Supplementary Online Content

Brustad N, Garland J, Thorsen J, et al. Effect of high-dose vs standard-dose vitamin D supplementation in pregnancy and bone mineralization in offspring until age 6 years. *JAMA Pediatr.* Published online February 24, 2020. doi:10.1001/jamapediatrics.2019.6083

eMethods.

Anthropometric measurements

Weight was measured without clothes and diaper using calibrated digital weight scales. Length was measured until 2 years using an infantometer (Kiddimeter; Raven Equipment Ltd, Dunmow, Essex, England); thereafter, height was measured with a stadiometer calibrated yearly (Harpenden; Holtain Ltd, Crymych, Dyfed, Wales). Head circumference was assessed using a tape measuring the largest diameter of the head, waist was measured at the umbilicus level, and thorax circumference was measured at the processus xiphoideus level after an exhalation.

Maternal 25(OH)D Levels

Maternal serum 25-hydroxyvitamin D (25(OH)D) level was measured at trial entry in pregnancy week 24 and at completion of the RCT 1 week postpartum[1] allowing for analyses of effect modification by maternal vitamin D status at entry defined as insufficient (<75 nmol/l) or sufficient (≥75 nmol/l)[2]. The laboratory used for analyzing the blood samples participated in the proficiency testing program Vitamin D External Quality Assessment Scheme (DEQAS).

eReferences.

1. Højskov, C. S., Heickendorff, L. & Møller, H. J. High-throughput liquid-liquid extraction and LCMSMS assay for determination of circulating 25(OH) vitamin D3 and D2 in the routine clinical laboratory. *Clin. Chim. Acta Int. J. Clin. Chem.* **411**, 114–116 (2010).

2. DeLuca, H. F. Overview of general physiologic features and functions of vitamin D. *Am. J. Clin. Nutr.* **80**, 1689S–96S (2004).

eTable 1. Baseline characteristics of the mother-child pairs in the vitamin D trial.

Baseline characteristic	High-dose Vitamin D (n=297)	Placebo (n=287)
Mothers		
Maternal age at birth (years), mean (SD)	32.5 (4.3)	32.0 (4.3)
Serum 25(OH)D level (nmol/L) at gestation week 24, mean (SD)	76.6 (25)	76.4 (25)
Serum 25(OH)D level (nmol/L) at week 1 postpartum, mean (SD)	106.3 (36)	73.1 (32)
Mothers with serum 25(OH)D levels ≥ 75 nmol/L at gestation week 24, n(%)	155 (53)	148 (52)
Mothers with serum 25(OH)D levels ≥50 and <75 nmol/L at gestation week 24, n(%)	91 (31)	104 (36)
Mothers with serum 25(OH)D levels ≥30 and <50 nmol/L at gestation week 24, n(%)	41 (14)	27 (9)
Mothers with serum 25(OH)D levels <30 nmol/L at gestation week 24, n(%)	7 (2)	7 (2)
Smoking during pregnancy, n (%)	8 (3)	15 (5)
Social circumstances*, mean (SD)	0.1 (1.02)	-0.07 (0.95)
Antibiotic use during pregnancy, n (%)	103 (35)	103 (36)
Children		
Sex (male), n (%)	157 (53)	144 (50)
Age at 3 years DXA-scan (years), mean (SD)	3.3 (0.2)	3.3 (0.1)
Age at 6 years DXA-scan (years), mean (SD)	6.2 (0.2)	6.2 (0.2)
No. of anthropometric assessments through age 6 years, mean (SD)	11.5 (1.2)	11.5 (1.2)
Births		
Gestational age (days), mean (SD)	279 (11)	279 (10.5)
Marsál percentile§, mean (SD)	48.6 (28.7)	48.7 (28.5)
SGA, n (%)	6 (2)	5 (2)
Caesarean section, n (%)	70 (24)	60 (22)
Emergency	40 (14)	33 (12)
Elective	30 (10)	27 (10)
Season of birth, n (%)		
Winter	108 (36)	103 (36)
Spring	53 (18)	53 (18)
Summer	63 (21)	57 (20)
Fall	73 (25)	74 (26)
Apgar score at 5 min <10, n (%)	14 (5)	12 (4)
Birth weight, kg, mean (SD)	3.55 (0.6)	3.53 (0.5)
Birth length, cm, mean (SD)	51.9 (2.5)	51.9 (2.3)
Birth head circumference, cm, mean (SD)	35 (1.7)	35 (1.6)

DXA = dual-energy X-ray absorptiometry; SD = standard deviation.

