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

**Supplementary Information**

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**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/m2), 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/m2), 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 (cm2) | 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/cm2) | 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** | **Placebo** | **Cholecalciferol****(1000IU/day)** | **p- differencea** | **p-interactionb** |
| **nmol/l** | **Mean (95%CI)** | **Mean (95%CI** |  |  |
|  | *BA (cm2)* |  |  |
| < 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/cm2)* |  |  |
| < 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 |   |
| Season of birth | **Placebo** | **Cholecalciferol (1000 IU/day)** | **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 |

Other than the greater number of cases of severe postpartum haemorrhage in the placebo than treatment group, there were no differences in safety measures between groups.

**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**

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**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**

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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](#_ENREF_1). Also consistent with findings from the Southampton Women’s Survey[2](#_ENREF_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<0.001for 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](#_ENREF_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.

1. Placebo



1. 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.