**Manuscript title:** Prenatal exposure to vitamin D from fortified margarine and risk of fractures in late childhood. Period and cohort results from 222 000 subjects in the D-tect observational study.

**Authors:** Mina Nicole Händel1,2, Peder Frederiksen2, Clive Osmond3, Cyrus Cooper3, Bo Abrahamsen1,4, Berit Lilienthal Heitmann2,5,6,7.

**1** Department of Clinical Research, University of Southern Denmark, Odense Patient Data Explorative Network (OPEN), Odense University Hospital, 5000 Odense C, Denmark.

2 Research Unit for Dietary Studies, The Parker Institute and the Institute of Preventive Medicine, Bispebjerg and Frederiksberg Hospital, 2000 Frederiksberg, Denmark.

3 Medical Research Council Lifecourse Epidemiology Unit, University of Southampton, Southampton SO16 6YD, United Kingdom.

4 Department of Medicine, Holbæk Hospital, DK-4300 Holbæk, Denmark.

5Section for General Practice, Department of Public Health, Copenhagen University, Copenhagen, Denmark.

6The Boden Institute, Charles Perkins Centre, University of Sydney, Sydney, Australia.

7National Institute of Public Health, University of Southern Denmark, Copenhagen, Denmark.

**Corresponding author:**

Mina N. Händel

Research Unit for Dietary Studies, Parker Institute

Nordre Fasanvej 57, Hovedvejen, entrance 5, ground floor

2000 Frederiksberg, Denmark

Phone: +45 38 16 30 53

E-mail: [Mina.Nicole.Holmgaard.Handel@regionh.dk](mailto:Mina.Nicole.Holmgaard.Handel@regionh.dk)

**Running head:** Vitamin D fortification and fracture risk in youth.

**Keywords:** epidemiology,vitamin D, fracture risk, fortification, children

**Word count:** abstract: 234; manuscript: 2961.

**Number of pages:** 21

**Number of tables:** 2

**Number of figures:**5 (supplementary figures: 12)

**Abbreviations:** Avon Longitudinal Study of Parents and Children (ALSPAC); bone mineral content (BMC); bone area (BA); bone mineral density (BMD); Confidence interval (CI); Dual-energy x-ray absorptiometry (DXA); National Patient Registry (NPR); Rate ratio (RR)

**Abstract**

Prenatal low **v**itamin D may have consequences for bone health. By means of a nationwide mandatory vitamin D fortification program, we examined the risk of fractures among 10-18 year old children from proximate birth cohorts born around the date of the termination of the program. For all subjects born in Denmark during 1983-1988, civil registration numbers were linked to the Danish National Patient Registry for incident and recurrent fractures occurring at ages 10-18 years. Multiplicative Poisson models were used to examine the association between birth cohort and fracture rates. The variation in fracture rates across birth cohorts was analyzed by fitting an age-cohort model to the data. We addressed the potential modification of the effect of vitamin D availability by season of birth. The risk of fractures was increased **a**mong both girls and boys who were born before the vitamin D fortification terminated in 1985 (rate ratio (RR) exposed vs. non-exposed girls: 1.15 (95% confidence interval (CI): 1.11, 1.20; RR exposed vs. non-exposed boys: 1.11 (95% CI: 1.07, 1.14). However, these associations no longer persisted after including the period effects. There was no interaction between season of birth and vitamin D availability in relation to fracture risk.The study did not provide evidence that prenatal exposure to extra vitamin D from a mandatory fortification program of 1.25 µg vitamin D/100 g margarine was sufficient to influence the risk of fractures in late childhood, regardless of season of birth. Replication studies are needed.

## Introduction

Based on serum 25(OH)D3 measurements, studies have shown a pronounced seasonal variation in vitamin D status in Denmark and other countries with latitudes above 35 degrees North and South (1;2), most likely related to insufficient actinic vitamin D synthesis during the darker part of the year in such countries. Consequently, the vitamin D status will then depend more on diet, supplementation and/or fortification alone. In Denmark, the re-introduction of fortified foods to improve the general population’s vitamin D status is currently being considered and its justification is part of an ongoing international debate.

Until June 1st 1985, vitamin D fortification of margarine was mandatory in Denmark, but fortification was abolished, related to an unsupported assumption that the amounts added to margarine were too small to impact the dietary needs of vitamin D in the Danish population (3). We used this historical change in fortification legislation to examine the influence of extra prenatal vitamin D exposure from fortified margarine on the risk of fractures during pubertal-related growth spurts, which has the highest fracture incidence(4).

Vitamin D and its metabolites play essential roles in regulating the calcium homeostasis in the intestine, kidney and bone. The evidence for linking maternal vitamin D insufficiency to offspring fracture rates is sparse, and is based on results from animal studies(5), as well as observational studies suggesting that maternal vitamin D status influence fetal bone growth and mineralization(6), and perhaps also long-term bone health among offspring (7-12). Given that low BMD is predictive of increased fracture risk, as shown in both case-control and prospective studies though principally in adults (13-20), maternal vitamin D insufficiency may potentially also influence fracture risk.

Among children and adolescents the most common fracture site are forearm fractures (21-25), followed by the carpal bones, clavicle and foot/ankle(26). Across the European countries, the seasonality of fractures exhibit notable similarities, with peaks during summer, with a notable drop in the month of July and a nadir during winter (4;22;24;25;27;28).

We hypothesized that individuals born during the 2 last years of the mandatory vitamin D fortification had a reduced risk of sustaining fractures of the forearm, wrist or scaphoid bone, clavicle and ankle in late childhood, compared with those born 2 years after the termination of vitamin D fortification, allowing for a washout period after termination. In addition, it was hypothesized that the vitamin D fortification during sun-deprived months of gestation would be associated with the greatest risk reduction of offspring childhood fractures.

### Materials and Methods

**Study design**

The D-tect study design(29) relies on a natural experiment, defined as an exposure to an event or intervention, which has not been manipulated by the researcher(30). The D-tect study is based on the fact that until June 1st 1985 it was mandatory in Denmark to fortify all margarine with vitamin D. Margarine was fortified with 1.25 µg/100g and around 13% (3-29%) of all dietary vitamin D is estimated to have come from the fortified margarine(31).

