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Influence of maternal vitamin D status on obstetric outcomes and the foetal skeleton

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Abstract

Vitamin D status is increasingly associated with wide ranging clinical outcomes. There is now a wealth of observational studies reporting on its associations with obstetric complications, including preeclampsia, gestational diabetes and mode and timing of delivery. The findings are inconsistent and currently there is a lack of data from high quality intervention studies to confirm a causal role for vitamin D in these outcomes. This is similarly true with regards to fetal development, including measures of fetal size and skeletal mineralisation. Overall, there is an indication of possible benefits of vitamin D supplementation during pregnancy for offspring birthweight, calcium concentrations and bone mass, and for reduced maternal pre-eclampsia. However, for none of these outcomes is the current evidence base conclusive, and the available data justify the instatement of high-quality randomised placebo controlled trials in a range of populations and health care settings to establish potential efficacy and safety of vitamin D supplementation to improve particular outcomes.

Introduction

The classical role of Vitamin D is in calcium and phosphate homeostasis: it is without doubt that severe vitamin D deficiency (VDD) can result in rickets, osteomalacia and hypocalcaemia. However, there is increasing suggestion that VDD is associated with wide ranging clinical outcomes, including pregnancy complications and adverse fetal development. As a result, a number of national guidelines recommend vitamin D supplementation during pregnancy[1-3], although this is not currently supported by the World Health Organisation (WHO)[4]. Here, we review the evidence basis for antenatal vitamin D supplementation to prevent obstetric complications, and the influence of vitamin D on fetal growth and skeletal development.

Literature search

This review is based on literature identified through our recently published systematic review of vitamin D in pregnancy (in relation to be both maternal and offspring outcomes), in which published and grey literature were comprehensively searched over many maternal and offspring health outcomes across a wide range of databases from their inception until 2012[5]. A full systematic update was outside the scope of the current review, but we aimed to identify important additional studies using the US National Library of Medicine National Institutes of Health (www.pubmed.com) with the search terms “vitamin D” AND “pregnancy”, up to August 2014.

Vitamin D physiology and epidemiology in pregnancy

Vitamin D can be derived from the diet, as ergocalciferol (vitamin D_2) from plant sources, or cholecalciferol (vitamin D_3) from animal sources. However, the majority is formed endogenously within the skin from the action of ultraviolet B (290-315nm wavelength) to

convert 7-dehydrocholesterol to pre-vitamin D_3. Hydroxylation within the liver produces 25-hydroxyvitamin D [25(OH)D]. This is the main circulating form of vitamin D, found either bound to vitamin D binding protein (VDP), albumin or in the free form. 25(OH)D acts as a reservoir for conversion to 1,25-dihydroxyvitamin D [$1,25(OH)_2D$]), primarily in the renal proximal tubular cells, but also within bone, the parathyroid gland and placenta. Whilst $1,25(OH)_2D$ is the active metabolite, its production is regulated in response to serum calcium and its half life is short at 4-6 hours. Conversely, hepatic 25-hydroxylation is not physiologically regulated and 25(OH)D has a half-life of approximately 2-3 weeks[6]. Therefore, serum 25(OH)D is currently considered the best marker of vitamin D status[7].

The primary function of $1,25(OH)_2D$ is in calcium and phosphate homeostasis, which occurs in conjunction with parathyroid hormone (PTH). Thus, low serum ionised Ca^{2}+ stimulates PTH release, which simultaneously increases renal calcium reabsorption in the distal tubule of the kidney, decreases proximal tubule phosphate reabsorption, and increases $1,25(OH)_2D$ synthesis. The main action of $1,25(OH)_2D$ is to increase uptake of dietary calcium through the intestinal enterocytes, but it also enables PTH induced mobilisation of calcium and phosphate from bone mineral[8].

During pregnancy alterations to calcium and phosphate metabolism occur to allow the accretion of calcium within the fetal skeleton, particularly during the last trimester[9]. This occurs through increased maternal intestinal calcium absorption[10, 11] and mobilization of calcium within the maternal skeletal[12], but without alteration to maternal serum ionized calcium concentration. Maternal calcitropic hormones, including $1,25(OH)_2D$, likely have an important role in these adaptations, as total $1,25(OH)_2D$ increases during the second and third trimesters[10, 13], although this could also reflect the increase in VDP from early through to late pregnancy[11, 14]. The increase in $1,25(OH)_2D$ appears to be independent of PTH, which remains within the normal adult range throughout pregnancy[9]. However PTH-related protein (PTHrP) is elevated in the maternal circulation from early pregnancy and might contribute to the rise in $1,25(OH)_2D$[13]. The effect of pregnancy on 25(OH)D however is less well

understood: Zhang et al. observed a reduction in 25(OH)D in late compared with early pregnancy, however as all subjects were recruited in summer months this might reflect seasonal variation[14]. In contrast, Ritchie et al. reported no significant differences in 25(OH)D measured in 14 women before pregnancy, in each trimester and during lactation[11]. Nonetheless, biochemically low levels of 25(OH)D are highly prevalent: In a cohort of predominantly Caucasian women in the United Kingdom (UK), 31% had a serum 25(OH)D less than 50nmol/l, which is widely considered to be insufficient, and 18% less than 25nmol/l, often considered deficient[15]. However in an ethnically more diverse UK population, 36% of women had a 25(OH)D <25nmol/l at pregnancy booking[16]. Indeed, dark skin pigmentation and extensive skin covering (eg for religious or cultural reasons) are the strongest risk factors for vitamin D deficiency. Obesity is also associated with biochemically low 25(OH)D levels, whereas in pregnancy, use of vitamin D supplements may prevent deficiency[15]. Maternal 25(OH)D in pregnancy is an important consideration as the fetus is entirely dependent on the mother for 25(OH)D. 25(OH)D readily crosses the placenta, and maternal and umbilical cord venous blood 25(OH)D are moderately-highly correlated, with umbilical cord concentrations typically lower than that of maternal blood, although the reported correlation coefficient does vary markedly between studies (r=0.44-0.89[17-20]). Randomised controlled trials have clearly demonstrated that vitamin D supplementation in pregnancy can increase umbilical cord venous and neonatal serum 25(OH)D compared to placebo[21-28].

