Vitamin D levels were significantly higher during and after lifestyle intervention in pregnancy

A randomized controlled trial

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Vitamin D levels were significantly higher during and after lifestyle intervention in pregnancy: a randomised controlled trial

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DMJ has served as a speaker and investigator for Novo Nordisk. MSA has served as an investigator for Novo Nordisk, Novartis, and Sonics. PGO is financially supported by the Novo Nordisk Foundation. No other potential conflicts of interest relevant to this article are reported.
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ABSTRACT

Introduction: Vitamin D deficiency is common in pregnancy, especially in obese women. Lifestyle intervention could potentially result in higher levels of Vitamin D. We therefore aimed to study the effect of lifestyle intervention during pregnancy on serum levels of 25-hydroxyvitamin D (25(OH)D). Material and methods: 360 obese women were randomised before gestational age 14 weeks to lifestyle intervention (diet and exercise) or routine clinical follow-up (controls). Clinical outcomes and levels of 25(OH)D were determined three times: At gestational age 12-15 weeks (baseline), gestational age 28-30 weeks and six months postpartum. Results: A total of 304 (84%) women completed the intervention study, and 238 (66%) attended postpartum follow-up. Vitamin D levels were similar in the two groups at baseline. At gestational age 28-30 weeks and 6 months postpartum, 25(OH)D levels were significantly higher in the intervention group compared to controls (75.6 vs 66.8 nmol/L, \(P = 0.009\)) and (54.8 vs 43.1 nmol/L, \(P = 0.013\)), respectively. Concurrently, vitamin D deficiency (25(OH)D <50 nmol/L) was less frequent in the intervention group compared to controls: 15% vs. 25% \((P = 0.038)\) at gestational age 28-30 and 45% vs. 63% \((P = 0.011)\) six months postpartum, respectively. Conclusions: Lifestyle intervention during pregnancy was associated with significantly increased vitamin D levels in late pregnancy and postpartum compared to controls.

Keywords:
lifestyle intervention, vitamin D, pregnancy, obesity, obese women, randomised controlled trial
Abbreviations
BMI; Body Mass Index
GA; gestational age
GWG; gestational weight gain
HOMA-IR; homeostasis model assessment of insulin resistance
LiP; Lifestyle in Pregnancy
25(OH)D; 25-hydroxyvitamin D

Key message
Lifestyle intervention with diet and exercise among obese pregnant women increases the level of vitamin D in late pregnancy and postpartum.

INTRODUCTION

Vitamin D (25-hydroxyvitamin D, 25(OH)D) is well known for its importance in calcium homeostasis and bone health. However, many tissues have vitamin D receptors, and during recent years an increasing awareness of extra-skeletal effects of vitamin D has emerged. There is no consensus on cut-off limit for 25OHD insufficiency, but vitamin D levels of <25 nmol/L and <50 nmol/L have been considered as insufficiency and deficiency, respectively (1).

During pregnancy, links between insufficient vitamin D status and gestational diabetes or impaired glucose tolerance (2, 3), preeclampsia (2, 3), low birthweight (3) and early miscarriage (4) have been reported. Offspring exposed to low levels of vitamin D during fetal life may be at risk of developing childhood obesity and increased fat mass (5), insulin resistance (6) and lower bone mass (7). Thus, achieving and maintaining sufficient vitamin D level are important both outside and during pregnancy. Vitamin D deficiency is, however, common in pregnancy (4, 8, 9).

The main sources of vitamin D are exposure to sunlight, dietary intake of fatty fish and eggs as well as vitamin supplementation. Vitamin D levels are also influenced by body mass index (BMI), lifestyle and possibly physical activity (10-12). In a recent US study, it was concluded that physical activity decreased the risk of vitamin D deficiency (11). Increased BMI is negatively associated with vitamin D status, also during pregnancy (8, 13). As vitamin D is
fat-soluble it has been suggested that a greater storage in fatty tissue decreases bioavailability in obese individuals (14). Theoretically, loss of fat mass could increase vitamin D levels in the circulation (15, 16). Therefore could an increase in circulating vitamin D be a marker of improved metabolic health during lifestyle intervention.

The Danish Lifestyle in Pregnancy (LiP) study was a randomised controlled trial with lifestyle intervention in obese pregnant women (17). In this secondary analysis, we aimed to investigate whether a lifestyle intervention program aiming to restrict gestational weight gain (GWG) and improve diet quality and physical activity resulted in higher levels of vitamin D during late pregnancy and postpartum.

