Association of vitamin D status with arterial blood pressure and hypertension risk

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


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.1,2 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.¹⁴,¹⁵ The largest of the randomised controlled studies was the Women’s Health Initiative trial (n=36,282), the results of which did not show any changes in blood pressure or hypertension after 7 years of follow-up.¹⁶ 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 doses⁷ and those investigating individuals with cardio-metabolic risk; however, as Elamin and colleagues have previously noted,⁸ 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.⁹ 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\n
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.¹¹\n
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

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.¹²–¹⁶ 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=8,591) 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)¹⁸ 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)¹⁹ and Global Blood Pressure Genetics (Global BPGen) (n=34,433)²⁰ 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.²¹ 25(OH)D concentrations were available for 19 of the 35 studies in the D-CarDia collaboration (n=51,122), with values expressed in nmol/L for all studies. The appendix (pp 2–19) includes details about the methods used to measure 25(OH)D concentration in each study.

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, with two SNPs in genes located upstream (DHCR7 and CYP2R1) and two downstream (GC and CYP24A1) of the 25(OH)D metabolite.²² 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=1.84×10⁻⁹ 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 rs10741657.²³ 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.
### Sample size

<table>
<thead>
<tr>
<th>Study</th>
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<th>Men</th>
<th>Women</th>
<th>All participants</th>
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</thead>
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<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><strong>Studies in adults</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NFBC 1966</td>
<td>4488 (2270 men, 2218 women)</td>
<td>31 (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 (5.1)</td>
<td>126.8 (14.2)</td>
<td>79.5 (11.8)</td>
</tr>
<tr>
<td>NESDA 1717</td>
<td>1712 (551 men, 1166 women)</td>
<td>47 (12.3)</td>
<td>145.1 (19.5)</td>
<td>85.1 (12.0)</td>
</tr>
<tr>
<td>PREVEND 3649</td>
<td>7152 (3525 men, 3672 women)</td>
<td>45 (0.0)</td>
<td>133.7 (15.6)</td>
<td>82.6 (10.8)</td>
</tr>
<tr>
<td>DPP 1998</td>
<td>1466 (1465 men, 0 women)</td>
<td>45 (7.1)</td>
<td>143.3 (19.3)</td>
<td>93.4 (12.3)</td>
</tr>
<tr>
<td>FHS 5654</td>
<td>267 (267 men, 297 women)</td>
<td>46 (12.9)</td>
<td>125.7 (17.1)</td>
<td>79.3 (10.4)</td>
</tr>
<tr>
<td>LifeLines 13235</td>
<td>552 (2532 men, 7703 women)</td>
<td>49 (11.7)</td>
<td>135.6 (16.1)</td>
<td>79.7 (10.0)</td>
</tr>
<tr>
<td>SPLIT 498</td>
<td>213 (213 men, 285 women)</td>
<td>47 (15.2)</td>
<td>134.4 (18.9)</td>
<td>80.9 (11.9)</td>
</tr>
<tr>
<td>Twins UK 2392</td>
<td>189 (189 men, 2203 women)</td>
<td>48 (12.4)</td>
<td>130.2 (14.1)</td>
<td>80.3 (9.9)</td>
</tr>
<tr>
<td>PREVEND 3649</td>
<td>1880 (1880 men, 1769 women)</td>
<td>51 (13.0)</td>
<td>136.0 (20.0)</td>
<td>78.0 (11.0)</td>
</tr>
<tr>
<td>DOPS 1684</td>
<td>0 (men, 1684 women)</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>GENMETS 868</td>
<td>421 (421 men, 447 women)</td>
<td>49 (10.4)</td>
<td>131.6 (18.8)</td>
<td>83.2 (10.9)</td>
</tr>
<tr>
<td>DPP 1998</td>
<td>1996 (691 men, 1307 women)</td>
<td>55 (10.9)</td>
<td>129.7 (16.5)</td>
<td>81.7 (11.0)</td>
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<tr>
<td>MRC NSHD 2674</td>
<td>(1340 men, 1334 women)</td>
<td>53 (0.0)</td>
<td>141.8 (21.3)</td>
<td>88.5 (13.1)</td>
</tr>
<tr>
<td>ORCADES 718</td>
<td>334 (334 men, 384 women)</td>
<td>54 (15.7)</td>
<td>137.5 (19.8)</td>
<td>80.0 (10.7)</td>
</tr>
<tr>
<td>NPHSII 2771</td>
<td>(2771 men, 0 women)</td>
<td>56 (3.4)</td>
<td>129.7 (20.5)</td>
<td>85.4 (12.2)</td>
</tr>
<tr>
<td>KORCULA 901</td>
<td>(332 men, 569 women)</td>
<td>57 (14.2)</td>
<td>146.3 (22.1)</td>
<td>86.3 (10.5)</td>
</tr>
<tr>
<td>Vibo 782</td>
<td>(33 men, 450 women)</td>
<td>56 (15.0)</td>
<td>141.6 (23.1)</td>
<td>86.4 (14.2)</td>
</tr>
<tr>
<td>Tromsø 9467</td>
<td>(4482 men, 4985 women)</td>
<td>58 (13.1)</td>
<td>146.4 (22.5)</td>
<td>85.7 (13.5)</td>
</tr>
</tbody>
</table>