Obstetric Complications

Observational studies

There are numerous observational studies reporting associations between either vitamin D intake in pregnancy or serum measurement of 25(OH)D and pregnancy complications, including gestational hypertension (GHT) and preeclampsia (PET), gestational diabetes (GDM), timing and mode of delivery. The interpretation and comparison of these studies is

limited by the timing of 25(OH)D measurements, ranging from first trimester to delivery, definition used for both VDD and the outcome, covariates adjusted for and study design (eg prospective cohort, case-control).

Gestational hypertension & preeclampsia

Although the aetiology of PET is poorly understood and likely multifactorial, there is some evidence that maternal calcium status might be important, and calcium supplementation can reduce PET risk, particularly in women with low calcium intake[29]. Thus, exploring a role for calcitropic hormones, including vitamin D, is a sensible approach. Several case-control and prospective cohort studies have demonstrated that women who developed PET had lower serum 25(OH)D compared to controls in early[30-32], mid[33, 34] or late pregnancy[30, 35, 36], and that VDD increases the risk of PET[30, 35, 37]. One case-control study suggested women with serum 25(OH)D<37.5nmol/l measured at less than 22 weeks gestation have a 5-fold higher risk of PET than women with a 25(OH)D>37.5nmol/l, independent of ethnicity, season, gestational age at sampling, pre-pregnancy body mass index (BMI), and educational achievement[30]. Similarly, in a cohort of 23,425 pregnant women in Norway, lower vitamin D intake estimated from a food frequency questionnaire at 22 weeks gestation was associated with a significantly increased risk of PET[38]. The lower vitamin D intake in women who developed PET was mostly due to a difference in vitamin D obtained from supplements, suggesting supplementation might prevent PET. However, these findings are not supported by all studies[32, 39-46], and indeed in a prospective cohort of 1591 women, for each additional 25nmol/l increment in 25(OH)D in early pregnancy, the risk of GHT (without PET) increased by 30%, but no effect on PET risk was observed[43], highlighting possible detrimental effects of higher vitamin D status.

In recent years, there have been several published meta-analyses of the relationship between maternal vitamin D status and PET risk, as shown in table 1[5, 47-52]. Similarly to the

observational studies, the conclusions of these are inconsistent. In our own meta-analysis, we found no significant reduction in the risk of PET with higher vitamin D status (Figure 1)[5]. In contrast, Aghajafari et al. found that the increased risk of PET in VDD was only observed in studies in which blood sampling was later than 16 weeks gestation and when VDD was defined as 25(OH)D<75nmol/l and not <50nmol/l[49]. However, Tabesh et al., including a larger number of studies defining VDD as less than 50nmol/l, did demonstrate an increased risk of PET, which was not found when deficiency was defined as less than 38nmol/l[50]. Importantly, the total number of women included in these meta-analyses varied from 610-2485 (excluding those based on intake only and the most recent meta-analyses which included novel data[47]). However, between January 2013 and July 2014 at least a further 14 case-control or prospective cohort studies with measurement of serum 25(OH)D and assessing PET risk have been published[32, 36, 37, 44-47, 52-59]. These newer studies include data for a further 21,000 women, considerably more than were included in the published meta-analyses.

Gestational Diabetes

Similarly to PET, conflicting findings have been reported for 25(OH)D status in case-control and prospective cohort studies of GDM risk: both lower[52, 60-65] and similar serum 25(OH)D[66, 67] during pregnancy in women with and without GDM have been reported. One study of women referred for GDM screening did not find a difference in the prevalence of GDM in women with 25(OH)D above and below 50nmol/l, but the women with 25(OH)D<50nmol/l did have higher fasting blood glucose, HBA_{1C} and insulin resistance. However these women also had higher BMI, lower physical activity and were less likely to be Caucasian, which might have confounded the findings[68]. Three separate meta-analyses of published studies all concluded that women with GDM had significantly lower mean 25(OH)D than normoglycaemic women[49, 51, 69] with the mean difference in 25(OH)D ranging from 3.9 to 7.4nmol/l. Furthermore, these meta-analyses suggested that the risk of GDM was increased

by 40-60% in women with VDD[49, 51, 69], as shown in Figure 2[49]. However, similarly to studies assessing PET risk, there is now substantially more data available than was used for these meta-analyses[44, 52, 62-65, 67, 70] and whilst many of the smaller studies would support the previous conclusions, a large prospective cohort of women in Australia, including 5109 women, of whom 7.4% developed GDM, first trimester VDD (defined either as <25nmol/l or <37.5nmol/l) was not associated with increased risk of GDM compared to 25(OH)D 50-75nmol/l after adjustment for age, parity, smoking during pregnancy, maternal weight, previously diagnosed hypertension, diabetes, season at sampling, country of birth, or socioeconomic disadvantage[52]. Furthermore in 1953 women in Southern China vitamin D sufficiency (25(OH)D>75nmol/l) at 16-20 weeks gestation was associated with a small, but statistically significant, increased risk of GDM (OR 1.02, 95%CI 1.00, 1.04)[44].

Caesarean Delivery

Unsurprisingly, in recent years, there has also been an increase in studies reporting maternal vitamin D status in relation to mode and timing of delivery. Again, these are inconsistent. After adjustment for potential confounding factors three studies which assessed 25(OH)D in early pregnancy, when attending for GDM screening, and at delivery, reported an increased risk of Caesarean delivery[68, 71, 72]. Conversely, two studies, which measured 25(OH)D in the first trimester demonstrated no increased risk[42, 44]. Assessment of the influence of VDD on mode of delivery is further complicated by the underlying cause for intervention; however Savvidou et al. additionally categorised women requiring emergency caesarean delivery due to failure to progress and for fetal distress. Neither group had significantly different serum 25(OH)D in early pregnancy compared to women who delivered vaginally[73].

