggested that these high levels might be explained, at least in part, by regular use of vitamin D supplements and/or vitamin D fortified foods or drinks by 75% of the infants. A school-based lifestyle study among 7 year old Icelandic children reported lower vitamin D intake and also lower levels of 25(OH)D than among the infants [18]. This is in line with reports of lower adherence to nutritional guidelines and decreased use of supplements as children grow older [19–21]. Also, several cross-sectional epidemiological studies have reported lower 25(OH)D levels among older children and adolescents than infants and younger children [22–24]. It is unknown to what extent the apparent decrease in 25(OH)D with age can be explained by background characteristics due to different study groups, decreased vitamin D intake, or other factors. Studies are lacking that measure both vitamin D intake and status at more than one time-point in the same individuals, thus giving the possibility to study maintenance or tracking of vitamin D status from younger age to childhood. Following up with infants from our population-based longitudinal infant and child cohort [17], our aims were to study vitamin D intake and status in healthy 6 year old children and to track vitamin D status from 1 year of age.

## 2. Materials and Methods

### 2.1. Study Design and Subjects

The study population, recruitment and data collection have previously been described in detail [25,26]. In brief, a random sample of 250 Icelandic infants born in 2005 (6% of live born infants) was collected and followed throughout the first year of life. At 6 years, 172 participated in a follow-up. In this current analysis, eligible subjects were those with vitamin D status measured at 6 years, in total 139 children (81% of children in the follow-up). Data on vitamin D status at both 1 and 6 years of age were available for 74 children. The basic characteristics of eligible subjects did not differ from other subjects in the follow-up. Informed written consent was obtained from the parents, and all individual information was processed with strict confidentiality. The study was approved by the Icelandic Bioethics Committee and Ethical Committee at Landspitali University Hospital and registered at the Icelandic Data Protection Authority.

### 2.2. Vitamin D Measurement

Fasting blood samples were collected when the children were 1 year old (January–December 2006) and 6 years old (June–December 2011). Serum samples were stored at −80 °C until analyzed for 25(OH)D with an electro-chemiluminescence immunoassay (Modular Analytics E170, Roche Diagnostis, Mannheim, Germany) (precision 0.1 nmol/L). The main cut-point explored and used to describe vitamin D sufficiency was ⩾50.0 nmol/L. Other cut-points were ⩾75.0 nmol/L, 30.0−49.9 nmol/L and <30.0 nmol/L [8,27]. To classify the children according to expected contribution of cutaneous synthesis to vitamin D status at 6 years, three categories were constructed; blood samples collected in July−August (major contribution; "summer"), September−October (moderate contribution; "autumn"), and November−December and June (minor contribution; "winter/spring").

### 2.3. Covariates

Potential confounders were gender, breastfeeding, vitamin D intake and supplement use, serum levels of triglycerides (TG), total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), body mass index (BMI), body weight, physical activity assessed at 6 years, and maternal age, maternal BMI and maternal education, reported when the child was 6 years [5,28–30]. Information on breastfeeding duration and exclusivity was gathered monthly in the first year of life. Dietary data were obtained from 3-day weighed food records (HR 2385 scales, Philips, Hungary or Austria) (precision 1 g) registered at 1 and 6 years of age. Total vitamin D intake included vitamin D from food and supplements. Serum TG and TC were analyzed using an enzymatic colorimetric test (Cholesterol CHOD-PAP, Roche Diagnostics, Mannheim, Germany) (precision 0.1 nmol/L). Serum HDL-C was analyzed using the same method after precipitation and centrifugation. Serum LDL-C was calculated using the Friedewald formula [31]. BMI was calculated using height and weight measured with a Marel M series 1100 scale (Reykjavik, Iceland) (precision 0.1 kg) and an Ulmer stadiometer (Prof. Heinze, Ulm, Germany) (precision 0.5 cm). Information on children's daily duration of physical activity, maternal age, maternal BMI and maternal education was obtained from parent questionnaires. Maternal education was defined as the highest completed level and categorized as primary (10 years), secondary (approx. 14 years) and tertiary (>16 years) education.

### 2.4. Statistical Analysis

Statistical analysis was performed with SAS (Enterprise Guide 4.3; SAS Institute Inc, Cary, NC, USA). Variables were examined for normality using Quantile Quantile-plots and described using mean and standard deviations (SD) or medians and 25th to 75th centiles. For comparison between groups, we used the *t*-test, Mann Whitney U-test or a chi-square. Missing values were replaced by the median value for each covariate (1%–4%) except for vitamin D supplement use and maternal education. Multivariate logistic regression was used to assess variables associated with vitamin D sufficiency (25(OH)D $\geqslant$ 50.0 nmol/L) at 6 years. Model 1 included gender and variables that were found to be significantly different between vitamin D sufficient children and children with lower status. In model 2 we examined the importance of including 25(OH)D at 1 year. Further adjustments for other covariates (serum TG, TC, LDL-C, HDL-C, BMI, body weight, physical activity, or maternal age, maternal BMI, or maternal education) did not contribute significantly to the models. The mean change in vitamin D intake and status from 1 to 6 years of age was calculated as the difference between the values at 6 years and 1 year. Correlations for vitamin D intake and status from 1 to 6 years were evaluated with Spearman and Pearson correlations, respectively. When categorizing subjects into tertiles based on serum 25(OH)D levels, the cut-off values of $\leqslant$81.3 nmol/L and $\leqslant$119.1 nmol/L at 1 year and $\leqslant$47.3 nmol/L and $\leqslant$63.2 nmol/L at 6 years were used. Linear regression models were used to test for predictors of change in vitamin D status from 1 to 6 years. Two sided *p*-value of <0.05 was considered statistically significant.