MATERIAL AND METHODS

The LiP-study was conducted between October 2007 and October 2010 in two university hospitals in Denmark. A total of 360 Caucasian Danish-speaking women with a pre-gestational BMI of 30–45 kg/m² were included. Women were excluded if they had chronic medical disorders, prior serious obstetric complications, positive oral glucose tolerance test (OGTT) in early pregnancy or had a multiple pregnancy. Thus, the women included in the LiP study were from a selected group of obese but otherwise healthy women (17). The participants were randomised before 14 weeks of gestation to either lifestyle intervention or control (routine prenatal care) in a ratio of one to one. Randomisation was carried out using computer-generated numbers in closed envelopes. Subsequently, there was no blinding to participants or healthcare professionals.

The intervention consisted of two major components: dietary counseling and physical activity. Dietary counseling was performed by trained dietitians on four face-to-face separate occasions during pregnancy. The aim was to limit GWG to five kg. The counseling included dietary advice based on the official Danish recommendations (18). Energy requirements for each participant were individually estimated according to weight and level of activity (6300-7500 KJ). At the last visit before delivery, the dietetic counseling also considered the postpartum period and information on nutritional requirements during breastfeeding was provided. Women in the intervention group were encouraged to be moderately physically active 30–60 min daily and were equipped with a pedometer to motivate and improve daily activity. Women in this group also had free full-time membership in a fitness center for 6 months, where they had closed training classes with physiotherapists for one hour each week.

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All participants, including controls, were offered free multi vitamins during pregnancy (GraVitamin, Ferrosan®, Denmark) containing 400 IU (10 micrograms) vitamin D and recommended doses of other vitamins and minerals.

The primary outcomes for the LiP study have been published previously (17, 19, 20), and included GWG and a composite score of gestational diabetes, preeclampsia/pregnancy induced hypertension, cesarean delivery, infants born large for gestational age and infants admitted to intensive care unit. The outcomes reported here are all secondary and include median vitamin D level and percentages of vitamin D deficiency during pregnancy and postpartum.

On two occasions during pregnancy [gestational ages (GA) 12-15 (baseline) and 28–30 weeks] and at six months postpartum, a sample of serum was stored at -80ºC for later analysis of vitamin D, which was performed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The method has been described in details previously (13). Both 25(OH)D2 and 25(OH)D3 were measured, however the reported level of 25(OH)D was equal to the S-25(OH)D3 as D2 content was negligible. Levels of vitamin D were dichotomized into season, i.e. summer and winter. Summer was defined as May through October and winter was defined as November through April.

Fasting plasma glucose was measured using enzymatic reference method with hexokinase (Integra 700; Roche, Mannheim, Germany). Blood-glucose values as part of a 2-h 75-g oral glucose tolerance test were measured using capillary blood and analysed photometrically in a HemoCue analyser (HemoCue, Ängelholm, Sweden). Serum levels of insulin were analysed by time-resolved fluoro-immunoassay (AutoDELFIA; Wallac Oy, Turku, Finland). Insulin resistance was estimated using the homeostasis model assessment of insulin resistance (HOMA-IR) according to Matthews et al. (21) and calculated with the following formula: (fasting plasma insulin in mU/ml × fasting plasma glucose in mmol/ml)/22.5.

At the 6 months postpartum visit questionnaires on breastfeeding were obtained. Information about initiation, duration and intensitivity of breastfeeding was collected. Full breastfeeding was defined as breastfeeding at 6 months postpartum without the introduction of formula.
feeding or solid food. For breastfeeding women, outcome data used for statistical analysis were presented as either any or full breastfeeding at 6 months.

GWG was calculated as measured weight at a 35-week visit minus measured weight at study entry. Postpartum weight retention was calculated as measured weight 6 months postpartum minus measured weight at study entry.

Statistical analyses
All analyses were conducted using Stata version 13.0 software (StataCorp, College Station, TX, USA) and a significance level of 0.05 (two-sided) was chosen. Differences between groups were analysed with the $\chi^2$-test for categorical variables. The Student $t$-test was used for continuous variables with normal distribution; otherwise the Mann–Whitney $U$-test was used. The sample size was predetermined from the original LiP study, which originally was based on detecting a difference in a composite clinical outcome score (gestational diabetes, preeclampsia/hypertension, emergency cesarean section, large for gestational age, and intensive care unit admission). To detect a significant difference in this score (Wilcoxon rank-sum test, 85% power), 180 women were required in each arm ($P < 0.05$) (17).