Preterm Delivery

More studies have concluded that maternal 25(OH)D status is not related to preterm birth[39, 42, 52, 74-78], than have shown VDD increases this risk[68, 79, 80]. Furthermore, Zhou et al reported women with higher vitamin D status at 16-20 weeks gestation had a higher odds of preterm delivery[44], and similarly Hossain et al. found that cord blood 25(OH)D was higher in preterm (<37 weeks gestation) deliveries (mean 55nmol/l) compared to term pregnancies (mean 40nmol/l, p=0.009) in women in Pakistan[81]. Interestingly, two of the studies which suggest VDD increased the risk of preterm delivery used a definition of less than 35 weeks gestation for preterm[79, 80], whereas all, but one[78], of the studies reporting either no relationship or VDD reduced the risk considered preterm delivery to be at less than 37 weeks gestation. Whilst this might suggest that VDD is particularly associated with an increased risk of very preterm birth, Schneuer et al, who prospectively studied first trimester 25(OH)D status in over 5000 women, found VDD did not increase the risk of either, all, or spontaneous, preterm birth <34 weeks gestation, before or after adjustment for potential confounding factors[52]. However, differences in timing of 25(OH)D assessment, and one study showing increased risk including only twin pregnancies[79], could account for these different findings. Furthermore, Bodnar et al. observed that only non-white mothers had an increased risk of preterm birth with low 25(OH)D at 26 weeks gestation[80], suggesting stratification of women by ethnicity in future intervention studies might be necessary.

Intervention studies of vitamin D supplementation to reduce obstetric complications

Observational data cannot confirm a causal effect of vitamin D or justification for population wide supplementation, particularly as some studies have suggested possible detrimental effects of higher 25(OH)D[43, 44, 81]. As 25(OH)D status is primarily determined by environmental factors, confounding and reverse causality need to be considered, and differences in covariates included in multivariate models might explain the inconsistent

findings. For example, obese individuals have lower 25(OH)D status, and a higher incidence of GDM, GHT, PET, caesarean section and preterm delivery[82, 83]. Similarly African-American women are more likely to require delivery by Caesarean section and to experience pre-eclampsia and preterm labour[84]. Whether these outcomes can truly be attributed to lower 25(OH)D compared to Caucasian women and therefore prevented by vitamin D supplementation must be established through intervention studies.

Despite the expanse of observational data, there are currently few trials of antenatal vitamin D supplementation reporting on maternal outcomes other than maternal/neonatal vitamin D and calcium status[85]. In three of the five studies, the interventional product contained only vitamin D[26, 86, 87], whereas a further two assessed the effects of combined vitamin D and calcium supplementation[88, 89] (Table 2). The interpretation of these two studies with regards to GHT and PET is limited as calcium supplementation is known to reduce the risk of PET[29]. Nonetheless, high dose vitamin D supplementation, with or without calcium supplementation, did not improve the incidence of GHT, PET, GDM, or preterm delivery compared to either usual care or low dose supplementation[26, 86-89]. However these studies were most likely underpowered to detect a difference in these outcomes. GDM complicates approximately 4.5% of pregnancies in the UK[90]. Thus, to detect a 50% reduction in this incidence with 80% power at the 5% significance level, 1010 women would be needed in each study arm. As PET occurs in 2-3% of pregnancies, even larger study numbers are needed.

Although trials of vitamin D supplementation have not yet demonstrated a reduction in the incidence of PET or GDM, there is some evidence to support effects on blood pressure and glucose metabolism when considered as continuous outcomes. For example, Marya et al. demonstrated a reduction in both systolic and diastolic BP in women randomised to vitamin D and calcium supplementation compared to those who received usual care[89]. Confirmation of this finding using vitamin D alone is now needed. Three studies have assessed the effects of vitamin D supplementation on insulin resistance. In an unblinded study of 113

Iranian women randomised to one of three treatment groups (200 IU/day, 50,000 IU/month, 50,000 IU/fortnight) from 12 weeks gestation until delivery, insulin resistance, assessed by HOMA-IR, increased significantly from baseline to delivery in all three groups, but the rise was significantly less in women randomised to 50,000 IU/fortnight than in women who received 200 IU/day[91]. In contrast, Yap et al found no difference in either fasting blood glucose or that measured two hours post glucose load in women randomised to either 400 IU/day or 5000 IU/day cholecalciferol, with similar results for HOMA-IR[87]. Finally, in a small study of 54 women with a diagnosis of GDM, two doses of 50,000IU cholecalciferol 3 weeks apart did improve fasting blood glucose and insulin resistance compared to placebo. However the women randomised to vitamin D supplementation had significantly higher insulin resistance at baseline making these results difficult to interpret[92]. Nonetheless, these findings support the need for further high quality large randomised controlled trials, and to concurrently determine if any effects on maternal physiology might also have beneficial effects on maternal and/or fetal morbidity, for example macrosomia or neonatal hypoglycaemia.

Fetal Development

Early rickets and symptomatic neonatal hypocalcaemia have been reported in infants born to mothers with VDD[93-95]. However, these outcomes are rarely reported in infants of white mothers, and most commonly occur in those born to mothers with dark skin pigmentation, extensive skin covering and profound VDD. The fetus is dependent on the mother for accretion of approximately 30g of calcium to enable skeletal development. As such, a subclinical role for vitamin D and/or calcium in fetal growth and bone development has been considered, yet maternal supplementation with calcium alone does not appear to have beneficial effects on fetal bone mineral accrual[85].

Size at birth

There are now a number of intervention studies assessing the effect of vitamin D supplementation on birth anthropometry, although the dose and timing of introduction of vitamin D varied widely (Table 3). Most studies trialled supplementation with vitamin D alone and did not find a significant effect on birth weight, length or head circumference (Table 1) However, interestingly, vitamin D in combination with calcium did increase birth weight in three studies despite women in the control group also receiving calcium supplementation in two of these studies[88, 96, 97]. Indeed the prevalence of VDD at baseline and mean 25(OH)D achieved was similar in a study of women in Bangladesh, who received 35,000 IU/day cholecalciferol from 26-30 weeks gestation[24], to women participating in a study of 50,000 IU cholecalciferol per week in addition to 200mg elemental calcium supplementation in Iran[97]. Both studies included a similar number of women. However, in the former study birth weight was similar in both intervention and control groups, whereas in the latter study mean birth weight in the intervention group was 170g greater than that in the control group. These differing findings might suggest that the effect of vitamin D is be dependent on the availability of calcium, or could result from genetic/racial variation in response to vitamin D supplementation, but nonetheless highlight the importance of using data obtained from an appropriate population in the development of antenatal supplementation policies.