## 3. Results

At 6 years, 89 children (64%) were classified as vitamin D sufficient (25(OH)D $\geqslant$ 50.0 nmol/L). From these, 17 children (12%) had levels $\geqslant$75.0 nmol/L. Fourty-two children (30%) had 25(OH)D levels 30.0–49.9 nmol/L and 8 children (6%) had levels <30.0 nmol/L. Children using vitamin D supplements, cod liver oil being most common, received a median of 11.9 μg/day vitamin D (25th, 75th percentiles: 7.4, 15.9), thereof 8.8 μg/day (25th, 75th percentiles: 5.3, 13.3) from supplements. Vitamin D intake from food did not differ between children using and not using vitamin D supplements. The main food sources for vitamin D were breakfast cereals (median 0.8 μg/day; 25th, 75th percentiles: 0.4, 1.6), butter (median 0.3 μg/day; 25th, 75th percentiles: 0.1, 0.5), fish (median 0.2 μg/day; 25th, 75th percentiles: 0.0, 0.4), meat (median 0.1 μg/day; 25th, 75th percentiles: 0.1, 0.2) and milk (median 0.1 μg/day; 25th, 75th percentiles: 0.1, 0.1). No dietary vitamin D sources apart from supplements were associated with vitamin D status. Table 1 presents the characteristics of eligible subjects. Vitamin D sufficient 6 year old children had been breastfed longer, were more likely to follow recommendations on vitamin D supplement use and therefore had higher total vitamin D intake than children with lower vitamin D status. Their blood samples were more likely to have been obtained during summer and less likely to have been obtained during winter or spring. From those children following vitamin D supplement recommendations (10 μg/day) were 83% vitamin D sufficient, with no differences in status between season of blood sampling ($p > 0.05$). Season was an important predictor of vitamin D sufficiency among children not receiving the recommended 10 μg/day of vitamin D. Among those children, 25% having blood drawn during winter or spring were vitamin D sufficient, compared with 52% of children measured during autumn and 76% of children measured during summer ($p < 0.001$).

**Table 1.** Characteristics of eligible subjects.

| Variable | All Subjects ($n$ = 139) [1] | 25(OH)D < 50 nmol/L ($n$ = 50) | 25(OH)D ⩾ 50 nmol/L ($n$ = 89) | $p$-Value |
|---|---|---|---|---|
| Male gender, $n$ (%) | 70 (50) | 22 (31) | 48 (69) | 0.26 [2] |
| Female gender, $n$ (%) | 69 (50) | 28 (41) | 41 (59) | 0.26 [2] |
| Breastfeeding, month, median (25th, 75th centile) | 8 (6, 10) | 7 (4, 9) | 9 (7, 10) | 0.017 [3] |
| Exclusive breastfeeding, month, median (25th, 75th centile) | 4 (2, 5) | 4 (2, 5) | 4 (3, 5) | 0.14 [3] |
| **Children at 6 years** | | | | |
| Age, year, mean ± SD | 6.1 ± 0.3 | 6.1 (0.2) | 6.2 (0.3) | 0.60 [4] |
| BMI, kg/m$^2$, median (25th, 75th centile) | 15.5 (14.8, 16.5) | 15.5 (15.0, 16.8) | 15.5 (14.7, 16.4) | 0.49 [3] |
| Physical activity, h, median (25th, 75th centile) | 1.6 (1.0, 2.5) | 1.6 (1.0, 2.0) | 1.6 (1.1, 2.7) | 0.16 [3] |
| Total vitamin D intake, μg/day, median (25th, 75th centile) | 5.0 (2.3, 12.1) | 3.2 (2.1, 6.6) | 7.5 (2.7, 13.7) | 0.003 [3] |
| Vitamin D from food, μg/day, median (25th, 75th centile) | 2.3 (1.6, 3.3) | 2.4 (1.7, 3.0) | 2.3 (1.6, 3.4) | 0.69 [3] |
| Vitamin D from supplements, μg/day, median (25th, 75th centile) | 1.5 (0.0, 10.0) | 0.0 (0.0, 4.0) | 4.7 (0.0, 10.0) | 0.002 [3] |
| No vitamin D supplement use, $n$ (%) | 63 (47) | 31 (49) | 32 (51) | 0.003 [2] |
| Vitamin D supplement use < 10 μg/day, $n$ (%) | 37 (27) | 11 (30) | 26 (70) | 0.36 [2] |
| Vitamin D supplement use ⩾ 10 μg/day, $n$ (%) | 35 (26) | 6 (17) | 29 (83) | 0.007 [2] |
| Blood samples in winter/spring, $n$ (%) | 38 (27) | 21 (55) | 17 (45) | 0.004 [2] |
| Blood samples in autumn, $n$ (%) | 34 (25) | 13 (38) | 21 (62) | 0.75 [2] |
| Blood samples in summer, $n$ (%) | 67 (48) | 16 (24) | 51 (76) | 0.004 [2] |
| Serum 25(OH)D, nmol/L, mean ± SD | 56.5 ± 17.9 | 39.3 ± 9.5 | 66.1 ± 13.8 | <0.001 [4] |
| Serum TG, mmol/L, mean ± SD | 0.6 ± 0.2 | 0.7 ± 0.3 | 0.6 ± 0.2 | 0.26 [4] |
| Serum TC, mmol/L, mean ± SD | 4.4 ± 0.6 | 4.4 ± 0.6 | 4.4 ± 0.7 | 0.57 [4] |
| Serum LDL-C, mmol/L, mean ± SD | 2.5 ± 0.6 | 2.6 ± 0.5 | 2.5 ± 0.6 | 0.47 [4] |
| Serum HDL-C, mmol/L, mean ± SD | 1.6 ± 0.3 | 1.6 ± 0.3 | 1.6 ± 0.3 | 0.80 [4] |
| **Mothers of 6 year old children** | | | | |
| Age, year, mean ± SD | 36.6 ± 5.0 | 36.2 ± 5.4 | 36.8 ± 4.8 | 0.50 [4] |
| BMI, kg/m$^2$, median (25th, 75th centile) | 24.4 (21.8, 27.8) | 24.8 (23.3, 28.1) | 24.4 (21.5, 27.3) | 0.16 [3] |
| Primary education, $n$ (%) | 18 (13) | 9 (50) | 9 (50) | 0.18 [2] |
| Secondary education, $n$ (%) | 31 (23) | 11 (35) | 20 (65) | 0.95 [2] |
| Tertiary education, $n$ (%) | 87 (64) | 29 (33) | 58 (67) | 0.40 [2] |

Abbreviations: 25(OH)D—25-hydroxyvitamin D; BMI—body mass index; HDL-C—high-density lipoprotein-cholesterol; LDL-C—low-density lipoprotein-cholesterol; TC—total cholesterol; TG—triglycerides; [1] For vitamin D supplement use and maternal education: $n$ = 135 and $n$ = 136, respectively; [2] Chi-square used to determine the $p$-value; [3] The Mann Whitney U-test used to determine the $p$-value; [4] The $t$-test used to determine the $p$-value.

Multivariate logistic regression models (Table 2) confirmed that vitamin D intake at 6 years (intake range: 1 to 26 μg/day) and seasons were both independent predictors of vitamin D sufficiency at 6 years. As an example, increasing total vitamin D intake by 10 μg/day in model 1 was associated with around 4-fold higher odds of having serum 25(OH)D above 50 nmol/L. This estimate was comparable to what was seen to seasonal differences. Vitamin D status at 1 year was also found to be an independent predictor of vitamin D sufficiency at 6 years (model 2). Including the vitamin D status at 1 year in the model strengthened the estimates for vitamin D intake and seasons.

