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META-ANALYSIS

Vitamin D and high blood pressure: causal association or epiphenomenon?

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Abstract High plasma levels of vitamin D are associated with a reduced risk of high blood pressure, but whether this association is causal remains to be ascertained. We performed a meta-analysis of randomized clinical trials, to examine the effect of vitamin D supplementation on both systolic blood pressure (SBP) and diastolic blood pressure (DBP) and supplemented these results with a Mendelian randomization analysis to investigate the causal relationship between vitamin D status (25-hydroxyvitamin D [25(OH)D]) and BP. Pooled random effects meta-analysis of weighted mean differences across 16 trials of vitamin D supplementation showed a non-significant reduction in SBP (−0.94, 95 % CI −2.98, 1.10 mmHg) and DBP (−0.52, 95 % CI −1.18, 0.14 mmHg), with evidence of heterogeneity ($I^2 = 67.9$ %, $P < 0.001$) and publication bias ($P = 0.02$) among trials of SBP. There was a significant reduction in DBP (−1.31, 95 % CI −2.28, −0.34 mmHg, $P = 0.01$) in participants with pre-existing cardiometabolic disease. Variants at three published loci (*GC*, *DHCR7*, *CYP2R1*, and *CYP24A1*) for 25(OH)D, were not significantly associated with BP, but rs6013897 in *CYP24A1* gene region had nominally significant associations with both SBP and DBP ($P < 0.05$). Evidence from the associations of the genetic variants with the risk of vitamin D deficiency (defined as a 25(OH)D level < 50 nmol/L) and BP showed that the causal effects of a doubling of genetically-elevated risk of vitamin D deficiency were 0.14 mmHg (95 % CI −0.19, 0.47, $P = 0.42$), and 0.12 mmHg (95 % CI −0.09, 0.33, $P = 0.25$) on SBP and DBP respectively. Additional evidence from genetic data are directionally consistent with clinical trial data, though underpowered to reliably demonstrate a strong causal effect of vitamin D status on BP. Further investigation may be warranted.

Keywords Vitamin D · Blood pressure · Clinical trial · Meta-analysis · Single nucleotide polymorphism · Mendelian randomisation

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Introduction

Vitamin D is pivotal in regulating calcium and bone homeostasis [1] and is associated with several biological processes, including modulation of blood pressure (BP). Amongst the proposed mechanistic pathways for the development of high BP, vitamin D inhibits the renin-angiotensin-aldosterone system [2], alters proliferation of vascular endothelial smooth muscle cells [3], and is essential for insulin secretion [4]. Several prospective studies and meta-analyses have consistently shown an inverse association between vitamin D status (as measured by 25-hydroxyvitamin D [25(OH)D]) and BP [5, 6]. As observational epidemiological studies are beset by residual confounding and reverse causation, it is difficult to infer causality from these findings. From a public health perspective, it is crucial to address this issue as the therapeutic modification of circulating vitamin D levels can be achieved through supplementation or therapy, more so as

both vitamin D deficiency and high BP have risen to pandemic proportions, individually affecting over 1 billion people worldwide [7, 8]. High BP has been shown to be continuously and linearly associated with cardiovascular risk over several decades ago [9] and is the most common modifiable risk factor for cardiovascular disease (CVD) [10], which represents a worldwide epidemic and is the leading cause of death globally [11]. Vitamin D deficiency may increase CVD risk by activating an inflammatory cascade, which results in endothelial dysfunction and increased arterial stiffness, both of which contribute to high BP and are risk markers for CVD risk [12–14].

Randomized clinical trials (RCTs) of vitamin D supplementation offer the highest clinical evidence for establishing whether vitamin D deficiency is causally related to high BP. However, findings from previous trials have failed to demonstrate significant reductions in BP and results of prior meta-analyses have been inconclusive [15–17]. In the absence of such trials, Mendelian Randomization (MR) [18] studies utilizing genetic variants which specifically alter levels of vitamin D may provide another route to help judge the causal relevance of vitamin D to BP. An MR study utilises the fact that since the presence of particular genetic variants or alleles are randomly allocated at conception (gamete formation) and such allocation is expected to be independent of any behavioural and environmental factors, the associations of such variants with levels of the exposure (in this case vitamin D status) or with disease outcome (in this case high BP) are not likely to be affected by potential confounding or reverse causation [19]. If lower vitamin D status is causally related to high BP, then a genetic variant associated with lower 25(OH)D levels should be associated with a higher risk of high BP. Since the last previous review [17], several interventions studies evaluating the effects of vitamin D supplementation on BP outcomes have been published and their results have been inconsistent. Against this background, we aimed to assess the potential causal relevance of vitamin D deficiency to high BP by updating the evidence on the effect of vitamin D supplementation on circulating levels of 25(OH)D and its impact on both systolic and diastolic BP. We have also supplemented this with evidence from published genetic association studies of 25(OH)D levels and BP and applied a MR approach [20] using summarized published data on four genetic variants of vitamin D status [as measured by 25(OH)D].

Methods

Data sources and study selection

This review was conducted using a predefined protocol and in accordance with PRISMA guidelines [21] (Appendix 1). We systematically searched MEDLINE, EMBASE, Web of Science, and Cochrane Central Register of Controlled Trials from inception up to November, 2013 for RCTs of vitamin D supplementation (cholecalciferol [vitamin D3] or ergocalciferol [vitamin D2]) on systolic blood pressure (SBP) and diastolic blood pressure (DBP). The searches combined terms for vitamin D and blood pressure and no language restrictions were imposed (Appendix 2). Additional studies were sought from the reference lists of recovered articles and previous review articles, by hand searching of relevant journals, and by correspondence with authors of included studies. We included only RCTs that aimed to study the effects of oral vitamin D supplementation alone. Studies in which the intervention was calcitriol or one of its analogues and those with participants not receiving an intervention to raise their vitamin D [25(OH)D] levels were excluded. The primary outcome was the difference in office or ambulatory SBP and DBP among treatment and control groups compared with baseline BPs. Additionally, lead single nucleotide polymorphisms (SNPs) exclusively associated with circulating levels of 25(OH)D were identified by searching the original publications of genome-wide association studies (GWASs) for vitamin D that have been indexed by the National Human Genome Research Institute (NHGRI) GWAS catalogue [22]. Single nucleotide polymorphisms were considered for inclusion if they were associated with levels of 25(OH)D at genome-wide significant levels ($P < 5 \times 10^{-8}$ unless otherwise specified) and were uncorrelated.