Skeletal Development

Currently, the data relating maternal 25(OH)D status to offspring bone development is largely observational in nature, but does span antenatal measurements to peak bone mass. Indeed, using gestational ultrasound, smaller femoral volumes[98] and widening of the distal femoral metaphysis relative to femur length has been demonstrated in fetuses of mothers with low levels of serum 25(OH)D[99].

A number of studies have demonstrated associations between maternal 25(OH)D status in pregnancy and offspring bone mineralisation in the neonatal period. In 71 Korean neonates, those born in summer (July-September) had 6% higher whole body bone mineral content (BMC) than infants born in winter (January-March), and neonatal 25(OH)D at delivery was correlated with whole body BMC in all children ($r=0.24$, $p=0.05$)[100]. However, in three similar studies by the same author in North America a reversed pattern was observed with whole body BMC 8-12% lower in infants born in summer[101]. The authors suggest that this difference reflects low uptake of vitamin D supplementation throughout pregnancy in Korea, but only during the first trimester in North America, thereby suggesting early pregnancy during winter might impact on skeletal development[101]. However, Weiler et al. studied 50 Canadian infants born between August and April, with the majority of mothers taking vitamin D supplementation in pregnancy. Infants with a cord blood 25(OH)D<37.5nmol/l (n=18) were heavier and longer than those with a cord blood 25(OH)D above this cut-point, but skeletal size was not relatively increased, such that whole body and femur BMC relative to body weight were significantly lower[102]. In a Finnish study, peripheral quantitative computed tomography (pQCT) was used to assess both BMC and bone geometry of the tibia in 98 neonates. In this analysis, the mean of two maternal 25(OH)D measurements in early pregnancy and 2 days postpartum was used to define maternal vitamin D status, and the median for the cohort used to establish two groups. BMC and bone cross-sectional area (CSA) were 13.9% and 16.3% higher, respectively, in infants of mothers with higher 25(OH)D[103]. When these children were reassessed at 14 months of age, the difference in tibial BMC was no longer present, but the greater CSA persisted[104]. Conversely, in 125 Gambian mother-offspring pairs, no significant relationships were observed between maternal 25(OH)D at either 20 or 36 weeks gestation and offspring whole body BMC or bone area at 2, 13 or 52 weeks of age[105]. However, in contrast to the other studies, no mother had a 25(OH)D less than 50nmol/l, consistent with the notion that poorer skeletal mineralisation might only occur in fetuses of mothers with the lowest vitamin D levels.

There is evidence to support the persistence of these relationships outside of the neonatal period, although the data are less consistent. In the first study to report on the relationship between maternal 25(OH)D status and offspring bone mineralisation in childhood, Javaid et al demonstrated positive associations between late pregnancy 25(OH)D and offspring whole body and lumbar spine BMC, bone area and areal bone mineral density (aBMD) measured at 9 years (Figure 3)[15]. Positive relationships with umbilical venous calcium concentration were also observed, suggesting that the effect of vitamin D on skeletal development might be mediated through placental calcium transport[15]. This was initially supported by data from the Avon Longitudinal Study of Parents and Children (ALSPAC), in which maternal estimated ultraviolet B exposure in late pregnancy, used as a proxy measure of vitamin D status, was positively associated with offspring whole body less head (WBLH) BMC and bone area at 9-10 years of age in 6955 children[106]. However, subsequent re-analysis in a more limited subset of the ALSPAC cohort using serum 25(OH)D measured in pregnancy demonstrated no association with WBLH BMC or bone area[107]. Interestingly there was strong collinearly between maternal gestational UVB exposure and offspring age at bone assessment, which limits the interpretation of these studies[108]. Finally, data from the Raine cohort in Western Australia provide support for a positive relationship between maternal gestational vitamin D status and offspring bone development to peak bone mass: In this study, whole body BMC and aBMD were 2.7% and 1.7% lower, respectively, at 20 years of age in offspring of mothers with 25(OH)D<50nmol/l (compared with offspring of mothers >50nmol/l) at 18 weeks gestation after adjustment for sex, age, height and body composition at 20 years, maternal height and prepregnancy weight, age at delivery, parity, education, ethnicity, smoking during pregnancy, and season of maternal blood sampling[109].

Currently there is only one intervention study of the effect of vitamin D supplementation in pregnancy on offspring bone mineralisation. Congdon et al. assessed forearm BMC using single photon absorptiometry in 64 infants of Asian mothers living in the UK who participated in a non-randomised study of vitamin D and calcium supplementation in pregnancy[110]. 19

334 women received 1000IU vitamin D and a calcium supplement (of unknown strength) during
335 the last trimester, and were compared to 45 women who did not receive any supplement. No
336 significant differences were identified between these two groups, but interpretation of the
337 study findings is limited by the small study size, lack of randomisation and technique used to
338 assess BMC. The ongoing Maternal Vitamin D Osteoporosis Study (MAVIDOS) in which
339 over 1000 women were randomised to 1000 IU cholecalciferol or placebo daily from 14
340 weeks gestation till delivery, with assessment of offspring bone mineralisation at birth and 4
341 years of age by dual energy X-ray absorptiometry (DXA)[111], will provide much needed high
342 quality evidence on the role of vitamin D supplementation in pregnancy in fetal skeletal
343 development[112].