**Table 2.** Multivariate logistic regression analysis of factors associated with vitamin D sufficiency (serum 25(OH)D ⩾ 50 nmol/L) at 6 years of age.

| | **Model 1 ($n$ = 139)** | | **Model 2 ($n$ = 74)** | |
|---|---|---|---|---|
| Variable | OR | 95% CI | OR | 95% CI |
| Female gender | 0.68 | 0.31–1.48 | 0.59 | 0.18–1.94 |
| Breastfeeding, month | 1.07 | 0.95–1.22 | 1.10 | 0.91–1.34 |
| Total vitamin D intake, 10 μg/day | 3.85 | 1.73–8.58 | 10.93 | 2.21–53.99 |
| Blood samples in winter/spring | reference | | reference | |
| Blood samples in autumn | 3.03 | 1.04–8.84 | 5.64 | 1.16–27.32 |
| Blood samples in summer | 5.84 | 2.12–16.07 | 8.88 | 1.83–43.23 |
| Serum 25(OH)D at 1 year, nmol/L | | | 1.02 | 1.002–1.04 |

Abbreviations: 25(OH)D—25-hydroxyvitamin D; CI—confidence interval; OR—Odds Ratio; Model 2: Same as model 1 as well as serum 25(OH)D at 1 year as independent variable.

The basic characteristics of the 74 subjects with vitamin D status measured both at 1 and 6 years did not differ from other eligible subjects (see Table 1). As presented in Table 3, vitamin D intake and status measured as 25(OH)D dropped from 1 to 6 years. Vitamin D intake at 1 and 6 years did not correlate, neither when explored as μg/day nor μg/kg/day, while the correlation for serum 25(OH)D levels at 1 and 6 years was 0.34 ($p$ = 0.003). The relationship between the serum 25(OH)D levels assessed at 1 year and 6 years is shown in Figure 1.

**Table 3.** Vitamin D intake and status of children with vitamin D status measured at two time points ($n$ = 74).

| Variable | Age 1 Year | Age 6 Years | Change from 1 to 6 Years |
|---|---|---|---|
| Total vitamin D intake μg/day, median (25th, 75th centile) | 6.4 (3.5, 11.6) | 4.5 (2.1, 11.0) | −0.9 (−8.1, 4.4) |
| Total vitamin D intake, μg/kg/day, median (25th, 75th centile) | 0.7 (0.3, 1.2) | 0.2 (0.1, 0.5) | −0.3 (−1.0, 0.0) |
| Serum 25(OH)D, nmol/L, mean ± SD | 97.5 ± 32.4 | 55.8 ± 17.9 | −41.6 ± 31.2 |

Abbreviations: 25(OH)D—25-hydroxyvitamin D.

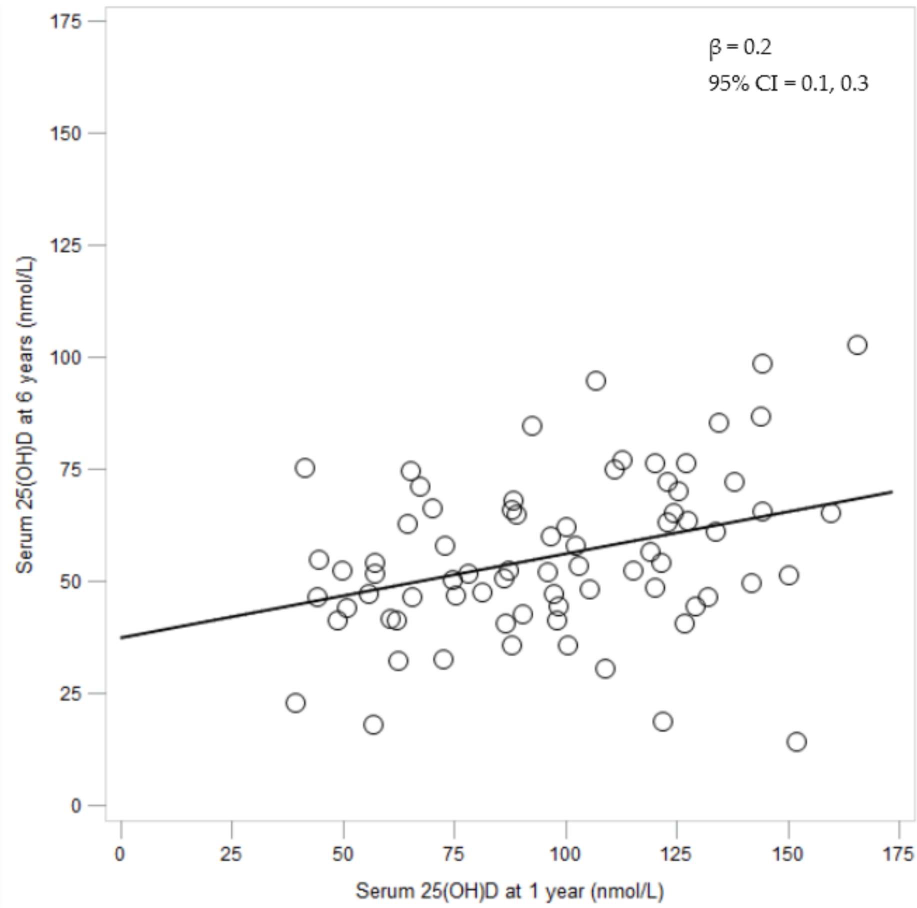


**Figure 1.** Relationship between the serum 25(OH)D levels assessed at 1 year and 6 years ($n$ = 74).

As shown in Table 4, 40%–54% of subjects stayed within the same vitamin D tertile at 1 and 6 years. Only 16%–21% moved from the lowest or highest tertile to the other extreme. The four children moving from tertile 1 at 1 year to tertile 3 at 6 years all had an increase in total vitamin D intake from 1 to 6 years (range 1.2 to 4.7 μg/day and 0.0 to 0.1 μg/kg/day). The five children moving from tertile 3 at

1 year to tertile 1 at 6 years all had a decrease in total vitamin D intake from 1 to 6 years (range −27.4 to −1.8 μg/day and −2.1 to −0.3 μg/kg/day).

**Table 4.** Numbers of children with vitamin D status measured at two time points falling in different vitamin D tertiles at 1 and 6 years of age (*n* = 74).

| | **Serum 25(OH)D Tertile at 6 Years** | | |
|---|---|---|---|
| Serum 25(OH)D tertile at 1 year | 1, *n* (%) | 2, *n* (%) | 3, *n* (%) |
| 1, *n* (%) | 12 (48) | 9 (36) | 4 (16) |
| 2, *n* (%) | 8 (32) | 10 (40) | 7 (28) |
| 3, *n* (%) | 5 (21) | 6 (25) | 13 (54) |

Abbreviations: 25(OH)D—25-hydroxyvitamin D.