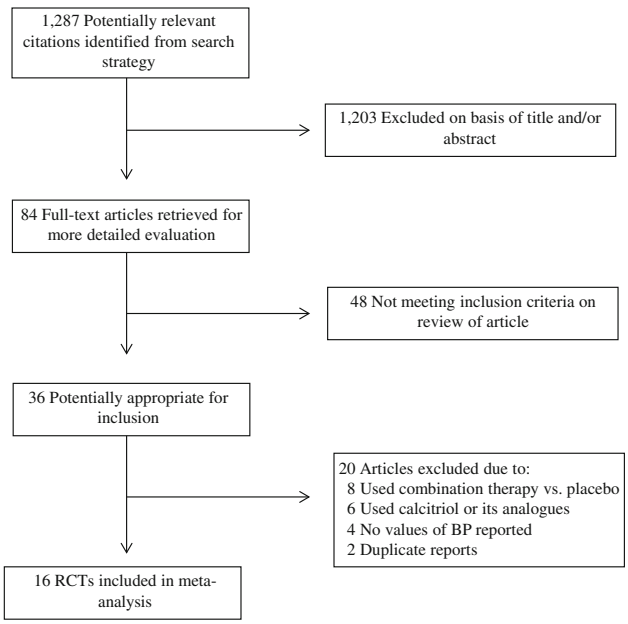


Fig. 1 Trial selection flow diagram. *BP* blood pressure, *RCT* randomized clinical trial

Table 1 Randomized controlled trials of the effect of vitamin D supplementation on 25(OH)D levels and blood pressure

Lead author, Year (Reference)	Location	Baseline age range (years)	Participants	Participants (n)	Mean baseline 25(OH) D concentration in intervention arm (ng/mL)	Mean final 25(OH) D concentration in intervention arm (ng/mL)	Interventions	Participants (n) intervention/ placebo	Study duration	Primary outcome	Effect of vitamin D vs. placebo
Scragg, 1995 [32]	UK	63–76	Elderly	189	13.0	20.2	Cholecalciferol 2.5 mg single dose	95/94	5 weeks	Blood pressure	↔ SBP change ↔ DBP change
Pfeifer, 2001 [33]	Germany	≥70	Healthy	148	10.3	26.0	Cholecalciferol 800 IU daily; 1,200 mg Ca daily	74/74	8 weeks	Blood pressure	↓ SBP ↔ DBP change
Schleithoff, 2006 [34]	Germany	55*	CHF patients	123	14.4	41.2	Cholecalciferol 2,000 IU daily; 500 mg Ca daily	61/62	9 months	Survival rates; biochemical variables	↔ SBP change ↔ DBP change
Nagpal, 2009 [35]	India	≥35	Healthy	71	14.6	28.7	Cholecalciferol 120,000 IU every other week	35/36	6 weeks	Insulin sensitivity	↔ SBP 0.60 change ↔ DBP change
Sugden, 2008 [3]	UK	64*	Type 2 diabetes	34	16.1	25.3	Ergocalciferol 100,000 IU once	17/17	8 weeks	Endothelial function and blood pressure	↓ SBP ↔ DBP change
Jorde, 2010 [36]	Norway	21–70	Healthy overweight or obese	438	23.2	40.5	Cholecalciferol 40,000 IU weekly; 20,000 IU weekly 500 mg Ca daily	150/139/149	12 months	Blood pressure and lipids	↑ SBP ↔ DBP change
Witham, 2010 [37]	UK	≥18	Type 2 diabetes	61	19.2	31.7	Cholecalciferol 200,000 or 100,000 IU as a single dose	20/19/22	16 weeks	Endothelial function	↔ SBP change ↔ DBP change
Shab-Bidar, 2011 [38]	Iran	25–70	Type 2 diabetes	100	15.4	28.8	Cholecalciferol 1,000 IU daily; 340 mg daily	50/50	12 weeks	Glycemic status, lipids, and endothelial biomarkers	↔ SBP change ↔ DBP change
Larsen, 2012 [39]	Denmark	61*	Hypertensive	112	23.0	44.0	Cholecalciferol 3,000 IU daily	55/57	20 weeks	24-hour SBP	↔ SBP change ↔ DBP change

Table 1 continued

Lead author, Year (Reference)	Location	Baseline age range (years)	Participants	Participants (n)	Mean baseline 25(OH) D concentration in intervention arm (ng/mL)	Mean final 25(OH) D concentration in intervention arm (ng/mL)	Interventions	Participants (n) intervention/ placebo	Study duration	Primary outcome	Effect of vitamin D vs. placebo
Witham, 2012 [40]	UK	66.9*	History of stroke	56	15.5	21.6	Ergocalciferol 100,000 as a single dose	29/27	8 weeks	Blood pressure and vascular health markers	↔ SBP change ↔ DBP change
Gepner, 2012 [41]	US	63.9*	Post-menopausal women	110	30.3	46.0	Cholecalciferol 2500 IU daily	55/55	4 months	Measures of endothelial function and arterial stiffness	↔ SBP change ↔ DBP change
Wood, 2012 [42]	UK	60–70	Post-menopausal women	305	13.0	30.2	Cholecalciferol 400 or 1,000 IU daily	101/101/102	12 months	Lipid profile, inflammatory markers, and blood pressure	↔ SBP change ↔ DBP change
Forman, 2013 [43]	US	44–59	Healthy	283	15.6	45.9	Cholecalciferol 1,000 IU daily	68/73/70/72	3 months	Blood pressure	↓ SBP ↔ DBP change
Witham, 2013 [44]	UK	≥70	Elderly with isolated systolic hypertension	159	18.0	28.0	Cholecalciferol 100,000 U every 3 months	80/79	3 months	Systolic blood pressure	↔ SBP change
Witham, 2013 [45]	UK	≥18	Healthy South Asian Women	50	10.4	14.4	Cholecalciferol 100,000 U for 8 weeks	25/25	8 weeks	Change in endothelial function	↔ SBP change ↔ DBP change
Wamberg, 2013 [46]	Denmark	18–50	Healthy obese adults with low plasma 25(0H)D levels	52	13.2	44.2	Cholecalciferol 7,000 IU daily	26/26	26 weeks	Fat distribution and obesity complications	↔ SBP change ↔ DBP change