344 ## Conclusions

345 There is now a wealth of observational data relating vitamin D status in pregnancy to
346 obstetric complications, fetal growth and offspring bone development. The findings of these
347 studies are inconsistent and whilst justifying the need for assessment of vitamin D
348 supplementation in high quality randomised controlled trials, observational data alone should
349 not be used as a basis for population wide vitamin D supplementation in pregnancy. Indeed
350 it is possible that the variability in findings of both observational and the few intervention
351 studies reflects the wide heterogeneity in the populations studied (including prevalence of
352 VDD, calcium status and ethnic diversity), dose of vitamin D, timing of initiation or
353 assessment of 25(OH)D status and definition used for the outcomes considered. Thus any
354 public health recommendations need to be based on an appropriate population.
355 Furthermore, whilst currently available data does not suggest any short term detrimental
356 effects for the mother or fetus, the long term safety of vitamin D supplementation, particularly
357 at supra-physiological doses remains to be established.
358

Figure Legends

Figure 1: Forest plot of the association between maternal vitamin D status and risk of pre-eclampsia (observational studies)

Reproduced from Harvey N, Holroyd C, Ntani G, Javaid M, Cooper P, Moon R, Cole Z, Tinati T, Godfrey K, Dennison E, Bishop N, Baird J & Cooper C. Vitamin D supplementation in pregnancy: a systematic review. *Health Technol Assess* 2014 **18**.

Figure 2: Meta-analysis of maternal serum 25(OH)D in pregnancy and gestational diabetes.

Reproduced from Association between maternal serum 25-hydroxyvitamin D level and pregnancy and neonatal outcomes: systematic review and meta-analysis of observational studies, Aghajafari F et al, BMJ 2013;346:f1169 doi: 10.1136/bmj.f1169 with permission from BMJ Publishing Group Ltd.

Figure 3: Maternal 25(OH)D concentration in late pregnancy and childhood bone mass at age 9 years

Reprinted from The Lancet, Vol 367, Javaid MK et al, Maternal vitamin D status during pregnancy and childhood bone mass at age 9 years: a longitudinal study, Pages 36–43, Copyright (2014), with permission from Elsevier

Table Legends

Table 1: Meta-analyses of maternal vitamin D status (intake and serum 25-hydroxyvitamin D level) and risk of pre-eclampsia

Table 2: Intervention studies of vitamin D supplementation (alone, and in combination with calcium supplementation) in pregnancy to reduce obstetric complications

Table 3: Intervention studies of the effect of vitamin D supplementation in pregnancy on offspring anthropometry at birth

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Table 1: Meta-analyses of maternal vitamin D status (intake and serum 25-hydroxyvitamin D level) and risk of pre-eclampsia.

Author	Publication cut-off	Number of studies included	Number of women included	Comparison	Risk of preeclampsia with low vitamin D status	
					Direction of effect	Reported odds ratio (95%CI)
Vitamin D intake						
Thorne-Lyman, 2012 [43]	June 2011	2	25141	Highest vs lowest category of vitamin D intake	↔	0.95 (0.86,1.06)
Hypponen, 2013 [42]	March 2013 + inclusion of novel data	2	77165	Self-supplementation vs unsupplemented	↑	1.23 (1.15, 1.33)
Serum 25(OH)D						
Aghajafari, 2013 [44]	August 2012	2	697	Serum 25(OH)D ≥50nmol/l vs <50nmol/l	↔	1.27 (0.67, 2.42)
		5	1165	Serum 25(OH)D ≥75nmol/l vs <75nmol/l	↑	2.11 (1.36, 3.27)
		7	1862	Higher serum 25(OH)D as defined by each study vs lower serum 25(OH)D	↑	1.79 (1.25, 2.58)
		7	1862	Higher serum 25(OH)D as defined by each study vs lower serum 25(OH)D, adjusted for "critical confounders"	↔	1.51 (0.89, 2.57)
Hypponen, 2013 [42]	March 2013 + inclusion of novel data	6	6864	Higher serum 25(OH)D as defined by each study vs lower serum 25(OH)D	↑	1.92 (1.12, 3.33)
Tabesh, 2013 [45]	December 2012	4	931	Serum 25(OH)D ≥38nmol/l vs <38nmol/l	↔	Actual odds ratios not reported
		5	1775	Serum 25(OH)D ≥50nmol/l vs <50nmol/l	↑	
		8	2485	Higher serum 25(OH)D as defined by each study vs lower serum 25(OH)D	↑	
Wei, 2013 [46]	October 2012	6	610	Serum 25(OH)D ≥50nmol/l vs <50nmol/l	↑	2.09 (1.50, 2.90)
		5	802	Serum 25(OH)D ≥75nmol/l vs <75nmol/l	↑	1.78 (1.23, 2.56)
Harvey, 2014 [47]	June 2012	4	628	Each 25nmol/l increase in serum 25(OH)D	↔	0.78 (0.59-1.05)

Table 2: Intervention studies of vitamin D supplementation (alone, and in combination with calcium supplementation) in pregnancy to reduce obstetric complications.

Study	Population	Gestation at random-isation	Interventional medicinal product (IMP)	Control	Effect of IMP vs control on incidence of obstetric events					
					Hypertensive disorders GHT	Hypertensive disorders PET	GDM	Preterm delivery	Caesarean section	Intrauterine death/ stillbirth
Vitamin D supplementation										
Hossain, 2014 [82] (Karachi, Pakistan)	N=178	20 weeks	4000 IU/day oral cholecalciferol	Usual care	↔	↔		↔	↔	↓ (0 vs 1 case, p=0.05)
Wagner, 2013 [21] (South Carolina, USA)[1]	N=504	12-16 weeks	2000 IU/day oral cholecalciferol (n=201) 4000 IU/day oral cholecalciferol (n=193)	400 IU/day oral cholecalciferol (n=111)	↔ ↔		↔ ↔	↔ ↔		
Yap, 2014 [83] (Sydney, Australia)	N=179 25(OH)D< 80nmol/l at baseline	< 20 weeks	5000 IU/day oral cholecalciferol	400 IU/day oral cholecalciferol		↔	↔	↔	↔	
Vitamin D + Calcium supplementation										
Kalra, 2011 [84] (Lucknow, India)	N=140	12-24 weeks	Group 1: 60,000 IU single dose oral cholecalciferol at recruitment + 1g elemental Ca/day until delivery (n=48) Group 2: 120,000 IU oral cholecalciferol at recruitment and 28 weeks gestation + 1g elemental Ca/day until delivery (n=49)	Usual care (n=43)	↔				↔	↔
Marya, 1987 [85] (Rothak, India)	N=400	20-24 weeks	1200 IU/day vitamin D + 375mg calcium	Usual care		↔				

