

Vitamin D Intervention in Infants (VIDI) trial

PROTOCOL AND STATISTICAL ANALYSIS PLAN

PROTOCOL

Vitamin D Intervention in Infants (VIDI) is a large randomised trial that aims to evaluate effects of two vitamin D supplemental doses in early childhood on bone strength, infections, immunity, allergy, atopy and asthma, neurologic and cognitive development, and genetic regulation of mineral homeostasis.

This protocol comprises the applied methods to evaluate the effects of vitamin D supplementation on the primary outcomes of the study: bone strength and incidence of infections during the first two years of life. Methods for additional outcomes have been described in Helve et al, 2017.¹

Background

Both cutaneously synthesised and vitamin D obtained from diet and supplements contribute to circulating 25-hydroxyvitamin D concentrations. The optimal 25-hydroxyvitamin D concentration is still under discussion. In 2011, Institute of Medicine guidelines stated that 25-hydroxyvitamin D concentrations above 50 nmol/L are required for normal body functions, including linear growth and bone mass accrual.² According to the Endocrine Society, concentrations above 75 nmol/L may be necessary to achieve optimal long-term health benefits.³

In Finland, Vitamin D supplementation has been recommended to all infants since the 1940's, but in line with the declining prevalence of rickets in our country, the recommended doses have gradually decreased. The present Finnish Nutritional Council guidelines recommend 10 µg (400 IU) of vitamin D₃ supplementation daily for all infants from the age of 2 weeks to 2 years. Despite these

recommendations, 20 % of 14-month-old children are shown to be vitamin D deficient (< 50 nmol/L).⁴

Normal bone development and growth requires adequate intake of minerals, such as calcium and phosphate. Parathyroid hormone and biologically active form of vitamin D, 1,25-dihydroxyvitamin D, regulate calcium and phosphate concentrations. In addition, 1,25-dihydroxyvitamin D has direct effect on bone cells. Insufficient vitamin D supply results in inadequate bone mineralisation at growth plates and leads to rickets.⁵ In infants, severe vitamin D deficiency (< 25 nmol/L) associates with poor linear growth and delayed motor development possibly due to muscle weakness.^{6,7} Correction of vitamin D deficiency has been shown to improve growth velocity and prevent stunted growth.^{8,9} Data on the relationship between vitamin D and bone mineral density (BMD) in children are contradictory and further studies are essential.^{10,11}

In addition to its skeletal effects, vitamin D modulates both the innate and adaptive immune systems. Immune cells express vitamin D receptor (VDR) and have local enzymatic capacity to synthesise active 1,25-dihydroxyvitamin D.^{12,13} Vitamin D induces production of antimicrobial peptides, such as cathelicidin, by monocytes and macrophages, and is involved in regulation of T-lymphocyte function.¹⁴ In epidemiological studies, vitamin D deficiency has been associated with increased risk of infections both in adults and children.^{15,16} Results from randomised trials are inconclusive. For example, in Japan, a daily supplemental vitamin D₃ dose of 30 µg (1200 IU) for 4 months resulted in a significant reduction of influenza A infections, and in Mongolia, daily 7.5 µg (300 IU) of vitamin D₃ reduced the risk of acute respiratory infections in schoolchildren.^{17,18} However, in Afghan infants, the incidence of pneumonia was not affected by 18 months of vitamin D supplementation.¹⁹ Further studies are

needed to determine whether vitamin D supplementation provides benefits against infections in healthy children.

Primary outcomes

Primary outcomes of the study are bone strength measured by peripheral quantitative computed tomography (pQCT) and incidence of parent-reported infections at age 2 years.

Participants and methods

A total of 1 000 families are recruited and informed consent is obtained 1-2 days after the delivery at the Kätilöopisto Maternity Hospital in Helsinki. We include white northern European women with a singleton pregnancy and without regular medication. Healthy infants born at term (37-42 weeks) and with weight appropriate for gestational age are included in the study. Exclusion criteria for the infants are: seizures, need for early antibiotic treatment, need for nasal continuous positive airway pressure > 24 hours, extended phototherapy > 72 hours, need for nasogastric tube > 24 hours or intravenous glucose infusion. Data on family background (parents' socio-economic status, health, lifestyle factors) and maternal dietary status are documented with a questionnaire. At birth, a cord blood sample (20 ml) is taken and stored for later analyses.