There was a strong negative association between the serum 25(OH)D level assessed at 1 year and the change in serum 25(OH)D levels from 1 to 6 years of age (β = −0.8, 95% CI = −0.9, −0.7). The only other baseline variable associated with 25(OH)D change over time in a univariate linear model was vitamin D intake at 1 year (β = −1.9, 95% CI = −3.2, −0.6 when explored as μg/day and β = −19.1, 95% CI = −32.4, −5.7 when explored as μg/kg/day). The change in vitamin D intake from 1 to 6 years of age was also associated with the change in serum 25(OH)D levels from 1 to 6 years of age (β = 1.3, 95% CI = 0.4, 2.2 when explored as μg/day and β = 18.6, 95% CI = 6.5, 30.7 when explored as μg/kg/day). Gender, breastfeeding, anthropometrics, or maternal factors were not associated with 25(OH)D change over time.

## 4. Discussion

Our main finding was the tracking of vitamin D status during a 5-year period from 1 to 6 years of age, with a clear regression towards the mean. A Norwegian study on adults (mean age at baseline 57 years) reported tracking of vitamin D status during a 14-year period [32] but to the best of our knowledge this is the first study to present tracking of vitamin D status from infancy to childhood. Tracking is used in public health and epidemiology to describe the stability of a variable over time, attempting early identification of subjects at risk for diseases later in life [33]. The correlation we observed in serum 25(OH)D levels from 1 to 6 years of age was similar in strength as that observed for other biochemical variables in children, e.g., serum lipid and apolipoprotein levels from infancy to 4 years of age in a Swedish study [34]. We believe that our results merely provide motivation for further studies on tracking of vitamin D status in infancy and childhood and its clinical significance. They may provide support for the importance of monitoring vitamin D status.

Serum 25(OH)D dropped from high levels at 1 year (mean 97.5 nmol/L) to lower levels at 6 years (mean 56.5 nmol/L). At 1 year, 92% of the infants were vitamin D sufficient (⩾50.0 nmol/L) and 24% had levels that may be considered adversely high (>125 nmol/L) [17] while at 6 years only 64% of the children were vitamin D sufficient (⩾50.0 nmol/L). This is in line with cross-sectional epidemiological studies reporting higher 25(OH)D levels in younger children than in older children and adolescents [22–24]. This might call into question using the same cut-off value to describe vitamin D sufficiency in infants as in older children. It should also be kept in mind that while 50 nmol/L (20 ng/mL) is frequently used as indicative of vitamin D sufficiency [8,35,36] higher cut-off values, e.g., 75 nmol/L (30 ng/mL) have been suggested for endpoints other than bone health [27].

Our study found that the higher the serum 25(OH)D level was at 1 year, the more it changed (decreased) over the 5-year period to 6 years of age, lessening our concerns about possible adverse effects due to high levels observed in infancy. A Polish infant study reported similar findings in the age period 6 to 12 months [37]. The explanations for our findings may be complex. Firstly, the decrease in 25(OH)D levels may be explained by a substantial decrease in vitamin D intake from 1 to 6 years, especially when measured in μg per kg body weight. Although age and body weight have been shown to be important predictors of variation in 25(OH)D levels [38,39], current vitamin

D recommendations do not take this into account [8,13]. Secondly, it has been suggested that high serum 25(OH)D levels at an earlier point may stimulate a negative feedback, resulting in lower levels at a later point [37]. Thirdly, there is a possibility that serum 25(OH)D levels in part of the children at 1 year were overestimated, *i.e.*, due to the possible presence of C-3 epimers [40–42]. Other studies have shown higher concentrations of C-3 epimers of 25(OH)D in infants under 1 year of age than older children and adults [41,42]. However, we do not know whether, or to what extent, these were present in our subjects.

As presented in our previous publication, vitamin D intake, but not breastfeeding or season, was associated with vitamin D status at 1 year [17]. In the current study we found that vitamin D status at 1 year and vitamin D intake at 6 years were associated with vitamin D status at 6 years. Season was only associated with vitamin D status among children not receiving the recommended intake of vitamin D. In our study population increasing vitamin D intake by 10 μg/day (amount corresponding to the daily recommended intake) and seasonal differences had similar influence on the odds of having serum 25(OH)D above 50 nmol/L. Vitamin D intake has been positively associated with serum 25(OH)D levels in international systematic reviews and meta-analyses [43,44], supporting a public health emphasis on vitamin D intake in regions and among groups of people where sufficient cutaneous synthesis of vitamin D is not guaranteed. This is the case in Iceland. Few foods are naturally rich in vitamin D [45] and consumption of vitamin D fortified foods and drinks, such as milk, dairy products or orange juice was not common in Iceland at the time of the study. Therefore vitamin D supplements are important vitamin D sources in Iceland and are included in the food-based dietary guidelines for children and adults [16]. As in our study, season has clearly been shown to affect vitamin D status in our neighboring countries [23,29,46]. Consequently, official guidelines emphasize vitamin D supplement use during winter but less so during summer [16]. However, mean serum 25(OH)D levels in June (spring) were lowest of all months with vitamin D status measured (45.3 nmol/L) and still only three-quarters of children were vitamin D sufficient in July–August (summer). Therefore, to ensure sufficient vitamin D status all year round it might be important to use vitamin D supplements throughout the year in Iceland. Both genetic and socio-demographic factors have been associated with vitamin D status in other studies [29,30,47,48]. Our finding of longer breastfeeding duration in the first year of life among vitamin D sufficient 6 year olds possibly reflects long-term effects of socio-demographic factors on vitamin D status. Although maternal education was not associated with vitamin D sufficiency in the current analysis, breastfeeding duration is associated with maternal education in this cohort, as previously presented [49].

The main strength of the study is that vitamin D status was measured at two different times in the subjects, giving tracking data from 1 to 6 years of age. Another strength is the detailed information on the cohort, *i.e.*, infant and socio-demographic data, and simultaneous analysis of vitamin D intake and status. We have previously reported that the original sample is population based and therefore representative of Icelandic children [25,50]. However, as the current analysis on vitamin D status at 6 years is a secondary analysis in the original study, the interpretations of the results are subject to some limitations. Vitamin D status was not measured all months of the year among the 6 year old children. This may possibly affect the observed magnitude of tracking. Data on vitamin D intake from food on the individual level may be subject to underreporting as intake of fish, especially fatty fish, may be hard to capture with three diet registration days [51]. Frequent fish consumption in Iceland as well as the likelihood of a lower day to day variance in the diet of young children compared to adults may diminish this potential problem. The parent questionnaire was not developed to assess cutaneous vitamin D synthesis and our best available indicator on sun exposure may be the reported physical activity. Information about time spent outdoors, weather conditions, skin pigmentation and habitual clothing and sunscreen use would have been a valuable addition [52].