↔ no effect shown, ↓vitamin D supplementation reduced the incidence of the outcome; GHT – gestational hypertension; PET – preeclampsia; GDM – gestational diabetes mellitus. (1) This reported a combined analysis of data collected in two previous studies.[22,109]

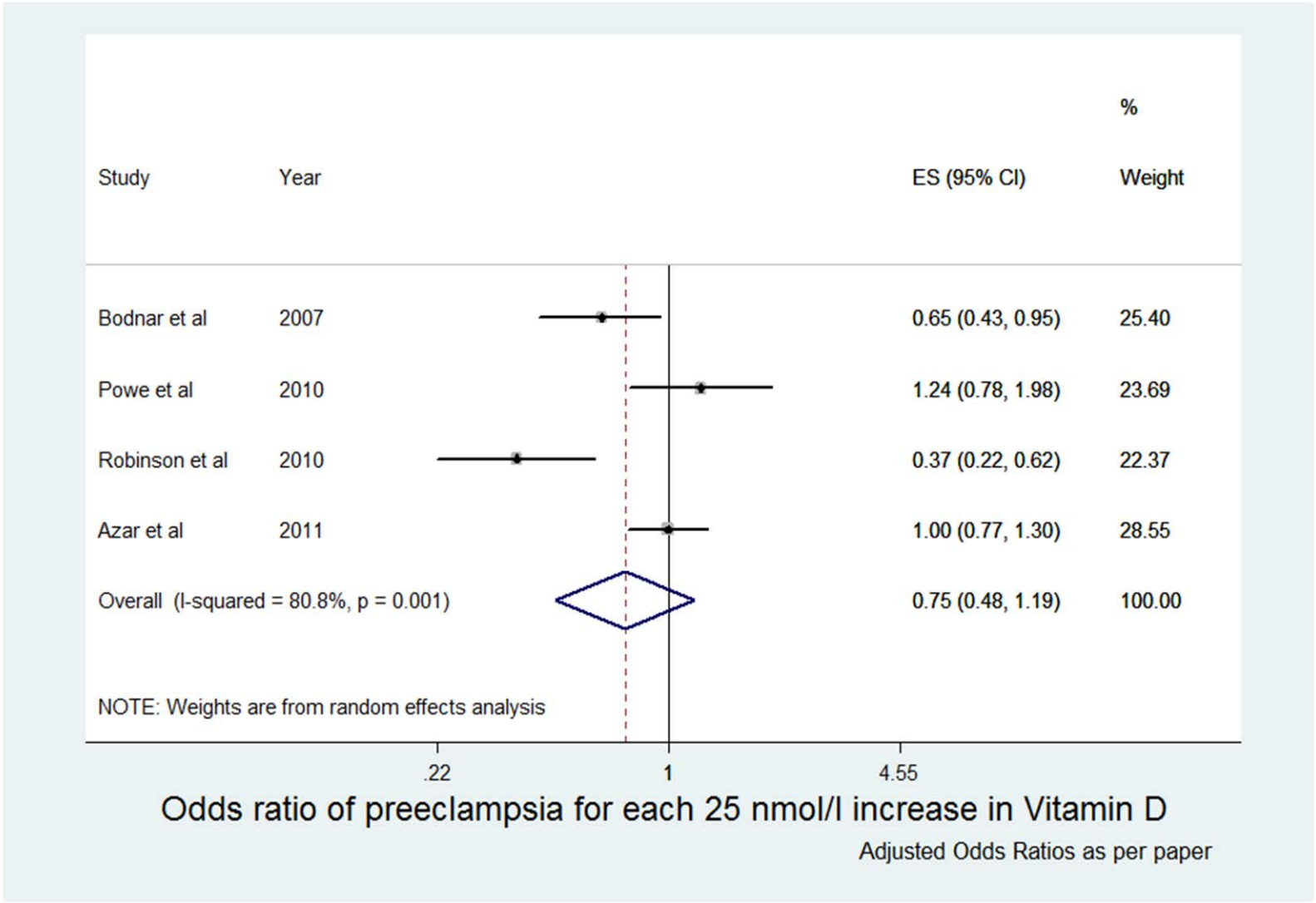
Table 3: Intervention studies of the effect of vitamin D supplementation in pregnancy on offspring anthropometry at birth

Study	Population	Gestation at Allocation/ Random-isation	Interventional medicinal product (IMP)	Control	Effect of vitamin D supplementation		
					Birth Weight	Birth Length	Head Circumference
Vitamin D only							
Brooke 1980 [16] (London, UK)	126 Asian women	28-32 weeks	1000 IU/day oral vitamin D	Placebo	↔	↔	↔
Mallet 1986 [18] (France)	68 women	Last trimester	Group A: 1000 IU/day oral vitamin D Group B: 200,000 IU single dose in 7th month of pregnancy	Usual care	↔		
Marya 1988 [110] (Rohtak, India)	200 Indian women	7 months	Single dose of 600000IU cholecalciferol in months 7 and 8 of pregnancy	Usual care	↑	↑	↑
Yu 2009 [20] (London, UK)	180 women	27 weeks	Group A: 800 IU/day oral cholecalciferol Group B: 200000IU oral cholecalciferol single dose at 27 weeks gestation	Usual care	↔		
Dawodu 2013 [23] (Al Ain, UAE)	192 Arab women	12-16 weeks	Group A: 4000 IU/day oral cholecalciferol Group B: 2000 IU/day oral cholecalciferol	400 IU/day oral cholecalciferol	↔	↔	↔
Grant 2013 [17] (Auckland, New Zealand)	260 women	26-30 weeks	Group A: 1000IU/day oral cholecaclciferol Group B: 2000IU/day oral cholecalciferol	Placebo	↔		
Wagner 2013 [21] (USA)	Combined analysis of two trials including a total of 513 women	12-16 weeks	Group A: 2000IU/day oral cholecalciferol Group B: 4000IU/day oral cholecalciferol	400 IU/day oral cholecalciferol	↔		
Roth, 2013 [19] (Dhaka, Bangladesh)	148	26-30 weeks	35000 IU/week oral cholecalcfierol	Placebo	↔	↔	↔
Vitamin D + calcium							