We did not identify other abrupt societal changes during 1983-1988 that potentially could influence our results, neither in relation to fortification practices in other food products for consumption(31;32), in relation to margarine intake in the Danish population(33) or in relation to national recommendations for vitamin D supplementation to pregnant women or infants. Therefore, any confounding, would be expected to have influenced the exposed and non-exposed individuals from the two groups similarly, making them fully comparable. Hence, the prenatal exposure to extra vitamin D from fortification is assumed to be the only parameter that separates the individuals in the two exposure groups.

**Study population**

All individuals born alive in Denmark from January 1st 1983 to December 31st 1988 were included in the study. We divided individuals from the birth cohort into different exposure groups. The exposed individuals were defined with birth dates from June 1st 1983 to May 31st 1985, and the non-exposed individuals were those with birth dates from September 1st 1986 to August 31st 1988. Between the exposed and non-exposed group we included a washout period (9 months of pregnancy plus 6 months of margarine shelf-life) in the time period from June 1st 1985 to August 31st 1986. In order to use the full potential of the dataset available we included a run-in period with individuals born from January 1st 1983 to May 31st 1983, and a late period running from September 1st 1988 to December 31st 1988 (**Figure 1**). Hence, individuals born before the termination of the mandatory fortification were exposed prenatally, but not during childhood (the exposed group) and those born after the fortification termination were neither exposed prenatally nor during childhood (the non-exposed group).

Follow-up time for fractures for each participant started at age 10 (or the age at January 1st 1996 if the participant at that date was older than 10 years) and ended at death, emigration, disappearance or age 18, whichever came first. An overview of the study population is presented in **table 1**.

Using the civil registration numbers, each individual was linked to the Danish National Patient Registry (NPR) for incident and recurrent diseases. The register contains information about hospital contacts, including diagnosis codes and procedure codes for all treatment at Danish hospitals(34). The main study outcome was fracture of the forearm, wrist or scaphoid bone (ICD-10: S52, S62.0); fracture of the clavicle (ICD-10: S42.0); and fracture of the ankle (ICD-10: S82.5, S82.6, S82.8). From 1994 ICD-10 diagnoses were classified according to the WHO International Classification of Diseases, and from January 1st 1995 the outpatient and emergency room contacts were mandatorily included in the registers(35). Due to this incompleteness in NPR, the individuals are only considered at risk from their current age per January 1st 1996 and onward. Recurrent fracture events were allowed, but if an individual had more than one fracture admission per event, we counted only the first admission and embedded a washout period of 6 months after a fracture at the same anatomical location, in order to avoid inflating the fracture risk estimates by including re-admissions.

Risk time (person-years) and the number of fracture events were classified by sex, date of birth, and age and calendar time during follow-up in monthly classes in a Lexis diagram(36).

There are no ethical considerations regarding the epidemiological aspects of the study since only pre-existing databases and registries that are already approved have been used. According to Danish law, ethical approval is not required for purely register-based studies. Permission to conducting the study has been granted by the Danish Data Protection Agency (J. no.: 2012-41-1156).

**Statistical Analysis**

Multiplicative Poisson models were used to examine the association between birth cohort and fracture rates. Multiplicative Poisson models were used to examine the association between birth cohort and fracture rates. Rates were analysed by models for the number of fractures with the log-person-years as offset. With the Poisson model it is possible to explicitly model the underlying rates, and thus getting estimates describing the fracture rates by age at occurrence, time of occurrence, and time of birth (age, period, cohort). The analysis was performed for fractures overall, and for fracture subtypes, separately for each sex. Power calculations for the present study have previously been published(29). In brief, as the follow-up period varies, the final prevalence (= incidence x duration) was used and varied from 0.5-8%. The significance level was α=0.05 and power β=0.80. The least detectable relative risk of outcome after change in fortification (assuming that either one (at least 100,000 individuals) or two birth cohorts are included) varied from 1.19 for a prevalence of 0.5% and 1.05 for a prevalence of 8%.

First, we described the variation in facture rates across birth cohorts by fitting an age-cohort model to the data. In a second series of models the birth cohorts were divided into the five exposure groups mentioned above (run-in, exposed, washout, non-exposed, late period). In a third series of models we addressed the potential modification of the effect of vitamin D availability by season of birth, with winter defined as births in November, December or January. We tested for interaction between season of birth and exposure in relation to fracture risk by likelihood ratio tests. In a fourth series of models we included period effects. First we fitted an age-period model to the data (with June 2001 as reference period) and subsequently a cohort-only model to the residuals (i.e. using the fitted values from the age-period model as offset). The estimated cohort effect is then the ratio between the observed number of fractures within the cohort, and the expected number of fractures, where the expected number is based on the predicted rates from the age-period model. This sequentially approach to age-period-cohort modelling corresponds to fixing the cohort effects to have no overall trend(36).

All data management was performed in Stata version 13.1 and statistical analyses were performed in R Statistical Software (Foundation for Statistical Computing, Vienna, Austria).

**Results**

In total, 327 254 children contributed with risk time in ages 10-18 years. We identified 104 406 individuals in the exposure period, of which approximately 51 % were boys and 113 577 individuals in the non-exposed period of which approximately 52 % were boys. In the run-in, washout and late periods there were 21 432, 68 888, and 18 951 individuals, respectively. In total, 12 330 exposed and 16 058 non-exposed individuals sustained a fracture with an overall fracture rate of 19.4 (95% confidence interval (CI): 19.1, 19.7) and 18.4 (95% CI: 18.1, 18.7) per 1000 person years among the exposed and non-exposed individuals, respectively. The fracture type with the highest incidence was forearm, wrist or scaphoid bone with 9315 events in the exposed group and 12 469 events in the non-exposed group (**Table 1**).

**Fracture risk across age groups and seasonality in fracture occurrence**

As expected, the fracture rates differed according to age and there was a rate difference between boys and girls. Among the boys, the overall average fracture rate was 22.5 per 1000 person years, with a peak fracture rate of 35.1 per 1000 person years between ages 13 to14 years (**Figure 2-3**). Among the girls, the overall average fracture rate was 14.6 per 1000 person years, with a peak fracture rate of 27.9 per 1000 person years between ages 11 to 12 years (**Figure 4-5**).

