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Associations of childhood 25-hydroxyvitamin D_2 and D_3 and cardiovascular risk factors in adolescence: prospective findings from the Avon Longitudinal Study of Parents and Children

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Abstract

Background—Studies of the associations of circulating total 25-hydroxyvitamin D (25(OH)D) with cardiovascular disease risk factors in adults have reported inconsistent findings. We aimed to compare prospective associations of two analogues of childhood 25(OH)D ($25(OH)D_2$ and $25(OH)D_3$) with cardiovascular risk factors measured in adolescence.

Methods and results—We examined associations of childhood (ages 7–12 years) $25(OH)D_2$ and 25-$25(OH)D_3$ with a range of cardiovascular risk factors (blood pressure, fasting lipids, glucose, insulin and C-reactive protein (CRP)) determined in adolescence (mean age 15.4 years). Data were from 2470 participants of the Avon Longitudinal Study of Parents and Children (ALSPAC), a prospective population-based cohort. After adjustments for age, gender, socioeconomic position and BMI, there were no associations of $25(OH)D_2$ with cardiovascular risk factors. There was a positive association of season-adjusted (and unadjusted) $25(OH)D_3$ with high-density lipoprotein cholesterol (HDL-C) (mean change per doubling of $25(OH)D_3$: 0.03 mmol/l; 95% confidence interval (CI): 0.001 to 0.05, $p = 0.02$) and an inverse association with fasting insulin (relative difference of −4.59% per doubling; 95% CI: −8.37 to −0.59, $p = 0.03$). Participants with total 25(OH)D concentration <50 nmol/l had 0.04 mmol/l lower HDL-C (95% CI: −0.07 to −0.01) and 5.54% higher fasting insulin (95% CI: 0.82 to 10.47) compared with participants with total 25(OH)D ≥72 nmol/l.

Conclusions—In the first prospective study of children/adolescents, we have shown that higher $25(OH)D_3$ concentrations in childhood are associated with higher levels of HDL-C and lower fasting insulin in adolescence.

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Keywords

Vitamin D; cardiovascular diseases; paediatrics; ALSPAC

Introduction

Several studies have reported inverse associations of circulating total 25-hydroxyvitamin D (25(OH)D) and cardiovascular disease incidence, mortality and levels of cardiovascular risk factors in adult populations.[1–3] Whilst the associations of total 25(OH)D with cardiovascular risk factors have been studied cross-sectionally in adolescence, with inconsistent findings,[4–8] prospective studies are lacking.

Recently, a need to distinguish between the two analogues of circulating 25(OH)D has been recognised.[9] Despite recommendations suggesting 25(OH)D deficient patients can be treated with supplementation by either vitamin D_2 or D_3[10], a recent meta-analysis found vitamin D_3, but not D_2, supplementation to decrease all-cause mortality.[11] 25(OH)D_2 and D_3 have differing potencies in their effects on bone metabolism,[9] but it is unclear whether the difference in molar potency translates to differences in strengths of associations with cardiovascular risk factors. Previous randomised controlled trials have been criticised on the basis that dosages or supplement type may not have been sufficient to give meaningful increases in 25(OH)D status.[12] Thus, it is necessary to establish whether 25(OH)D_2 and 25(OH)D_3 status are differentially associated with cardiovascular risk factors. Recently, we conducted a cross-sectional study in children (mean age 9.9 years) and found some evidence of differences in associations of 25(OH)D_2 and 25(OH)D_3 with cardiovascular risk factors.[13] We were unable to explore associations with fasting glucose and insulin, which have been related to 25(OH)D concentrations in adults,[2,14] and prospective studies in this area are important for examining associations with less possibility that these may have resulted from reverse causality.

Our aim here is to build on that work by estimating and comparing prospective associations of 25(OH)D_2 and 25(OH)D_3 measured in childhood with a range of cardiovascular risk factors (blood pressure, fasting lipids, insulin, glucose and C-reactive protein (CRP)) measured during adolescence at a mean age of 15.4 years.

Methods

Participants

The Avon Longitudinal Study of Parents and Children (ALSPAC) is a prospective birth cohort that recruited pregnant women (n = 14,541) in the former county of Avon, South West England. Women with an expected delivery date between 1 April 1991 and 31 December 1992 were eligible to be included. Full details of the study have been published[15,16] and are found online at http://www.alspac.bris.ac.uk. Live born children (n = 13,972) who survived past age one year have been followed up alongside their mothers with questionnaires during early childhood, and at regular assessments from age seven years. Ethical approval was granted by the ALSPAC Law and Ethics committee and the local research ethics committee, in compliance with the Declaration of Helsinki. Written informed consent/assent was obtained from both parents/guardians and the children. For this study, we used measures of 25(OH)D from blood samples collected from children at the follow-up assessments at seven, nine or 11 years, and cardiovascular risk factors measured when the children attended the year 15 follow-up assessment (mean follow-up: 5.5 years). A total of 2470 children had valid 25(OH)D measures, attended the year 15 assessment and had

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complete information on exposures, outcomes and co-variables, and these form our main study sample (see Supplementary Figure 1).