Marya 1981 [92] (Rohtak, India)	120 Hindu women	Last trimester	Group A: 1200IU/day vitamin D + 375mg calcium during third trimester Group B: 600000IU vitamin D orally in the 7th and 8th months of pregnancy (n=20)	Usual care	↑		
Kalra 2011 [84] (Lucknow, India)	140 women	12-24 weeks	Group A: 60,000IU oral cholecalciferol single dose at randomisation + 1g/day calcium carbonate Group B: 120,000IU oral cholecalciferol at randomisation and at 28 weeks gestation + 1g/day calcium carbonate	1g calcium carbonate/day	↑	↑	↑
Hashemipour 2014 [93] (Qazin, Iran)	109 women, 25(OH)D<75 nmol/l	24-26 weeks	50,000 IU/week cholecalciferol for 8 weeks in addition to the supplement received by control group	400IU/day oral cholecalciferol; 200mg elemental calcium	↑	↑	↑
Hossain 2014 [82] (Karachi, Pakistan)	198	20 weeks	4000IU/day oral cholecalciferol, 600mg calcium lactate & 200mg ferrous sulphate	600mg calcium lactate & 200mg ferrous sulphate	↔	↔	↔

↔ no effect shown, ↑vitamin D supplementation increased the outcome, ↓vitamin D supplementation the outcome

Figure 1


Study
Year
ES (95% CI)
%
Weight
Bodnar et al
2007
0.65 (0.43, 0.95)
25.40
Powe et al
2010
1.24 (0.78, 1.98)
23.69
Robinson et al
2010
0.37 (0.22, 0.62)
22.37
Azar et al
2011
1.00 (0.77, 1.30)
28.55
Overall (I-squared = 80.8%, p = 0.001)
0.75 (0.48, 1.19)
100.00
NOTE: Weights are from random effects analysis
.22
1
4.55
Odds ratio of preeclampsia for each 25 nmol/l increase in Vitamin D
Adjusted Odds Ratios as per paper