The peak age of the ankle and clavicle fracture rates occurred later compared to the overall fracture, but was similar for both girls (range of 12-14 years) and boys (range of 15-16 years) (**Supplementary Figure 1-8**). The overall average fracture rate was 2.7 per 1000 person years for ankle and 1.7 per 1000 person years for clavicle, respectively. Forearm, wrist or scaphoid bone fractures showed a similar pattern as the overall analysis, resulting from the high incidence rate of forearm, wrist or scaphoid bone fractures in children, which was the main contribution to the fracture outcome (**Supplementary Figures 9-12**).

The estimates from the age-period model revealed a seasonal fracture pattern with increased risk during spring and early fall and with nadir during winter in the period from 1996-2007 (**Figure 2-5**).

A summary of the month by month periodicity is presented in **Table 2**. For both girls and boys, we observed an almost twofold increase in the fracture rate when comparing the months with the highest rates (April, May or August) and the month with the lowest rate (December).

**Fracture rates compared between individuals potentially exposed to vitamin D fortification and non-exposed individuals**

Among girls, the rate ratio for the exposed was 1.15 (95% CI: 1.11, 1.20) compared to the non-exposed. Among boys, the rate ratio for exposed compared to the non-exposed was 1.11 (95% CI: 1.07, 1.14) (**Figure 2-5**). However, these associations no longer persisted after including the period effects. The downward trend seen in the estimates from the age-cohort model has successfully been assigned as a period effect and there appears to be no systematic variation left in the cohort term based on the residuals. The relative risk for the exposed girls was 1.01 (95% CI: 0.96, 1.05) compared to the non-exposed and the relative risk for exposed boys compared to the non-exposed was 1.01 (95% CI: 0.98, 1.04) (**Figure 2-5**).

For both girls and boys there was no interaction between season of birth and the exposure to vitamin D fortification in relation to overall fracture risk (girls: *p* = 0.23; boys: *p* = 0.44).

**Discussion**

This study assessed the long term risk of childhood fractures from the natural experiment, of terminating a mandatory margarine fortification program that until June 1st 1985 fortified all margarine with vitamin D in Denmark. The study did not provide evidence that prenatal exposure to extra vitamin D from fortification was sufficient to influence the risk of fractures in late childhood, regardless of season of birth.

Interestingly, the fortification program may have taken place against a long term trend of a decreasing fracture risk, including these particular birth cohorts under study. Thus, we cannot rule out whether the birth cohort results reflect a true adverse effect of vitamin D on bone, and one previous study did indeed report borderline significant inverse associations between maternal late pregnancy 25(OH)D concentrations and offspring forearm fractures(12). Also, in the Danish National Birth Cohort, mid-pregnancy maternal supplementation use above 10 µg vitamin D/day was associated with a 30 % higher risk of offspring forearm fractures(37).

Nonetheless, the decreasing trend may have been affected by influences more powerful than the likely impact of vitamin D in the modest amounts of 1.25 µg vitamin D/100 g margarine(31). By comparing individuals from entire adjacent birth cohorts that were, or were not exposed to extra vitamin D from margarine fortification prenatally only, but all unexposed thereafter, we assume that all potential confounders are equally distributed in both groups, and hence that control for confounding is not needed. Though, we are unaware of such changes, we cannot exclude the possibility that other societal, environmental or behavioral change coinciding with the change in fortification practice took place. However, changes in potential risk factors for pediatric fractures during the study period 1996-2012 may also be considered, such as secular trends in weight status, in high impact sports, better safety on play grounds, onset of puberty etc. Nonetheless, recent studies have found tendencies for a levelling off of in the trend in overweight and obesity among Danish adolescents (age range 11-16 years) during 2002-2010(38;39). Similarly, the rate of injuries among children and the youth occurring during home and leisure activities (as a proxy of physical activity) i.e. at play grounds and in sports participation, were stable during the period of interest(40). An increasing number of Danish children are diagnosed with precocious puberty from mid 1990ties and onward(41), although very few cases in total have been registered in the period 1993 to 2001 (n=670)(42). Also other Danish studies have suggested a decline of the age of onset of puberty both among girls and boys from 1991 to 2008 (43;44). However, the decline was around 3 to 4 months over a 15 year period, and thus considered minor in regards to the adjacent birth cohorts we studied with a maximum year span of 5 years. Hence the unchanging fluctuations in the trend of these major risk factors of fractures cannot explain the decreasing drift seen in our data.

The decreasing fracture trend may potentially be explained by the marked decrease in bicycle and pedestrian accidents related to road traffic injuries during 1990-2009(40), especially since fractures of shoulders, arms, hands and wrist were those represented in our dataset, and are the body parts most likely to be injured in bicycle accidents(40). Also, the seasonal pattern in bicycle accidents with particularly high rates during spring and summer compared to winter and autumn, were similar to the fracture rate pattern in our study as well as in other studies(45-52). Climatically, Denmark has large seasonal variation in daylight length, temperature and precipitation. This seasonal variation might also contribute to periodic changes in outdoor physical activities among children during the year(53;54).

