Association of vitamin D status with arterial blood pressure and hypertension risk: a mendelian randomisation study


Summary

Background Low plasma 25-hydroxyvitamin D (25[OH]D) concentration is associated with high arterial blood pressure and hypertension risk, but whether this association is causal is unknown. We used a mendelian randomisation approach to test whether 25(OH)D concentration is causally associated with blood pressure and hypertension risk.

Methods In this mendelian randomisation study, we generated an allele score (25(OH)D synthesis score) based on variants of genes that affect 25(OH)D synthesis or substrate availability (CYP2R1 and DHCR7), which we used as a proxy for 25(OH)D concentration. We meta-analysed data for up to 108 173 individuals from 35 studies in the D-CarDia collaboration to investigate associations between the allele score and blood pressure measurements. We complemented these analyses with previously published summary statistics from the International Consortium on Blood Pressure (ICBP), the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium, and the Global Blood Pressure Genetics (Global BPGen) consortium.

Findings In phenotypic analyses (up to n=49 363), increased 25(OH)D concentration was associated with decreased systolic blood pressure (β per 10% increase, −0.12 mm Hg, 95% CI −0.20 to −0.04; p=0.003) and reduced odds of hypertension (odds ratio [OR] 0.98, 95% CI 0.97−0.99; p=0.0003), but not with decreased diastolic blood pressure (β per 10% increase, −0.02 mm Hg, −0.08 to 0.03; p=0.37). In meta-analyses in which we combined data from D-CarDia and the ICBP (n=146 581), after exclusion of overlapping studies, each 25(OH)D-increasing allele of the synthesis score was associated with a change of −0.10 mm Hg in systolic blood pressure (−0.21 to −0.001; p=0.0498) and a change of −0.08 mm Hg in diastolic blood pressure (−0.15 to −0.02; p=0.01). When D-CarDia and consortia data for hypertension were meta-analysed together (n=142 255), the synthesis score was associated with a reduced odds of hypertension (OR per allele, 0.98, 0.96−0.99; p=0.001). In instrumental variable analysis, each 10% increase in genetically instrumented 25(OH)D concentration was associated with a change of −0.29 mm Hg in diastolic blood pressure (−0.52 to −0.07; p=0.01), a change of −0.37 mm Hg in systolic blood pressure (−0.73 to 0.003; p=0.052), and an 8.1% decreased odds of hypertension (OR 0.92, 0.87–0.97; p=0.002).

Interpretation Increased plasma concentrations of 25(OH)D might reduce the risk of hypertension. This finding warrants further investigation in an independent, similarly powered study.

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Introduction Low vitamin D status has been associated with an increased risk of cardiovascular disease and all-cause mortality, and the possible benefits of vitamin D supplementation are being actively investigated and debated.12 In observational studies, low plasma 25-hydroxyvitamin D (calcidiol, 25(OH)D) concentration is associated with an increased risk of hypertension.3
However, few large randomised controlled trials of vitamin D supplementation with primary cardiovascular outcomes have been done, and secondary analyses from other trials have provided little evidence to support an effect of vitamin D supplementation on cardiovascular outcomes.\(^4\) The largest of the randomised controlled studies was the Women’s Health Initiative trial\(^4\) (n=36 282), the results of which did not show any changes in blood pressure or hypertension after 7 years of follow-up.\(^4\) However, the vitamin D dose used in that trial was quite small (400 IU per day), and women in both treatment and placebo groups were allowed to take up to 1000 IU per day of additional open-label vitamin D supplementation. Some evidence for possible effects of vitamin D supplementation on blood pressure has been obtained from randomised controlled trials with higher doses\(^5\) and those investigating individuals with cardiovascular metabolic risk;\(^6\) however, as Elamin and colleagues have previously noted,\(^7\) the quality of the available evidence is "low to moderate at best".

In this study, we explored the possible causal relation between vitamin D status and blood pressure and hypertension using a genetic approach. Mendelian randomisation exploits the fact that individual genotypes are assigned randomly at meiosis, so the effect of genetics on disease is generally unaffected by confounding or reverse causality.\(^8\) Recent genome-wide association studies (GWAS) have identified several variants that affect circulating concentrations of 25(OH)D.\(^9\) If 25(OH)D concentrations are causally involved in determining blood pressure or the risk of hypertension, then the genetic variants that affect circulating concentrations of 25(OH)D could be expected to affect blood pressure and hypertension risk. This assumption seems to be valid for at least two of the genes that affect 25(OH)D, namely CYP2R1 (encoding cytochrome P450, family 2, subfamily R, polypeptide 1) and DHCR7 (encoding 7-dehydrocholesterol reductase). These genes function upstream of 25(OH)D production and affect vitamin D synthesis or substrate availability.\(^10,11\) Two further downstream variants affect 25(OH)D, GC (encoding group-specific component [vitamin D binding protein]) and CYP24A1 (encoding cytochrome P450, family 24, subfamily A, polypeptide 1), but both are known to have pleiotropic effects.\(^12,13\) In this study, we used genetic variants that affect vitamin D synthesis as proxy markers for lifelong differences in vitamin D status to test for a causal association with blood pressure and hypertension.