Measures

For most participants $25(OH)D_2$ and $25(OH)D_3$ were measured on non-fasting blood samples taken during the year 9 assessments (n = 1965). When samples from this age were not available we used measures sampled from year 11 (n = 406) or year 7 (n = 99) assessments. Details of 25(OH)D, PTH, calcium and phosphate assays, as well as measurements of outcomes and co-variables, are included in the Supplementary Material.

Statistical analysis

$25(OH)D_2$ and $25(OH)D_3$ were $\log_e$ transformed to reduce heteroscedasticity. $25(OH)D_3$ was adjusted for seasonal variability, as previously described.[13] In order to include all children on whom $25(OH)D_2$ was assayed, those with a value below the detectable limit of the assay (1.25 nmol/l) (34.5%) were assigned a value of 1.25 nmol/l. Children with $25(OH)D_2$ at or below the detection limit were also indicated using a binary variable (i.e. 0 = measured $25(OH)D_2$ value; 1 = a value at or below the detection limit) in all association regression models of $25(OH)D_2$ with cardiovascular risk factors.

Multivariable linear regression models were used to examine the associations of $25(OH)D_2$ and season-adjusted $25(OH)D_3$ with cardiovascular risk factors and to adjust for potential confounding and mediating factors. Regression coefficients and 95% confidence intervals (95% CIs) were multiplied by $\log_e(2)$ so that results represent the mean unit change in outcome per doubling of $25(OH)D_2$ or season-adjusted $25(OH)D_3$. Coefficients for log-transformed outcomes (triglycerides, insulin and CRP) were expressed in terms of relative per cent change per doubling of the exposure, by reformatting ratios of geometric means and 95% CIs. All regression analyses were conducted using a non-parametric bootstrap procedure (following 1000 replications) in conjunction with ordinary least squares linear regression. The bootstrapping procedure allowed us to obtain a valid p-value testing the null hypothesis that the association of $25(OH)D_2$ with a given outcome does not differ from the association of $25(OH)D_3$ with this same outcome; these p-values were calculated from the bootstrap replicate distribution.

We conducted several multivariable linear regression models for each exposure–outcome association. In model 1, we adjusted for age and gender. Model 2 was additionally adjusted for potential confounding by family socioeconomic position and BMI (and repeated with waist circumference in place of BMI) measured at the time of 25(OH)D sampling. Because adiposity tracks across childhood and is strongly associated with 25(OH)D and cardiovascular risk factors we adjusted for both baseline and follow-up (at the time of cardiovascular risk factor assessment) BMI in model 2. Lastly, in model 3, we also adjusted for potential mediation by PTH, circulating calcium and phosphate, since vitamin D helps maintain circulating calcium and phosphate and suppresses production of PTH, and each of these has been independently associated with risk of cardiovascular disease in studies of adult populations;[17–20] and models for $25(OH)D_2$ also included adjustment for season-adjusted $25(OH)D_3$, and vice versa. Possible non-linearity of associations between exposures and outcomes was tested by examining fractional polynomial statistics and interpreting graphical plots.[21]

Additional analyses—We conducted multivariable regression analyses examining mean differences of cardiovascular risk factors in participants with total 25(OH) D (the sum of $25(OH)D_2$ and $25(OH)D_3$ without seasonal adjustment) <50 nmol/l and participants with

total 25(OH)D between 50 and 72 nmol/l, compared with risk factors in participants with total 25(OH)D ≥72 nmol/l.[22]

We repeated multivariable linear regression analyses for $25(OH)D_3$ without adjustment for season of sampling, and also for total 25(OH)D. These models enable us to observe the effect of seasonality on associations, and also to compare results with other studies which have not examined associations of 25(OH)D analogues with outcomes separately.

Data on physical activity, dietary total energy intake and pubertal status were available on only 1724 (69.8%) of the 2470 participants included in the main analyses. Thus, we performed an additional analysis to determine whether these might confound associations in this subsample with data on these variables.

We also conducted analyses adjusted for age and gender including participants who were missing data on one or more co-variables (and therefore excluded from main analyses), to see if associations were similar when these participants were included.