Ethical approval
The study was approved by the local ethics committee of the Region of Southern Denmark (May 13, 2007; S-20070058) and the trial was registered at clinicaltrials.gov as NCT00530439. However, this secondary analysis of vitamin D levels was not registered as part of the original study. All participants were given full explanation of the purpose and content of the trial and gave written consent to participate.

RESULTS
A total of 304 women completed the LiP study until delivery, and 238 (78 %) attended the 6 months follow-up (Figure 1). Primary outcomes from the LiP trial have been published previously (17). In short, women from the intervention group had significantly lower GWG (7.0 vs 8.6 kg, $P = 0.028$) and lower HOMA-IR (2.7 vs 3.0, $P = 0.031$) at GA 28-30 compared to controls. We found no difference in any maternal or neonatal clinical outcomes. The two groups were similar regarding age, baseline BMI, smoking, parity, level of
education, breastfeeding patterns, postpartum weight retention and additional measures of glucose metabolism (Table 1) (17). The drop-out group was characterized by being older and having a higher percentage of BMI ≥40 kg/m² as well as a higher percentage of smokers compared to the completing groups. However, these differences were not statistically significant due to small numbers.

Concentrations of 25(OH)D were measured in 294 women at GA 12-15 weeks (97%), in 289 at GA 28-30 weeks (95%) and in 199 six months postpartum (65%), with a median of 61.9, 73.0 and 48.4 nmol/l, respectively. The presence of vitamin D deficiency (25(OH)D < 50 nmol/l) in the total study population was 30% at baseline, 20% at GA 28-30 weeks and 53% six months postpartum. Very few had vitamin D levels below 25 nmol/l during pregnancy (four and three %, respectively), whereas this number increased to 13% postpartum.

Vitamin D levels were similar in the two groups at baseline except for a slightly higher median 25(OH)D during summertime in the intervention group compared to controls (71.7 vs 66.3 nmol/l, \( P = 0.046 \)) (Table 2). At GA 28-30 weeks women in the intervention group had significantly higher median 25(OH)D compared to controls (75.6 vs 66.8 nmol/l, \( P = 0.009 \)), and 15 vs 25% (\( P = 0.038 \)) were vitamin D deficient. The difference in median 25(OH)D was mainly seen during wintertime (Table 2). Postpartum, the difference between randomised groups increased with intervention group having a median 25(OH)D of 54.8 vs 43.1 nmol/l in controls (\( P = 0.013 \)), and 45 vs 63 % were vitamin D deficient (\( P = 0.011 \)). Also postpartum, the difference in 25(OH)D was predominantly seen during wintertime.

Vitamin D levels increased from baseline to GA 28-30 in both groups (63.4 vs. 75.6 nmol/l, \( P < 0.001 \)) and (60.9 vs. 66.8 nmol/l, \( P < 0.001 \)), intervention and controls, respectively. The increase was higher in the intervention group compared to controls with median (interquartile range) delta values of 18.5 (-35.4 to 62.9) vs. 7.8 (-5.1 to 25.1) nmol/l, \( P = 0.026 \).

Postpartum vitamin D levels were lower than early pregnancy levels in both groups (54.8 vs. 63.4 nmol/l, \( P = 0.000 \)) and (43.1 vs. 60.9 nmol/l, \( P = 0.000 \)), intervention and controls, respectively. Concurrently, the median (interquartile range) delta values (from baseline to 6 months postpartum) were -10.3 (-20.8 to 1.2) in intervention group, compared to -14.7 (-24.1 to -5.3) nmol/l in controls, \( P = 0.089 \).
DISCUSSION

To our knowledge, this is the first study to show an association between lifestyle intervention during pregnancy and improved vitamin D levels in obese women. Using data from a randomised controlled trial, we found that obese women receiving lifestyle intervention with dietary advice and physical activity promoting initiatives during pregnancy had higher levels of vitamin D and lower prevalence of vitamin D deficiency in the early third trimester and postpartum compared to controls.