*Social circumstances in household defined as first component of principal component analysis on household income, maternal age and maternal level of education.

§Calculation was based on Marsál's intrauterine growth curves

eTable 2. Repeated measurements mixed effect model analyses of anthropometrics from age 1 week to 6 years and analyses adjusted for birth season, LCPUFA and mothers baseline 25(OH)D levels at age 6 years in the high-dose vitamin D vs. placebo group. WHO age and sex specific z-scores for BMI, height and weight.

Measurement	Estimate, mean difference (95% CI) age 0-6 years	P value	Adjusted estimate**, mean difference (95% CI) age 6 years	P value
BMI, z score	-0.08 (-0.18;0.03)	0.16	-0.09 (-0.23;0.05)	0.21
Length/height, z score	-0.01 (-0.15;0.14)	0.9	-0.03 (-0.20;0.14)	0.71
Weight, z score	-0.06 (-0.18;0.07)	0.36	-0.07 (-0.23;0.08)	0.35
Waist*, cm	-0.17 (-0.51;0.18)	0.34	-0.30 (-0.94;0.35)	0.37
Head*, cm	0.07 (-0.12;0.26)	0.46	0.08 (-0.17;0.32)	0.54
Thorax circumference*, cm	-0.36 (-0.86;0.14)	0.16	-0.35 (-0.86;0.17)	0.19

*Adjusted for age and sex

**Adjusted for birth season, LCPUFA and mothers baseline 25(OH)D levels

eTable 3. Characteristics of children with available DXA scan compared with children with no available DXA scan at age 3 and 6 years.

	3 years				6 years			
	Available DXA scan (n=244)	**Clinical follow-up with no available DXA scan**	**n**	**p value**	**Available DXA scan (n=383)**	**Clinical follow-up with no available DXA scan**	**n**	**p value**
Gestational age; days, mean (SD)	279 (11)	279 (11)	340	0.82	280 (11)	279 (11)	201	0.30
Birth weight, kg	3.54 (0.54)	3.54 (0.54)	340	0.93	3.55 (0.54)	3.53 (0.52)	201	0.63
Sex (male), n (%)	120 (49)	181 (53)	340	0.33	190 (50)	111 (55)	201	0.20
Randomized to vitamin D intervention, n (%)	119 (49)	178 (52)	340	0.4	187 (49)	110 (55)	201	0.18
Randomized to LCPUFA intervention, n (%)	115 (47)	167 (49)	340	0.64	186 (49)	96 (48)	201	0.85
Mothers smoking during pregnancy, n (%)	5 (2)	18 (5)	340	**0.05**	14 (4)	9 (4)	201	0.63
Weight at DXA scan, kg	14.7 (1.6)	14.7 (1.7)	289	0.97	21.4 (2.9)	21.7 (3)	134	0.30
Height at DXA scan, cm	96 (3.7)	96.3 (3.8)	285	0.25	117.7 (5)	118.7 (4.4)	134	**0.03**
Diagnosed with persistent wheeze/asthma at time of DXA scan, n (%)	49 (20)	47 (15)	323	0.08	35 (9)	7 (4)	162	0.05

eTable 4: Analyses stratified by maternal vitamin D pre-intervention levels (nmol/L: <75 insufficient, ≥75 sufficient)