The relationship between vitamin D status during fetal life and long-term bone health has not previously been widely examined, and results from earlier observational studies showed either no or a direct association. In the Avon Longitudinal Study of Parents and Children (ALSPAC) study, UVB-exposure during the third trimester of pregnancy was used as a proxy of vitamin D status, and the exposure was directly associated with bone mineral content (BMC), bone area (BA) and bone mineral density (BMD) among 6955 children at age 9.9 years(8). However later, another report from the ALSPAC study, showed no association with maternal 25(OH)D and total body BMC or spine BMC among 3960 offspring children, after adjusting for offspring age at dual-energy x-ray absorptiometry (DXA) scan(10). Correspondingly, Danish Cohort studies, showed no association between maternal 25(OH)D concentrations during pregnancy and fractures among the offspring at ages 0-18 and 0-21 years(12;37). Findings from Southampton Women Survey suggested that both 25(OH)D and estimated UV-B radiation during late pregnancy (mean gestation week of 34) were directly associated with bone-mineral accrual among 198 children up to age 9(10). Finally, the Western Australian Pregnancy Cohort (Raine) which followed 341 offspring up to the age of 20 years, showed a direct association between maternal 25(OH)D measured between 16-20 weeks of gestation and total body BMC and BMD at age 20 years(11). The diverse results from these studies potentially relates to differences in sample sizes, or the covariates adjusted for, and the number and timing of serum 25(OH)D concentration measurement during pregnancy. No previous studies have examined the effect of prenatal food fortification. Intervention studies with supplementation of vitamin D in pregnancy are currently ongoing(9;55). One of them, the MAVIDOS study, recently reported that maternal supplementation with 1000IU/day cholecalciferol during pregnancy increased bone mass in winter born infants, only (56), however it will be more than a decade before the first bone fracture outcome data in the children will be available to properly inform health policy.

A number of methodological issues deserve attention. From systematic review and meta-analysis of randomized controlled trials, there is evidence that vitamin D fortified food generally improve vitamin D status among both children and adults in a dose-dependent manner(57-59). The Danish margarine fortification program, has been shown to account for approximately 0.36-0.57 µg vitamin D per person per day(60). These estimates are based on food disappearance data (food availability for human use), where the average purchasing of margarine was estimated to be 16,000-17,000 g margarine per person per year during the period 1983-1988(33), which is equal to 44-46 g margarine per person per day, and the dose-equivalents for vitamin D from fortified margarine is on average 0.55-0.57 µg per person per day(33). Furthermore, from the Danish dietary habit survey from 1985, the median vitamin D intake was 2.8 µg/day among women aged 23-50 year(61). Margarine consumption contributed to 13 % of this vitamin D intake, which is equivalent to 0.36 µg(61). It is possible that this amount was too small to influence fracture risk at population level, even when we confined the analysis to those pregnancies with habitually low vitamin D during late trimesters i.e. those giving birth during winter months. Unfortunately, we lack information on vitamin D status among Danish women in the reproductive age during the fortification period, but to compare, a study showed among 850 pregnant women recruited from the second-largest city in Denmark in 1988-1989 (the period without fortification) that only 6.3 of the pregnant women had a serum 25-hydroxy vitamin D concentrations ≤ 25 nmol/L(12); the median concentrations of 76.2 nmol/L (95% CI: 23.0, 152.1) were also above the “optimal level” as stated by the US Endocrine Society(62;63). For replication, the Finish vitamin D fortification program (initiated in 2003) may be an option because Finland, like Denmark, has access to nationwide individual registration of fractures(64).

There are also limitations to the statistical model. The age-period analysis assumed that the overall trend in the fractures rates could be attributed as a period effect. Bearing in mind the identifiability problem between age, period, and cohort, the data cannot tell whether the age-period approach is more appropriate than the age-cohort analysis. To help elucidate the “true” period effect, we suggest at this point, a National trend analysis of pediatric fractures for birth cohorts from several decades and not only births from 1983-1988.

The strength of the present study lies with the use of comprehensive registers covering the entire Danish population. This enables us to capture all fracture information in the study population from NPR, which is a high quality national mandatory registration system initiated in 1977. The accuracy of NPR has not been formally assessed as regards paediatric bone ICD coding. Fracture diagnosis coding has high precision in adults (65), and treatment of childhood fractures takes place at the same hospital units as treatment of fractures in adulthood, with very little extent of fracture treatment in general practice.

**Conclusion**

The study did not provide evidence that prenatal exposure to extra vitamin D from a mandatory fortification program adding1.25 µg vitamin D/100 g margarine, was sufficient to influence the risk of fractures in late childhood, regardless of season of birth. Replication studies are needed. There was a decreasing trend in fracture events occurring in the birth cohort of 1983-1988, which might be explained by secular trends of bicycle accidents in the period rather than differences in the birth cohorts.

**Acknowledgment**

**Author Contribution:** BLH conceived and designed the D-tect observational study. MNH, BA and BLH conceived this sub-study. MNH, PF and CO performed the statistical analysis. MNH, PF, BA and BLH wrote the paper with contributions from all authors. MNH has primary responsibility for the final content. All authors have read and approved the final manuscript.

**Financial support:** This work was supported by the Danish Council for Strategic Research [11-116213]; and the University of Southern Denmark. The sources of funding had no influence on the manuscript.

**Conflict of interest:** Professor Cyrus Cooper has received consultancy, lecture fees and honoraria from Alliance for Better Bone Health, Amgen, Eli Lilly, GSK, Medtronic, Merck, Novartis, Pfizer, Roche, Servier, Takeda and UCB. Professor Bo Abrahamsen conducts epidemiological studies through research contracts between his institution and Novartis and UCB Pharma. All other authors have nothing to disclose

Reference List

(1) Woolcott CG, Giguere Y, Weiler HA, Spencer A, Forest JC, Armson BA, et al. Determinants of vitamin D status in pregnant women and neonates. Can J Public Health 2016 Dec 27;107(4-5):e410-e416.

(2) Andersen R, Brot C, Jakobsen J, Mejborn H, Molgaard C, Skovgaard LT, et al. Seasonal changes in vitamin D status among Danish adolescent girls and elderly women: the influence of sun exposure and vitamin D intake. Eur J Clin Nutr 2013 Mar;67(3):270-4.

(3) Executive order on margarine no. 196 20 May 1985. Act on margarine no. 189. Comments on the bill., Ministry of Food, (1984).

(4) Randsborg PH, Gulbrandsen P, Saltyte BJ, Sivertsen EA, Hammer OL, Fuglesang HF, et al. Fractures in children: epidemiology and activity-specific fracture rates. J Bone Joint Surg Am 2013 Apr 3;95(7):e42.

(5) Anderson PH, Atkins GJ, Turner AG, Kogawa M, Findlay DM, Morris HA. Vitamin D metabolism within bone cells: effects on bone structure and strength. Mol Cell Endocrinol 2011 Dec 5;347(1-2):42-7.

