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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Cooper C, Harvey NC, Bishop NJ, et al, and the MAVIDOS Study Group. Maternal gestational vitamin D supplementation and offspring bone health (MAVIDOS): a multicentre, double-blind, randomised placebo-controlled trial. *Lancet Diabetes Endocrinol* 2016; published online M+arch 1. http://dx.doi.org/10.1016/S2213-8587(16)00044-9.

Maternal gestational vitamin D supplementation and offspring bone health: a multicentre randomised, double-blind, placebo controlled trial (MAVIDOS)

Supplementary Information

Supplementary Table 1: Comparison of baseline characteristics between mothers whose offspring underwent DXA assessment and mothers whose offspring did not.

	Without DXA	With DXA	p difference
N	469	665	
Age (years), mean±SD	30.0 ± 5.4	30.8 ± 5.0	0.01
Ethnicity, % Caucasian	92.9	95.0	0.15
Parity, % nulliparous	44.5	43.2	0.68
Current smoker, %	10.2	6.9	0.05
Educational attainment ≥ A level, %	73.7	78.6	0.07
Walking speed at least fairly brisk, %	38.7	40.2	0.63
Strenuous exercise ≥ once week, %	14.5	15.1	0.81
Height (cm), mean±SD	165.7 ± 6.6	165.6 ± 6.4	0.77
Weight (kg), median (IQR)	70.4 (63-82.3)	69.3 (61.8-79.6)	0.1
BMI (kg/m^2), median (IQR)	25.6 (22.8-30.2)	24.9 (22.5-29.0)	0.1
Sum of all skinfold (mm), mean±SD	84.7 ± 27.4	80.0 ± 28.1	0.01
25(OH)D (nmol/l), mean±SD	47.1 ± 18.6	45.8 ± 16.5	0.22
25(OH)D>50nmol/l, %	41.1	37.8	0.28

Supplementary Table 2: Baseline characteristics by randomisation group and season.

	Placebo	Cholecalciferol (1000 IU/day)	Placebo	Cholecalciferol (1000 IU/day)	Placebo	Cholecalciferol (1000 IU/day)	Placebo	Cholecalciferol (1000 IU/day)
	WINTER		SPRING		SUMMER		AUTUMN	
N	102	104	126	120	130	122	128	133
Age (years), mean±SD	30.5 ± 5.4	30.5 ± 5.4	30.7 ± 5.0	30.4 ± 5.5	30.4 ± 5.1	31.2 ± 4.6	31.1 ± 5.3	30.9 ± 5.0
Ethnicity, % Caucasian	93.1	96.2	94.4	95.0	92.1	96.7	97.3	94.1
Parity, % nulliparous	43.6	46.2	46.0	47.1	41.3	37.2	45.5	41.2
Current smoker, %	10.0	6.8	7.1	11.8	6.3	5.0	7.1	8.3
Educational attainment ≥ A level, %	71.3	75.7	80.0	79.8	77.6	76	77.5	80.8
Walking speed at least fairly brisk, %	40.0	37.4	46.4	44.9	42.5	29.8	39.8	38.4
Strenuous exercise ≥ once week, %	20.0	20.9	14.4	12.8	13.4	15.7	10.7	16.2
Height (cm), mean±SD	165.3 ± 6.9	164.7 ± 6.1	166.5 ± 7.2	166.1 ± 6.8	165.7 ± 6.3	165.3 ± 5.7	165.3 ± 6.1	165.9 ± 6.3
Weight (kg), median (IQR)	68.4 (62.7-80.7)	68.7 (61.2-77.7)	70.5 (63.8-80.5)	69.5 (61.0-81.6)	70.7 (63.3-80.2)	67.1 (59.7-78.3)	72.7 (63.1-83.0)	69.0 (61.3-77.0)
BMI (kg/m^2), median (IQR)	25.0 (22.7-26.7)	24.6 (22.4-28.8)	25.0 (22.6-28.5)	24.9 (22.1-28.9)	25.6 (23.0-29.3)	24.9 (22.5-28.3)	26.5 (23.1-30.3)	24.6 (22.1-28.7)
Sum of all skinfold (mm), mean±SD	78.8 ± 27.6	80.6 ± 25.6	83.7 ± 27.4	81.0 ± 30.4	85.1 ± 27.6	78.1 ± 28.8	85.8 ± 27.9	79.8 ± 27.7
25(OH)D (nmol/l), mean±SD	53.7 ± 16.1	54.7 ± 18.9	43.6 ± 15.7	46.2 ± 16.7	40.7 ± 16.8	42.8 ± 17.1	46.4 ± 16.7	44.7 ± 14.9
25(OH)D>50nmol/l, %	61.0	56.4	33.6	38.5	22.8	32.5	35.0	38.6

Supplementary Table 3: Neonatal anthropometry, bone mineralisation and body composition by season of birth in infants born to mothers randomised to placebo or 1000IU/day cholecalciferol in pregnancy. Shown as mean (SD), unless otherwise stated.