Differences between the randomisation groups were predominantly seen during winter and cannot be explained by differences in sunlight exposure, as there a minimum of synthesis of vitamin D in the skin from October to March in Denmark. Winter is thus a time where individuals are at increased risk of hypovitaminosis D. A recent Norwegian longitudinal study studied seasonal variations in measures of vitamin D during pregnancy and found lower levels of free and total 25(OH)D during winter (9). Interestingly, these variations were independent of supplementation with 400 IU of vitamin D, suggesting that this standard dose is too low to battle the risk of vitamin D deficiency during wintertime in the Nordic countries.

In our study, all participants received free multivitamin supplements containing 400 IU of vitamin D. It is unlikely that the difference between the groups is due to supplementation, as they were treated equally in this aspect.

We therefore suggest that the combined advice on physical activity and healthy diet in the lifestyle intervention group resulted in the improved vitamin D status. Previous studies have shown positive associations between physical activity (10-12) and vitamin D as well as between loss of weight/fat mass and vitamin D (15, 16). It could be speculated that a healthier body composition due to increased physical activity and less calorie dense diet contributed to the elevated levels of vitamin D during intervention, that is, a reduction in visceral fat and hence increased mobilization of vitamin D from fat deposits. In our study, we found a significant difference in GWG (7.0 vs. 8.6 kg) between the intervention and the control group. The reduced GWG did, however, not result in lower BMI six months postpartum, where the differences in vitamin D levels were still evident. BMI does not measure fat mass, muscle mass or fat distribution, and is not necessarily reflective of a healthy body.

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composition. Therefore, measurements of body composition could have been beneficial in further exploring the possible role of improved lifestyle.

Another possible explanation for the difference between the groups could be an increased consumption of vitamin D containing foods such as fatty fish and eggs in the intervention group. Unfortunately, we have no food frequency questionnaires or food diaries to further explore this possibility.

Vitamin D deficiency has been associated with a number of adverse outcomes such as poor bone health, autoimmune disease, some cancers and type 2 diabetes (22). However, whether improved levels during pregnancy and early postpartum can confer long-term benefits for the mother and child is unknown. As the difference in GWG did not result in improved outcomes, it is likely that the effect size of increased vitamin D levels is too little to show long term clinical results. To answer this question a properly designed, larger long-term follow-up study with dietary information would be required.

Previous randomised controlled trials on lifestyle intervention in pregnancy have not investigated associations with vitamin D levels. In the European multicenter study “Vitamin D and lifestyle intervention for the prevention of gestational diabetes mellitus” (DALI) obese women were randomised to healthy eating, physical activity or both with or without supplementation of vitamin D/placebo or to a control group. Primary results on the effect of lifestyle changes showed significantly reduced GWG (23), which is in accordance with our study. Data on the results of vitamin D supplementation is awaited.

Vitamin D deficiency was observed in 30% at baseline, which is comparable to results from recent Danish studies (13, 24). Vitamin D levels increased during pregnancy as shown by others (25-27). The physiological increase in vitamin D may be due to increased bioavailability and metabolic adaptations, as well as adherence to intake of supplements, during normal pregnancy. However, as the prevalence of vitamin D insufficiency was significantly lower in the intervention group compared to controls (14.8 vs. 24.5, \( P = 0.038 \)), our study indicates, that it is possible to enhance this naturally occurring increase with lifestyle intervention in obese women. We found a substantially lower median 25(OH)D postpartum compared to early pregnancy (54.8 vs. 63.4 nmol/l \( P = 0.000 \)) in the intervention group and 43.1 vs. 60.9 nmol/l \( p = 0.000 \) in the control group. Concurrently, the...
prevalences of vitamin D deficiency increased in both the intervention and control group (45% and 63%, $P = 0.011$), respectively. This is in contrast to recent Scandinavian studies, where prevalences of deficiency postpartum are reported lower (23-33%) (24-27). Two longitudinal studies found that mean levels of vitamin D increased during pregnancy, and reversed to early pregnancy levels postpartum (26, 27). The differences in prevalences between our results and the above mentioned studies could be due to different BMI categories, as the other studies were mainly comprised of normal weight women. The reason for decrease in vitamin D status postpartum in our study is not known. Breastfeeding does not seem to be the answer, as it is not associated with increased or decreased vitamin D levels postpartum (24, 25). However, possible explanations might be discontinued supplements of vitamin D, less sun exposure due to sleep deprivation and more in-door life with a baby.