(continued on next page)
<table>
<thead>
<tr>
<th>Sample size</th>
<th>Men</th>
<th>Women</th>
<th>All participants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age (years)</td>
<td>Systolic blood pressure (mm Hg)</td>
<td>Diastolic blood pressure (mm Hg)</td>
</tr>
<tr>
<td>Whitehall II</td>
<td>5146</td>
<td>60.8 (5.9)</td>
<td>132.6 (18.2)</td>
</tr>
<tr>
<td>HBICS</td>
<td>1728</td>
<td>61.4 (2.8)</td>
<td>152.9 (22.2)</td>
</tr>
<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 (20.7)</td>
</tr>
<tr>
<td>ELSA</td>
<td>5054</td>
<td>65.9 (9.4)</td>
<td>138.0 (18.7)</td>
</tr>
<tr>
<td>InCHIANTI</td>
<td>1210</td>
<td>67.2 (25.4)</td>
<td>149.6 (21.8)</td>
</tr>
<tr>
<td>PVUS</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 (21.3)</td>
</tr>
<tr>
<td>MoS Sweden</td>
<td>2911</td>
<td>75.4 (3.2)</td>
<td>155.2 (23.9)</td>
</tr>
</tbody>
</table>

(Continued from previous page)

Studies in adults

<table>
<thead>
<tr>
<th>Sample size</th>
<th>Men</th>
<th>Women</th>
<th>All participants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age (years)</td>
<td>Systolic blood pressure (mm Hg)</td>
<td>Diastolic blood pressure (mm Hg)</td>
</tr>
<tr>
<td>bvD acute</td>
<td>5460</td>
<td>16.0 (0.0)</td>
<td>121.1 (12.3)</td>
</tr>
<tr>
<td>bvD chronic</td>
<td>1289</td>
<td>16.3 (0.66)</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
Figure 1: Flow chart showing the sample sizes available at each stage of the meta-analyses

25(OH)D=25-hydroxyvitamin D. ICBP=International Consortium for Blood Pressure. CHARGE=Cohorts for Heart and Aging Research in Genomic Epidemiology. Global BPGen=Global Blood Pressure Genetics. "Did not contribute data to analyses with the synthesis score single-nucleotide polymorphisms (SNPs) because of unavailability of the CYP2R1 SNP. Did not contribute data to analyses with the metabolism score SNPs because of unavailability of GC or CYP24A1 SNPs.

CYP24A1; score range 0–4) of the 25(OH)D metabolite.11,11 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 it consists of variants that directly affect substrate availability or synthesis of 25(OH)D. Components included in the metabolism score are relevant for the transfer and clearance of 25(OH)D and could provide insights into the effect of vitamin D metabolism on blood pressure. However, the use of the metabolism score as a formal instrument in mendelian randomisation analyses is not possible because of problems with quantification of expected associations, pleiotropic effects,12,13 and the metabolic feedback loops associated with the clearance of vitamin D-related metabolites by CYP24A1.17 Investigations with the vitamin D metabolism score were therefore exploratory only.