In conclusion, vitamin D status in infancy as well as current vitamin D intake and season are clear predictors of vitamin D status in early school age Icelandic children. To our best knowledge this is the first study to present tracking of vitamin D status from infancy to childhood, providing motivation for further studies on tracking and its clinical significance.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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# Paper IV

# Feeding in infancy, vitamin D and IgE sensitization to food allergens at 6 years: a longitudinal cohort

Birna Thorisdottir[1] bth50@hi.is, Ingibjorg Gunnarsdottir[1] ingigun@landspitali.is, Anna Gudrun Vidarsdottir[2] annavid@landspitali.is, Sigurveig Sigurdardottir[2,3,4] veiga@landspitali.is, Bryndis Eva Birgisdottir[1] beb@hi.is, Inga Thorsdottir[5] ingathor@hi.is

[1]*Unit for Nutrition Research, Faculty of Food Science and Nutrition, University of Iceland & Landspitali University Hospital, Eiriksgata 29, 101 Reykjavik, Iceland; +354 5438410*

[2]*Department of Immunology, Landspitali University Hospital, Hringbraut, 101 Reykjavik, Iceland; +354 5431000*

[3]*Faculty of Medicine, University of Iceland, Vatnsmyrarvegi 16, 101 Reykjavik, Iceland; +354 5254000*

[4]*The Allergy Outpatient Department, Landspitali University Hospital, Fossvogi, 108 Reykjavik, Iceland; +354 5431000*

[5]*School of Health Sciences, University of Iceland, Vatnsmyrarvegi 16, 101 Reykjavik, Iceland; +354 5254000*

**Corresponding author:** Birna Thorisdottir, Unit for Nutrition Research, Eiriksgata 29, 101 Reykjavik, Iceland. Phone numbers: +354 5438410, +354 8464681; Fax number: +354 5521331; E-mail address: bth50@hi.is. ORCID identifier: 0000-0001-7320-0116.

# Feeding in infancy, vitamin D and IgE sensitization to food allergens at 6 years: a longitudinal cohort


## Abstract

***Aim:*** Our aim was to compare feeding in infancy and vitamin D intake between sensitized and non-sensitized 6-year-old children.

***Methods:*** In a prospective Icelandic cohort, serum-specific IgE-antibodies against food were analyzed at 6 years (cut-off specific IgE ≥0.35 kUA/L). Serum 25(OH)D was analyzed at 12 months and 6 years. Dietary information until 4 months was gathered with dietary recall and at 5-12 months and at 6 years with food records. Weight, length/height and head circumference was measured in infancy and at 6 years.

***Results:*** Out of 144 6-year-old children, 14 were IgE-sensitized (10%). At 4 months, 57% sensitized vs. 23% non-sensitized children ($p<0.01$) had received solid food. At 12 months, sensitized children had a lower intake of vitamin D (median (25th,75th percentiles); 3.9 μg/d (3.2,7.2) vs. 8.1 μg/d (4.4,12.3), $p=0.03$) and at 6 years, they were less likely to use vitamin D supplements (23% vs. 56%, $p=0.03$). From birth to 2 months, sensitized compared to non-sensitized children had greater increase in weight (mean±SD; 2.2±0.4 kg vs. 1.8±0.5 kg, $p=0.04$) and head circumference (4.9±1.2 cm vs. 4.2±1.0 cm, $p=0.02$) and at 6 years, sensitized children were more likely overweight or obese (29% vs. 10%, $p=0.04$). No significant difference was seen in serum 25(OH)D.

***Conclusions:*** The results suggest that it might not be justified to consider recommending solid foods before the age of 4 months and support encouraging vitamin D intake from the diet and supplements in infant and children populations without sufficient sun exposure.



**Keywords:** nutrition; infant; child; sensitization; complementary feeding; solid food; vitamin D

## Introduction

Dietary approaches to prevent food allergies are currently the topic of much research focus and have implications for infant nutrition recommendations (1, 2). While most official bodies recommend exclusive breastfeeding for either the full first 6 months or at least 4-6 months (3-7), there is a debate regarding the optimal age of introduction of solid foods to decrease the risk of food allergy (1, 2). The evidence regarding introduction of peanuts has resulted in special recommendations for peanut introduction to infants (8, 9). The evidence for other food allergies remains inconclusive. Cow's milk allergy is the most common food allergy among European children (10).

Immunologically active nutrients, including vitamin D and omega-3 polyunsaturated fatty acids (n-3 PUFA), are considered of interest for food allergy prevention (2). A recent Icelandic observational study found associations between fish liver oil use in infancy and decreased food sensitization and clinical food allergies up to 2.5-years-of-age (11). Furthermore, in the same study, children starting to receive fish liver oil already in their first half year of life were significantly more protected than those with later introduction (11). While vitamin D supplements are recommended for infants up to 1-year-of-age in Europe and North America (12, 13), there is a special opportunity in Iceland to study intakes of both vitamin D and fish oil supplements, the latter including both vitamin D and n-3 PUFA, as official guidelines recommend that fish liver oil should replace vitamin D drops when complementary feeding starts (14). Furthermore, vitamin D supplements, e.g. fish liver oil, are recommended throughout life in Iceland (15).

The detection of serum specific IgE to common food allergens indicates a state of sensitization but does not in isolation prove the existence of clincial allergy (16). Some studies have suggested that about half of sensitized children have food allergy, clinically proven by food challenge (17). It may be worthwhile to study early markers of allergy, and according to a model by Grimshaw et al., primary prevention of allergy should focus on preventing immunological sensitization (2). Therefore, we decided to test for serum specific IgE to common food allergens at 6-years-of-age in a cohort with detailed information of timing of solid food introduction and repeated information on use of vitamin D supplements, including fish liver oil, throughout the first year of life and in a follow-up at 6-years-of-age. Our aim was to compare feeding in infancy and vitamin D intake between sensitized and non-sensitized children.