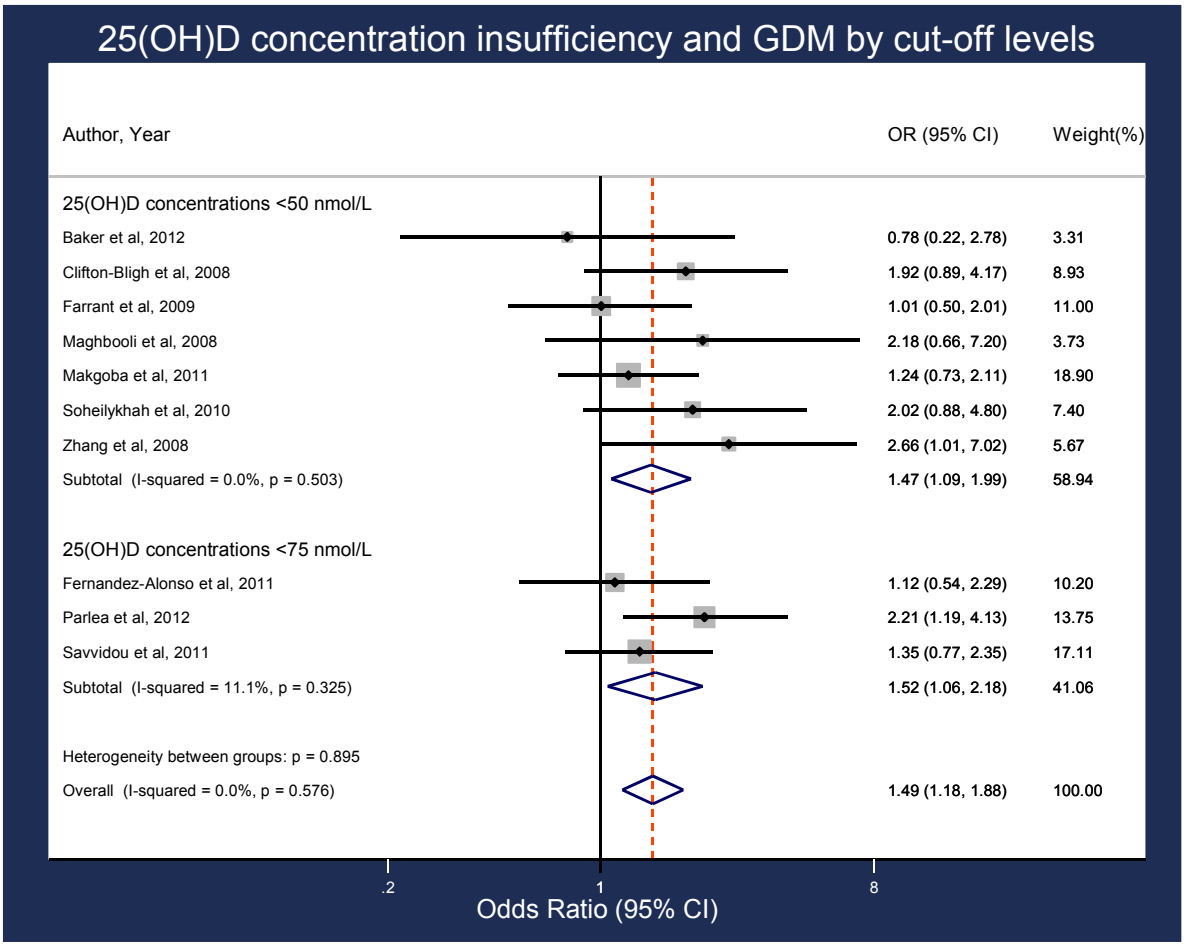
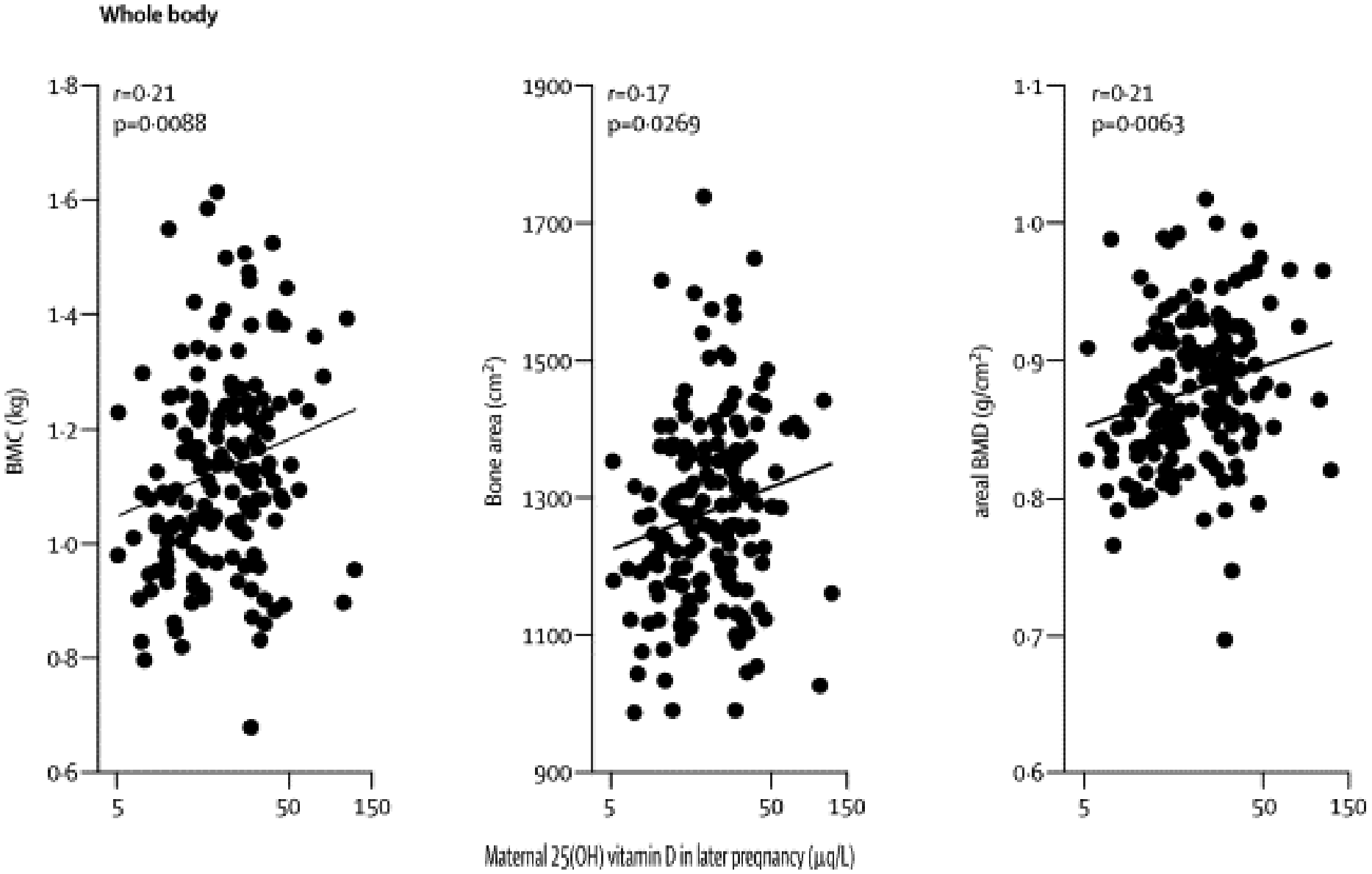

25(OH)D concentration insufficiency and GDM by cut-off levels
Author, Year
OR (95% CI)
Weight(%)
25(OH)D concentrations <50 nmol/L
Baker et al, 2012
0.78 (0.22, 2.78)
3.31
Clifton-Bligh et al, 2008
1.92 (0.89, 4.17)
8.93
Farrant et al, 2009
1.01 (0.50, 2.01)
11.00
Maghbooli et al, 2008
2.18 (0.66, 7.20)
3.73
Makgoba et al, 2011
1.24 (0.73, 2.11)
18.90
Soheilykhah et al, 2010
2.02 (0.88, 4.80)
7.40
Zhang et al, 2008
2.66 (1.01, 7.02)
5.67
Subtotal (I-squared = 0.0%, p = 0.503)
1.47 (1.09, 1.99)
58.94
25(OH)D concentrations <75 nmol/L
Fernandez-Alonso et al, 2011
1.12 (0.54, 2.29)
10.20
Parlea et al, 2012
2.21 (1.19, 4.13)
13.75
Savvidou et al, 2011
1.35 (0.77, 2.35)
17.11
Subtotal (I-squared = 11.1%, p = 0.325)
1.52 (1.06, 2.18)
41.06
Heterogeneity between groups: p = 0.895
Overall (I-squared = 0.0%, p = 0.576)
1.49 (1.18, 1.88)
100.00
.2
1
8
Odds Ratio (95% CI)

Figure 3


Whole body
r=0·21
p=0·0088
BMC (kg)
1·8
1·6
1·4
1·2
1·0
0·8
0·6
5
50
150
r=0·17
p=0·0269
Bone area (cm²)
1900
1700
1500
1300
1100
900
r=0·21
p=0·0063
areal BMD (g/cm²)
1·1
1·0
0·9
0·8
0·7
0·6
Maternal 25(OH) vitamin D in later pregnancy (µg/L)