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, andivial blood pressure outcomes, but no 25(OH)D measurements)

Summary

D-CarDia studies in adults
31 studies, n=99 582; 50% of participants with 25(OH)D measurements
D-CarDia studies in adults and ICBP (overlapping studies removed)
53 studies, n=146 581; 32% of participants with 25(OH)D measurements
D-CarDia studies in adults, CHARGE, and Global BPGen (overlapping studies removed)
50 studies, n=147 255; 33% of participants with 25(OH)D measurements
D-CarDia studies in adolescents (secondary analyses)
Four studies, n=8591; 21% of participants with 25(OH)D measurements

Consortia contributing data to genotype-outcome analyses only (with SNPs and blood pressure outcomes, but no 25(OH)D measurements)
(CarDia (29 studies, n=69 395)
CHARGE (6 studies, n=29 116)
Global BPGen (16 studies, n=24 433)

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

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
SIBIC (UK, n=7 152)
DOPS (Denmark, n=1 684)
DPP (USA, n=1 998)
ESTHER I (Germany, n=8 199)
FHS (USA, n=5 654)
GENETICS (Finland, n=8 668)
Health ABC (USA, n=15 564)
HCS (UK, n=2 906)
InCHIANTI (Italy, n=1 229)
LURIC (Germany, n=2 287)
MrOS (Sweden, n=2 911)
NFBC66 (Finland, n=4 488)
PPUS (Sweden, n=995)
TROMSO (Norway, n=9467)
TWINUK (UK, n=2 392)
ULSAM (Sweden, n=1125)
Young Finns (Finland, n=2 443)
17 studies, total n=58 337 (with 25(OH)D measurements, n=49 346)
Studies in adolescents (secondary analyses)
GINILISA (Germany, n=901)
GOOD (Sweden, n=941)
Two studies, total n=1 842 (with 25(OH)D measurements, n=1 276)

Studies in adults
EAS (UK, n=907)
ELSA (UK, n=5 054)
GOYA (Denmark, n=1 465)
HBKS (Finland, n=1 728)
KORICLA (Croatia, n=901)
Lifelines (Netherlands, n=13 235)
NHSNI (Netherlands, n=17 177)
NPHS2 (UK, n=2 771)
MRIC NSHD (UK, n=2 674)
ORKKEY (UK, n=718)
PREVEND (Netherlands, n=36 492)
SPLIT (Croatia, n=498)
VIS (Croatia, n=782)
WHRi (UK, n=5 146)
14 studies, total n=41 245
Studies in adolescents (secondary analyses)
NFBC66 (Finland, n=5 460)
TRAILS (Netherlands, n=1139)

C M T Tiesler PhD, V Vitart PhD, S McLachlan MD, and MRC Human Genetics Unit, Institute of Genetics and Molecular Medicine (V Vitart PhD, P Navarro PhD, J E Huffman MSc, C Hayward PhD, Prof A F Wright PhD, University of Edinburgh, Edinburgh, UK; Department of Public Health and Primary Care, Trinity College Dublin, Dublin, Ireland (L Zgaga PhD), Prof B Carrell, D Theodoratou PhD, M Fraser PhD, J Wilson DPhil, Prof I Rudan MD, J Price MD, S McLachlan MD), and MRC Human Genetics Unit, Institute of Genetics and Molecular Medicine (V Vitart PhD, P Navarro PhD, J E Huffman MSc, C Hayward PhD, Prof A F Wright PhD, University of Edinburgh, Edinburgh, UK; Department of Public Health and Primary Care, Trinity College Dublin, Dublin, Ireland (L Zgaga), Institute of Epidemiology I, Helmholtz Zentrum München—German Research Center for Environmental Health, Neuherberg, Germany (E Thiering MSc, C M T Tiesler MSc, J Heinrich PhD); Division of Nutritional Medicine (E Thiering), and Institute of Medical Informatics, Biometry and Epidemiology (E Thiering), Ludwig Maximilian University of Munich, Dr von Hauner Children’s Hospital, Munich, Germany; Oxford Centre for Diabetes, Endocrinology and Metabolism (Prof M I McCarthy), Wellcome Trust Centre for Human Genetics (M I McCarthy, Prof E Ingelsson MD), and NIHR Articles