(6) Galthen-Sorensen M, Andersen LB, Sperling L, Christesen HT. Maternal 25-hydroxyvitamin D level and fetal bone growth assessed by ultrasound: a systematic review. Ultrasound Obstet Gynecol 2014 Dec;44(6):633-40.

(7) Javaid MK, Crozier SR, Harvey NC, Gale CR, Dennison EM, Boucher BJ, et al. Maternal vitamin D status during pregnancy and childhood bone mass at age 9 years: a longitudinal study. Lancet 2006 Jan 7;367(9504):36-43.

(8) Sayers A, Tobias JH. Estimated maternal ultraviolet B exposure levels in pregnancy influence skeletal development of the child. J Clin Endocrinol Metab 2009 Mar;94(3):765-71.

(9) Viljakainen HT, Korhonen T, Hytinantti T, Laitinen EK, Andersson S, Makitie O, et al. Maternal vitamin D status affects bone growth in early childhood--a prospective cohort study. Osteoporos Int 2011 Mar;22(3):883-91.

(10) Lawlor DA, Wills AK, Fraser A, Sayers A, Fraser WD, Tobias JH. Association of maternal vitamin D status during pregnancy with bone-mineral content in offspring: a prospective cohort study. Lancet 2013 Jun 22;381(9884):2176-83.

(11) Zhu K, Whitehouse AJ, Hart P, Kusel M, Mountain J, Lye S, et al. Maternal Vitamin D Status During Pregnancy and Bone Mass in Offspring at 20 Years of Age: A Prospective Cohort Study. J Bone Miner Res 2014 Nov 5;29(5):1088-95.

(12) Petersen SB, Olsen SF, Molgaard C, Granstrom C, Cohen A, Vestergaard P, et al. Maternal vitamin D status and offspring bone fractures: prospective study over two decades in Aarhus City, Denmark. PLoS One 2014;9(12):e114334.

(13) Clark EM, Tobias JH, Ness AR. Association between bone density and fractures in children: a systematic review and meta-analysis. Pediatrics 2006 Feb;117(2):e291-e297.

(14) Manias K, McCabe D, Bishop N. Fractures and recurrent fractures in children; varying effects of environmental factors as well as bone size and mass. Bone 2006 Sep;39(3):652-7.

(15) Mayranpaa MK, Viljakainen HT, Toiviainen-Salo S, Kallio PE, Makitie O. Impaired bone health and asymptomatic vertebral compressions in fracture-prone children: a case-control study. J Bone Miner Res 2012 Jun;27(6):1413-24.

(16) Olney RC, Mazur JM, Pike LM, Froyen MK, Ramirez-Garnica G, Loveless EA, et al. Healthy children with frequent fractures: how much evaluation is needed? Pediatrics 2008 May;121(5):890-7.

(17) Clark EM, Ness AR, Bishop NJ, Tobias JH. Association between bone mass and fractures in children: a prospective cohort study. J Bone Miner Res 2006 Sep;21(9):1489-95.

(18) Ferrari SL, Chevalley T, Bonjour JP, Rizzoli R. Childhood fractures are associated with decreased bone mass gain during puberty: an early marker of persistent bone fragility? J Bone Miner Res 2006 Apr;21(4):501-7.

(19) Flynn J, Foley S, Jones G. Can BMD assessed by DXA at age 8 predict fracture risk in boys and girls during puberty?: an eight-year prospective study. J Bone Miner Res 2007 Sep;22(9):1463-7.

(20) Cheng S, Xu L, Nicholson PH, Tylavsky F, Lyytikainen A, Wang Q, et al. Low volumetric BMD is linked to upper-limb fracture in pubertal girls and persists into adulthood: a seven-year cohort study. Bone 2009 Sep;45(3):480-6.

(21) Maasalu K, Raukas M, Märtson A. Children´s fractures in Estonia: Population based study. Bone 45, S59-S111. 2009.

(22) Hedstrom EM, Svensson O, Bergstrom U, Michno P. Epidemiology of fractures in children and adolescents. Acta Orthop 2010 Feb;81(1):148-53.

(23) Baker R, Orton E, Tata LJ, Kendrick D. Epidemiology of poisonings, fractures and burns among 0-24 year olds in England using linked health and mortality data. Eur J Public Health 2016 May 31.

(24) Mayranpaa MK, Makitie O, Kallio PE. Decreasing incidence and changing pattern of childhood fractures: A population-based study. J Bone Miner Res 2010 Dec;25(12):2752-9.

(25) Cooper C, Dennison EM, Leufkens HG, Bishop N, van Staa TP. Epidemiology of childhood fractures in Britain: a study using the general practice research database. J Bone Miner Res 2004 Dec;19(12):1976-81.

(26) Moon RJ, Harvey NC, Curtis EM, de VF, van ST, Cooper C. Ethnic and geographic variations in the epidemiology of childhood fractures in the United Kingdom. Bone 2016 Apr;85:9-14.

(27) Lyons RA, Delahunty AM, Kraus D, Heaven M, McCabe M, Allen H, et al. Children's fractures: a population based study. Inj Prev 1999 Jun;5(2):129-32.

(28) Lyons RA, Sellstrom E, Delahunty AM, Loeb M, Varilo S. Incidence and cause of fractures in European districts. Arch Dis Child 2000 Jun;82(6):452-5.

(29) Jacobsen R, Abrahamsen B, Bauerek M, Holst C, Jensen CB, Knop J, et al. The influence of early exposure to vitamin D for development of diseases later in life. BMC Public Health 2013;13:515.

(30) Craig P, Cooper C, Gunnell D, Haw S, Lawson K, Macintyre S, et al. Using natural experiments to evaluate population health interventions: new Medical Research Council guidance. J Epidemiol Community Health 2012 Dec;66(12):1182-6.

(31) Haradsdóttir J, Thaarup S. Tilsætning af vitaminer og mineraler til levnedsmidler [The fortification of foods with vitamins and minerals]. Nordic Council of Ministers; 1989. Report No.: 45.