	Placebo					Cholecalciferol (1000IU/day)				
Season of birth	**Winter**	**Spring**	**Summer**	**Autumn**	**p**	**Winter**	**Spring**	**Summer**	**Autumn**	**p**
Obstetric data										
Males, N(%)	53 (52.0)	68 (54.0)	65 (50.0)	65 (50.8)	0.93	59 (56.7)	55 (45.8)	66 (54.1)	78 (58.7)	0.20
Birth weight (g)	3407 ± 430	3560 ± 560	3575 ± 493	3508 ± 551	0.07	3507 ± 545	3462 ± 622	3486 ± 510	3473 ± 496	0.94
Crown-heel length (cm)	50.6 ± 1.9	50.5 ± 2.5	51.0 ± 2.2	51.1 ± 2.3	0.11	50.6 ± 2.2	50.3 ± 3.1	50.7 ± 2.8	50.9 ± 1.9	0.51
Head circumference (cm)	35.0 ± 1.3	35.4 ± 1.4	35.6 ± 1.6	35.6 ± 1.4	**0.02**	35.4 ± 1.2	35.4 ± 1.5	35.4 ± 1.4	35.4 ± 1.4	0.99
Abdominal circumference (cm)	32.0 ± 2.0	32.7 ± 2.5	33.0 ± 2.3	32.8 ± 2.3	0.06	32.6 ± 2.2	32.9 ± 2.3	33.2 ± 2.3	32.9 ± 2.2	0.39
DXA										
Bone Area (cm^2)	289.4 ± 35.3	299.0 ± 39.9	301.2 ± 37.6	299.6 ± 35.2	0.25	300.9 ± 31.3	305.2 ± 40.6	303.9 ± 32.6	296.5 ± 33.4	0.36
BMC (g)	57.5 ± 10.9	60.8 ± 12.1	60.7 ± 10.6	62.3 ± 10.4	0.07	63.0 ± 10.8	62.3 ± 14.1	61.2 ± 10.4	60.2 ± 11.0	0.45
BMD (g/cm^2)	0.200 ± 0.019	0.202 ± 0.019	0.201 ± 0.020	0.207 ± 0.019	0.11	0.208 ± 0.024	0.202 ± 0.024	0.200 ± 0.019	0.202 ± 0.019	0.17
Lean (g)	2955 ± 415	2968 ± 472	3073 ± 423	3049 ± 411	0.26	3074 ± 428	3063 ± 478	3063 ± 396	3023 ± 393	0.87
Fat (g), median (IQR)	324 (210-428)	440 (267-617)	378 (243-497)	379 (268-501)	**0.02**	378 (271-516)	373 (264-645)	360 (211-553)	302 (218-470)	0.27

Amongst infants born to mothers who received placebo, statistically significant differences in head circumference and total fat mass across the birth seasons were identified such that head circumference and fat mass were lower in winter. In contrast, these differences were not observed in infants of mothers who received vitamin D supplementation

Supplementary Table 4: Mean (95% CI) neonatal whole body bone mineral content by treatment group and month of birth [winter= December, January, February]

	Placebo			1000 IU/d		
Month of Birth	**n**	**Mean**	**95%CI**	**n**	**Mean**	**95%CI**
December	16	56.7	45.1-65.4	17	62.3	55.1-67.3
January	27	56.2	50.4-61.9	29	62.6	58.8-66.6
February	21	59.7	52.6-67.6	28	63.7	57.2-69.1
March	26	59.8	50.3-67.4	24	59	52.8-67.5
April	27	60.8	53.4-67.4	29	58.2	47.3-69.2
May	45	61.3	52.5-71.8	31	68.6	59.6-79.2
June	33	61.1	56.1-68.1	40	62.3	54.7-67.1
July	18	62	57.0-66.5	25	60.1	55.0-65.9
Aug	28	59.4	51.6-65.9	26	60.7	53.5-68.1
Sep	31	60	54.0-66.2	32	55.7	48.2-60.9
Oct	25	63	57.8-68.3	23	65.8	58.3-74.0
Nov	30	64.1	58.1-71.9	34	60.6	52.4-69.3

Supplementary Table 5: Mean (95%CI) bone outcomes in cholecalciferol and placebo groups stratified by 14 week 25(OH)D above versus below the median (45.1 nmol/l).

14 week 25(OH)D nmol/l	Placebo Mean (95%CI)	Cholecalciferol (1000IU/day) Mean (95%CI	p- difference[a]	p-interaction[b]
	BA (cm^2)			
< 45.1	297.0 (290.9-303.1)	302.5 (297.2-307.7)	0.18	0.20
≥ 45.1	298.6 (293.0-304.2)	300.8 (295.3-306.2)	0.58	
	BMC (g)			
< 45.1	60.9 (59.2-62.7)	61.9 (60.2-63.7)	0.42	0.67
≥ 45.1	60.1 (58.4-61.8)	61.3 (59.5-63.1)	0.36	
	BMD (g/cm^2)			
< 45.1	0.204 (0.201-0.207)	0.203 (0.200-0.206)	0.60	0.53
≥ 45.1	0.201 (0.198-0.204)	0.202 (0.199-0.206)	0.59	
	BMC for height (g)			
< 45.1	61.2 (59.4-63.0)	62.2 (60.4-63.9)	0.45	0.57
≥ 45.1	59.9 (58.2-61.6)	61.1 (59.3-62.9)	0.33	

[a] p-value for difference between cholecalciferol and placebo groups; [b] p-value for interaction between treatment and baseline 25(OH)D.