* Mean baseline age; Ca, Calcium; CHF, congestive heart failure; DBP, diastolic blood pressure; IU, international unit; 25(OH)D, plasma or serum 25-hydroxyvitamin D; SBP, systolic blood pressure; U.K., United Kingdom; U.S, United States; -, Not available; ↔, no difference (no statistical significance); ↓, decreased (statistical significance); ↑, increased (statistical significance)

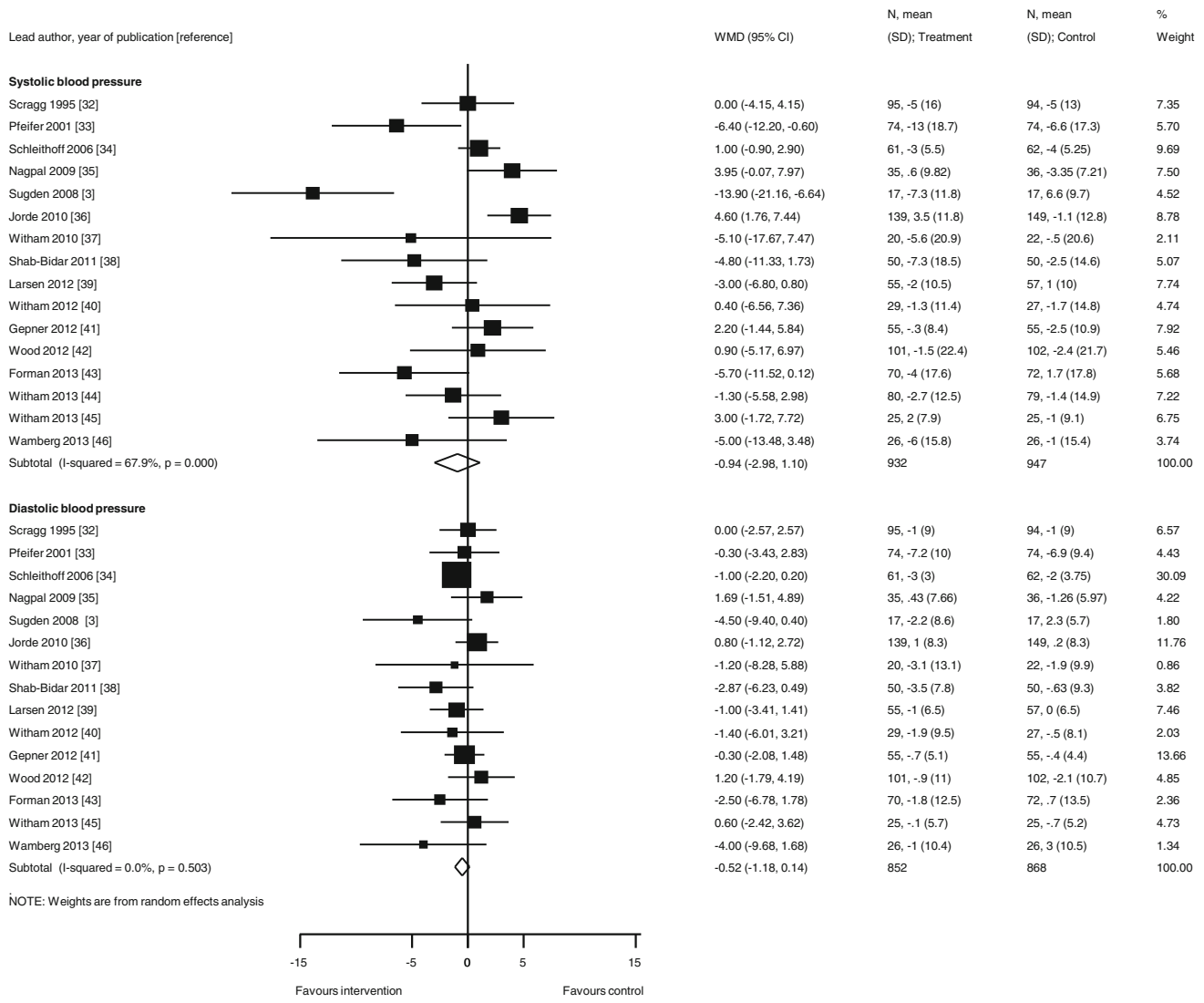


Fig. 2 Meta-analysis of effects of vitamin D supplementation on blood pressure in eligible randomized controlled trials. The summary estimates presented were calculated using random effects models; *CI* confidence interval (*bars*), *SD* standard deviation, *WMD* weighted mean difference

Data extraction and quality assessment

Data on the following characteristics were extracted independently by two investigators who used standardised protocols: number of participants, sampling population, geographical location (defined as Europe, North America, and the Asia–Pacific region); age range of participants at baseline, gender; duration of intervention; type and formulation of vitamin D supplementation, daily dose of supplementations, composition of placebo, and mean BP and standard deviation, or the mean difference were abstracted. Discrepancies were resolved by discussion and by adjudication of a third reviewer. The Cochrane Collaboration's tool for assessing risk of bias was used to assess the validity of the trials. This tool uses the following methodological features most relevant to the control of bias: randomization, random allocation concealment, masking of treatment allocation and outcome assessments, incomplete outcome data, selective reporting, and other bias [23]. For each individual domain, studies were classified into low, unclear and high risk of bias.