Participating infants are randomised to receive either the currently recommended vitamin D₃ supplementation of 10 µg (400 IU) daily or a higher dose of 30 µg (1200 IU) daily from age 2 weeks to 2 years. Boys and girls are randomised separately in blocks of 50. Randomisation is performed by the

Helsinki University Hospital Pharmacy. The study is double-blinded. Vitamin D is administered orally as vitamin D₃, with a dose of 5 drops a day for both concentrations.

Follow-up

Participants are assessed at a study outpatient clinic by a study nurse and/or pediatrician at 6 months, 1 year and 2 years of age. Growth parameters (length, weight, head circumference) are measured and compared with Finnish growth charts.²⁰ Blood samples are taken for biochemistry. Bone strength of participants is evaluated by pQCT at 1 and 2 years of age. Families are provided study diaries where they keep daily records on dosing of vitamin D₃ supplement and on all infections of the participating child. Nutrient intake from food is evaluated from 3-day food record at 1 year and a Food Frequency Questionnaire (FFQ) at 2 years of age.

Specific methods

Biochemical markers

Serum 25-hydroxyvitamin D concentration is measured from serum samples with an automated IDS-iSYS analyser (IDS Ltd., Bolton, UK) which employs a chemiluminescence immunoassay (CLIA) with high sensitivity, a fast protocol, with a 10- μ l specimen volume. The method is validated against LC-MS in-house as well as by the manufacturer. Analyses are performed at the Pediatric Research Centre laboratory in Biomedicum, University of Helsinki (Helsinki, Finland). Reproducibility is ensured by adhering to the Vitamin D External Quality Assessment Scheme (DEQAS, Charing Cross Hospital, London UK). The IDS-iSYS immunoassay will also be used to analyse serum intact parathyroid

hormone concentration from serum samples.

Ionised calcium (adjusted to pH 7.40, normal range 1.16-1.39 and 1.17-1.35 mmol/L for age groups 1-12 months and 24 months respectively) is analysed from capillary blood samples at 6 months and from serum samples at 12 and 24 months at the Central Laboratory of Helsinki University Hospital (HUSLAB) using ABL 90 FLEX or ABL 835 FLEX blood gas analysers. HUSLAB is an accredited laboratory adhering to international (T055) SFS-EN ISO 15189 and SFS-EN ISO/IEC 17025 standards.

Bone strength

Bone strength is measured by pQCT from the distal left tibia using a XCT-2000 scanner (Stratec Medizintechnik GmbH, Pforzheim, Germany).

Infections

Data on infections are collected prospectively from daily diaries kept by parents of participating infants. Parents record the time and duration of the infection, symptoms, required medication, physician visit or hospitalisation in the daily diaries. Every 3-6 months, new diaries are provided to the families by mail or at follow-up visits, and completed diaries are returned to the study nurse.

Dietary vitamin D intake

At recruitment, maternal diet is evaluated retrospectively with a semi quantitative 22-item Food Frequency Questionnaire, for the time-period of one month before delivery.

At age 1 years, data on nutrient intake from food is collected with a three-day food record and analysed using AivoDiet software (Aivo Oy Finland, Turku, Finland). At 2 years, the dietary habits of

the child are collected with 47-item Food Frequency Questionnaire. If the child attends daycare, the personnel are asked to complete a separate two-day food record.

Ethical issues and research permits

A recent intervention study confirmed the safety of vitamin D supplementation in infants with a daily dose of 50 µg (2000 IU) daily.²¹ Furthermore, we conducted a pilot study in 113 healthy newborns in order to evaluate short-term effects and safety of 3 different vitamin D₃ doses (10 µg, 30 µg, 40 µg daily).²² No adverse events occurred and all doses were deemed safe. Based on the results, we chose daily doses of 10 µg and 30 µg for the intervention study.