	3 years of age						6 years of age					
	Vitamin D		Placebo				Vitamin D		Placebo			
Measurement	**n**	**Mean (SD)**	**n**	**Mean (SD)**	**Adjusted* estimate (95% CI)**	**P**	**n**	**Mean (SD)**	**n**	**Mean (SD)**	**Adjusted* estimate (95% CI)**	**P**
Insufficient maternal pre-intervention vitamin D												
Total BMD, g/cm^2	29	0.62 (0.03)	49	0.6 (0.04)	0.010 (-0.004;0.025)	0.16	90	0.72 (0.04)	94	0.71 (0.05)	0.0125 (0.001;0.024)	**0.04**
Total BMC, g,	29	537.5 (38.5)	49	513.6 (66.2)	14.0 (1.3;26.8)	**0.03**	90	834.0 (98)	94	817.3 (101.3)	17.9 (2.6;33.1)	**0.02**
Head BMD, g/cm^2	38	1.18 (0.08)	57	1.14 (0.1)	0.038 (0.003;0.072)	**0.03**	90	1.43 (0.12)	94	1.40 (0.11)	0.045 (0.012;0.077)	**0.01**
Head BMC, g,	38	232 (17.9)	57	227.3 (26.5)	4.2 (-3.7;12.0)	0.29	90	300.7 (32.8)	94	294.9 (30.1)	7.5 (-0.7;15.7)	0.07
Sufficient maternal pre-intervention vitamin D												
Total BMD, g/cm^2	51	0.61 (0.04)	45	0.6 (0.03)	0.002 (-0.012;0.016)	0.80	95	0.72 (0.05)	101	0.71 (0.04)	0.006 (-0.006;0.017)	0.35
Total BMC, g,	51	521.8 (69)	45	513.3 (51.2)	5.6 (-9.7;20.9)	0.47	95	834.5 (120)	101	818.3 (101.8)	10.5 (-4.9;25.0)	0.18
Head BMD, g/cm^2	65	1.15 (0.11)	55	1.14 (0.09)	-0.009 (-0.043;0.024)	0.57	95	1.43 (0.13)	101	1.40 (0.12)	0.025 (-0.008;0.059)	0.14
Head BMC, g,	65	225.6 (28.3)	55	223.2 (22.4)	-3.7 (-11.4;3.6)	0.33	95	301.4 (35.9)	101	292.5 (30.3)	5.7 (-2.1;13.6)	0.15

*Adjusted for age, sex, height and weight

eTable 5: Combined mixed effects model analyses additionally adjusted for LCPUFA intervention and p-values of interaction between vitamin D and LCPUFA.

	0-6 years of age		
Measurement	**Adjusted estimate** (95% CI)	**P value**	**P value of interaction** (vitamin D*LCPUFA)
TBLH BMD*, g/cm^2	0.005 (-0.001;0.01)	0.11	0.28
TBLH BMC*, g	7.5 (1.5;13.5)	0.01	0.56
Head BMD*, g/cm^2	0.023 (0.003;0.042)	0.03	0.02
Head BMC*, g	3.0 (-1.8;7.9)	0.22	0.16
Total BMD*, g/cm^2	0.007 (-0.001;0.014)	0.08	0.07
Total BMC*, g	11.4 (2.3;20.7)	0.02	0.36
Total lean mass**, g	-5.7 (-196.8;184.7)	0.95	0.98
Total fat mass**, g	-38.9 (-243.7;-165.9)	0.71	0.40

*Adjusted for age, sex, height, weight and LCPUFA

**Adjusted for age, sex, height, height^2 and LCPUFA

eTable 6: Head BMD analyses of the high-dose vitamin D and LCPUFA treatment groups combined in a mixed effects model and differences between groups at age 3 and 6 years.