The strengths of this study include the randomised design and the high follow-up rate (78%). The women in the drop-out group did not differ statistically from the follow-up attendees regarding baseline outcomes, and they were distributed evenly in the randomisation groups. Therefore, we do not believe that a systematical bias is present due to loss to follow-up. Vitamin D levels were assessed with serial measures both during pregnancy and postpartum, which is a considerable strength. The measurements were performed using liquid chromatography-tandem mass spectrometry (LC-MS/MS), the most reliable method for measuring vitamin D (28). The women were all Caucasians, limiting bias due to skin pigmentation and clothing habits.

Blinding was not possible due to the nature of the intervention. And unfortunately, no measurement of body composition was performed, which could have been beneficial in exploring if the difference in vitamin D levels was conjoined by a shift in regional fat deposition indicating improved metabolic status. Further, no dietary assessment was performed, therefore we cannot conclude on whether the participants improved their dietary habits.

CONCLUSION

To our knowledge this is the first randomised controlled trial with lifestyle intervention during pregnancy to show higher levels of vitamin D and lower prevalence of vitamin D deficiency in the early third trimester and postpartum compared to controls. As we do not
have data on the women’s diet or adherence to advice on vitamin supplements we can only speculate on the differences between the groups. The data needs to be further explored and confirmed by large scale studies in obese and non-obese women. So far the evidence to guide recommendations for prenatal vitamin D supplementation is insufficient (29), however, the results of our study highlights the importance of attention towards vitamin D insufficiency in obese pregnant women and the possible effects of improved lifestyle.

References


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Legends

Figure 1. Participation rates in the Lifestyle in Pregnancy study

Table 1. Baseline, pregnancy and postpartum outcomes in trial groups from the Lifestyle in Pregnancy study. Data are given as median (interquartile range) or number (%). Differences between groups were analysed with the X² test for categorical variables. The students t test was used for continuous variables with normal distribution; otherwise, the Mann-Whitney U test was used. At a significance level of 0.05 (two-sided), there were no statistical differences in any variables between the intervention and control groups. HOMA-IR; homeostasis model assessment of insulin resistance, GA; gestational age, h; hour.

Table 2. Vitamin D levels at baseline (gestational age 12-15 weeks), 28-30 gestational weeks and six months postpartum in trial groups from the Lifestyle in Pregnancy study. Data are given as median (interquartile range) or number (%). Differences between groups were analysed with the X² test for categorical variables. The students t test was used for continuous variables.
variables with normal distribution; otherwise, the Mann-Whitney $U$ test was used. Winter was defined as November through April and summer was defined as May through October. 25(OH)D; 25-hydroxyvitamin D.
Table 1. Baseline, pregnancy and postpartum outcomes in trial groups from the Lifestyle in Pregnancy study

Table 1 legend: Data are given as median (interquartile range) or number (%). Differences between groups were analysed with the $X^2$ test for categorical variables. The students $t$ test was used for continuous variables with normal distribution; otherwise, the Mann-Whitney $U$ test was used. At a significance level of 0.05 (two-sided), there were no statistical differences in any variables between the intervention and control groups. HOMA-IR; homeostasis model assessment of insulin resistance, GA; gestational age, h; hour