An external clinical research institute monitors the study and possible adverse effects. As a safety protocol, the infants are monitored for hypercalcemia at follow-up visits. If the calcium concentration exceeds the upper reference limit of ionised calcium by $\geq 10\%$, defined as ionised calcium concentration above 1.53 mmol/L at 6 and 12 months and 1.48 mmol/L at 2 years follow-up, the ionised calcium and 25-hydroxyvitamin D concentrations will be repeatedly measured, symptoms indicative of hypercalcemia will be evaluated, and, if necessary, dosing of vitamin D supplementation adjusted.

Blood samples are taken as follows: A) 20 ml from the umbilical vein after cord clamping at birth, B) 15 ml at age 1 year, and C) ≤ 20 ml at age 2 years. These volumes are clearly below the allowed maximal volumes for research sampling (approximate limits for research purposes are for ages 1 year: 24 ml and 2 years: 36 ml; i.e. 3% of circulating blood volume).

The radiation exposure from pQCT measurements is estimated to be 30 μSv , and exposure from whole-body DXA 50 μSv . This total dose of approximately 80 μSv equals radiation exposure during an overseas flight or 2 weeks' background radiation, and can thus be regarded as insignificant.²³ Similar methods have previously been used to study bone variables in newborns.²²

The vitamin D₃ supplements are provided by Orion Pharmaceuticals free of charge. The study is researcher initiated and independent.

Informed consent is obtained from the parents at recruitment. An ethical approval has been obtained from the Research Ethics Committee of the Hospital District of Helsinki and Uusimaa (ID 107/13/03/03/2012) including permission to keep a research register of collected data where the anonymity of all participants is secured with an identification number. Research permits from Children's Hospital are valid until 2018. The project protocol is registered into ClinicalTrial.com (NCT01723852).

References

1. Helve O, Viljakainen H, Holmlund-Suila E, et al. Towards evidence-based vitamin D supplementation in infants: vitamin D intervention in infants (VIDI) - study design and methods of a randomised controlled double-blinded intervention study. *BMC Pediatr* 2017;17:91,017-0845-5.
2. Ross AC. The 2011 report on dietary reference intakes for calcium and vitamin D. *Public Health Nutr* 2011;14:938-9.
3. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Guidelines for preventing and treating vitamin D deficiency and insufficiency revisited. *J Clin Endocrinol Metab* 2012;97:1153-8.

4. Viljakainen HT. Maternal vitamin D status affects bone growth in early childhood-a prospective cohort study. *Osteoporosis Int* 2011;22:883-91.
5. Elder CJ, Bishop NJ. Rickets. *Lancet* 2014;383:1665-76.
6. Pettifor JM, Prentice A. The role of vitamin D in paediatric bone health. *Best Pract Res Clin Endocrinol Metab* 2011;25:573-84.
7. Soliman A, Salama H, Alomar S, Shatla E, Ellithy K, Bedair E. Clinical, biochemical, and radiological manifestations of vitamin D deficiency in newborns presented with hypocalcemia. *Indian J Endocrinol Metab* 2013;17:697-703.
8. Soliman AT, El-Dabbagh M, Adel A, Al Ali M, Aziz Bedair EM, Elalaily RK. Clinical responses to a mega-dose of vitamin D3 in infants and toddlers with vitamin D deficiency rickets. *J Trop Pediatr* 2010;56:19-26.
9. Kumar GT, Sachdev HS, Chellani H, et al. Effect of weekly vitamin D supplements on mortality, morbidity, and growth of low birthweight term infants in India up to age 6 months: randomised controlled trial. *BMJ* 2011;342:d2975.
10. Viljakainen HT, Saarnio E, Hytinantti T, et al. Maternal vitamin D status determines bone variables in the newborn. *J Clin Endocrinol Metab* 2010;95:1749-57.
11. Mayranpaa MK, Tamminen IS, Kroger H, Makitie O. Bone biopsy findings and correlation with clinical, radiological, and biochemical parameters in children with fractures. *J Bone Miner Res* 2011;26:1748-58.
12. Holick MF. Vitamin D deficiency. *N Engl J Med* 2007;357:266-81.
13. Di Rosa M, Malaguarnera M, Nicoletti F, Malaguarnera L. Vitamin D3: a helpful immunomodulator. *Immunology* 2011;134:123-39.