**Methods**

**Study design and population**

We used a mendelian randomisation approach to investigate the association between genetic variants that affect concentrations of circulating 25(OH)D and blood pressure measurements. We meta-analysed data from 35 studies in the D-CarDia collaboration, with results complemented by previously published summary statistics from other large-scale consortium efforts.\(^14-16\) D-CarDia is a collaboration of studies, consisting of cohorts of European ancestry from Europe and North America, that investigates the association of vitamin D and the risk of cardiovascular disease and related traits.\(^17\) We meta-analysed directly genotyped and imputed single-nucleotide polymorphisms (SNPs) from 31 adult (aged 31–92 years, n=99 582) and four adolescent (aged 10–20 years, n=85 911) cohorts in the D-CarDia collaboration (table 1, figure 1). All participants provided written informed consent, and all participating studies received approval from local research ethics committees. The appendix (pp 2–19) includes descriptions of all the studies included in the analysis.

To further increase the statistical power of our study, we meta-analysed our results in adults with data from the International Consortium for Blood Pressure (ICBP)\(^14\) when examining systolic or diastolic blood pressure as the outcome (n=146 581, after exclusion of overlapping studies; figure 1). At the time of the study, hypertension had not been formally examined as an outcome in the ICBP consortium, and related coefficients were not available. Therefore, we used summary data from Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE; n=29 136)\(^16\) and Global Blood Pressure Genetics (Global BPGen) (n=34 433)\(^16\) consortia when examining hypertension as the outcome (n=142 255 after exclusion of overlapping studies; figure 1).

**Phenotypic measures**

Hypertension was defined as systolic blood pressure of 140 mm Hg or higher, diastolic blood pressure of 90 mm Hg or higher, or current use of antihypertensive drugs. For participants taking antihypertensive drugs, we added 15 mm Hg to systolic and 10 mm Hg to diastolic blood pressure to correct for the effect of the treatment.\(^14\) When examining systolic or diastolic blood pressure as the outcome (n=142 255 after exclusion of overlapping studies; figure 1).

**Selection of SNPs and allele scores**

To create vitamin D allele scores, we selected four vitamin D-related SNPs (DHCR7 rs12785878, CYP2R1 rs12794714, GC rs2282679, and CYP24A1 rs6013897) based on the results of the GWAS by the SUNLIGHT Consortium,\(^13\) with two SNPs in genes located upstream (DHCR7 and CYP2R1) and two downstream (GC and CYP24A1) of the 25(OH)D metabolite.\(^16\) All but one (CYP2R1) were selected as the top hit; for CYP2R1 we used an alternative SNP also identified by the GWAS by the SUNLIGHT Consortium (p=1.84×10⁹ for association with 25(OH)D concentration) because it was a functional variant in moderate linkage disequilibrium (r²=0.41) with the first-stage GWAS top hit rs10741657. The appendix (pp 2–25) includes a detailed description of the genotyping and imputation methods and effect allele frequencies for all the studies included in the meta-analysis.
## Studies in adults

<table>
<thead>
<tr>
<th>Study</th>
<th>Sample size</th>
<th>Men</th>
<th>Women</th>
<th>All participants</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Age (years)</td>
<td>Systolic blood pressure (mm Hg)</td>
<td>Diastolic blood pressure (mm Hg)</td>
<td>Participants with hypertension</td>
</tr>
<tr>
<td>NFB 1966</td>
<td>4488 (2270 men, 2218 women)</td>
<td>31.2 (0.4)</td>
<td>130.5 (13.2)</td>
<td>80.4 (11.7)</td>
</tr>
<tr>
<td>Young Finns</td>
<td>2443 (1123 men, 1320 women)</td>
<td>37.6 (5.1)</td>
<td>126.8 (14.2)</td>
<td>79.5 (11.8)</td>
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<tr>
<td>NEDSA</td>
<td>1717 (551 men, 1166 women)</td>
<td>43.7 (12.3)</td>
<td>145.1 (19.5)</td>
<td>85.1 (12.0)</td>
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<tr>
<td>195 BBC</td>
<td>752 (3525 men, 3672 women)</td>
<td>45.0 (0.0)</td>
<td>133.7 (15.6)</td>
<td>82.6 (10.8)</td>
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<tr>
<td>GOYA</td>
<td>1465 (1465 men, 0 women)</td>
<td>45.6 (7.1)</td>
<td>143.3 (19.3)</td>
<td>93.4 (12.3)</td>
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<tr>
<td>FHS</td>
<td>5654 (267 men, 2977 women)</td>
<td>46.7 (12.9)</td>
<td>125.7 (17.7)</td>
<td>79.3 (10.4)</td>
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<tr>
<td>LifeLines</td>
<td>13235 (5532 men, 7703 women)</td>
<td>49.0 (11.7)</td>
<td>135.6 (16.1)</td>
<td>79.7 (10.0)</td>
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<tr>
<td>SPLIT</td>
<td>496 (213 men, 285 women)</td>
<td>47.9 (15.5)</td>
<td>134.4 (18.9)</td>
<td>80.9 (11.9)</td>
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<td>Twins UK</td>
<td>2392 (189 men, 2203 women)</td>
<td>48.6 (12.4)</td>
<td>130.2 (14.1)</td>
<td>80.3 (9.9)</td>
</tr>
<tr>
<td>PREVEND</td>
<td>3649 (1880 men, 1769 women)</td>
<td>51.0 (13.0)</td>
<td>136.0 (20.0)</td>
<td>78.0 (11.0)</td>
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<tr>
<td>DOPS</td>
<td>1684 (0 men, 1684 women)</td>
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<td>...</td>
<td>...</td>
</tr>
<tr>
<td>GENMETS</td>
<td>868 (421 men, 447 women)</td>
<td>49.2 (10.4)</td>
<td>131.6 (18.8)</td>
<td>83.2 (10.9)</td>
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<td>DPP</td>
<td>1998 (691 men, 1307 women)</td>
<td>55.1 (10.9)</td>
<td>129.7 (16.5)</td>
<td>81.7 (11.0)</td>
</tr>
<tr>
<td>MRC NSHD</td>
<td>2674 (1340 men, 1334 women)</td>
<td>53.0 (0.0)</td>
<td>141.8 (21.3)</td>
<td>88.5 (13.1)</td>
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<tr>
<td>ORCADIES</td>
<td>718 (334 men, 384 women)</td>
<td>54.3 (15.7)</td>
<td>137.5 (19.8)</td>
<td>80.0 (10.7)</td>
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<tr>
<td>NPHSII</td>
<td>277 (2771 men, 0 women)</td>
<td>56.1 (3.4)</td>
<td>129.7 (20.5)</td>
<td>85.4 (12.2)</td>
</tr>
<tr>
<td>KORCULA</td>
<td>901 (332 men, 569 women)</td>
<td>57.5 (14.2)</td>
<td>160.3 (22.1)</td>
<td>86.3 (10.5)</td>
</tr>
<tr>
<td>VIS</td>
<td>782 (323 men, 459 women)</td>
<td>56.0 (15.0)</td>
<td>141.6 (23.1)</td>
<td>86.4 (14.2)</td>
</tr>
<tr>
<td>Tronse</td>
<td>9467 (4482 men, 4985 women)</td>
<td>58.7 (13.1)</td>
<td>146.4 (22.5)</td>
<td>85.7 (13.5)</td>
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</table>