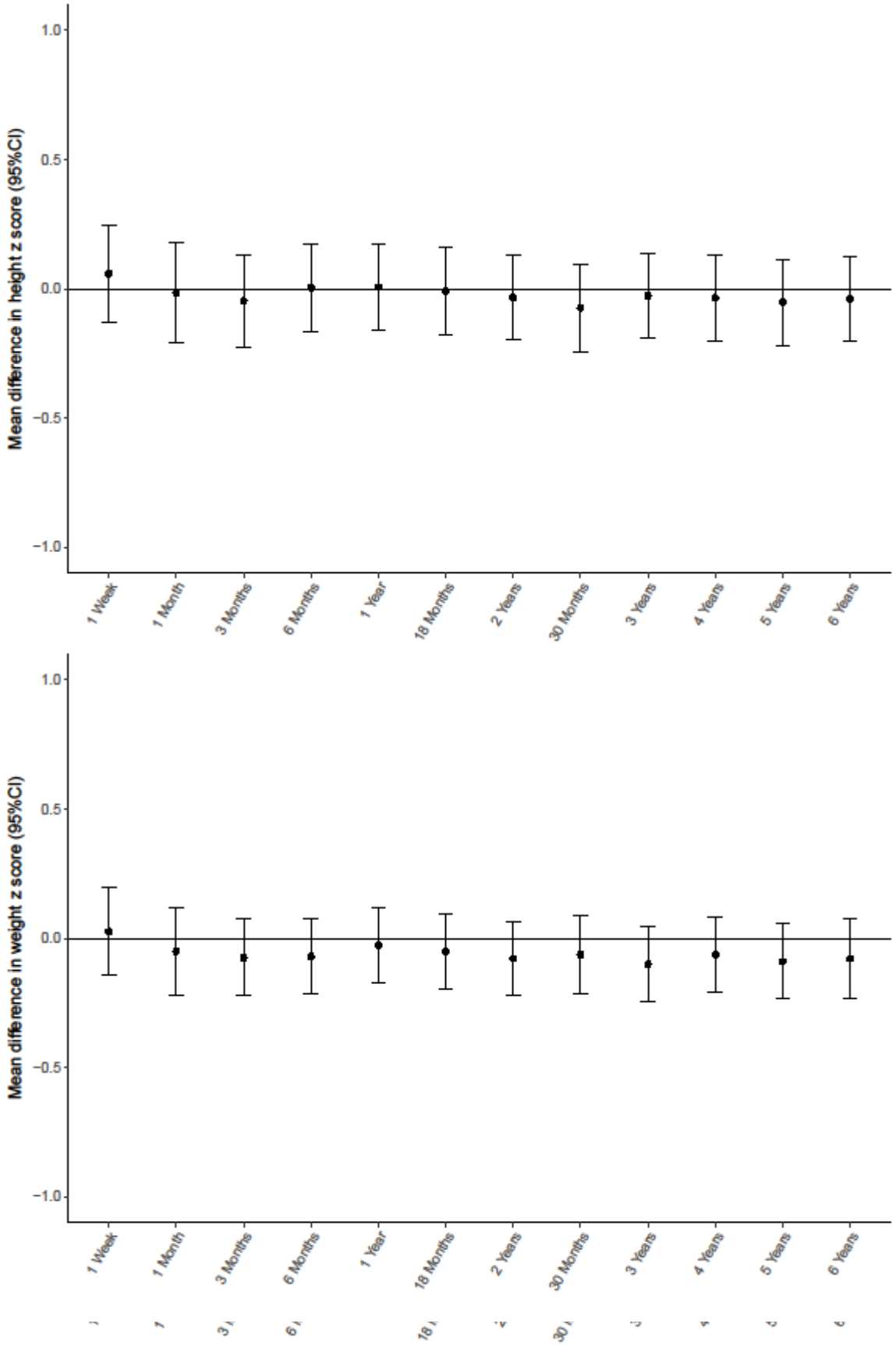
	Head BMD age 3 years Mean (SD) g/cm^2	**P value**	**Head BMD age 6 years Mean (SD) g/cm^2**	**P value**	**Estimate, mean difference (95% CI) age 3 + 6 years**
Standard-dose vitamin D and olive oil.	1.13 (0.09)	REF	1.39 (0.11)	REF	REF
Standard-dose vitamin D and LCPUFA	1.16 (0.10)	0.08	1.41 (0.12)	0.19	0.02 (-0.01;0.05)
High-dose vitamin D and olive oil	1.17 (0.11)	0.03	1.45 (0.13)	<0.001	0.05 (0.02;0.08)
High-Dose vitamin D and LCPUFA	1.15 (0.09)	0.18	1.41 (0.12)	0.14	0.02 (-0.01;0.05)