## Methods

### *Participants and Design*

The study population, recruitment and data collection have previously been described in detail (18, 19). In brief, a random sample of 250 Icelandic infants born in year 2005 (6% of live-born infants) was followed throughout their first year of life. The inclusion criteria were Icelandic parents, singleton birth, gestational length of 37-41 weeks, birth weight within the 10th and 90th percentiles, no birth defects or congenital long-term diseases and that the mother had regular antenatal care. The current study includes 144 children who participated in blood sampling in the follow-up at 6 years and had allergen-specific IgE levels analyzed (84% of the children in the follow-up). As previously described, children participating in the blood sampling did not differ from the original study sample in terms of birth size and breastfeeding (20) and the basic characteristics of subjects included in this study did not differ from other subjects in the follow-up. Informed written consent from the parents was obtained for the study and all individual information was processed with strict confidentiality. The study was conducted according to the Declaration of Helsinki, approved by the National Bioethics Committee (VSNb2005040019/037; VSNb2011010008/037) and registered at the Data Protection Authority (S2449/2005; S5099/2011) in Iceland.

### *Dietary Assessments*

Detailed information about infant feeding from birth until 4 months (17 weeks) including breastfeeding, introduction of infant formula and solid food, and use of vitamin D or other dietary supplements, was gathered with one structured dietary recall facilitated by a trained interviewer (mean infant age at dietary recall 21 weeks). The exact age when the first infant formula and first solid food was introduced to the diet, along with the type of formula or solid was registered. One-day non-weighed food records, using common household measures, were kept at 5, 6, 7, 8, 10 and 11 months of age. At 9 months, 12 months and 6 years weighed food records were kept for 3 consecutive days (72-h). All food and fluids were weighed on accurate scales (Philips type HR 2385, Hungary and Austria) with 1-g precision. Dietary supplements and medicines were also registered. An average daily consumption of food and nutrients was calculated using the Icelandic food composition database (21) which included infant products, such as porridges, purées and cereals. Information on breastfeeding duration was collected from food records and retrospectively confirmed with parents.

***Blood Sampling and Measurements***

At 12 months and 6 years, venous blood samples were collected in the morning in fasting state at Landspitali University Hospital by a certified paediatrician. Separated serum samples were stored at -80°C at the biobank at Landspitali. Specific IgE levels were analyzed for ImmunoCAP® allergen Fx5 by using ImmunoCAP fluoroenzyme immunoassay (Phadia 250, Thermo Scientific, Uppsala, Sweden) (precision 0.01 kUA/L). The ImmunoCAP® allergen Fx5 is a food mix test detecting specific IgE to six major food allergens (cow's milk, egg white, cod, wheat, soy bean and peanut). In the case of a positive Fx5 test, specific IgE was further analyzed for each of the six major food allergens. Tests were performed according to the manufacturer's instructions and classified as a positive test, using the term "sensitized", when specific IgE $\geq$0.35 kUA/L. Serum 25(OH)D levels were analyzed in samples from 12-month-old infants and 6-year-old children by using an electro-chemiluminescence immunoassay (Elecsys Vitamin D total assay, Modular Analytics E170, Roche Diagnostics, Mannheim, Germany) (precision 0.1 nmol/L).

***Other Variables***

Information on number of siblings and parental variables including age, self-reported weight and height (from which BMI was calculated) and smoking after birth was gathered from structured questionnaires answered by parents (usually the mother) when the children were 12 months old. Some parents did not answer individual questions and therefore have missing values. Birth months were categorized as 'winter/spring' (November-April) and 'summer/autumn' (May-October) based on the expected contribution of sunlight exposure to maternal vitamin D status around birth (22). During follow-up at 6-years-of-age, parents answered another questionnaire including questions on children's regular use of antihistamines and asthma inhalers.

Information about weight, length and head circumference at birth was gathered from the maternity wards. The primary healthcare provided measurements on weight, length and head circumference as close to the 2, 6, 10 and 12 months birthday as possible. Weight was registered with 5 g precision, length measured on length boards and registered with 0.1 cm precision and head circumference was registered with 0.1 cm precision. At follow-up (mean age 73.4±3.2 months) weight (Marel M series 110, Iceland) (precision 0.1 kg) and height (Ulmer stadiometer, Professor Heinze, Busse design, Ulm, Germany) (precision 0.1 cm) were measured at Landspitali University Hospital. Children were classified as being normal weight or overweight/obese according to the International Obesity Task Force cut-off points for overweight defined to pass through body mass index (BMI) of 25 $kg/m^2$ at 18 years of age (23). Cut-off points of 17.55 $kg/m^2$ and 17.34 $kg/m^2$ were applied for 6-year-old boys and girls, respectively. Gain in weight, length or head circumference were calculated for each participant as the difference in measurements between two-time points.

***Statistical Analysis***

Statistical analysis was performed with SAS (Enterprise Guide 4.3; SAS Institute Inc, Cary, NC, USA). Variables were examined for normality using Quantile Quantile-plots and presented as n (%), mean and standard deviations (SD) or medians and 25th to 75th percentiles. For comparison between groups we used a chi-square, two-sided t-test or Mann Whitney U-test. Logistic regression analyses was used to examine possible associations with sensitization at 6 years, adjusting for maternal smoking during infancy, and presented as the odds ratio (OR) with its 95% confidence intervals (CI). The level of significance was set at ≤0.05.

## Results

Fourteen 6-year-old children (10%) were sensitized to food allergens; thereof eight to cow's milk only; two to cow's milk and eggs; two to peanuts only; one to peanuts and eggs; and one to peanuts, wheat and soy. Table 1 presents the characteristics of participants included in this study. Significant differences according to sensitization were observed in maternal smoking during infancy, more common among sensitized children. Four sensitized children and one non-sensitized child were reported regular users of asthma inhalers or antihistamine at six years. [*Table 1 near here*]

Breastfeeding initiation rate was high (99%), and 88% of infants were still breastfed when introduced to solid food. Figure 1 shows the feeding style during the first half year of life among non-sensitized and sensitized children. Feeding style in the first 4 months did differ as higher proportion of sensitized vs. non-sensitized 6-year-old children had received solid foods at 1, 2, 3 and 4 months of age. By 4 months-of-age (17 weeks), 57% of sensitized and 23% of non-sensitized children had received solid food. Introduction of solid foods prior to 4 months increased the odds for sensitization, OR=4.9 (95% CI=1.4-16.6), adjusted for maternal smoking. No significant differences were seen in breastfeeding or use of formula. [*Figure 1 near here*]

By 1 month of age, 50% of infants had received vitamin D supplements and this proportion increased to 75% at 2 months and 85% at 3 months, with no significant differences according to sensitization. In the first 4 months of life, AD drops were used and no infant received fish liver oil prior to 5 months. Figure 2 shows the proportion of participants with vitamin D supplements (AD drops/vitamin D tablets in grey and fish liver oil in black) in infancy and at 6 years according to sensitization. [*Figure 2 near here*] No significant differences between sensitized and non-sensitized children were observed in infancy, neither when looking at type of vitamin D supplement nor when specifically looking at fish liver oil use. 72% of non-sensitized and 57% of sensitized participants received some fish liver oil in infancy (non-significant, p=0.2). There was no significant difference in age of fish liver oil introduction in infancy according to sensitization. Vitamin D supplement use at 6 years was however significantly less common among sensitized (23%) than non-sensitized (56%) participants (p=0.03). Vitamin D supplement use at 6 years was mainly in the form of fish liver oil, used by 23% of sensitized children and 52% of non-sensitized children (p=0.04). Vitamin D supplement use at 6 years decreased the odds for sensitization, OR=0.2 (95% CI=0.1-0.98), adjusted for maternal smoking.