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Sciences, Division of Public Health Sciences (K K Lohman MStat), Wake Forest School of Medicine, Winston-Salem, NC, USA; Metabolic Genetics, Novo Nordisk Foundation Centre for Basic Metabolic Research (T S Ahluwalia PhD, Prof T I A Sørensen DrMedSci), and Copenhagen Prospective Studies on Asthma in Childhood (T S Ahluwalia), Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; Institute of Preventive Medicine, Bispebjerg and Frederiksberg Hospital, Copenhagen, Denmark (T I A Sørensen); Danish Pediatric Asthma Center, Gentofte Hospital, Copenhagen, Denmark (T S Ahluwalia); Genetics of Complex Traits, University of Exeter Medical School, Exeter, UK (Dorota Pasko MSc, Prof T M Frayling PhD), Centre for Population Health Sciences (L Zgaga PhD), Prof H Campbell MD, E Theodoratou PhD, R M Fraser PhD, J Wilson DPhil, Prof I Rudan MD, J Price MD, S McLachlan MD), and MRC Human Genetics Unit, Institute of Genetics and Molecular Medicine (V Vitart PhD, P Navarro PhD, J E Huffman MSc, C Hayward PhD, Prof A F Wright PhD, University of Edinburgh, Edinburgh, UK; Department of Public Health and Primary Care, Trinity College Dublin, Dublin, Ireland (L Zgaga), Institute of Epidemiology I, Helmholtz Zentrum München—German Research Center for Environmental Health, Neuherberg, Germany (E Thiering MSc, C M T Tiesler MSc, J Heinrich PhD); Division of Nutritional Medicine (E Thiering), and Institute of Medical Informatics, Biometry and Epidemiology (E Thiering), Ludwig Maximilian University of Munich, Dr von Hauner Children’s Hospital, Munich, Germany; Oxford Centre for Diabetes, Endocrinology and Metabolism (Prof M I McCarthy), Wellcome Trust Centre for Human Genetics (M I McCarthy, Prof E Ingelsson MD), and NIHR
hypertension 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
laboratory batch, as relevant.

With respect to the phenotypic analyses, confounding
factors that affect 25(OH)D concentrations were assessed
previously with the 1958 British birth cohort 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. 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-
geneity in the associations between the SNPs and
25(OH)D concentration with systolic blood pressure,
diastolic blood pressure, and hypertension, the
association of the vitamin D synthesis allele score with
25(OH)D concentration as the outcome were additionally
adjusted for in the additive models. We tested for
heterogeneity with univariate meta-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).

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
categories; appendix p 33). As previously reported, 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 3.8% 4.67–6.08, p=5.93×10⁻³⁰,
R²=1.4%; appendix p 33). There was no evidence for
heterogeneity in the association between the synthesis
instrumental variable ratio method. 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.
Increased 25(OH)D concentrations were associated with reduced systolic blood pressure (β per 10% change, –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); however, we did not see an association between 25(OH)D concentration and diastolic blood pressure (β –0.02 mm Hg, –0.08 to 0.03; p=0.37; appendix p 40). Despite evidence for heterogeneity in the phenotypic association between 25(OH)D concentration and the outcomes within the studies done in adults (systolic blood pressure, I²=73%, p=9.19 × 10⁻⁹; diastolic blood pressure, I²=78%, p=5.00 × 10⁻¹⁰; hypertension, I²=62%, 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 –0.21 to 0.00; p=0.08), diastolic blood pressure (β –0.07 mm Hg, –0.15 to 0.01; p=0.07), or hypertension (OR 0.99, 95% CI 0.97–1.01; 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 0.0001; p=0.0498) and diastolic blood pressure (β –0.08 mm Hg, –0.12 to 0.0001).