<table>
<thead>
<tr>
<th></th>
<th>Intervention, n = 150</th>
<th>Control, n = 154</th>
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<tbody>
<tr>
<td><strong>Baseline</strong></td>
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<tr>
<td>Prepregnancy BMI (kg/m$^2$)</td>
<td>33.4 (31.7-36.5)</td>
<td>33.3 (31.7-36.9)</td>
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<td>Age (years)</td>
<td>29 (27-32)</td>
<td>29 (26-31)</td>
<td>0.257</td>
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<td>Smoking in pregnancy</td>
<td>11 (7.3)</td>
<td>18 (11.7)</td>
<td>0.196</td>
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<tr>
<td>Primiparous</td>
<td>79 (52.7)</td>
<td>84 (54.6)</td>
<td>0.743</td>
</tr>
<tr>
<td>School ≥ 12 years</td>
<td>111 (74)</td>
<td>100 (64.9)</td>
<td>0.086</td>
</tr>
<tr>
<td>Further education ≥3 years</td>
<td>75 (43.5)</td>
<td>67 (50.0)</td>
<td>0.257</td>
</tr>
<tr>
<td>Gainfully employed</td>
<td>102 (68.8)</td>
<td>106 (68.0)</td>
<td>0.876</td>
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<tr>
<td>Fasting glucose (mmol/l)</td>
<td>4.9 (4.7-5.1)</td>
<td>4.9 (4.8-5.2)</td>
<td>0.204</td>
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<tr>
<td>2-h capillary blood glucose (mmol/l)</td>
<td>6.2 (5.6-7.0)</td>
<td>6.5 (5.7-7.4)</td>
<td>0.018</td>
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<td>HOMA-IR</td>
<td>2.2 (1.5-2.9)</td>
<td>2.2 (1.6-3.0)</td>
<td>0.812</td>
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<tr>
<td><strong>Pregnancy outcomes</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Fasting glucose (mmol/l), GA 28-30</td>
<td>4.8 (4.6-5.2), n=145</td>
<td>4.9 (4.7-5.2), n=150</td>
<td>0.068</td>
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<td>2-h capillary blood glucose (mmol/l), GA 28-30</td>
<td>6.3 (5.5-7.2), n=141</td>
<td>6.5 (5.6-5.2), n=142</td>
<td>0.415</td>
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<tr>
<td>HOMA-IR, GA 28-30</td>
<td>2.7 (2.0-3.6), n=144</td>
<td>3.0 (2.2-4.4), n=147</td>
<td>0.031</td>
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<td>Gestational weight gain (kg)</td>
<td>7.0 (4.7-10.6), n=144</td>
<td>8.6 (5.7-11.5), n=148</td>
<td>0.028</td>
</tr>
<tr>
<td><strong>Six months postpartum</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>BMI</td>
<td>33.8 (31.8-36.8), n=123</td>
<td>33.9 (31.9-36.4), n=115</td>
<td>0.883</td>
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<tr>
<td>Postpartum Weight Retention (kg)</td>
<td>0.2 (-3.5 – 2.5), n=123</td>
<td>-1.1 (-4.0 – 2.9), n=115</td>
<td>0.546</td>
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<tr>
<td>Fasting v-p glucose (mmol/l)</td>
<td>5.2 (5.0-5.5), n=110</td>
<td>5.3 (5.0-5.5), n=97</td>
<td>0.486</td>
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<td>2-h capillary blood glucose (mmol/l)</td>
<td>6.3 (5.7-7.0), n=106</td>
<td>6.2 (5.5-6.9), n=93</td>
<td>0.532</td>
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<td>HOMA-IR</td>
<td>2.3 (1.7-3.1), n=108</td>
<td>2.3 (1.6-3.5), n=94</td>
<td>0.906</td>
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<td>Initiation of breastfeeding</td>
<td>117 (95.1%), n=123</td>
<td>104 (90.4%), n=115</td>
<td>0.161</td>
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<td>Breastfeeding to any extent</td>
<td>68 (55.3%), n=123</td>
<td>62 (53.9%), n=115</td>
<td>0.832</td>
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<tr>
<td>Full breastfeeding</td>
<td>31 (25.2%), n=123</td>
<td>31 (26.9%), n=115</td>
<td>0.758</td>
</tr>
</tbody>
</table>
Table 2. Vitamin D levels at baseline (gestational age 12-15 weeks), 28-30 gestational weeks and six months postpartum in trial groups from the Lifestyle in Pregnancy study

Table 2 legend: Data are given as median (interquartile range) or number (%). Differences between groups were analysed with the X² test for categorical variables. The students t test was used for continuous variables with normal distribution; otherwise, the Mann-Whitney U test was used. Winter was defined as November through April and summer was defined as May through October. 25(OH)D; 25-hydroxyvitamin D.