Data synthesis and analysis

Random-effects models were used to pool the weighted mean differences (WMDs) across trials. Heterogeneity was assessed with the I^2 statistic, with $I^2 > 50$ % considered to be important. Study-level characteristics including geographical location, gender, number of participants, baseline population (presence or absence of pre-existing

Table 2 Subgroup analysis of vitamin D supplements and systolic blood pressure

Group	No. of participants intervention/control	Weighted mean difference (95 % CI)	*P* value	Heterogeneity (I^2 %)	*P*-value for meta-regression
Location					
Europe	722/734	−1.17 (−3.59, 1.25)	0.34	69.5	0.90
North America	125/127	−1.41 (−9.12, 6.30)	0.72	80.3	
Asia	85/86	−0.03 (−8.57, 8.51)	0.99	80.0	
Gender					
Female	255/256	0.26 (−3.60, 4.12)	0.89	59.0	0.29
Male	35/36	3.95 (−0.07, 7.97)	0.05	–	
Mixed	642/655	−2.05 (−4.71, 0.60)	0.13	72.0	
Number of participants					
≥120 participants	620/632	−0.36 (−2.97, 2.26)	0.79	67.9	0.66
<120 participants	312/315	−1.68 (−5.10, 1.75)	0.34	70.4	
Baseline population					
Healthy	700/712	0.30 (−2.07, 2.67)	0.81	62.2	0.12
Pre-existing cardiometabolic disease	232/235	−3.53 (−7.69, 0.63)	0.10	74.0	
Intervention duration (weeks)					
≥15	457/473	0.73 (−1.62, 3.07)	0.54	55.7	0.23
<15	475/474	−2.27 (−5.57, 1.03)	0.18	71.6	
Intervention dose (IU/day)					
<2,000	396/396	−2.94 (−6.58, 0.71)	0.11	63.5	0.11
≥2,000	536/551	0.56 (−1.69, 2.80)	0.63	65.2	
Intervention type					
Cholecalciferol	886/903	−0.19 (−2.03, 1.66)	0.84	58.9	0.07
Ergocalciferol	46/44	−6.71 (−20.72, 7.30)	0.35	87.1	

cardiometabolic disease), duration of intervention, daily dose of supplementation, and type of vitamin D supplement were pre-specified as characteristics for assessment of heterogeneity, which was conducted using stratified analysis and random effects meta-regression [24]. We assessed the potential for publication bias through formal tests, namely Begg's funnel plots [25] and Egger's regression symmetry test [26]. The associations of exclusive SNPs identified from GWASs of 25(OH)D levels and other published reports [27–30], were queried with both systolic and diastolic BP using data from the International Consortium of Blood Pressure GWAS (ICBPGWAS), which has been described in detail elsewhere [31]. Briefly, the ICBPGWAS involves a meta-analysis of GWAS data evaluating the associations between 2.5 million genotyped or imputed SNPs and SBP and DBP in 69,395 individuals of European ancestry from 29 studies. Mendelian randomization analyses were conducted using a likelihood-based method for combining summarized genetic association estimates [20] into single estimates of the causal effects of vitamin D status on SBP and DBP. All analyses were conducted using Stata version 12 (Stata Corp, College Station, Texas) and R version 2.15.3 (R Foundation, Vienna, Austria).

Results

Figure 1 shows the number of studies assessed and excluded through the stages of the meta-analysis. A total of 16 trials (comprising 1,879 participants) reported the effect of vitamin D supplementation on SBP, of which 15 reported on DBP [3, 32–46]. Duration of vitamin D supplementation varied from 5 weeks to 12 months. Risk of bias assessment in each trial is reported in Appendix 3. All trials had low risk of bias for the random sequence generation, blinding of both participants and personnel, and the selective reporting domains. One trial had an unclear risk of bias for allocation concealment and another had a high risk of bias. Eleven trials had an unclear risk of bias for blinding of outcome assessments. One trial had a high risk of bias for incomplete outcome data and risk of other bias was unclear in five trials. There was considerable variability in study populations which included healthy participants as well as participants with pre-existing conditions such as diabetes, hypertension, and cardiovascular disease. All trials used vitamin D supplementation of more than 600 IU per day (which is the US Institute of Health Recommended Dietary Allowance [47]), with the doses varying from 800 to 8,571 IU per day. Comparing follow-up with baseline

Table 3 Subgroup analysis of vitamin D supplements and diastolic blood pressure

Group	No. of participants intervention/control	Weighted mean difference (95 % CI)	*P*-value	Heterogeneity (I^2 %)	*P*-value for meta-regression
Location					
Europe	642/655	−0.50 (−1.26, 0.25)	0.19	0.0	0.98
North America	125/127	−0.63 (−2.27, 1.02)	0.46	0.0	
Asia	85/86	−0.56 (−5.03, 3.91)	0.81	73.0	
Gender					
Female	255/256	0.12 (−1.13, 1.37)	0.85	0.0	0.15
Male	35/36	1.69 (−1.51, 4.89)	0.30	–	
Mixed	562/576	−0.92 (−1.71, −0.12)	0.02	0.0	
Number of participants					
≥120 participants	540/553	−0.37 (−1.22, 0.48)	0.40	0.0	0.58
<120 participants	312/315	−0.78 (−1.86, 0.31)	0.16	4.8	
Baseline population					
Healthy	620/633	0.16 (−0.74, 1.05)	0.73	0.0	0.03
Pre-existing cardiometabolic disease	232/235	−1.31 (−2.28, −0.34)	0.01	0.0	
Intervention duration (weeks)					
≥15	457/473	−0.47 (−1.26, 0.32)	0.24	0.0	0.80
<15	395/395	−0.69 (−1.97, 0.60)	0.30	11.0	
Intervention dose (IU/day)					
<2,000	316/317	−0.72 (−2.15, 0.72)	0.33	5.4	0.77
≥2,000	536/551	−0.47 (−1.22, 0.28)	0.22	0.0	
Intervention type					
Cholecalciferol	806/824	−0.43 (−1.10, 0.24)	0.21	0.0	0.16
Ergocalciferol	46/44	−2.86 (−6.22, 0.51)	0.10	0.0	

Table 4 Associations of identified vitamin D polymorphisms with 25(OH)D levels and blood pressure

Chromosome	Gene	Lead SNP	% change in 25(OH)D per effect allele(95% CI)	% change in variation in 25(OH)D levels	Association with SBP (*P*-value)	Association with DBP (*P*-value)
4	*GC*	rs2282679	−8.5 (−9.1 to −7.8)	1.18	0.467	0.640
11	*DHCR7*	rs12785878	−3.7 (−4.3 to −3.1)	0.35	0.703	0.121
11	*CYP2R1*	rs10741657	−3.1 (−3.7 to −2.6)	0.21	0.998	0.587
20	*CYP24A1*	rs6013897	−1.9 (−2.5 to −1.2)	0.07	0.045	0.023