(32) Jensen CB, Stougard M, Sorensen TI, Heitmann BL. Does prenatal exposure to vitamin D-fortified margarine and milk alter birth weight? A societal experiment - CORRIGENDUM. Br J Nutr 2016 May 19;1-3.

(33) Fagt S, Trolle E. Forsyning af fødevarer 1955-1999. Udvikling i danskernes kost - forbrug, indkøb og vaner (The supply of food from 1955 to 1999. Overview of the developments in the Danish diet - consumption, purchasing and habits). Søborg, Denmark: Danish Veterinary and Food Administration; 2001.

(34) Nickelsen TN. [Data validity and coverage in the Danish National Health Registry. A litterature review]. Ugeskr Laeger 2001 Dec;164(1):33-7.

(35) Lynge E, Sandegaard JL, Rebolj M. The Danish National Patient Register. Scand J Public Health 2011 Jul;39(7 Suppl):30-3.

(36) Carstensen B. Age-period-cohort models for the Lexis diagram. 2007.

(37) Petersen SB, Strom M, Maslova E, Granstrom C, Vestergaard P, Molgaard C, et al. Predicted vitamin D status during pregnancy in relation to offspring forearm fractures in childhood: a study from the Danish National Birth Cohort. Br J Nutr 2015 Dec 14;114(11):1900-8.

(38) Pearson S, Hansen B, Sorensen TI, Baker JL. Overweight and obesity trends in Copenhagen schoolchildren from 2002 to 2007. Acta Paediatr 2010 Nov;99(11):1675-8.

(39) Schmidt MC, Rokholm B, Sjoberg BC, Schou AC, Geisler AL, Rasmussen M, et al. Trends in prevalence of overweight and obesity in danish infants, children and adolescents--are we still on a plateau? PLoS One 2013;8(7):e69860.

(40) Møller H, Damm M, Laursen B. Accidents in Denmark 1990-2009 [Ulykker i Danmark 1990-2009]. Copenhagen: National Institute of Public Health, University of Southern Denmark; 2012 Mar.

(41) Mogensen SS, Aksglaede L, Mouritsen A, Sorensen K, Main KM, Gideon P, et al. Diagnostic work-up of 449 consecutive girls who were referred to be evaluated for precocious puberty. J Clin Endocrinol Metab 2011 May;96(5):1393-401.

(42) Teilmann G, Pedersen CB, Jensen TK, Skakkebaek NE, Juul A. Prevalence and incidence of precocious pubertal development in Denmark: an epidemiologic study based on national registries. Pediatrics 2005 Dec;116(6):1323-8.

(43) Aksglaede L, Sorensen K, Petersen JH, Skakkebaek NE, Juul A. Recent decline in age at breast development: the Copenhagen Puberty Study. Pediatrics 2009 May;123(5):e932-e939.

(44) Sorensen K, Aksglaede L, Petersen JH, Juul A. Recent changes in pubertal timing in healthy Danish boys: associations with body mass index. J Clin Endocrinol Metab 2010 Jan;95(1):263-70.

(45) Sinikumpu JJ, Pokka T, Serlo W. The Changing Pattern of Pediatric Both-Bone Forearm Shaft Fractures among 86,000 Children from 1997 to 2009. Eur J Pediatr Surg 2013 Feb 26.

(46) Bell SW, McLaughlin D, Huntley JS. Paediatric forearm fractures in the west of Scotland. Scott Med J 2012 Aug;57(3):139-43.

(47) Heideken J, Svensson T, Blomqvist P, Haglund-Akerlind Y, Janarv PM. Incidence and trends in femur shaft fractures in Swedish children between 1987 and 2005. J Pediatr Orthop 2011 Jul;31(5):512-9.

(48) Ryan LM, Teach SJ, Searcy K, Singer SA, Wood R, Wright JL, et al. Epidemiology of pediatric forearm fractures in Washington, DC. J Trauma 2010 Oct;69(4 Suppl):S200-S205.

(49) Kalenderer O, Gurcu T, Reisoglu A, Agus H. [The frequency and distribution of fractures in children presenting to the emergency service]. Acta Orthop Traumatol Turc 2006;40(5):384-7.

(50) Lautman S, Bergerault F, Bonnard C, Laumonier F, Bronfen C, Mallet JF, et al. [Epidemiological survey of wrist fractures in children]. Rev Chir Orthop Reparatrice Appar Mot 2003 Sep;89(5):399-403.

(51) Ljungberg E, Rosberg HE, Dahlin LB. Hand injuries in young children. J Hand Surg Br 2003 Aug;28(4):376-80.

(52) Wareham K, Johansen A, Stone MD, Saunders J, Jones S, Lyons RA. Seasonal variation in the incidence of wrist and forearm fractures, and its consequences. Injury 2003 Mar;34(3):219-22.

(53) Kolle E, Steene-Johannessen J, Andersen LB, Anderssen SA. Seasonal variation in objectively assessed physical activity among children and adolescents in Norway: a cross-sectional study. Int J Behav Nutr Phys Act 2009;6:36.

(54) Kristensen PL, Korsholm L, Moller NC, Wedderkopp N, Andersen LB, Froberg K. Sources of variation in habitual physical activity of children and adolescents: the European youth heart study. Scand J Med Sci Sports 2008 Jun;18(3):298-308.

(55) Harvey NC, Javaid K, Bishop N, Kennedy S, Papageorghiou AT, Fraser R, et al. MAVIDOS Maternal Vitamin D Osteoporosis Study: study protocol for a randomized controlled trial. The MAVIDOS Study Group. Trials 2012;13:13.

(56) Cooper C, Harvey NC, Bishop NJ, Kennedy S, Papageorghiou AT, Schoenmakers I, et al. Maternal gestational vitamin D supplementation and offspring bone health (MAVIDOS): a multicentre, double-blind, randomised placebo-controlled trial. Lancet Diabetes Endocrinol 2016 Mar 1.

(57) Black LJ, Seamans KM, Cashman KD, Kiely M. An updated systematic review and meta-analysis of the efficacy of vitamin D food fortification. J Nutr 2012 Jun;142(6):1102-8.