Total vitamin D intake from supplements including fish liver oil, fortified formula and food at 12 months was lower among sensitized than non-sensitized 6-year-old children (Table 2). [*Table 2 near here*]

Table 3 shows gain in weight, length and head circumference from birth to 2 months of age, from 2 to 6 months and 6 to 12 months. [*Table 3 near here*] From birth to 2 months of age, sensitized vs. non-sensitized children had greater increase in weight (mean±SD; 2.2±0.4 kg vs. 1.8±0.5 kg, p=0.04) and head circumference (4.9±1.2 cm vs. 4.2±1.0 cm, p=0.02). Sensitized children had larger crude head circumference than non-sensitized children from 2 to 10 months, but not at 12 months. Sensitized children were more likely overweight/obese at 6 years compared with non-sensitized children (29% vs. 10%, p=0.04). All sensitized children consumed cow's milk at 12 months and 6 years. Compared with non-sensitized children, sensitized children more commonly consumed regular cow's milk at 12 months (100% vs. 56%, p<0.01) and less frequently fortified formula at 12 months (30% vs. 68%, p=0.02).

Mean serum 25(OH)D did not differ between sensitized and non-sensitized children, neither at 12 months (96.8±33.6 vs. 99.3±32.2 nmol/L, respectively), nor at 6 years (59.3±15.9 vs. 56.0±16.7 nmol/L, respectively). Sensitized children were not more likely than others to be in the lowest or highest quartiles for vitamin D status.

## Discussion

In this study in a population of Icelandic children, solid food introduction prior to four months and less vitamin D intake at 12 months were associated with increased risk of sensitization at 6 years of age. Also, vitamin D supplement use, mainly in the form of fish liver oil, at 6 years was less common among sensitized children. Although this study cannot determine causality, the results add to the present literature due to the detailed and accurate information on feeding and supplement use in infancy and at follow-up. If prevention of sensitization is considered important for prevention of food allergy (2), our results support current vitamin D recommendations and avoidance of solid foods prior to 4 months of age.

While some studies (9, 24-26) but not all (27, 28) propose that introduction of allergenic solid food from 3-4 months of age is safe and may lead to tolerance and protection against IgE-mediated food allergy, associations between solid food introduction prior to 4 months, faster infant growth and childhood obesity have been reported (29-33). Furthermore, it has been hypothesized that childhood obesity may promote immunological changes increasing the risk for allergy (34) but the evidence remains uncertain (35). Our finding of greater weight gain from birth to 2 months of age might be a result of earlier introduction of solid foods. Faster weight gain in infancy has been associated with increased risk of allergic rhinitis (36), asthma (37-40), impaired lung function (41-43) and wheezing (36, 44). Larger head circumference at birth, or a high ratio of head circumference relative to birth weight has been associated with atopy (45-52). Although very rare, maternal smoking during infancy was more common among sensitized than non-sensitized children in our study, in the same line as literature on associations between passive smoking and increased risk for food allergy (53).

A recent study reported associations between postnatal fish liver oil consumption and decreased food sensitization and clinical food allergy in infants (11). In our study, fish liver oil consumption was very rare in the first six months of life and did not become more common than AD drops until age 9 months. Our findings of less vitamin D intake at 12 months among sensitized children, and sensitized children being less likely to use vitamin D supplements (fish liver oil) at 6 years of age are interesting and warrant further study. Fish liver oil is a good source of both vitamin D and n-3 PUFA. Vitamin D modulates the action of immune cells, such as T and B cells, monocytes and dendritic cells, in an interplay between the innate and adaptive immune systems (54). Omega-3 can influence cell membrane structure, cell signaling and antigen presentation and thereby exert anti-inflammatory effects (55).

However, we did not see difference in vitamin D status according to sensitization. While some studies suggest that vitamin D insufficiency increases the risk of IgE-mediated food allergy or food sensitization in infants and children (56-59), other studies suggest that high-dose vitamin D supplementation in infancy may increase the risk of food allergy (60). Recent systematic reviews and meta-analyses have reported no significant associations between vitamin D status and food allergy in children (61). A U-shaped relationship between serum 25(OH)D and allergy has been proposed, with both very low and very high levels associated with increased risk (62-64). To detect possible U-shaped associations a great sample size might be needed.

The strength of our study lies in the detailed longitudinal data on infant feeding and vitamin D supplement use. Our cohort is population-based and the prevalence of atopic disease among Icelandic children is similar as in other European countries (65). We acknowledge that the sample size is a major limitation in our study. Still the cohort's size was sufficient for the findings we observed. Studies on sensitization, like the current one, may be important to study determinants of and monitor changes in the prevalence of allergic sensitization. This is for example done in US and European studies (66, 67).

## Conclusions

Avoidance of solid food before the age of 4 months and intake of vitamin D, mainly fish liver oil, were associated with decreased risk of sensitization at 6 years. The results suggest that it might not be justified to consider recommending solid foods before the age of 4 months. Furthermore, while the present literature is unclear on the associations between vitamin D and food allergy, the results support encouraging vitamin D intake from the diet and supplements in infant and children populations without sufficient sun exposure.


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**Table 1. Characteristics of the participants included in the study.**

| Variable | Non-sensitized (n=130) | Sensitized (n=14) |
|---|---|---|
| Male sex, n (%) | 65 (50) | 9 (64) |
| Birth in winter/spring, n (%) | 70 (54) | 8 (57) |
| Urban living, n (%) | 91 (70) | 11 (79) |
| First born, n (%) | 42 (36) | 2 (17) |
| Exclusive breastfeeding, months | 4 (2, 5) | 4 (0, 4) |
| Any breastfeeding, months | 8 (6, 10) | 8 (7, 11) |
| Maternal smoking[1], n (%) | 4 (3) | 2 (15)[2] |
| Paternal smoking[1], n (%) | 18 (16) | 3 (23) |
| Maternal age[1], years | 31.3 ± 4.8 | 33.2 ± 5.4 |
| Paternal age[1], years | 34.1 ± 5.7 | 36.5 ± 6.6 |
| Maternal BMI[1], $kg/m^2$ | 23.5 (21.4, 26.5) | 26.6 (21.8, 33.2) |
| Paternal BMI[1], $kg/m^2$ | 26.0 (24.3, 28.1) | 26.6 (23.4, 28.7) |

Data presented as n (%), mean ± SD or median (25th, 75th percentiles). Chi-square, two sided t-test or Mann Whitney U-test used for comparison between groups.