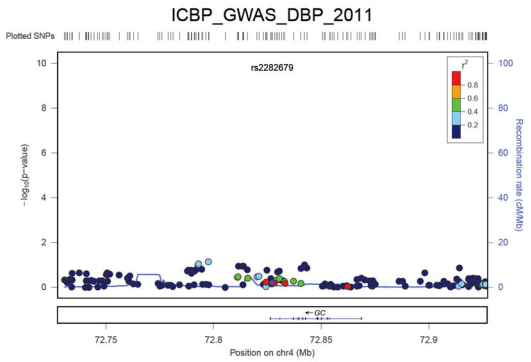
DBP diastolic blood pressure, *SNP* single nucleotide polymorphisms, *SBP* systolic blood pressure, *25(OH)D* 25-hydroxyvitamin D

assessment, circulating levels of 25(OH)D increased substantially in the intervention arms in all the included trials (Table 1).

Effect of vitamin D supplementation on blood pressure

In pooled random effects meta-analysis of WMDs across eligible trials, vitamin D supplementation showed a non-significant reduction in SBP (−0.94, 95 % CI −2.98, 1.10 mmHg, $P = 0.37$; $I^2 = 67.9$ %, $P_{\text{for heterogeneity}} < 0.001$) and DBP (−0.52, 95 % CI −1.18, 0.14 mmHg, $P = 0.12$; $I^2 = 0.0$ %, $P_{\text{for heterogeneity}} = 0.50$) (Fig. 2). The heterogeneity among trials for SBP was not explained by differences in several study level characteristics (Table 2). In sensitivity analysis, we excluded the study by Sugden et al. [3] as it reported a significant decrease in SBP on vitamin D supplementation by about 14 mmHg compared with placebo, which may have unduly influenced our findings. The pooled random effects meta-analysis of WMDs excluding this study also showed a non-significant reduction in SBP (−0.13, 95 % CI −1.89, 1.63 mmHg, $P = 0.88$; $I^2 = 55.8$ %, $P_{\text{for heterogeneity}} = 0.004$). Subgroup analysis of trials of DBP showed a significant reduction in DBP with vitamin D supplementation in six trials involving participants with pre-existing cardiometabolic disease (−1.31, 95 % CI −2.28, −0.34 mmHg, $P = 0.01$; $I^2 = 0.0$ %, $P_{\text{for heterogeneity}} = 0.03$) (Table 3). Egger's test for publication bias among trials for SBP was

GC



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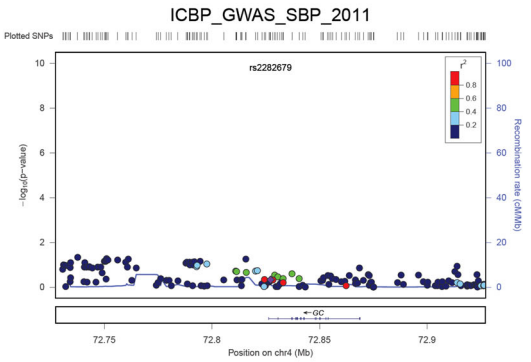
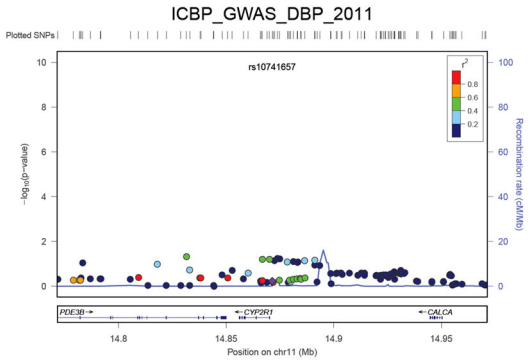


CYP2R1



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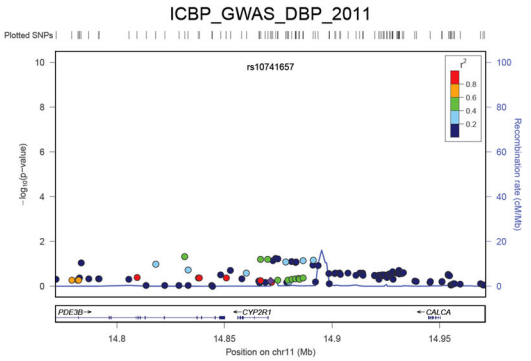
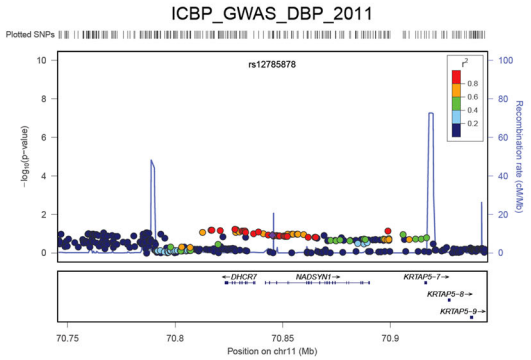


DHCR7



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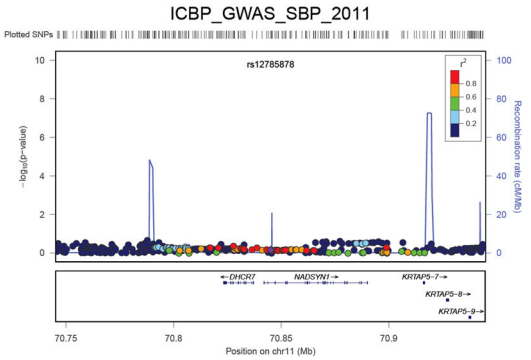
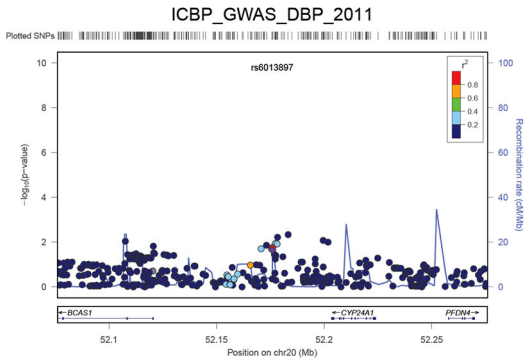
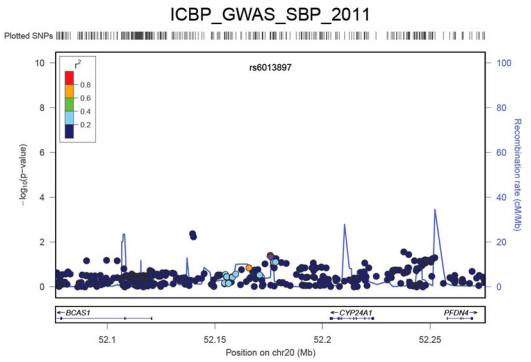