When stratified by median 14 week 25(OH)D in the whole cohort (45.1nmol/l), differences in BA, BMC and BMC for length did not achieve statistical significance either above or below the median. Tests for interaction between baseline 25(OH)D (above vs below median) and group (cholecalciferol 1000IU/day vs placebo) were also non-significant.

Supplementary Table 6: Mean (SD) 25(OH)-vitamin D concentration at 14 and 34 weeks in treatment and placebo groups by a) season of birth and b) by individual month of birth.

a	Placebo				1000 IU/d			
	14 week 25(OH)D		34 week 25(OH)D		14 week 25(OH)D		34 week 25(OH)D	
Season of birth	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Winter	53.7	16.1	30.3	15.6	54.7	18.9	62.4	22.6
Spring	43.6	15.7	32.9	19.7	46.2	16.7	64.5	24.6
Summer	40.7	16.8	48.9	18.3	42.8	17.1	72.1	19
Autumn	46.4	16.7	58.2	22.3	44.7	14.9	72	20.3

b	Placebo				1000 IU/d			
	14 week 25(OH)D		34 week 25(OH)D		14 week 25(OH)D		34 week 25(OH)D	
Month of Birth	Mean	SD	Mean	SD	Mean	SD	Mean	SD
January	57.3	14.2	33.0	16.1	54.3	18.0	65.2	16.6
February	50.4	18.0	24.5	14.5	52.9	19.0	56.2	25.7
March	45.4	16.5	31.7	19.6	49.2	17.2	57.4	25.0
April	46.5	15.1	30.3	21.7	49.2	17.5	67.8	23.3
May	40.6	15.3	34.7	18.8	40.8	14.3	66.8	25.1
June	39.4	16.9	39.3	17.0	41.3	16.9	68.4	17.9
July	44.1	18.3	51.6	15.4	40.8	16.7	69.2	16.3
August	39.2	15.3	56.6	17.8	46.7	17.7	79.0	20.7
September	42.1	16.4	62.8	25.6	44.7	15.1	73.0	18.9
October	45.7	15.7	59.8	19.3	43.9	14.9	75.9	21.7
November	52.2	16.7	52.2	20.8	45.3	14.8	68.0	20.5
December	52.2	15.9	32.9	14.8	57.4	20.2	66.6	23.5

Supplementary Table 7: Frequency of non-protocol vitamin D supplement use (up to 400 IU per day) by treatment group and season of birth.

	% who used additional vitamin D supplements		
	Placebo	**Cholecalciferol (1000 IU/day)**	
Season of birth			**p difference**
Winter	22.6	25.0	0.68
Spring	23.8	23.3	0.93
Summer	24.6	25.4	0.88
Autumn	26.6	27.1	0.93