(58) Hennessy A, Browne F, Kiely M, Walton J, Flynn A. The role of fortified foods and nutritional supplements in increasing vitamin D intake in Irish preschool children. Eur J Nutr 2016 Feb 19.

(59) Piirainen T, Laitinen K, Isolauri E. Impact of national fortification of fluid milks and margarines with vitamin D on dietary intake and serum 25-hydroxyvitamin D concentration in 4-year-old children. Eur J Clin Nutr 2007 Jan;61(1):123-8.

(60) Jacobsen R, Hypponen E, Sorensen TI, Vaag AA, Heitmann BL. Gestational and Early Infancy Exposure to Margarine Fortified with Vitamin D through a National Danish Programme and the Risk of Type 1 Diabetes: The D-Tect Study. PLoS One 2015;10(6):e0128631.

(61) Haraldsdóttir J, Holm L, Højmark Jensen J, Møller A. Dietary habits in Denmark 1985. 1. Main results. National Food Agency [Levnedsmiddelstyrelsen]; 1985.

(62) Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011 Jul;96(7):1911-30.

(63) Rosen CJ, Abrams SA, Aloia JF, Brannon PM, Clinton SK, Durazo-Arvizu RA, et al. IOM committee members respond to Endocrine Society vitamin D guideline. J Clin Endocrinol Metab 2012 Apr;97(4):1146-52.

(64) Pietinen P, Mannisto S, Valsta LM, Sarlio-Lahteenkorva S. Nutrition policy in Finland. Public Health Nutr 2010 Jun;13(6A):901-6.

(65) Vestergaard P, Mosekilde L. Fracture risk in patients with celiac Disease, Crohn's disease, and ulcerative colitis: a nationwide follow-up study of 16,416 patients in Denmark. Am J Epidemiol 2002 Jul 1;156(1):1-10.

**Figure 1**. Definition of the exposure groups. Vertical lines indicate the timing of the cohorts around the vitamin D fortification termination date (May 31st 1985). ■ Exposed; non-exposed; □ run-in, washout and late cohort.

**Figure 2.** Age and birth cohort effects for boys born in 1983-1988. Upper left: Age specific fracture rates pr. 1000 person years and 95% CI for boys born in September 1986. Upper right: Rate ratio relative to September 1986 cohort. Lower left: Rate ratios by birth cohort exposure groups (“Non-exposed” cohort is reference). Lower right: Rate ratios by birth cohort exposure group and season of birth (Birth season “August-October” and “Non-exposed” cohort is reference).

**Figure 3.** Age and period effects for boys with fractures occurring from 1996-2007. Upper left: Age specific fracture rates pr. 1000 person years and 95% CI for boys in June 2001. Upper right: Rate relative to the July 2001 rate. Lower left: Observed vs. expected number of fractures conditional on the estimated age and period rates. Lower right: Cohort effect by birth cohort exposure group relative to the cohort effect in the “Non-exposed” cohort.

**Figure 4.** Age and birth cohort effects girls born in 1983-1988. Upper left: Age specific fracture rates pr. 1000 person years and 95% CI for girls born in September 1986. Upper right: Rate ratio relative to September 1986 cohort. Lower left: Rate ratios by birth cohort exposure groups ( “Non-exposed” cohort is reference). Lower right: Rate ratios by birth cohort exposure group and season of birth (Birth season “August-October” and “Non-exposed” cohort is reference).

**Figure 5.** Age and period effects for girls with fractures occurring from 1996-2007. Upper left: Age specific fracture rates pr. 1000 person years and 95% CI for girls in June 2001. Upper right: Rate relative to the July 2001 rate. Lower left: Observed vs. expected number of fractures conditional on the estimated age and period rates. Lower right: Cohort effect by birth cohort exposure group relative to the cohort effect in the “Non-exposed” cohort.

**Supplementary Figure 1**. *Age and birth cohort effects on ankle fractures for boys born in 1983-1988*. Upper left: Age specific fracture rates pr. 1000 person years and 95% CI for boys born in September 1986. Upper right: Rate ratio relative to September 1986 cohort. Lower left: Rate ratios by birth cohort exposure groups (“Non-exposed” reference). Lower right: Rate ratios by birth cohort exposure group and season of birth (Birth season “August-October” and exposure group “Non-exposed” reference).

**Supplementary Figure 2**.*Age and period effects on ankle fractures for boys with fractures occurring from 1996-2007*. Upper left: Age specific fracture rates pr. 1000 person years and 95% CI for boys in June 2001. Upper right: Rate relative to the June 2001 rate. Lower left: Observed vs. expected number of fractures conditional on the estimated age and period rates. Lower right: Cohort effect by birth cohort exposure group relative to the cohort effect in the “Non-exposed” cohort.

**Supplementary Figure 3**.*Age and birth cohort effects on ankle fractures for girls born in 1983-1988*. Upper left: Age specific fracture rates pr. 1000 person years and 95% CI for girls born in September 1986. Upper right: Rate ratio relative to September 1986 cohort. Lower left: Rate ratios by birth cohort exposure groups (“Non-exposed” reference). Lower right: Rate ratios by birth cohort exposure group and season of birth (Birth season “August-October” and exposure group “Non-exposed” reference).

**Supplementary Figure 4**. *Age and period effects on ankle fractures for girls with fractures occurring from 1996-2007*. Upper left: Age specific fracture rates pr. 1000 person years and 95% CI for girls in June 2001. Upper right: Rate relative to the June 2001 rate. Lower left: Observed vs. expected number of fractures conditional on the estimated age and period rates. Lower right: Cohort effect by birth cohort exposure group relative to the cohort effect in the “Non-exposed” cohort.

**Supplementary Figure 5**. *Age and birth cohort effects on clavicle fractures for boys born in 1983-1988*. Upper left: Age specific fracture rates pr. 1000 person years and 95% CI for boys born in September 1986. Upper right: Rate ratio relative to September 1986 cohort. Lower left: Rate ratios by birth cohort exposure groups (“Non-exposed” reference). Lower right: Rate ratios by birth cohort exposure group and season of birth (Birth season “August-October” and exposure group “Non-exposed” reference).