CYP24A1



CYP24A1



◀**Fig. 3** Regional association plots of vitamin D related gene regions. Each panel spans 200 kb around the published vitamin D SNP in the region, which is highlighted with a purple diamond. The SNPs are coloured according to their linkage disequilibrium with the top variant based on the CEU Hap Map population (http://www.hapmap.org). Gene transcripts are annotated in the lower box. The association results for blood pressure were taken from the International Consortium of Blood Pressure Genome Wide Association Studies (ICBPGWAS)

significant ($P = 0.02$), consistent with observed funnel plot asymmetry.

Evidence from genome wide association studies

We identified genome-wide significant variants at *GC*, *DHCR7*, and *CYP2R1*and *CYP24A1* loci which together explained up to 1–4 % of the variation in 25(OH)D levels (Table 4). Regional association plots within 200 kb window of these vitamin D SNPs showed lack of significant associations (at a Bonferroni corrected $P = 1 \times 10^{-4}$ threshold; Fig. 3) with BP. However, the associations of rs6013897 on chromosome 20q13 in *CYP24A1* with SBP and DBP were nominally significant ($P < 0.05$).

Mendelian randomization analysis using published data

Estimates using genetic variants for the causal effect of vitamin D on BP were −0.11 mmHg (95 % CI −0.31, 0.09, $P = 0.27$) for systolic BP and −0.10 mmHg (95 % CI −0.22, 0.03, $P = 0.13$) for diastolic BP, based on a 10 % increase in 25(OH)D levels (Fig. 4). Using published data on the association of the genetic variants with the risk of vitamin D deficiency [defined as a 25(OH)D level < 50 nmol/L] and BP, we estimated the change in BP for an increase in the genetic component of the risk of vitamin D deficiency. The causal effect of a doubling of genetically-determined risk of vitamin D deficiency on systolic BP was 0.14 mmHg (95 % CI −0.19, 0.47, $P = 0.42$), and on diastolic BP was 0.12 mmHg (95 % CI −0.09, 0.33, $P = 0.25$).

Comment

Pooled results of the available clinical evidence were directionally suggestive of a reduction in both systolic and diastolic BP with vitamin D supplementation, but lacked statistical significance. Subgroup analysis of trials of DBP however, showed a significant reduction in DBP (by 1.3 mmHg) with vitamin D supplementation in participants with pre-existing cardiometabolic disease. In the published literature, [27–30] we identified several genome-wide significant variants at 4 unique loci, involved in 25(OH)D synthesis (*DHCR7*, *CYP2R1*) and metabolism (*GC*, *CYP24A1*), which have been suggested to be exclusively associated with vitamin D pathways. The variants together, explained up to 1–4 % of the variation in 25(OH)D levels. Utilizing data from ICBP GWAS [31], we demonstrated that vitamin D SNPs had small effects on BP but lacked statistical significance, except for one variant rs6013897 in *CYP24A1* gene region. All SNPs showed directionally concordant associations with 25(OH)D levels and BP, which were consistent with the clinical trial results. However, the causal effect estimates based on the available genetic evidence did not achieve statistical significance.

The current results argue against a strong causal role of vitamin D pathways in the aetiology of high BP, but cannot rule out a weak causal effect. There was evidence of a significant reduction in DBP in participants with pre-existing cardiometabolic disease on vitamin D supplementation. Several plausible reasons may explain this observation. Whiles, optimal vitamin D status is an excellent marker of good health [48], suboptimal vitamin D status may reflect chronic illnesses [15] such as cardiometabolic diseases. Though our results (Table 1) were not indicative of low baseline vitamin D status [25(OH)D levels] in participants with pre-existing cardiometabolic diseases, there is data to suggest that significant improvements in cardiometabolic outcomes (such as reductions in BP) with vitamin D supplement use may be seen only among those with vitamin D deficiency [15]. Further data are necessary to adjudicate this observation. The inconsistent results reported by the clinical trials have been attributed to several reasons as suggested by previous reviews [15, 16]. These include limited sample sizes to detect incremental differences in BP, heterogeneity in study populations, short follow-up periods, and the fact that majority of trials reported results from post hoc sub-group analyses. If there is a causal relationship between vitamin D deficiency and high BP, then establishing this may require carefully designed RCTs with large-sample sizes and long follow-up durations. The on-going VITamin D and OmegA-3 TriaL (VITAL), with over 20,000 healthy participants randomized to daily dietary supplements of vitamin D3 or omega-3 fatty acids [49] may offer useful insights. Whiles we await results of this trial, MR investigations using individual-level data may provide another efficient method to help establish causality. Such MR investigations have been used to investigate the causal relevance of risk markers, such as C-reactive protein and lipoprotein (a), to risk of coronary heart disease in the absence of interventions that specifically modify levels of these risk markers [50, 51]. Collective evidence from several studies demonstrates that variability in 25(OH)D levels is explained by both genetic and environmental factors. Heritability of 25(OH)D levels has been estimated to be as high as 80 % [52], and given this level of heritability, recent advances have been made in identifying