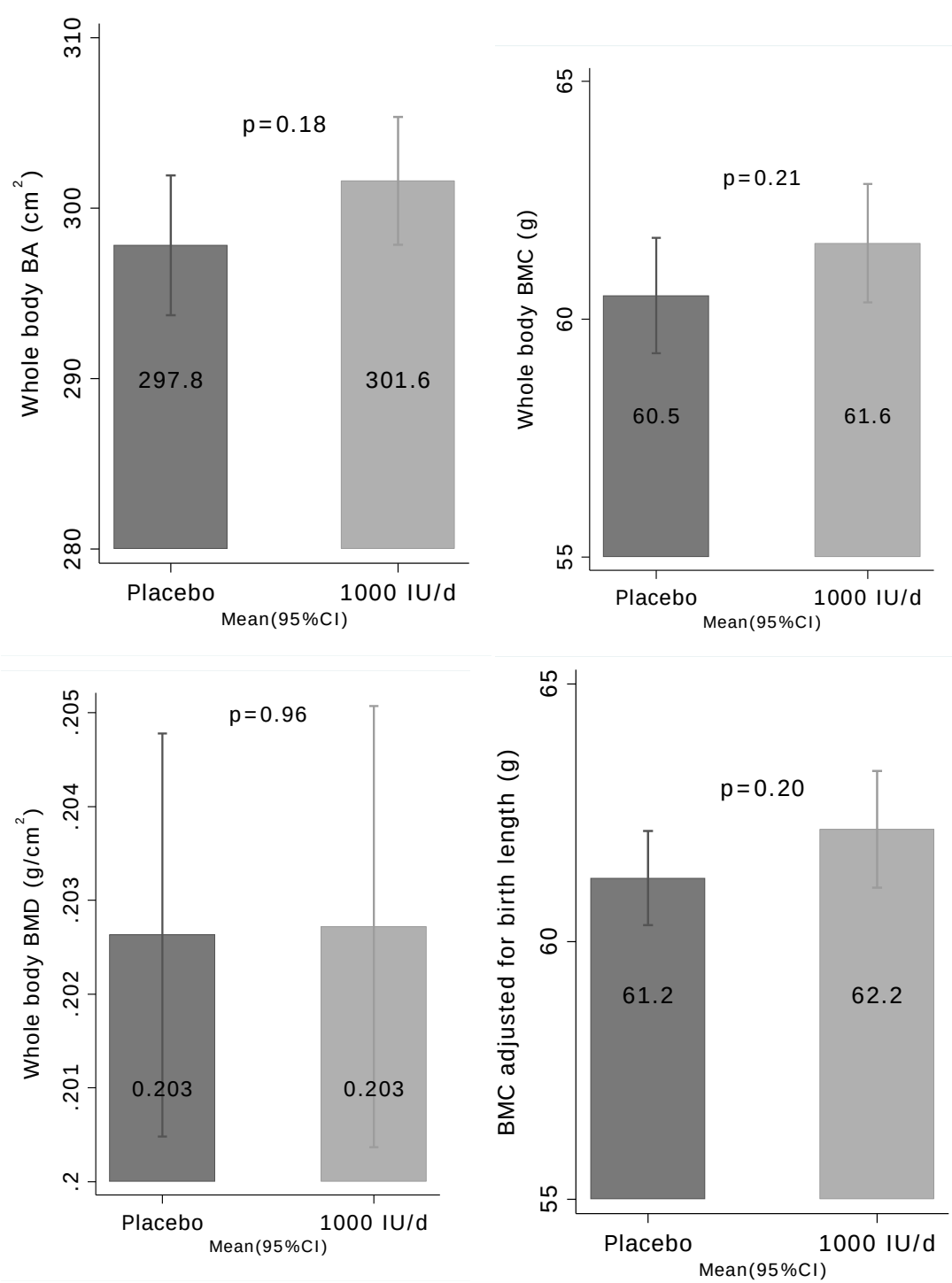
. **Supplementary Table 8:** Maternal weight and skinfold thicknesses at 34 weeks' gestation by treatment group and season of offspring birth. Data are the mean (95% CI) difference in the maternal measure between treatment and placebo group.

		Skinfold thickness (mm)			
	Weight (kg)	**Triceps**	**Biceps**	**Subscapular**	**Upper suprailiac**
Season of birth	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Winter	0.20 (-3.97 to 4.39)	0.65 (-1.45 to 2.75)	-0.42 (-2.09 to 1.25)	2.00 (-1.37 to 5.38)	2.07 (-0.94 to 5.08)
Spring	0.14 (-3.70 to 3.98)	-0.22 (-2.25 to 1.80)	0.19 (-1.21 to 1.59)	-0.48 (-3.58 to 2.62)	0.52 (-2.25 to 3.28)
Summer	-2.28 (-6.14 to 1.57)	-1.41 (-3.36 to 0.53)	-0.70 (-2.17 to 0.76)	-1.73 (-4.35 to 0.89)	-0.52 (-3.38 to 2.35)
Autumn	-4.62 (-7.93 to -1.31)	-1.61 (-3.43 to 0.21)	-1.62 (-3.12 to -0.11)	-1.34 (-4.01 to 1.32)	-2.13 (-4.70 to 0.44)
p interaction	0.22	0.37	0.38	0.34	0.23

Supplementary Table 9: Absolute numbers and percentages of adverse events and serious adverse events by treatment group.