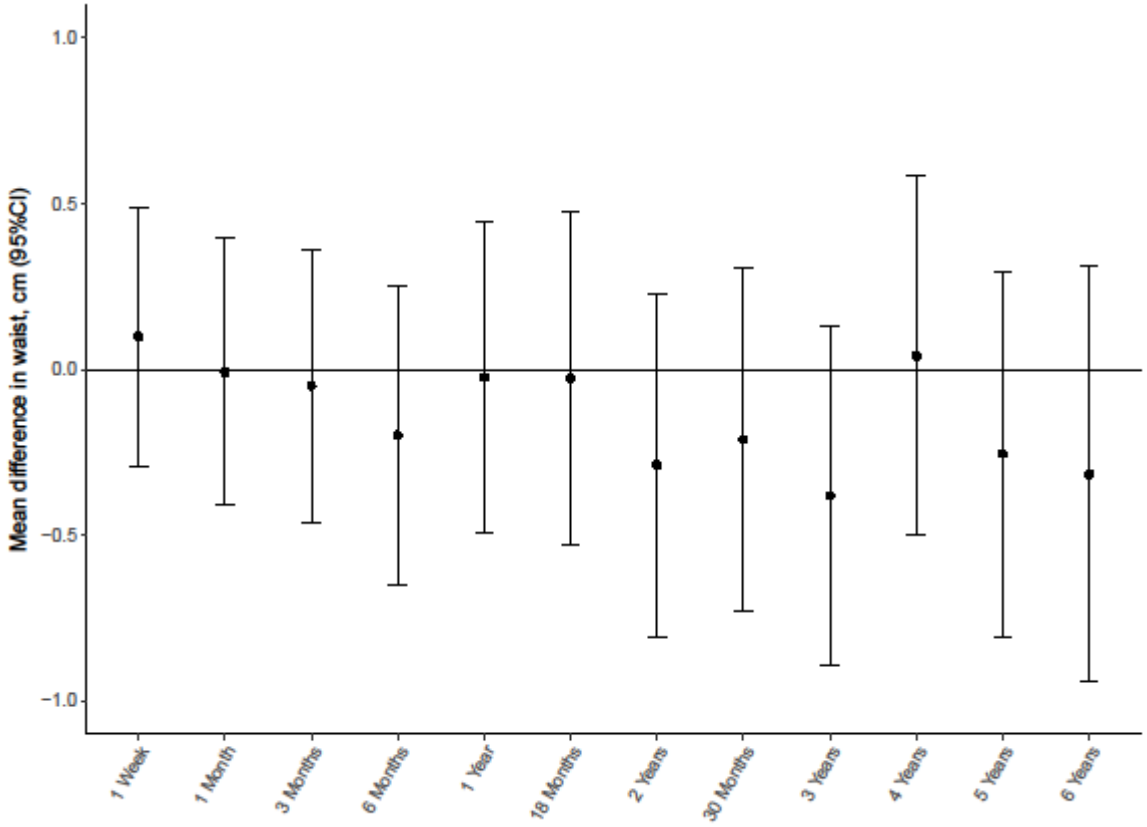
eTable 7: Age 6 years BMC values (g) stratified by birth seasons.

	Vitamin D mean (SD)	**n**	**Placebo mean (SD)**	**n**	**P value***	**Interaction P value (vitamin D*season)**
Season (low/high)						
Head BMC; low	300.6 (34)	105	290.3 (29)	122	**0.02**	Head $BMC_{low/high}$ **p=0.04**
Head BMC; high	301.4 (35)	82	299.7 (31)	74	0.45	
Total BMC; low	833.4 (121)	105	806.3 (102)	122	**0.03**	Total $BMC_{low/high}$ p=0.12
Total BMC; high	833 (94)	82	836.7 (97)	74	0.14	
Season (regular)						
Head BMC, winter	302.7 (29)	70	289.4 (36)	75	**0.03**	Head $BMC_{regular}$ **p=0.01**
Head BMC, spring	297.9 (26)	40	286 (31)	39	0.12	
Head BMC, summer	307.1 (36)	40	306.8 (31)	30	0.53	
Head BMC, fall	294.4 (36)	37	298.6 (28)	52	0.84	
Total BMC, winter	832.9 (131)	70	796.4 (99)	75	0.07	Total $BMC_{regular}$ p=0.09
Total BMC, spring	830.3 (93)	40	805.3 (96)	39	0.08	
Total BMC, summer	850.1 (99)	40	837.5 (108)	30	0.21	
Total BMC, fall	818.8 (93)	37	846.6 (97)	52	0.87	