Results

Table 1 shows the characteristics of the study sample alongside characteristics of those who were eligible but were excluded from analyses because of missing data for one or more variable. Children included in the study had on average higher mean age, waist circumference, energy intake, PTH, systolic blood pressure (SBP) and CRP, and were from higher socioeconomic backgrounds than those excluded because of missing data. The study sample also had lower mean unadjusted $25(OH)D_3$, diastolic blood pressure (DBP), triglycerides, low-density lipoprotein cholesterol (LDL-C) and fasting insulin. In most cases differences were small in magnitude.

There was a weak negative correlation between $25(OH)D_2$ and unadjusted $25(OH)D_3$ (Pearson's $r = -0.12$, $p < 0.001$) but not between $25(OH)D_2$ and season-adjusted $25(OH)D_3$ (Pearson's $r = -0.005$, $p = 0.81$). Unadjusted $25(OH)D_3$ was strongly positively correlated with both the season-adjusted measure (Pearson's $r = 0.80$, $p < 0.001$) and total 25(OH)D (Pearson's $r = 0.97$, $p < 0.001$). There were weak negative correlations between PTH and season-adjusted $25(OH)D_3$ (Pearson's $r = -0.12$, $p < 0.001$) and unadjusted $25(OH)D_3$ (Pearson's $r = -0.20$, $p < 0.001$), and a weak positive correlation between PTH and $25(OH)D_2$ (Pearson's $r = 0.04$, $p = 0.03$). Seven hundred and thirty-six (29.8%) participants had total 25(OH)D under 50 nmol/l and 1104 (44.7%) participants had 25(OH)D concentrations between 50 and 72 nmol/l.

Table 2 shows the multivariable associations of childhood $25(OH)D_2$ with cardiovascular risk factors measured in adolescence. $25(OH)D_2$ was not associated with any cardiovascular risk factors in any of the multivariable models.

Table 3 shows the multivariable associations of season-adjusted $25(OH)D_3$ and cardiovascular risk factors. In analyses adjusted for age and gender only (model 1), $25(OH)D_3$ was positively associated with high-density lipoprotein cholesterol (HDL-C) and inversely associated with fasting insulin. No associations with other risk factors were observed. Results remained the same after further adjustments for socioeconomic position and baseline and follow-up BMI (model 2), and also after adjustments for $25(OH)D_2$, PTH, calcium and phosphate (model 3).

There was statistical evidence that associations of $25(OH)D_2$ with HDL-C and insulin differed from associations of $25(OH)D_3$ with these risk factors in all models (p for differences = 0.003 for HDL-C; 0.004 for insulin). There was no strong statistical evidence

that associations of $25(OH)D_2$ and $25(OH)D_3$ with other cardiovascular risk factors differed in any model (all other $p \geq 0.27$).

There was no strong statistical evidence that any of the associations of $25(OH)D_2$ or $25(OH)D_3$ with outcomes were non-linear (all p values for deviation from linearity ≥0.09).

Table 4 shows mean differences in cardiovascular risk factors in those with total 25(OH)D <50 nmol/l and those with total 25(OH)D between 50 and 72 nmol/l, compared with those with total 25(OH)D ≥72 nmol/l. Consistent with the linear associations of $25(OH)D_3$ with outcomes, participants with lower concentrations of total 25(OH)D in childhood had lower HDL-C and higher fasting insulin compared with those in the high total 25(OH)D group, which remained after adjustment for potential confounders and mediators. Participants in the low 25(OH)D groups also had higher triglycerides compared with those in the high 25(OH)D group.

Supplemental Tables 1 and 2 show multivariable linear associations of unadjusted $25(OH)D_3$ and total 25(OH)D with cardiovascular risk factors, respectively. For both exposures, results were largely the same as those observed between season-adjusted $25(OH)D_3$ and cardiovascular risk factors. The one exception was that inverse associations were present between both $25(OH)D_3$ (unadjusted for season) and total 25(OH)D and triglycerides, which remained after adjusting for potential confounders and mediators. To further explore why we may have found a positive prospective association of $25(OH)D_3$ with triglycerides which is attenuated with seasonal adjustment, we compared the month of the year in which each participant attended the childhood assessment used for 25(OH)D sampling with the month in which they attended the assessment at 15 years. The rationale being that if children were called back to clinic at the same time each year, it is possible that both exposure and outcome are measured in the same season and that triglyceride levels are affected by season. However, we found little agreement (6.2%) between the two (compared with 8.6% agreement expected by chance; Kappa statistic = −0.03; $p = 1.0$).

Supplemental Tables 3 and 4 show associations of $25(OH)D_2$ and season-adjusted $25(OH)D_3$ in the subsample of participants who had data on physical activity, energy intake and pubertal status at the time of 25(OH)D sampling ($n = 1724$). Results from the subsample were very similar to those of the main analyses, and adjustment for the additional potential confounders did not appreciably change associations.