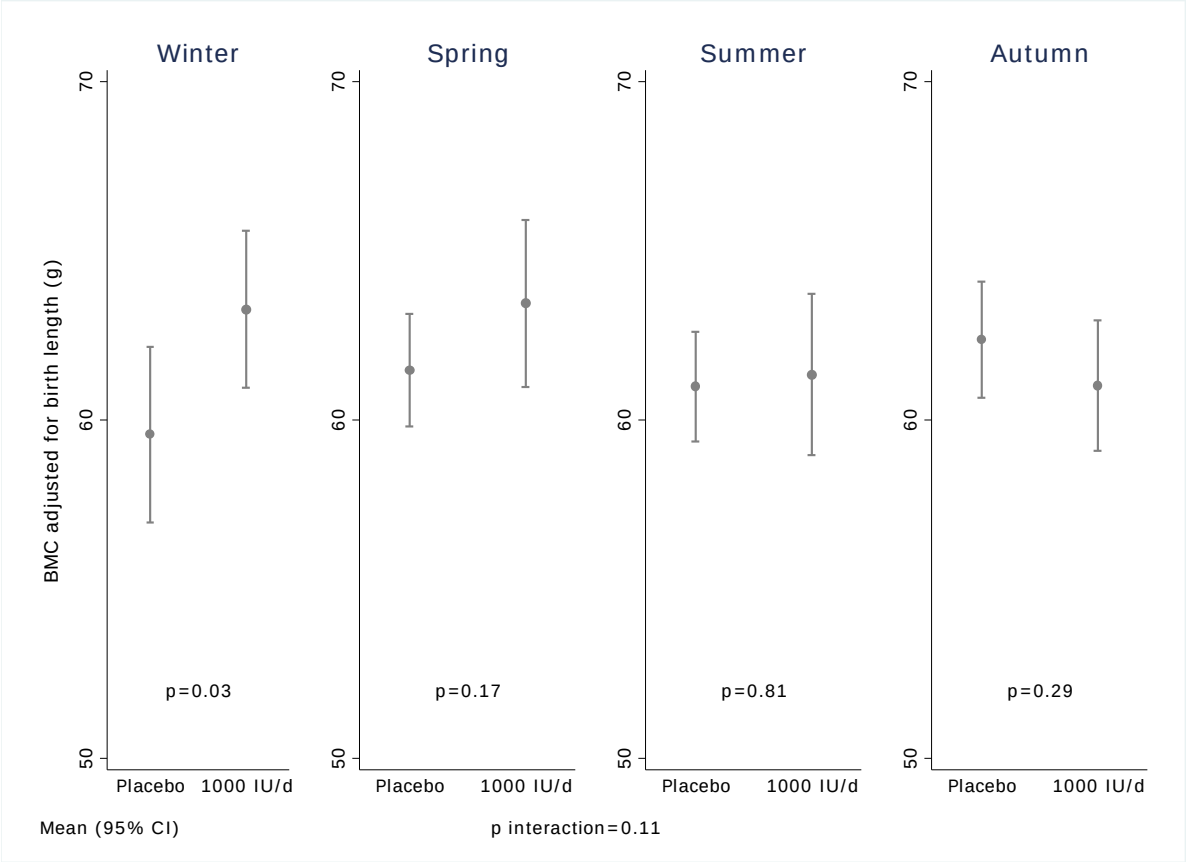
	Placebo	**Cholecalciferol (1000IU/day)**	**p- difference**
	N (%)	**N (%)**	
Adverse events			
Infection	17 (3.0)	17 (3.0)	0.98
Nausea/vomiting	7 (1.2)	6 (1.1)	0.79
Diarrhoea	4 (0.7)	6 (1.1)	0.52
Abdominal pain	19 (3.3)	16 (2.8)	0.62
Headache	9 (1.6)	8 (1.4)	0.82
Hypertension	15 (2.6)	13 (2.3)	0.72
Hypercalcaemia (≥ 2.75 mmol per l) at 34 weeks gestation	0	0	
Fetal growth retardation	0	0	
Severe adverse events			
Preterm delivery / Premature birth	10 (1.8)	16 (2.8)	0.23
Instrumental delivery	35 (6.2)	25 (4.4)	0.19
Severe postpartum haemorrhage	96 (16.9)	65 (11.5)	0.01
Intrauterine or neonatal death	3 (0.5)	1 (0.2)	0.32
Congenital abnormalities	5 (0.9)	8 (1.4)	0.4

Supplementary Figure 1: Neonatal whole body bone area; bone mineral content; bone mineral density; and bone mineral content adjusted for birth length, in offspring of women randomised to 1000IU/day cholecalciferol or placebo during pregnancy.



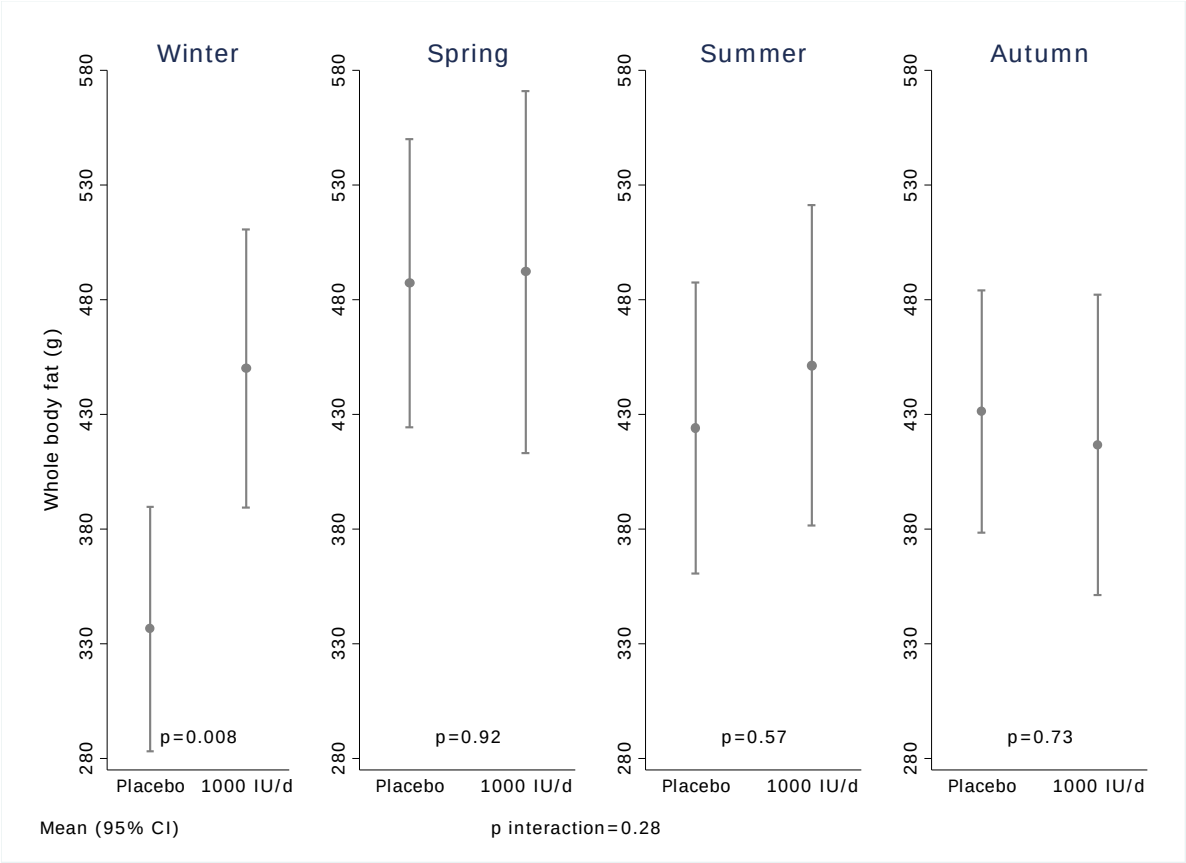
Supplementary Figure 2: Neonatal whole body bone mineral content adjusted for birth length in offspring of women randomised to 1000IU/day cholecalciferol or placebo during pregnancy, stratified by season.