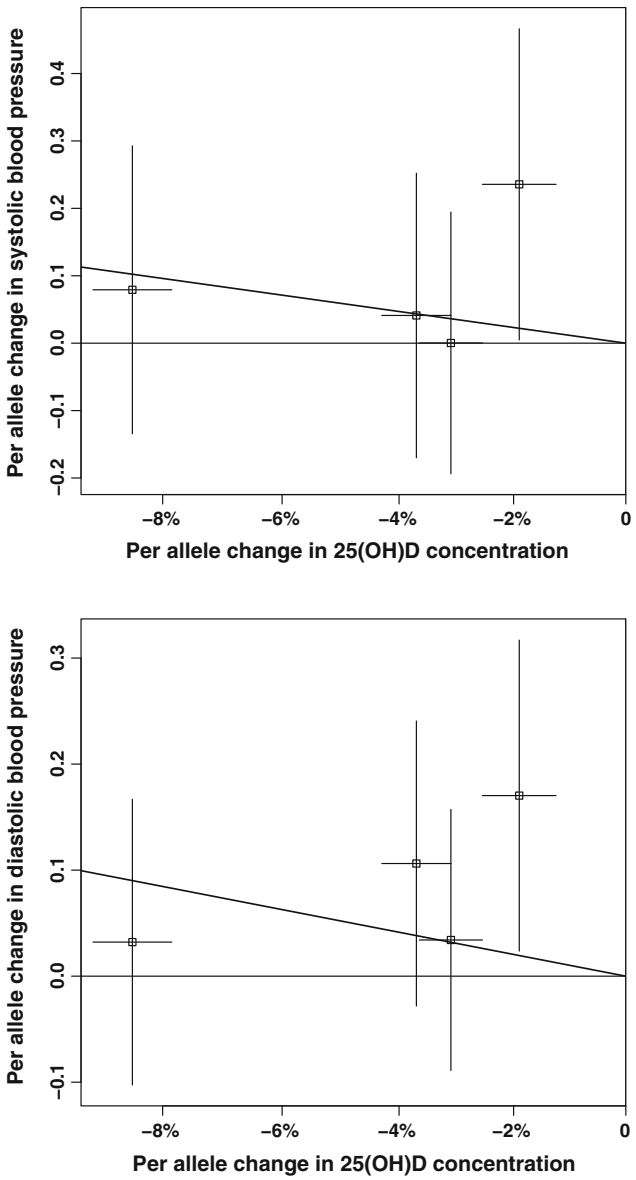


Fig. 4 Estimated effects on blood pressure change plotted against estimated effects on serum vitamin D levels, for four SNPs associated with vitamin D. SNP, single nucleotide polymorphism; Vertical and horizontal solid black lines show 95 % confidence intervals (CIs) for each individual SNP. Estimates of casual effect of vitamin D on blood pressure, by using a likelihood-based method for combining summarized genetic association estimates using all SNPs, are represented by solid black line with gradient. Using all SNPs, multi-SNP risk score analyses identified weak protective causal effects of vitamin D on blood pressure levels, −0.11 mmHg (95 % CI −0.31, 0.09, $P = 0.27$) for systolic blood pressure and −0.10 mmHg (95 % CI −0.22, 0.03, $P = 0.13$) for diastolic blood pressure, based on a 10 % increase in 25(OH)D levels

several genetic determinants of 25(OH)D levels. The four genes identified in the present analysis play important roles in the vitamin D metabolic pathway. *DHCR7* and *CYP2R1* function upstream of the 25(OH)D synthesis pathway, whiles *GC* and *CYP24A1* function downstream of the metabolism pathway [29]. The *DHCR7* gene encodes 7-dehydrocholesterol reductase, the enzyme that converts 7-dehydrocholesterol to cholesterol. *CYP2R1* is known to encode the enzyme that catalyzes the synthesis of 25(OH)D in the liver [53]. *GC*, the group-specific component gene (located on chromosome 4q12-q13), which encodes vitamin D-binding protein, harbors a set of SNPs which are associated with circulating levels of 25(OH)D levels at genome-wide significance. The strongest association with 25(OH)D levels has consistently been demonstrated for rs2282679 [28, 29]. The *CYP24A*1 gene encodes the enzyme which plays an important role in calcium homeostasis and the vitamin D endocrine system, where it acts at the initial stage of 25(OH)D catabolism [54]. Informative MR studies on vitamin D and BP are likely to be feasible given the potential specificity of the associations of these genetic variants with vitamin D. However, given the small fractions of the variances in vitamin D levels explained by these common variants, MR studies would require large sample sizes ($\sim$ 80,000 participants) to have sufficient power to establish causality [29]. Fine mapping and exome sequencing of the common gene regions involved in vitamin D pathways may help uncover rarer genetic variations with larger effects on vitamin D levels and may be better instrumental variables for MR.

The strengths and limitations of this study merit careful consideration. This study has provided a comprehensive systematic synthesis of available evidence by including data from different sources, evaluated the impact of vitamin D supplementation for several relevant subgroups in a consistent way, and has utilized genetic data to assess the causal relevance of vitamin D to high BP. The majority of trials included in this review appear to have low risk of bias; however, the current findings should be interpreted with some caution, owing to the potential differences in design and population characteristics of each trial. There was substantial heterogeneity among trials of SBP. Given this, it was debatable whether pooled estimates should be presented rather than reporting estimates in relevant subgroups, as the presence of heterogeneity makes pooling of risk estimates data somewhat controversial. We however systematically explored possible sources of heterogeneity using stratified analyses, metaregression techniques, and sensitivity analyses and also presented pooled estimates for the relevant subgroups. In general, there was a consistent trend of reduction in BP in subgroups assessed. Our findings for studies of SBP may have been over-estimated somewhat due to preferential publication of extreme findings, or, analogously, by selective reporting of striking results. Furthermore, in the current analyses, we employed an MR approach using summarized published data on multiple genetic variants for 25(OH)D levels, as individual-level data on large numbers of participants was unavailable. Though it has been

reported that causal estimates from summarized data are almost as precise as those obtained from individual-level data [20], there are several limitations to the use of summarized data. These include inability to (1) fully assess instrumental variable assumptions; (2) address population stratification; (3) test for the attenuation of genetic associations with the outcome on adjustment for the exposure of interest; and (4) assess parametric assumptions required by instrumental variable methods for effect estimation [20]. The results should therefore be interpreted in context of the limitations available.