(>Table 1 continues on next page)
<table>
<thead>
<tr>
<th>Sample size</th>
<th>Men</th>
<th>Women</th>
<th>All participants</th>
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<tr>
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<td>Age (years)</td>
<td>Systolic blood pressure (mm Hg)</td>
<td>Diastolic blood pressure (mm Hg)</td>
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<td><strong>Studies in adults</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Whitehall II</td>
<td>5146</td>
<td>60.8 (5.9)</td>
<td>132.6 (18.2)</td>
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<td>HBICS</td>
<td>1728</td>
<td>61.4 (2.8)</td>
<td>152.9 (22.2)</td>
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<tr>
<td>ESTHER</td>
<td>8199</td>
<td>62.1 (6.7)</td>
<td>139.4 (20.2)</td>
</tr>
<tr>
<td>LURIC</td>
<td>3287</td>
<td>61.2 (10.7)</td>
<td>154.2 (23.9)</td>
</tr>
<tr>
<td>EAS</td>
<td>907</td>
<td>64.6 (5.6)</td>
<td>141.7 (22.7)</td>
</tr>
<tr>
<td>HCS</td>
<td>2906</td>
<td>65.7 (2.9)</td>
<td>139.5 (21.8)</td>
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<tr>
<td>ELSA</td>
<td>7104</td>
<td>65.9 (9.4)</td>
<td>138.0 (18.7)</td>
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<td>InCHIANTI</td>
<td>1210</td>
<td>67.2 (5.4)</td>
<td>149.6 (21.8)</td>
</tr>
<tr>
<td>PVUUS</td>
<td>995</td>
<td>70.1 (0.2)</td>
<td>150.7 (25.4)</td>
</tr>
<tr>
<td>ULSAM</td>
<td>1129</td>
<td>71.0 (0.64)</td>
<td>150.8 (21.9)</td>
</tr>
<tr>
<td>Health ABC</td>
<td>1554</td>
<td>74.9 (2.9)</td>
<td>137.6 (23.1)</td>
</tr>
<tr>
<td>Mo5s Sweden</td>
<td>2911</td>
<td>75.4 (3.2)</td>
<td>155.2 (23.9)</td>
</tr>
<tr>
<td><strong>Studies in adolescents</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LIAPlus and GIMplus</td>
<td>901</td>
<td>10.2 (0.2)</td>
<td>108.5 (9.9)</td>
</tr>
<tr>
<td>NFBc 1986</td>
<td>5460</td>
<td>16.0 (0.0)</td>
<td>121.1 (12.3)</td>
</tr>
<tr>
<td>TRAILS</td>
<td>1289</td>
<td>16.2 (0.06)</td>
<td>122.1 (12.3)</td>
</tr>
<tr>
<td>GOOD</td>
<td>941</td>
<td>18.9 (0.6)</td>
<td>130.7 (13.3)</td>
</tr>
</tbody>
</table>

Data are n, mean (SD), or n (%).