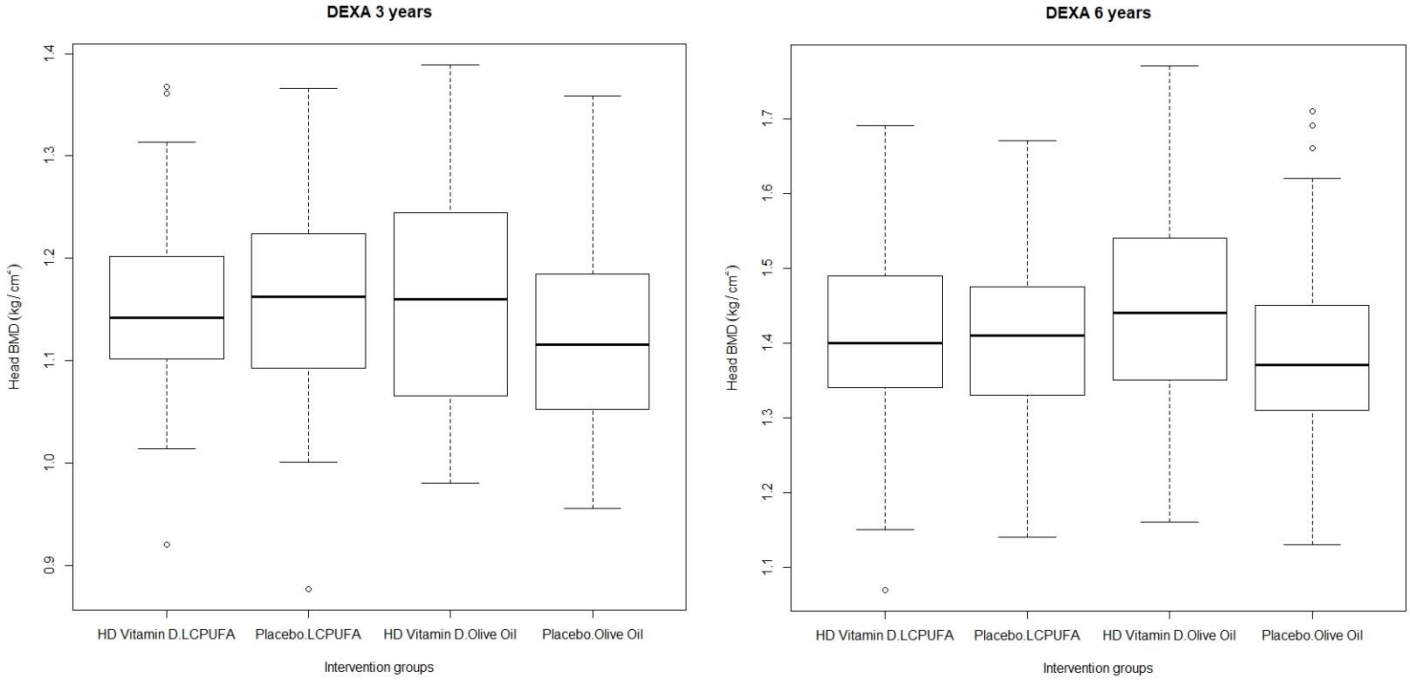
*Adjusted for age, sex, height and weight

eFigure 1: Effect of high-dose vitamin D supplementation on anthropometric measurements from age 1 week to 6 years.





eFigure 2: Boxplots showing head BMD outcomes at age 3 and 6 years stratified by the high-dose vs. LCPUFA intervention groups.



HD Vitamin D.LCPUFA *= High-Dose vitamin D and LCPUFA,* ***Placebo.LCPUFA*** *= Standard-dose vitamin D and LCPUFA,* ***HD Vitamin D.Olive Oil*** *= High-dose vitamin D and olive oil,* ***Placebo.Olive Oil*** *= Standard-dose vitamin D and olive oil.*

eFigure 3: Illustration of vitamin D levels measured at pregnancy week 24. Highest level measured at 8th of August, lowest level at 7th of February, p<0.001.

The baseline (week 24 of gestation) levels of 25(OH)D were investigated for seasonality with the season package in R for sinusoidal (parametric) seasonal pattern with one season cycle per year based on the day in year of sampling. From these high vs low level was derived. The analysis was based on Peter Baker, A. B. Seasonal Analysis of Health Data Package 'season' v. 0.3.8. (2018).