<table>
<thead>
<tr>
<th>Study</th>
<th>β (95% CI)</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIVUS</td>
<td>–1.27 (–3.01 to 0.48)</td>
<td>0.85 (0.72 to 1.00)</td>
</tr>
<tr>
<td>DOPPS</td>
<td>–1.00 (–2.05 to 0.05)</td>
<td>0.90 (0.82 to 0.99)</td>
</tr>
<tr>
<td>Young Finns</td>
<td>–0.84 (–1.43 to –0.25)</td>
<td>0.91 (0.85 to 0.97)</td>
</tr>
<tr>
<td>Goya</td>
<td>–0.73 (–1.68 to 0.23)</td>
<td>0.92 (0.83 to 1.03)</td>
</tr>
<tr>
<td>Whitehall II</td>
<td>–0.61 (–1.72 to –0.02)</td>
<td>0.93 (0.79 to 1.08)</td>
</tr>
<tr>
<td>NPHSII</td>
<td>–0.55 (–1.42 to 0.32)</td>
<td>0.94 (0.83 to 1.05)</td>
</tr>
<tr>
<td>Tromsø</td>
<td>–0.16 (–0.53 to 0.22)</td>
<td>0.95 (0.81 to 1.10)</td>
</tr>
<tr>
<td>ICBP</td>
<td>–0.11 (–0.25 to 0.03)</td>
<td>0.95 (0.81 to 1.11)</td>
</tr>
<tr>
<td>EAS</td>
<td>–0.10 (–1.85 to 1.66)</td>
<td>0.95 (0.81 to 1.10)</td>
</tr>
<tr>
<td>ESTHER</td>
<td>–0.10 (–0.54 to 0.36)</td>
<td>0.95 (0.81 to 1.10)</td>
</tr>
<tr>
<td>Lifelines</td>
<td>–0.01 (–0.31 to 0.35)</td>
<td>0.95 (0.81 to 1.10)</td>
</tr>
<tr>
<td>PREVEND</td>
<td>0.0000 (–0.63 to 0.63)</td>
<td>0.95 (0.81 to 1.10)</td>
</tr>
<tr>
<td>LURIC</td>
<td>0.02 (–0.92 to 0.95)</td>
<td>0.95 (0.81 to 1.10)</td>
</tr>
<tr>
<td>CROATIA-Korcula</td>
<td>0.03 (–1.35 to 1.40)</td>
<td>0.95 (0.81 to 1.10)</td>
</tr>
<tr>
<td>CROATIA-Split</td>
<td>0.11 (–1.41 to 1.64)</td>
<td>0.95 (0.81 to 1.10)</td>
</tr>
<tr>
<td>HBCs</td>
<td>0.17 (–0.97 to 1.32)</td>
<td>0.95 (0.81 to 1.10)</td>
</tr>
<tr>
<td>EAS</td>
<td>0.19 (–1.08 to 1.45)</td>
<td>0.95 (0.81 to 1.10)</td>
</tr>
<tr>
<td>GENMETS</td>
<td>0.21 (–0.96 to 1.21)</td>
<td>0.95 (0.81 to 1.10)</td>
</tr>
<tr>
<td>ELSA</td>
<td>0.40 (–0.21 to 1.01)</td>
<td>0.95 (0.81 to 1.10)</td>
</tr>
<tr>
<td>ULSAM</td>
<td>0.52 (–0.85 to 1.89)</td>
<td>0.95 (0.81 to 1.10)</td>
</tr>
<tr>
<td>MRCSNSHD</td>
<td>0.52 (–0.40 to 1.45)</td>
<td>0.95 (0.81 to 1.10)</td>
</tr>
<tr>
<td>NESDA</td>
<td>0.61 (–0.26 to 1.49)</td>
<td>0.95 (0.81 to 1.10)</td>
</tr>
<tr>
<td>mOSSweden</td>
<td>0.72 (–0.99 to 2.43)</td>
<td>0.95 (0.81 to 1.10)</td>
</tr>
<tr>
<td>Health ABC</td>
<td>0.86 (–0.29 to 2.00)</td>
<td>0.95 (0.81 to 1.10)</td>
</tr>
<tr>
<td>Overall</td>
<td>–0.10 (–0.21 to –0.0001)</td>
<td>0.95 (0.81 to 1.10)</td>
</tr>
</tbody>
</table>

Figure 2: Meta-analysis of D-CarDia studies with summary data from the ICBP, CHARGE, and Global BPGen consortia.
Instrumental variable ratio calculation done with the natural log of the odds ($\beta_{ZY}$). OR=odds ratio.