**Supplementary Figure 6**. *Age and period effects on clavicle fractures for boys with fractures occurring from 1996-2007*. Upper left: Age specific fracture rates pr. 1000 person years and 95% CI for boys in June 2001. Upper right: Rate relative to the June 2001 rate. Lower left: Observed vs. expected number of fractures conditional on the estimated age and period rates. Lower right: Cohort effect by birth cohort exposure group relative to the cohort effect in the “Non-exposed” cohort.

**Supplementary Figure 7**. *Age and birth cohort effects on clavicle fractures for girls born in 1983-1988*. Upper left: Age specific fracture rates pr. 1000 person years and 95% CI for girls born in September 1986. Upper right: Rate ratio relative to September 1986 cohort. Lower left: Rate ratios by birth cohort exposure groups (“Non-exposed” reference). Lower right: Rate ratios by birth cohort exposure group and season of birth (Birth season “August-October” and exposure group “Non-exposed” reference).

**Supplementary Figure 8**. *Age and period effects on clavicle fractures for girls with fractures occurring from 1996-2007*. Upper left: Age specific fracture rates pr. 1000 person years and 95% CI for girls in June 2001. Upper right: Rate relative to the June 2001 rate. Lower left: Observed vs. expected number of fractures conditional on the estimated age and period rates. Lower right: Cohort effect by birth cohort exposure group relative to the cohort effect in the “Non-exposed” cohort.

**Supplementary Figure 9**. *Age and birth cohort effects on forearm, wrist or scaphoid bone fractures for boys born in 1983-1988*. Upper left: Age specific fracture rates pr. 1000 person years and 95% CI for boys born in September 1986. Upper right: Rate ratio relative to September 1986 cohort. Lower left: Rate ratios by birth cohort exposure groups (“Non-exposed” reference). Lower right: Rate ratios by birth cohort exposure group and season of birth (Birth season “August-October” and exposure group “Non-exposed” reference).

**Supplementary Figure 10**. *Age and period effects on forearm, wrist or scaphoid bone fractures for boys with fractures occurring from 1996-2007*. Upper left: Age specific fracture rates pr. 1000 person years and 95% CI for boys in June 2001. Upper right: Rate relative to the June 2001 rate. Lower left: Observed vs. expected number of fractures conditional on the estimated age and period rates. Lower right: Cohort effect by birth cohort exposure group relative to the cohort effect in the “Non-exposed” cohort.

**Supplementary Figure 11**. *Age and birth cohort effects on forearm, wrist or scaphoid bone fractures for girls born in 1983-1988*. Upper left: Age specific fracture rates pr. 1000 person years and 95% CI for girls born in September 1986. Upper right: Rate ratio relative to September 1986 cohort. Lower left: Rate ratios by birth cohort exposure groups (“Non-exposed” reference). Lower right: Rate ratios by birth cohort exposure group and season of birth (Birth season “August-October” and exposure group “Non-exposed” reference).

**Supplementary Figure 12**. *Age and period effects on forearm, wrist or scaphoid bone fractures for girls with fractures occurring from 1996-2007*. Upper left: Age specific fracture rates pr. 1000 person years and 95% CI for girls in June 2001. Upper right: Rate relative to the June 2001 rate. Lower left: Observed vs. expected number of fractures conditional on the estimated age and period rates. Lower right: Cohort effect by birth cohort exposure group relative to the cohort effect in the “Non-exposed” cohort.

**Table 1.** Number of individuals contributing with risk time, person-years, and number of fracture events in the study population by birth cohort exposure groups and sex.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Run-in** | **Exposed** | **Washout** | **Non-exposed** | **Late** | **Total** |
| **BOYS**, total | 10 974 | 53 555 | 35 308 | 58 542 | 9 665 | 168 044 |
| Person-years | 54 035 | 321 870 | 263 149 | 446 284 | 74 230 | 1 159 568 |
| Fractures, total | 1 298 | 7 636 | 5 984 | 9 623 | 1 566 | 26 107 |
| Forearm, wrist or scaphoid bone | 950 | 5 706 | 4 521 | 7 361 | 1 221 | 19 759 |
| Ankle | 207 | 1 101 | 830 | 1 241 | 187 | 3 566 |
| Clavicle | 141 | 829 | 633 | 1 021 | 158 | 2 782 |
| **GIRLS**, total | 10 458 | 50 851 | 33 580 | 55 035 | 9 286 | 159 210 |
| Person-years | 52 875 | 313 792 | 255 056 | 426 974 | 72 442 | 1 121 138 |
| Fractures, total | 673 | 4 694 | 4 063 | 6 435 | 1 051 | 16 916 |
| Forearm, wrist or scaphoid bone | 490 | 3 609 | 3 258 | 5 108 | 878 | 13 343 |
| Ankle | 136 | 805 | 597 | 918 | 123 | 2 579 |
| Clavicle | 47 | 280 | 208 | 409 | 50 | 994 |

**Table 2.** Age adjusted facture rate ratio (RR) and 95 % CI by month of fracture (July is reference).

|  |  |  |
| --- | --- | --- |
|  | **Boys** | **Girls** |
| **Month of fracture** | RR (95% CI) | RR (95% CI) |
| January | 0.92 (0.86, 0.98) | 1.04 (0.96, 1.13) |
| February | 1.04 (0.97, 1.11) | 1.14 (1.05, 1.24) |
| March | 1.21 (1.13, 1.28) | 1.37 (1.27, 1.48) |
| April | 1.48 (1.40, 1.57) | 1.55 (1.43, 1.67) |
| May | 1.67 (1.58, 1.77) | 1.71 (1.58, 1.84) |
| June | 1.44 (1.36, 1.53) | 1.40 (1.29, 1.51) |
| July | 1 | 1 |
| August | 1.73 (1.63, 1.83) | 1.57 (1.45, 1.70) |
| September | 1.48 (1.40, 1.57) | 1.65 (1.53, 1.78) |
| October | 1.02 (0.96, 1.09) | 1.20 (1.11, 1.31) |
| November | 0.93 (0.87, 1.00) | 1.17 (1.08, 1.27) |
| December | 0.70 (0.65, 0.76) | 0.90 (0.82, 0.98) |