Table 1: Characteristics of the D-CarDia study cohorts, stratified by sex

We created two separate vitamin D allele scores: a synthesis allele score, created by summing the vitamin D-increasing alleles in the genes located upstream (DHCR7 and CYP2R1; score range 0–4), and a metabolism allele score, created by summing the vitamin D-increasing alleles in the genes located downstream (GC and
CYP24A1; score range 0–4) of the 25(OH)D metabolite. The synthesis allele score can be regarded as an instrument for 25(OH)D concentration when testing for causal association in mendelian randomisation analyses because of a small proportion of SNPs with the metabolic score SNPs because of unavailability of GC or CYP24A1 SNPs.

Figure 1: Flow chart showing the sample sizes available at each stage of the meta-analyses

Summary

D-CarDia studies contributing data to genotype-outcome analyses (with SNPs and blood pressure outcomes, but no 25(OH)D measurements)

Studies in adults

EAS (UK, n=907)
ELSA (UK, n=5054)
GOYA (Denmark, n=1465)
HIHCS (Finland, n=1728)
KORICULA (Croatia, n=901)
Lifelines (Netherlands, n=13235)
MESSA (Netherlands, n=1717)
NPIHES (UK, n=2771)
MRCS NSHD (UK, n=2674)
OIKONEY (UK, n=718)
PREVENI1 (Netherlands, n=3649)
SPLIT (Croatia, n=498)
VIS (Croatia, n=782)
WHR (UK, n=516)
14 studies, total n=41245

Studies in adolescents (secondary analyses)

NFBC66 (Finland, n=5460)
SPLIT (Croatia, n=498)
ORKNEY (UK, n=718)
MRC NSHD (UK, n=2674)
NPIHES (UK, n=2771)
PREVENI1 (Netherlands, n=3649)
SPLIT (Croatia, n=498)
VIS (Croatia, n=782)
WHR (UK, n=516)
14 studies, total n=41245

Studies in adults

EAS (UK, n=907)
ELSA (UK, n=5054)
GOYA (Denmark, n=1465)
HIHCS (Finland, n=1728)
KORICULA (Croatia, n=901)
Lifelines (Netherlands, n=13235)
MESSA (Netherlands, n=1717)
NPIHES (UK, n=2771)
MRCS NSHD (UK, n=2674)
OIKONEY (UK, n=718)
PREVENI1 (Netherlands, n=3649)
SPLIT (Croatia, n=498)
VIS (Croatia, n=782)
WHR (UK, n=516)
14 studies, total n=41245

Studies in adolescents (secondary analyses)

NFBC66 (Finland, n=5460)
SPLIT (Croatia, n=498)
ORKNEY (UK, n=718)
MRC NSHD (UK, n=2674)
NPIHES (UK, n=2771)
PREVENI1 (Netherlands, n=3649)
SPLIT (Croatia, n=498)
VIS (Croatia, n=782)
WHR (UK, n=516)
14 studies, total n=41245

Summary

D-CarDia studies contributing data to phenotype-outcome, genotype-outcome, and genotype-phenotype analyses (with SNPs, blood pressure outcomes, and 25(OH)D measurements)

Studies in adults

5BRC (UK, n=7152)
DOPS (Denmark, n=1684)
DPP* (USA, n=1998)
ESTHER1 (Germany, n=8199)
FHS (USA, n=8564)
GENIMETS (Finland, n=8564)
Health ABC (USA, n=1554)
HCS (UK, n=2906)
InC4AN1TI (Italy, n=1210)
LURIC (Germany, n=3287)
MrOs (Sweden, n=2912)
NFBC66 (Finland, n=4458)
PREDIX (Sweden, n=995)
TROMSO (Norway, n=8467)
TWIN SUK (UK, n=2392)
ULSAM (Sweden, n=1129)
Young Finns (Finland, n=2443)
17 studies, total n=58337
(with 25(OH)D measurements, n=49346)

Studies in adolescents (secondary analyses)

GINILISA (Germany, n=501)
GOOD (Sweden, n=941)
Two studies, total n=1882
(with 25(OH)D measurements, n=2776)

D-CarDia studies contributing data to genotype-outcome analyses only (with SNPs and blood pressure outcomes, but no 25(OH)D measurements)

Studies in adults

EAS (UK, n=907)
ELSA (UK, n=5054)
GOYA (Denmark, n=1465)
HIHCS (Finland, n=1728)
KORICULA (Croatia, n=901)
Lifelines (Netherlands, n=13235)
MESSA (Netherlands, n=1717)
NPIHES (UK, n=2771)
MRCS NSHD (UK, n=2674)
OIKONEY (UK, n=718)
PREVENI1 (Netherlands, n=3649)
SPLIT (Croatia, n=498)
VIS (Croatia, n=782)
WHR (UK, n=516)
14 studies, total n=41245

Studies in adolescents (secondary analyses)

NFBC66 (Finland, n=5460)
SPLIT (Croatia, n=498)
ORKNEY (UK, n=718)
MRC NSHD (UK, n=2674)
NPIHES (UK, n=2771)
PREVENI1 (Netherlands, n=3649)
SPLIT (Croatia, n=498)
VIS (Croatia, n=782)
WHR (UK, n=516)
14 studies, total n=41245

D-CarDia studies contributing data to genotype-outcome analyses (with SNPs and blood pressure outcomes, but no 25(OH)D measurements)