Whole body bone mineral content adjusted for birth length



Supplementary Figure 3: Neonatal whole body fat and lean in offspring of women randomised to 1000IU/day cholecalciferol or placebo during pregnancy, stratified by season.

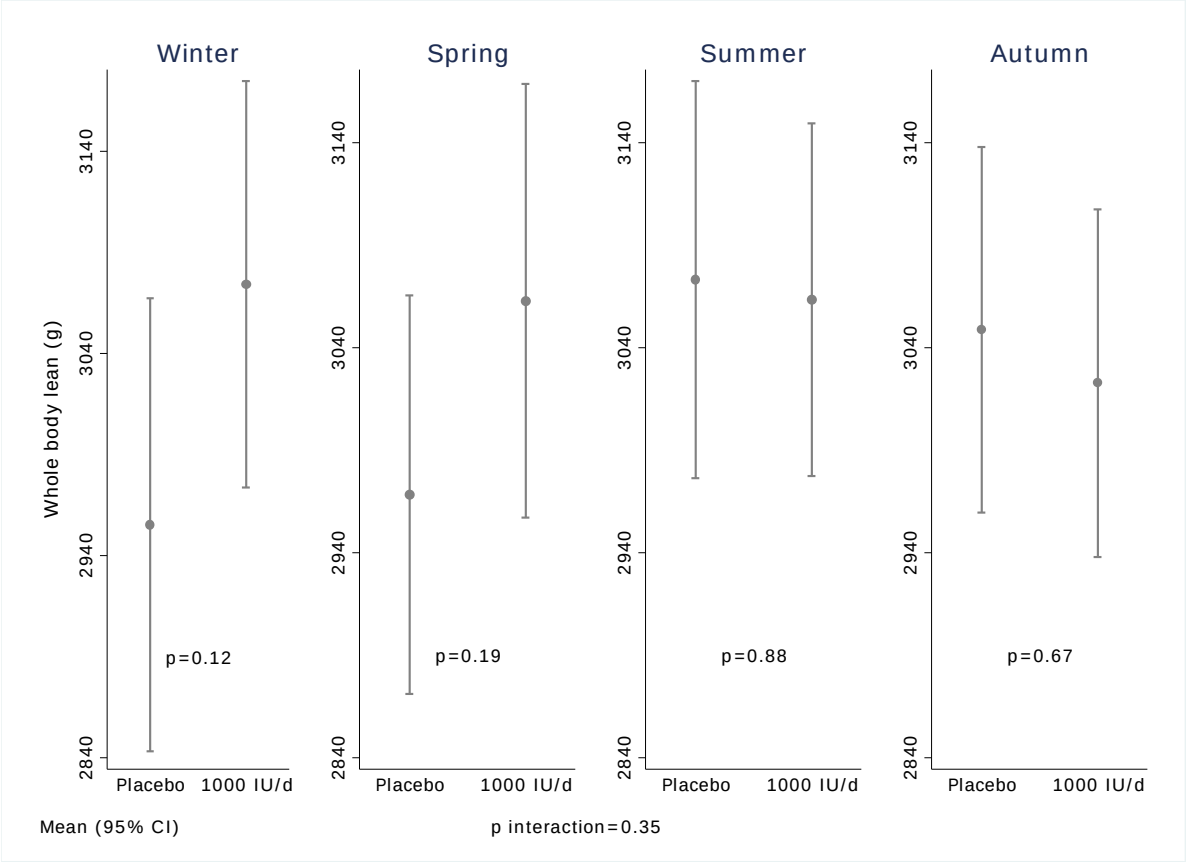
Whole body fat mass



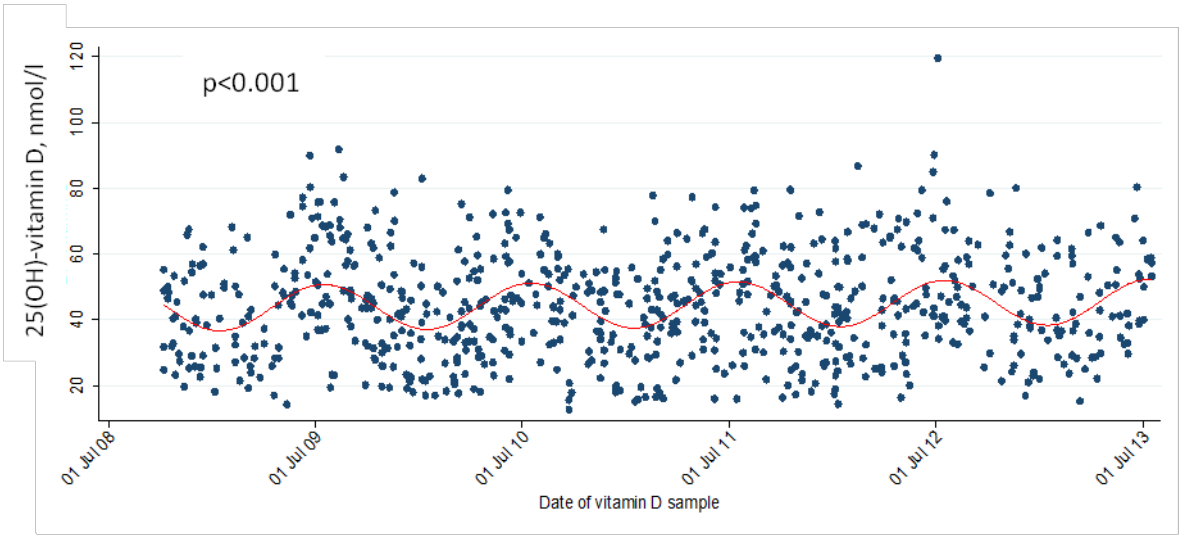
Whole body fat mass appeared similar amongst offspring of treatment and placebo mothers for births in spring, summer and autumn. However offspring of mothers in the cholecalciferol group who delivered during winter had greater whole body fat mass than offspring of mothers receiving placebo: 450 (95%CI: 389.4-510.6) g versus 336.4 (95%CI: 283.1-389.8) g, p=0.008. However, the test for interaction between treatment group and season on whole body fat was not statistically significant (p = 0.28). This result is consistent with previous findings from the Southampton Women's Survey in which positive associations between maternal 25(OH)D concentrations during pregnancy and offspring fat mass