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<th>Intervention, n = 150</th>
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</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D (nmol/L), all year</td>
<td>63.4 (47.7-77.8), n=143</td>
<td>60.9 (43.6-74.9), n=151</td>
<td>0.211</td>
</tr>
<tr>
<td>25(OH)D (nmol/L), winter (53.1%)</td>
<td>51.6 (37.0-8.6), n=75</td>
<td>53.1 (36.3-66.7), n=81</td>
<td>0.900</td>
</tr>
<tr>
<td>25(OH)D (nmol/L), summer (46.9%)</td>
<td>71.7 (61.2-85.5), n=68</td>
<td>66.3 (54.3-78.7), n=70</td>
<td>0.046</td>
</tr>
<tr>
<td>25(OH)D &lt; 50 nmol/L, all year</td>
<td>40 (28.0%), n=143</td>
<td>48 (31.8%), n=151</td>
<td>0.475</td>
</tr>
<tr>
<td>25(OH)D &lt; 50 nmol/L, winter</td>
<td>33 (44.0%), n=75</td>
<td>35 (43.2%), n=81</td>
<td>0.921</td>
</tr>
<tr>
<td>25(OH)D &lt; 50 nmol/L, summer</td>
<td>7 (10.3%), n=68</td>
<td>13 (18.6%), n=70</td>
<td>0.167</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gestational age 28-30 weeks</th>
<th>Intervention, n = 150</th>
<th>Control, n = 154</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D (nmol/L), all year</td>
<td>75.6 (58.4-94.5), n=142</td>
<td>66.8 (50.7-90.8), n=147</td>
<td>0.009</td>
</tr>
<tr>
<td>25(OH)D (nmol/L), winter (47%)</td>
<td>63.8 (48.4-82.3), n=64</td>
<td>55.5 (41.9-74.4), n=72</td>
<td>0.025</td>
</tr>
<tr>
<td>25(OH)D (nmol/L), summer (53%)</td>
<td>85.5 (70.3-101.1), n=78</td>
<td>84.9 (60.7-94.7), n=75</td>
<td>0.187</td>
</tr>
<tr>
<td>25(OH)D &lt; 50 nmol/L, all year</td>
<td>21 (14.8%), n=142</td>
<td>36 (24.5%), n=147</td>
<td>0.038</td>
</tr>
<tr>
<td>25(OH)D &lt; 50 nmol/L, winter (47%)</td>
<td>18 (28.1%), n=64</td>
<td>27 (37.5%), n=72</td>
<td>0.246</td>
</tr>
<tr>
<td>25(OH)D &lt; 50 nmol/L, summer (53%)</td>
<td>3 (3.85%), n=78</td>
<td>9 (12.0%), n=75</td>
<td>0.061</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Six months postpartum</th>
<th>Intervention, n = 150</th>
<th>Control, n = 154</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D (nmol/L), all year</td>
<td>54.8 (38.1-66.7), n=105</td>
<td>43.1 (28.1-58.6), n=94</td>
<td>0.013</td>
</tr>
<tr>
<td>25(OH)D (nmol/L), winter (56.3%)</td>
<td>41.5 (30.2-56.0), n=56</td>
<td>34.1 (23.2-48.3), n=56</td>
<td>0.037</td>
</tr>
<tr>
<td>25(OH)D (nmol/L), summer (43.7%)</td>
<td>63.0 (53.8-71.4), n=38</td>
<td>56.7 (38.2-72.2), n=49</td>
<td>0.250</td>
</tr>
<tr>
<td>25(OH)D &lt; 50 nmol/L, all year</td>
<td>47 (44.8%), n=105</td>
<td>59 (62.8%), n=94</td>
<td>0.011</td>
</tr>
<tr>
<td>25(OH)D &lt; 50 nmol/L, winter (56.3%)</td>
<td>37 (66.1%), n=56</td>
<td>45 (80.4%), n=56</td>
<td>0.088</td>
</tr>
<tr>
<td>25(OH)D &lt; 50 nmol/L, summer (43.7%)</td>
<td>14 (36.7%), n=38</td>
<td>10 (20.4%), n=49</td>
<td>0.089</td>
</tr>
</tbody>
</table>
Assessed for eligibility (n=1224)

Excluded (n=864)
- Not meeting inclusion criteria (n=493)
- Declined to participate (n=317)
- Other reasons (n=54) (miscarriage, malformations, multiple pregnancies)

Allocated to intervention (n=180)
Allocated to control (n=180)

Drop-out during pregnancy (n=30)
Drop-out postpartum (n=27)

Drop-out during pregnancy (n=36)
Drop-out postpartum (n=39)

Analyses

Pregnancy outcomes (n=150)
Postpartum outcomes (n=123)

Pregnancy outcomes (n=154)
Postpartum outcomes (n=115)