Mendelian randomisation triangulation for hypertension

25(OH)D=25-hydroxyvitamin D.

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Table 2: Summary of coefficients for instrumental variable ratio analyses, with the synthesis score as an difference in blood pressure (mm Hg) or the odds ratio.

with 25-hydroxyvitamin D (25[OH]D): coefficient per allele, 2·83% (95% CI 2·48–3·18). *Coefficient represents the

Results include the D-CarDia studies (in adults only) and consortium summary statistics from the International National Institute for Health Life Laboratory Uppsala University, Uppsala, Sweden; Department of Chronic Disease Prevention, National Institute for Health and Welfare, Turku, Finland (A Jula MD), Croatian Centre for Global Health, University of Split Medical School, Split, Croatia (O Polasek MD), National Institute for Health and Welfare, Helsinki, Finland (Prof V Salomaa MD, J Eriksson), Clinical and Molecular Osteoporosis Research Unit, Department of

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>Synthetic variable ratio analyses, per 10% increase in 25(OH)D concentration</th>
<th>Synthesis score with outcome, per allele</th>
<th>Instrumental variable ratio analyses, per 10% increase in 25(OH)D concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>coefficient* (95% CI)</td>
<td>p value</td>
<td>coefficient* (95% CI)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>coefficient* (95% CI)</td>
<td>p value</td>
<td>coefficient* (95% CI)</td>
</tr>
<tr>
<td>Hypertension (odds ratio)</td>
<td>coefficient* (95% CI)</td>
<td>p value</td>
<td>coefficient* (95% CI)</td>
</tr>
</tbody>
</table>

Results include the D-CarDia studies (in adults only) and consortium summary statistics from the International Consortium for Blood Pressure (ICBP), the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium, and the Global Blood Pressure Genetics (Global BPGen) consortium. Association of the synthesis score with 25-hydroxyvitamin D (25(OH)D): coefficient per allele, 2·83% (95% CI 2·48–3·18). *Coefficient represents the difference in blood pressure (mm Hg) or the odds ratio.

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. In subgroup analyses done within meta-analyses of these trials, 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. Although the investigators of one study 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 trial 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.

Our findings are biologically plausible. Inappropriate activation of the renin-angiotensin system increases blood pressure and the risk of cardiovascular disease.
Studies in animals have shown that 1,25-dihydroxyvitamin D (calcitriol, 1,25(OH)2D) suppresses the expression of the renin gene by a vitamin D receptor-dependent mechanism, thereby lowering blood pressure.27 In an open-label, blinded-endpoint trial28 in 101 patients with chronic heart failure who were randomly assigned to receive 2000 IU of oral vitamin D3 per day for 6 weeks or control (no treatment), treatment led to a significant decrease in plasma renin activity (p=0.002) and concentration (p=0.02). However, some findings have raised concerns about whether activation of the renin-angiotensin system has a role in the vitamin D-deficient state in human beings.29

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 in the protective and anti-inflammatory actions of vitamin D. 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 would therefore be less affected by temporal variations in individual characteristics.
Articles

Panel: Research in context

Systematic review

Investigators of several systematic reviews 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, but the quality of the available evidence has been criticised. 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.

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)D, and 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. 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 EH contributed to study design. RJ, AKD, HJLH, JE, DKH, PJVD, ETHi, VV, RMF, JEH, RADB, BS, K-US, MIM, SS, AP, JLIa, MEK, GN, ML, KJ, NA, JC, JA, RH, SR, LKB, JLaH, CoS, BWP, LR, KKl, RPS, DGH, LB, EHa, HM, EI, DM, OL, IT, SM, ETHe, CMTT, AJ, PN, AFW, OP, CH, JFW, IR, VS, JF, JHC, JFP, MK, LL, KM, SB, TMF, CAH, TIAS, SBK, BLL, JGE, JCF, TDS, TL, DK, SEH, CC, CoS, WM, MHD, Mki, MKi, TJW, CP, HB, GG, PVDH, HS, SP, M-Rj, and EH contributed to data collection. KSV, AC, DJB, ADH, JCW, and EH interpreted results. The writing group consisted of KSV, AC, SP, and EH. 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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References