Studies in adults

EAS (UK, n=907)
ELSA (UK, n=5054)
GOYA (Denmark, n=1465)
HIHCS (Finland, n=1728)
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OIKONEY (UK, n=718)
PREVENI1 (Netherlands, n=3649)
SPLIT (Croatia, n=498)
VIS (Croatia, n=782)
WHR (UK, n=516)
14 studies, total n=41245

Studies in adolescents (secondary analyses)

NFBC66 (Finland, n=5460)
SPLIT (Croatia, n=498)
ORKNEY (UK, n=718)
MRC NSHD (UK, n=2674)
NPIHES (UK, n=2771)
PREVENI1 (Netherlands, n=3649)
SPLIT (Croatia, n=498)
VIS (Croatia, n=782)
WHR (UK, n=516)
14 studies, total n=41245

Consortia contributing data to genotype-outcome analyses only (with SNPs and blood pressure outcomes, but no 25(OH)D measurements)

CIBP (29 studies, n=69395)
CHARGE (6 studies, n=29116)
Global BPGen (16 studies, n=34433)

Statistical analysis

Statistical analyses in each of the D-CarDia studies were done in accordance with a standard analysis plan. We used the natural-log transformation for 25(OH)D concentrations to achieve a closer approximation of the normal distribution, and to remove non-linearity in the association with the outcomes. Additive models with systolic blood pressure, diastolic blood pressure, and metabolic feedback loops associated with the clearance of vitamin D-related metabolites by CYP24A1. Investigations with the vitamin D metabolism score were therefore exploratory only.
hypothesis as outcomes were adjusted for age, age-
squared, BMI, sex, geographical region, or principal
components (as relevant for the study); models with
25(OH)D concentration as the outcome were additionally
adjusted for month of blood sample collection and
labatory batch, as relevant.

With regard to the phenotypic effects, confounding
factors that affect 25(OH)D concentrations were assessed
previously with the 1958 British birth cohort39 and in
selected D-CarDia studies with individual-level data
(appendix pp 26–28). To assess the association of
25(OH)D concentration with systolic blood pressure,
diastolic blood pressure, and hypertension, the
investigators of each of the D-CarDia studies did linear
regression analyses, adjusting for the key covariates as
adjusted for in the additive models, and the models were
repeated stratified by sex (appendix p 39).

With respect to genetic effects on 25(OH)D concen-
tration, the effect allele was the 25(OH)D-increasing
allele, as established by the SUNLIGHT Consortium.9
We tested the association of the four individual
vitamin D-related genetic markers, and the two vitamin D
allele scores, with 25(OH)D concentrations using linear
regression models, adjusting for the same covariates as
adjusted for in the additive models. We tested for
associations of the synthesis score, and its components,
with several confounders: age, sex, season, BMI, total
cholesterol, and triglycerides (appendix pp 29–32). To
examine variations that could affect the validity of the
instruments, we used meta-regression to assess hetero-
genesis in the association between the synthesis
and metabolism allele scores were strongly
associated with 25(OH)D concentrations (synthesis score
β 2·83%, 95% CI 2·48–3·18, p=2·70 × 10–⁵⁵, R²=0·5%;
comparison; appendix p 33). As previously reported,10,11
the synthesis and metabolism allele scores were strongly
associated with 25(OH)D concentrations (synthesis score
β 2·83%, 95% CI 2·48–3·18, p=2·70 × 10–⁵⁵, R²=0·5%;
metabolism score β 5·38%, 4·67–6·08, p=5·93 × 10–⁵⁰,
R²=1·4%; appendix p 33). There was no evidence for
heterogeneity in the association between the synthesis
instrumental variable ratio method.20 To estimate the
instrumental variable ratio for the effect of 25(OH)D
concentration on systolic blood pressure, diastolic blood
pressure, and hypertension, we divided the meta-analysed
association of the vitamin D synthesis allele score with
systolic blood pressure, diastolic blood pressure, and
hypertension by the association of vitamin D synthesis
allele score with 25(OH)D concentration. We estimated
the variance for the instrumental variable ratio using a
Taylor expansion.21

We used summary statistics for the four vitamin D SNPs
from the ICBP,46 Global BPGen65 and CHARGE65
consortia to increase the statistical power of our analyses
of the association between the vitamin D allele scores
and blood pressure outcomes. We used an approximation
method that has been previously described46 to combine
SNPs into the synthesis and metabolism allele scores.

In the presence of heterogeneity of association between
the studies, we used random-effects meta-analyses; other-
wise, we tested fixed-effects models. We investigated
sources of heterogeneity with univariable meta-regression
models.

Four studies in the D-CarDia collaboration are in
adolescents (aged 10–20 years, n=8591). The main meta-
analyses were restricted to adult populations (aged
31–92 years, n=99 582), and only exploratory analyses
were done in adolescents because of insufficient sample
sizes (appendix pp 20, 33, and 39).

We did additional sensitivity analyses to examine the
effect of adjusting for lipids (appendix p 34) of the quality
of genetic information (appendix p 35), and of the
adjustment applied for the use of antihypertensive drugs
(appendix p 36), as well as to compare the two-stage
instrumental variable ratio method with the meta-analysis
of study-specific instrumental variable ratios
(appendix p 37).

All meta-analyses were done at the UCL Institute
of Child Health (University College London, London, UK)
with Stata version 12.

Role of the funding source
The funders of the study had no role in study design,
data collection, data analysis, data interpretation, or writing
of the report. The corresponding author had full access to
summary data from all studies and had final responsibility
for the decision to submit for publication.