14. Hewison M. An update on vitamin D and human immunity. *Clin Endocrinol (Oxf)* 2012;76:315-25.
15. Ginde AA, Camargo CA, Jr, Shapiro NI. Vitamin D insufficiency and sepsis severity in emergency department patients with suspected infection. *Acad Emerg Med* 2011;18:551-4.
16. McNally JD, Leis K, Matheson LA, Karuananyake C, Sankaran K, Rosenberg AM. Vitamin D deficiency in young children with severe acute lower respiratory infection. *Pediatr Pulmonol* 2009;44:981-8.
17. Urashima M, Segawa T, Okazaki M, Kurihara M, Wada Y, Ida H. Randomized trial of vitamin D supplementation to prevent seasonal influenza A in schoolchildren. *Am J Clin Nutr* 2010;91:1255-60.
18. Camargo CA, Jr, Ganmaa D, Frazier AL, et al. Randomized trial of vitamin D supplementation and risk of acute respiratory infection in Mongolia. *Pediatrics* 2012;130:e561-7.
19. Manaseki-Holland S, Maroof Z, Bruce J, et al. Effect on the incidence of pneumonia of vitamin D supplementation by quarterly bolus dose to infants in Kabul: a randomised controlled superiority trial. *The Lancet* 2012;379:1419-27.
20. Saari A, Sankilampi U, Hannila ML, Kiviniemi V, Kesseli K, Dunkel L. New Finnish growth references for children and adolescents aged 0 to 20 years: Length/height-for-age, weight-for-length/height, and body mass index-for-age. *Ann Med* 2011;43:235-48.
21. Wicklow BA, Taback SP. Feasibility of a type 1 diabetes primary prevention trial using 2000 IU vitamin D3 in infants from the general population with increased HLA-associated risk. *Ann N Y Acad Sci* 2006;1079:310-2.
22. Holmlund-Suila E, Viljakainen H, Hytinantti T, Lamberg-Allardt C, Andersson S, Makitie O. High-dose vitamin d intervention in infants--effects on vitamin d status, calcium homeostasis, and bone strength. *J Clin Endocrinol Metab* 2012;97:4139-47.

23. Blake GM, Naeem M, Boutros M. Comparison of effective dose to children and adults from dual X-ray absorptiometry examinations. *Bone* 2006;38:935-42.

STATISTICAL ANALYSIS PLAN

Sample size calculation and statistical analyses

We aim at detecting a 0.2 SD unit difference between continuous bone strength parameters; bone mineral content and cross-sectional area. In order to reach a statistical power of 90% with significance level of 0.05, a total of 210 and 297 measurements, respectively, are necessary.¹ In addition, challenges in measuring infants with pQCT may occur. According to previous studies, small children suffer from an average of 6 infections annually.^{2,3} In order to detect a decrease from 12 to 9 infections during the 24-month study period, a sample size of 220 in each group is required. We estimate a possible drop-out rate of 20%. Taking into consideration all the aforementioned aspects, the trial is designed for 1000 subjects: 500 subjects in each intervention group.

Statistical methods

Comparisons between two intervention groups are analysed with independent samples t-test, Mann Whitney U-test or Pearson Chi-square as applicable. If possible, logarithmic transformation for non-normal variables is performed in order to achieve normal distribution. The impact of vitamin D supplementation, and relevant covariates, on serum 25-hydroxyvitamin D concentration is analysed with linear mixed model. For continuous variables (bone strength), differences between intervention groups are assessed with multivariate analysis of covariance (MANCOVA).

For evaluating the effect of vitamin D supplementation on the frequency of infections, negative binomial model is applied. Infection incidence is estimated as proportion of follow-up time as person-

months allowing utilisation of all available data. Main outcome measures are analysed according to intention-to-treat and protocol-based manner.

References

1. Ireland A, Rittweger J, Schönau E, Lamberg-Allardt C, Viljakainen H. Time since onset of walking predicts tibial bone strength in early childhood. *Bone*. 2014 Nov;68:76-84.
2. Wald ER, Guerra N, Byers C. Frequency and severity of infections in day care: three-year follow-up. *J Pediatr* 1991;118:509-14.
3. Denny FW, Collier AM, Henderson FW. Acute respiratory infections in day care. *Rev Infect Dis* 1986;8:527-32.