[1]When infant is 12 months old

[2]$p=0.05$

**Table 2. Vitamin D intake at 9 months, 12 months and 6 years.**

| | Non-sensitized, median ($25^{th}$,$75^{th}$perc) | Sensitized median ($25^{th}$, $75^{th}$ perc) |
|---|---|---|
| Vitamin D intake 9 mo, µg/d | 9.8 (5.6, 13.7) | 7.2 (3.2, 10.5) |
| Vitamin D intake 12 mo, µg/d | 8.1 (4.4, 12.3) | 3.9 (3.2, 7.2)[1] |
| Vitamin D intake 6 y, µg/d | 5.2 (2.3, 12.3) | 3.1 (2.1, 3.6) |

[1]p=0.03

**Table 3. Gain in weight, length and head circumference in selected periods.**

| Variable | Non-sensitized (n=130) | Sensitized (n=14) |
|---|---|---|
| Weight gain | | |
| 0-2 months, kg | 1.8 ± 0.5 | 2.2 ± 0.4[3] |
| 2-6 months, kg | 2.4 ± 0.7 | 2.3 ± 0.7 |
| 6-12 months, kg | 1.9 ± 0.6 | 1.7 ± 0.6 |
| Length gain[1] | | |
| 0-6 months, cm | 16.8 ± 2.0 | 17.8 ± 1.7 |
| 6-12 months, cm | 8.2 ± 1.5 | 7.3 ± 2.1[3] |
| Head circumference gain | | |
| 0-2 months, cm | 4.2 ± 1.0 | 4.9 ± 1.2[2] |
| 2-6 months, cm | 4.2 ± 0.7 | 3.9 ± 0.8 |
| 6-12 months, cm | 2.9 ± 1.1 | 2.7 ± 0.8 |

Data presented as mean ± SD. Two sided t-test used for comparison between groups.

[1]Length gain 0-2 mo and 2-6 mo not reported due to a large number of missing values for length at 2 months (missing n=56)

[2]p=0.02

[3]p=0.04

## Figures

**Figure 1. Feeding style in the first half year of life.** Proportion (%) of infants in each month receiving exclusively breastmilk (EBF); mixed breastmilk and infant formula or exclusively infant formula (MBF); or any solid food (SF).

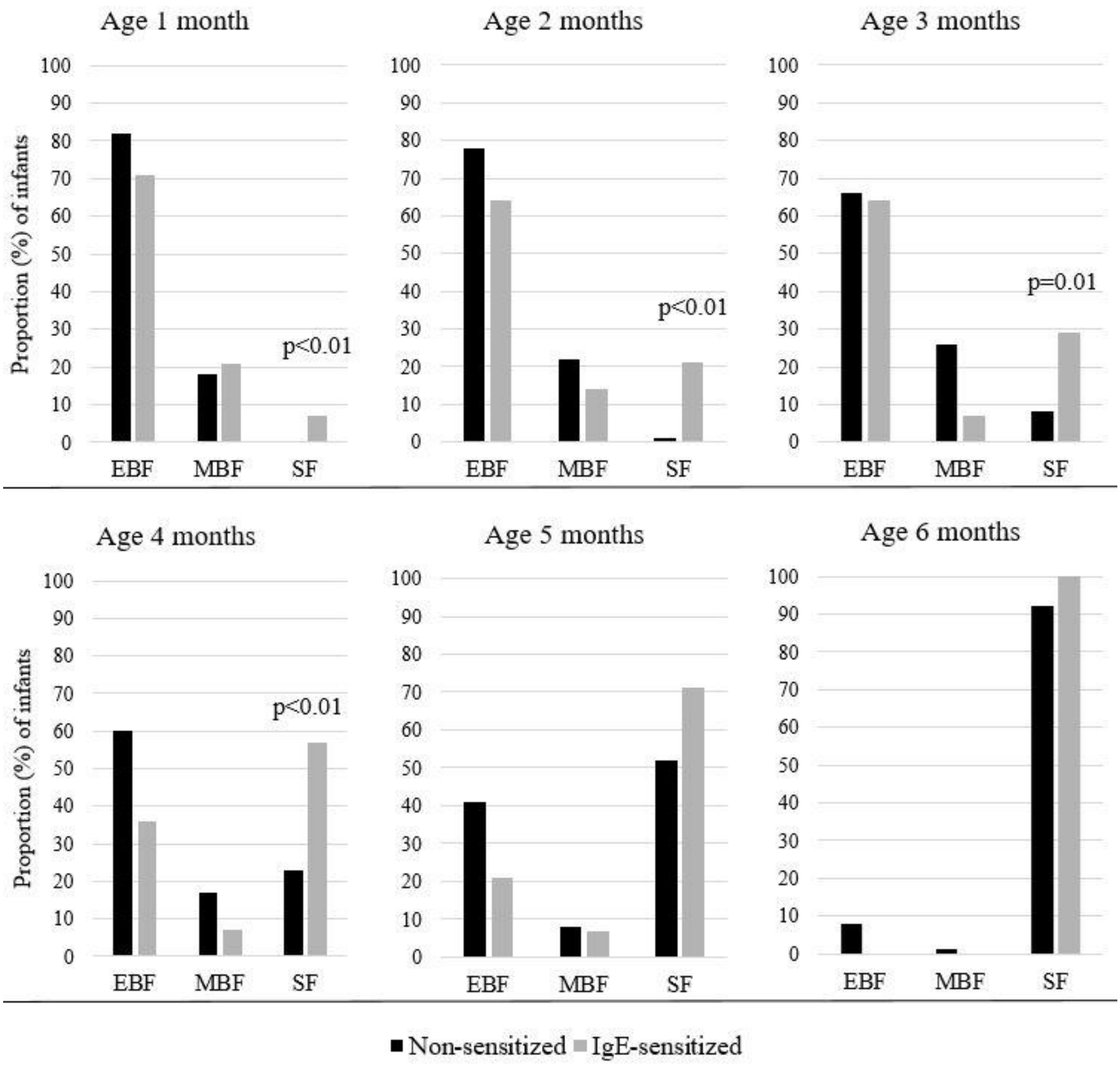

**Figure 2. Vitamin D supplement use in infancy and childhood.** Proportion (%) of infants and children receiving vitamin D supplements (AD drops/vitamin D tablets in grey and fish liver oil in black).