Results
All four vitamin D-related SNPs were strongly associated
with 25(OH)D concentrations (p<2·22 × 10⁻¹² for all
comparisons; appendix p 33). As previously reported,9,11
the synthesis and metabolism allele scores were strongly
associated with 25(OH)D concentrations (synthesis score
β 2·83%, 95% CI 2·48–3·18, p=2·70 × 10⁻⁵⁵, R²=0·5%;
metabolism score β 5·38%, 4·67–6·08, p=5·93 × 10⁻⁵⁰,
R²=1·4%; appendix p 33). There was no evidence for
heterogeneity in the association between the synthesis
Heterogeneity was seen for the synthesis allele score (I²=62%, p=0.003), with evidence of variation in the association between 25(OH)D concentration and the outcomes within the studies done in adults (systolic blood pressure, I²=73%, p=0.01; diastolic blood pressure, I²=78%, p=0.001). The observed association between 25(OH)D concentration and systolic blood pressure, diastolic blood pressure, or hypertension between studies did not vary by age (meta-regression p=0.09 for all comparisons), sex (meta-regression p=0.65), method of blood pressure measurement (meta-regression p=0.14), geographical region (meta-regression p=0.39), or BMI (meta-regression p=0.10). However, for the association between 25(OH)D concentration and diastolic blood pressure, there was variation across the proportion of hypertensive participants (meta-regression p=0.01).

In the meta-analyses of the D-CarDia studies (n=108 173), there was no association of the synthesis allele score with systolic blood pressure (β per 25(OH)D-increasing allele, −0.10 mm Hg, 95% CI −0.23 to 0.02; p=0.11), diastolic blood pressure (β −0.07 mm Hg, −0.15 to 0.01; p=0.07), or hypertension (OR 0.99, 95% CI 0.97–1.00; p=0.08). After increasing the sample size by meta-analysing the D-CarDia results with the summary data from the ICBP consortium (total n=146 581, after exclusion of overlapping studies), the precision of estimation was improved, but the estimated strengths of these associations remained unchanged. The synthesis score was associated with both systolic blood pressure (β −0.10 mm Hg, −0.21 to −0.001; p=0.0498) and diastolic blood pressure (β −0.08 mm Hg, 95% CI 0.96 to 1.09; p=0.07), with evidence of variation in the association between 25(OH)D concentration and the outcomes within the studies done in adults (systolic blood pressure, I²=73%, p=0.01; diastolic blood pressure, I²=78%, p=0.001). The observed association between 25(OH)D concentration and systolic blood pressure, diastolic blood pressure, or hypertension between studies did not vary by age (meta-regression p=0.09 for all comparisons), sex (meta-regression p=0.65), method of blood pressure measurement (meta-regression p=0.14), geographical region (meta-regression p=0.39), or BMI (meta-regression p=0.10). However, for the association between 25(OH)D concentration and diastolic blood pressure, there was variation across the proportion of hypertensive participants (meta-regression p=0.01).

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For hypertension as the outcome, we meta-analysed the summary results from the CHARGE and Global BPGen consortia with the study results from adults in the D-CarDia collaboration (total $n=142,255$, after exclusion of overlapping studies). This analysis showed that the synthesis score was associated with hypertension (OR for increase per allele, 0.98; 95% CI 0.96–0.99; $p=0.001$; figure 2). The metabolism allele score was not associated with any blood pressure outcomes. For analyses with maximum sample sizes, $\beta$ for systolic blood pressure was $-0.001$ mm Hg (95% CI $-0.12$ to $0.12$; $p=0.99$), $\beta$ for diastolic blood pressure was $0.005$ mm Hg ($-0.07$ to $0.08$; $p=0.90$), and the OR for hypertension was $0.99$ (0.98–1.01; $p=0.48$; appendix p 44).

In the instrumental variable analysis, in which the synthesis score was used as an instrument, the direction of association between 25(OH)D concentration and all outcomes was compatible with that suggested by the observational phenotypic analyses (table 2). Every 10% relative increment in genetically instrumented 25(OH)D concentration was associated with $0.29$ mm Hg lower diastolic blood pressure (95% CI $0.07$ to $0.52$; $p=0.01$) and a $0.37$ mm Hg lower systolic blood pressure ($-0.003$ to $0.73$; $p=0.052$). Every 10% increment in 25(OH)D concentration was associated with an 8.1% reduced odds of hypertension (OR 0.92; 95% CI $0.87$–$0.97$; $p=0.002$) in the instrumental variable ratio analyses (table 2, figure 3).

The CYP2R1 SNP was individually associated with reduced diastolic blood pressure (per allele, $\beta=-0.09$ mm Hg, 95% CI $-0.18$ to $-0.01$; $p=0.03$) and reduced odds of hypertension (OR for increase per allele, 0.98; 95% CI $0.96$–$1.00$; $p=0.02$), but no individual associations were seen for the DHC7 SNP, or either of the downstream metabolism SNPs (GC and CYP24A1), with any of the blood pressure outcomes (appendix pp 41–43).