at birth were observed. However the relationship was equivocal when the child was 4 years old, and became negative at 6 years, such that greater maternal gestational 25(OH)D was associated with reduced offspring fat mass in later childhood[1]. Also consistent with findings from the Southampton Women's Survey[2], whole body lean mass was greater (albeit not significantly) in offspring born to mothers randomised to cholecalciferol compared with placebo amongst winter and spring deliveries [winter: 3074 (95%CI: 2974-3175) g vs 2955 (95%CI: 2843-3067) g; p = 0.12) and spring: 3063 (95%CI: 2957-3169) g versus 2968 (2871-3065) g; p = 0.19]. There was no difference in lean mass by treatment group

amongst offspring born in summer and autumn months, and the treatment group by season interaction term for whole body lean did not achieve statistical significance (p = 0.35).

Whole body lean mass



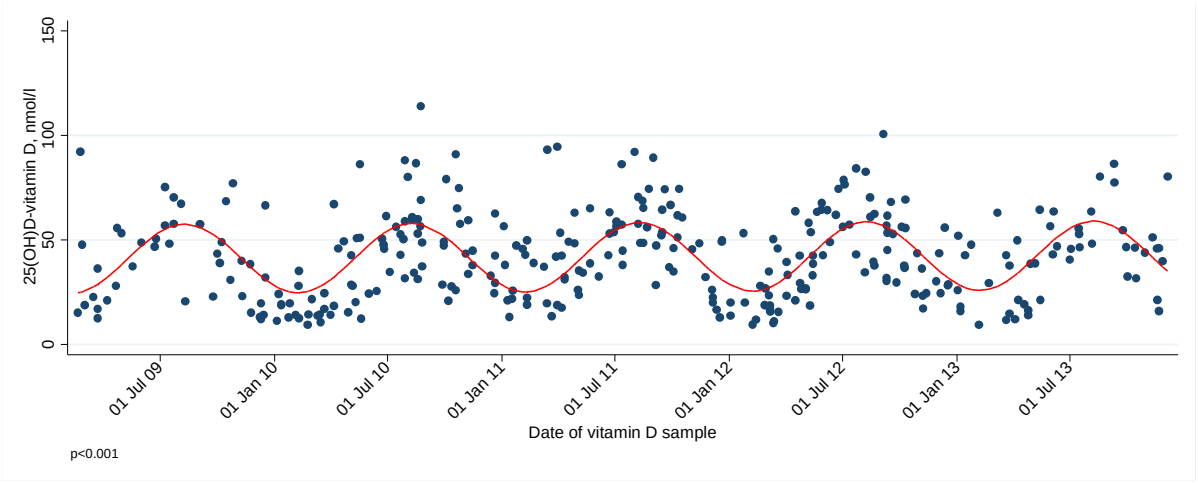
Supplementary Figure 4: Plasma 25(OH)D at 14 weeks' gestation by date of collection.