In conclusion, pooled results of relevant clinical trials provide non-significant reductions in both SBP and DBP on vitamin D supplementation, with evidence of considerable heterogeneity and publication bias among studies of SBP. Subgroup analysis however showed evidence of a significant reduction in DBP in participants with pre-existing cardiometabolic disease. Additional evidence from genetic data are directionally consistent with clinical trial data, though underpowered to reliably demonstrate a strong causal effect of vitamin D status on BP. Since vitamin D remains a promising though unproven strategy in the prevention of high BP (hypertension), further evaluation may be warranted to assess any causal association. Further research is also warranted to assess the evidence with more refined indices of BP such as heart rate, pulse pressure, and cardiac output.

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Appendix 1

See Table 5.

Table 5 PRISMA 2009 check-list

Section/topic	Item No	Checklist item	Reported on page No
Title			
Title	1	Identify the report as a systematic review, meta-analysis, or both	First page
Abstract			
Structured summary	2	Provide a structured summary including, as applicable, background, objectives, data sources, study eligibility criteria, participants, interventions, study appraisal and synthesis methods, results, limitations, conclusions and implications of key findings, systematic review registration number	First page
Introduction			
Rationale	3	Describe the rationale for the review in the context of what is already known	First and second pages
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS)	Second page
Methods			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (such as web address), and, if available, provide registration information including registration number	Not applicable
Eligibility criteria	6	Specify study characteristics (such as PICOS, length of follow-up) and report characteristics (such as years considered, language, publication status) used as criteria for eligibility, giving rationale	Second page
Information sources	7	Describe all information sources (such as databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched	Second page
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated	Appendix 2
Study selection	9	State the process for selecting studies (that is, screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis)	Second to fifth pages
Data collection process	10	Describe method of data extraction from reports (such as piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators	Fifth page
Data items	11	List and define all variables for which data were sought (such as PICOS, funding sources) and any assumptions and simplifications made	Fifth page

Table 5 continued

Section/topic	Item No	Checklist item	Reported on page No
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis	Fifth page
Summary measures	13	State the principal summary measures (such as risk ratio, difference in means).	Fifth page
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (such as I^2 statistic) for each meta-analysis	Fifth and sixth pages
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (such as publication bias, selective reporting within studies)	Fifth and sixth pages
Additional analyses	16	Describe methods of additional analyses (such as sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified	Fifth and sixth pages
Results			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram	Sixth page, Fig. 1
Study characteristics	18	For each study, present characteristics for which data were extracted (such as study size, PICOS, follow-up period) and provide the citations	Sixth and eighth pages, Table 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome-level assessment (see item 12).	Sixth page, Appendix 3
Results of individual studies	20	For all outcomes considered (benefits or harms), present for each study (a) simple summary data for each intervention group and (b) effect estimates and confidence intervals, ideally with a forest plot	Seventh page, Fig. 2
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency	Seventh page
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see item 15)	Sixth page, Appendix 3
Additional analysis	23	Give results of additional analyses, if done (such as sensitivity or subgroup analyses, meta-regression) (see item 16)	Seventh to ninth pages, Tables 2–3
Discussion			
Summary of evidence	24	Summarise the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (such as health care providers, users, and policy makers)	Ninth page
Limitations	25	Discuss limitations at study and outcome level (such as risk of bias), and at review level (such as incomplete retrieval of identified research, reporting bias)	Tenth page
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research	Eleventh page
Funding			
Funding	27	Describe sources of funding for the systematic review and other support (such as supply of data) and role of funders for the systematic review	None

Appendix 2: Literature search strategy

Relevant studies, published before November 30, 2013 (date last searched), were identified through electronic searches not limited to the English language using MEDLINE, EMBASE, and Cochrane databases. Electronic searches were supplemented by scanning reference lists of articles identified for all relevant studies (including review articles), by hand searching of relevant journals and by correspondence with study investigators. The computer-based searches combined search terms related to vitamin D supplementation and blood pressure without language restriction.

(i) MEDLINE strategy to identify relevant exposures: ("Vitamin D"[Mesh] OR "vitamin d"[All Fields] OR "25-hydroxyvitamin D"[All Fields] OR "25(OH)D"[All Fields] OR "calcidiol"[All Fields] OR "ergocalciferols"[Mesh] OR "ergocalciferols"[All Fields] OR "Vitamin D Supplementation"[Mesh])

(ii) MEDLINE strategy to identify relevant outcomes: ("Hypertension"[Mesh] OR "hypertension"[All Fields] OR "blood pressure"[Mesh])

(iii) MEDLINE strategy to identify relevant population: ("humans"[MeSH Terms])

Parts i, ii and iii were combined using 'AND' to search MEDLINE. Each part was specifically translated for searching alternative databases.

Appendix 3

See Table 6.

Table 6 Risk of bias assessments for included trials

Lead author, Year (Reference)	Random sequence generation	Allocation concealment	Blinding of participants & personnel	Blinding of outcome assessments	Incomplete outcome data	Selective reporting	Other bias
Scragg, 1995 [32]	Low	Low	Low	Unknown	Low	Low	Unknown
Pfeifer, 2001 [33]	Low	Low	Low	Unknown	Low	Low	Unknown
Schleithoff, 2006 [34]	Low	Unknown	Low	Unknown	Low	Low	Unknown
Nagpal, 2009 [35]	Low	Low	Low	Unknown	Low	Low	Low
Sugden, 2008 [3]	Low	Low	Low	Unknown	Low	Low	Unknown
Jorde, 2010 [36]	Low	Low	Low	Low	Low	Low	Low
Witham, 2010 [37]	Low	Low	Low	Unknown	Low	Low	Low
Shab-Bidar, 2011 [38]	Low	High	Low	Unknown	High	Low	Unknown
Larsen, 2012 [39]	Low	Low	Low	Low	Low	Low	Low
Witham, 2012 [40]	Low	Low	Low	Low	Low	Low	Low
Gepner, 2012 [41]	Low	Low	Low	Low	Low	Low	Low
Wood, 2012 [42]	Low	Low	Low	Unknown	Low	Low	Low
Forman, 2013 [43]	Low	Low	Low	Unknown	Low	Low	Low
Witham, 2013 [44]	Low	Low	Low	Low	Low	Low	Low
Witham, 2013 [45]	Low	Low	Low	Unknown	Low	Low	Low
Wamberg, 2013 [46]	Low	Low	Low	Unknown	Low	Low	Low

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