### Table 2: Summary of coefficients for instrumental variable ratio analyses, with the synthesis score as an instrumental variable

<table>
<thead>
<tr>
<th>Exposure (X)</th>
<th>Synthesis score</th>
<th>Outcome (Y)</th>
<th>Hypertension</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D concentration</td>
<td>$\beta_{1}=-2.83%$ (95% CI $2.48$–$3.18$)</td>
<td>$\beta_{2}=-2.83%$ (95% CI $2.48$–$3.18$)</td>
<td>$\beta_{0}=-0.98%$ (95% CI $0.97$–$0.99$)</td>
</tr>
</tbody>
</table>

In the instrumental variable ratio calculation done with the natural log of the odds ($\beta_{0}$), OR=odds ratio. 25(OH)D$\rightarrow$25-hydroxyvitamin D.

Discussion

The results of our mendelian randomisation analysis provide evidence for a causal effect of low vitamin D status on increasing blood pressure and risk of hypertension. This finding lends support to continued efforts to prevent vitamin D deficiency. In view of the costs and side-effects associated with antihypertensive drugs, the potential to reduce hypertension by vitamin D is very attractive. However, because we cannot exclude the possibility that our findings were caused by chance, they need to be replicated in an independent, similarly powered study.

Evidence from randomised controlled trials to assess the effectiveness of vitamin D supplementation in reducing blood pressure have not provided consistent evidence of a benefit.1 In subgroup analyses done within meta-analyses of these trials,2,3 some reductions in diastolic blood pressure were reported for participants with hypertension or cardiometabolic disease, and when studies that used higher doses were compared with those that used lower doses of vitamin D.21 Although the investigators of one study2 reported dose-dependent reductions in systolic blood pressure after 3 months of supplementation with 1000 IU, 2000 IU, and 4000 IU of vitamin D per day (0.66, 3.4, and 4.0 mm Hg, respectively), no effect was seen in another trial24 in which participants were given a bolus supplement of 100 000 IU every 3 months. These inconsistencies could be attributed to differences in the mode of administration, dose, and duration of supplementation, or to baseline differences in 25(OH)D concentrations or blood pressure, or other sources of heterogeneity between the studies. Thus, the evidence remains inconclusive. Nevertheless, these exploratory randomised controlled trials have paved the way for large trials (with upwards of 18 000 participants) that are being undertaken to examine the benefits of vitamin D for the prevention of cardiovascular disease outcomes.25

Our findings are biologically plausible. Inappropriate activation of the renin-angiotensin system increases blood pressure and the risk of cardiovascular disease.26
Furthermore, adipocyte inflammation has a crucial role and mortality in patients with chronic kidney disease. For vitamin D deficiency in cardiovascular morbidity interaction with parathyroid hormone in the regulation of calcium absorption from the gut and its effects through various other molecular mechanisms. Vitamin D metabolites could also exert antihypertensive effects through various other molecular mechanisms. Vitamin D is indirectly related to blood pressure through its regulation of calcium absorption from the gut and its interaction with parathyroid hormone in the maintenance of calcium homeostasis. The renoprotective and anti-inflammatory actions of vitamin D metabolites and their analogues suggest a possible role for vitamin D deficiency in cardiovascular morbidity and mortality in patients with chronic kidney disease. Furthermore, adipocyte inflammation has a crucial role in hypertension: in an in vitro study, 1,25(OH)2D inhibited lipopolysaccharide-stimulated cytokine secretion in two human adipocyte models through direct inhibition of nuclear factor-κB.

On the basis of our effect size estimates, the genetic associations of the synthesis allele score with systolic or diastolic blood pressure were less pronounced than that seen for hypertension (represented as a binary trait). This finding might suggest that adequate 25(OH)D concentrations are particularly important for the prevention of hypertension as a clinical outcome. This interpretation is also supported by the results of sensitivity analyses in normotensive individuals, which showed that both the phenotypic association and the genetically indexed association between 25(OH)D concentrations and blood pressure were substantially weaker in this subgroup than in the full sample (data not shown).

Although the mendelian randomisation approach is helpful in testing for underlying causality, an imbalance in the possible overestimation or underestimation of the genetic associations for the outcome and exposure could affect the quantification of the effect. The weaker association with blood pressure than with hypertension could also reflect a greater measurement error or heterogeneity in the assessment of gradual increases across the range of the blood pressure distribution, compared with the classification of hypertension by raised blood pressure or use of antihypertensive drugs. Such noise or heterogeneity in the blood pressure measurements in our meta-analyses could also account for why the estimated strength of the associations between 25(OH)D concentrations and blood pressure outcomes were weaker than we would have expected on the basis of previous observational analyses or of power calculations that were done with data from one of the D-CarDia studies (1958 British birth cohort, n=6877).

The main strength of our study is in the large sample size (up to n=146 581), which allowed us to assess the consistency of associations across several studies and to gain sufficient power for conclusive analyses. This study shows the benefits of the mendelian randomisation approach: although the phenotypic associations between 25(OH)D concentrations and blood pressure or hypertension were very heterogeneous across the studies, notably less heterogeneity was seen for the genetic associations. Age and adiposity are issues that would be expected to affect 25(OH)D concentrations and bias the phenotypic association it might have with blood pressure, but participating studies included both young and old cohorts, and both lean and obese participants. By contrast, genetic variants used in mendelian randomisation would be expected to reflect lifelong differences in 25(OH)D concentrations and therefore would be less affected by temporal variations in individual characteristics.