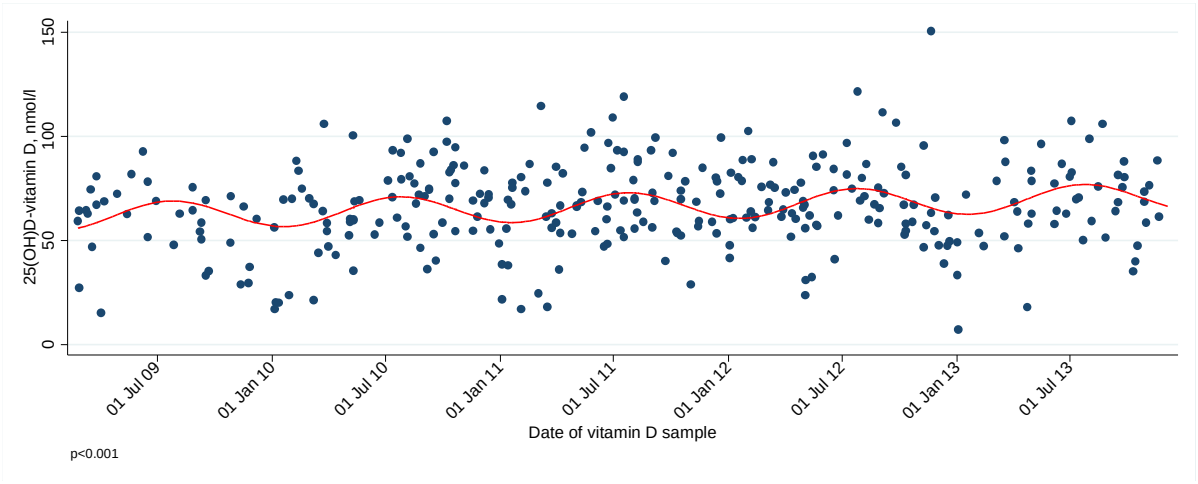
Baseline 25(OH)D status was similar in both groups and varied by season. Supplementary Figure 4 demonstrates 14 week plasma 25(OH)D concentration by date of sampling across the duration of recruitment into the study. There was a clear sinusoidal distribution in relation to season ($p<0.001$for fit to Fourier model) with summer peaks and winter nadirs. Fourier analysis undertaken as per Crozier et al., Am J Clin Nutr 2012: A form of Fourier analysis was used to model the association between vitamin D status and date of sample to take account of the cyclical variation in 25(OH)D concentration by season[1]. A variable was derived that described the number of years the sample was taken after the first sample in the study and multiplied by 2p to give a value h, in radians. Maternal serum 25(OH)D concentration was regressed on cos h and sin h (representing one cycle per annum), on cos 2h and sin 2h (representing 2 cycles per annum), and on cos 3h and sin 3h (representing 3 cycles per annum). The most parsimonious model was the regression of maternal serum 25(OH)D on cos h, sin h, cos 2h, and sin 2h.

Supplementary Figure 5: Plasma 25(OH)D concentrations at 34 weeks' gestation by date of collection, among mothers randomised to (a) placebo, and (b) vitamin D supplementation.

(a) Placebo



(b) Cholecalciferol 1000IU/day



The maternal plasma 25(OH)D concentrations in late pregnancy showed statistically significant sinusoidal distributions ($p<0.01$) using Fourier transformation in both treatment and placebo groups, but the supplemented mothers had higher average values in late pregnancy, and a marked reduction in seasonal amplitude resulting from correction in trough values during pregnancies which completed during winter months.

References

1. Crozier SR, Harvey NC, Inskip HM, Godfrey KM, Cooper C, Robinson SM. Maternal vitamin D status in pregnancy is associated with adiposity in the offspring: findings from the Southampton Women's Survey. *The American journal of clinical nutrition* 2012; **96**(1): 57-63.

2. Harvey NC, Moon RJ, Sayer AA, et al. Maternal Antenatal Vitamin D Status and Offspring Muscle Development: Findings From the Southampton Women's Survey. *The Journal of clinical endocrinology and metabolism* 2014; **99**(1): 330-7.