Potential limitations with the mendelian randomisation approach include the requirement of large sample sizes, the possibility of population stratification, canalisation, pleiotropy, and an inability to generalise findings to people in other ethnic groups. A limitation of our study was that we only looked at associations with blood pressure and hypertension, and whether these associations are related to hypertension in the risks of rarer disease outcomes remains uncertain. Population stratification is unlikely to have had a major effect on our main findings, since participants were all of European descent, and we adjusted for geographical region and principal components from the population stratification analysis in the statistical models.

The synthesis or metabolism SNPs might have led to biological adaptations during development (ie, canalisation), although this possibility seems unlikely in view of the similar associations with 25(OH)D seen in the analyses done in adolescents and adults. Pleiotropic effects, wherein the genetic instruments might affect other metabolic pathways independent of their influence on 25(OH)D concentration, are more likely. Our main instrument was a composite score consisting of two independent SNPs. Although differential associations between the components included in the score can suggest a possible pleiotropic effect, in our case the associations for CYP2R1 and DHCR7 were similar for all three outcomes (appendix p 38). Furthermore, because 25(OH)D is a secosteroid, we explicitly sought to exclude pleiotropic effects through lipid metabolism by adjusting for serum triglycerides and total cholesterol in addition to other covariates, and noted no differences in our findings (appendix p 34). However, pleiotropy is an issue with the metabolism variants included in our secondary analyses.
Panel: Research in context

Systematic review

Investigators of several systematic reviews1,2,5–7,11 have summarised evidence for the phenotypic association between 25-hydroxyvitamin D (25(OH)D) and blood pressure and assessed the cardiovascular effects of vitamin D supplementation in randomised controlled trials. A prospective phenotypic association is well established, but few large randomised controlled trials of vitamin D with primary cardiovascular outcomes have been done. Evidence is largely restricted to secondary analyses of mostly small trials that were initially established to assess the effects of vitamin D supplementation on bone health. Some effects have been reported from subgroup analyses of trials focused on individuals with cardiometabolic disease,3,4 but the quality of the available evidence has been criticised.5 Previous studies that have used mendelian randomisation analyses to examine the association of 25(OH)D and cardiovascular outcomes have been underpowered and evidence for causality of association is inconclusive.2,3

Interpretation

Our results suggest that people who have genetic variants associated with low endogenous production of 25(OH)D have an increased risk of hypertension, emphasising the need for further, well-designed randomised controlled trials to assess causality and the potential clinical benefits of vitamin D supplementation. In view of the costs and side-effects associated with antihypertensive drugs, the possibility of preventing or reducing hypertension with vitamin D supplementation is very attractive. However, because we cannot exclude the possibility that the findings from this study were caused by chance, they need to be replicated in an independent, similarly powered study.

The GC allele that is associated with increased 25(OH)D concentrations also leads to reduced bioavailability of active 1,25(OH)₂D,3,9 hence increased serum concentrations of the 25(OH)D substrate are a possible consequence of reduced uptake by the cells. CYP24A1 in turn acts as a hydroxylase for other vitamin D metabolites in addition to 25(OH)D, and the activity of the enzyme probably reflects the absolute 25(OH)D concentration, raising uncertainty about the association we would expect to see with the outcome. Indeed, we noted no evidence for associations between the metabolism SNPs and the blood pressure outcomes.

Overall, our study provides genetic evidence that increased 25(OH)D concentrations are causally associated with reduced blood pressure and hypertension risk (panel). If replicated in an independent, similarly powered study, these findings will strengthen the case for appropriately powered, well-designed randomised clinical trials to investigate the necessary vitamin D doses and appropriate target groups for the prevention or treatment of hypertension.

Contributors

KSV, AC, DJB, CL, ACA, ETI, AW, MM, KAJ, IMN, TSA, DP, LZ, JD, LP, and EHy took part in data analysis. KSV, AC, DJB, ADH, JCW, and EHy contributed to study design. RJ, AKD, HJLH, JE, DKKH, PJVDM, EH, VV, RMF, JEH, RADB, BS, K-US, MIM, SS, AP, JLaI, MEK, GN, ML, KJ, NA, JC, JA, RH, SR, LKB, JLab, COs, BWP, LR, KKL, RPS, DGH, LB, EHa, HM, EI, DM, OL, IT, SM, ETHe, CMTT, AJ, PN, AFW, OP, CH, JFW, IR, VS, JH, JHC, JFP, MK, LL, KM, SB, TMF, CAH, TIAS, SBK, BIL, JGE, JCF, TDS, TL, DK, SEH, CC, Coh, WM, MHDB, MKi, MKj, TJW, CP, HB, GG, PVDH, HS, SP, M-Rj, and EHy contributed to data collection. KSV, AC, DJB, ADH, JCW, and EHy interpreted results. The writing group consisted of KSV, AC, SP, and EHy. All the authors have read the report critically and approved the submitted version. A full list of study and consortia investigators is provided in the appendix (pp 45–48).

Declaration of interests

TJW is on the scientific advisory board for DiaSorin and has received research support from the company. JCW is employed by GlaxoSmithKline and holds shares in the company. JCF has received honoraria from Lilly for consulting. All other authors declare no competing interests.

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