Supplemental Table 5 shows age and gender adjusted associations of $25(OH)D_2$ and season-adjusted $25(OH)D_3$ with cardiovascular risk factors in participants who formed our main study sample, alongside associations in samples including participants who were excluded from main models because of missing data on one or more co-variable. All associations were very similar between the samples.

Discussion

In this prospective study, circulating $25(OH)D_3$ (either unadjusted or season-adjusted) measured in childhood was positively associated with HDL-C and inversely associated with fasting insulin measured in adolescence. Conversely, circulating $25(OH)D_2$ was not associated with any of the cardiovascular risk factors examined. Children in low total 25(OH)D concentration groups (<50 nmol/l, or 50 to 72 nmol/l) had lower HDL-C and higher fasting insulin than those with high concentrations (≥72 nmol/l).

In studies of adult populations, higher total 25(OH)D concentrations or measures of dietary vitamin D intake have been associated with lower levels of cardiovascular disease and cardioprotective levels of cardiovascular risk factors,[1,2,14,23] though current evidence from

randomised controlled trials that have examined these questions and a recent meta-analysis of them appears to be inconsistent.[24–26] With the exception of our recent cross-sectional study of ALSPAC children,[13] studies examining associations of 25(OH)D status and cardiovascular risk factors have assessed total 25(OH)D rather than separate components of 25(OH)D.[4,6–8,14] However, since $25(OH)D_3$ is the major constituent of circulating 25(OH)D, we would expect our results for $25(OH)D_3$ to be comparable to these previous studies. Thus, the positive prospective association of $25(OH)D_3$ (and total 25(OH)D) with HDL-C reported here is consistent with previous cross-sectional studies of total 25(OH)D in children and adolescents.[4,7] We also previously observed a positive, cross-sectional association of $25(OH)D_3$ with HDL-C measured in ALSPAC children at mean age 9.9 years.[13] The presence of both cross-sectional and prospective associations of childhood $25(OH)D_3$ with HDL-C measured at mean age 9.9 years and again at 15.4 years may in part reflect the positive correlation between HDL-C measures at the two time points ($r = 0.60$, $p < 0.001$). However, the prospective association observed in the current study remained when adjusted for childhood HDL-C, suggesting that the association of $25(OH)D_3$ with HDL-C is present throughout childhood and adolescence. If the association represents a causal relationship, mechanisms by which $25(OH)D_3$ could potentially influence HDL-C levels are unclear. *VDR*-knockout mice (with perturbed vitamin D receptor function) have a leaner phenotype than wildtype mice, with reduced circulating triglycerides and total cholesterol,[27] but also have lower expression of Apo-A1mRNA and possibly lower circulating HDL-C.[28] There could potentially be direct or indirect effects of $1,25(OH)_2D_3$ on HDL-C levels, with indirect effects taking place via effects on intestinal calcium absorption and/or by decreasing PTH,[29] but these links are speculative.

We also found an inverse association of $25(OH)D_3$ (and total 25(OH)D) with fasting insulin, which has been observed previously in children and adolescents,[8] although strong associations have not been observed universally in adolescents following adjustment for adiposity measures.[4] Our finding for insulin is in keeping with prospective studies in adults that have reported associations of low 25(OH)D status with increased risk of insulin resistance and type-2 diabetes.[2,30] Experimental studies have suggested a direct role of vitamin D metabolites in improving insulin-mediated glucose transport, by up-regulating expression of the human insulin receptor gene,[31] and also in the activation of transcription factors involved in glucose homeostasis.[32] There may also be indirect actions of vitamin D through effects on inflammatory processes, or on insulin-mediated intracellular processes via the regulation of calcium.[2,33] However, the lack of association of $25(OH)D_3$ with fasting glucose in the current study suggests that if there is any influence of the vitamin D system on insulin/glucose metabolism, it may not fully emerge until adulthood.

If higher $25(OH)D_3$ concentrations are causally related to lower insulin and higher HDL-C, this could reflect more potent effects of $25(OH)D_3$ relative to those of $25(OH)D_2$ or differences in the specific metabolic pathways influenced by these, via different abilities of D_3 and D_2 metabolites to bind to the vitamin D receptor, for example.[9] Unadjusted $25(OH)D_3$ and total 25(OH)D were also inversely associated with triglycerides, in contrast to the null-finding for season-adjusted $25(OH)D_3$. This finding is also in contrast to previous cross-sectional studies of children and adolescents, none of which reported associations of total 25(OH)D (or $25(OH)D_3$)[34] with triglycerides.[4,6,7] In our own previous cross-sectional study using data from ALSPAC, we found no strong statistical evidence of associations of either unadjusted and season-adjusted $25(OH)D_3$ with triglycerides.[13] Adjustment of total 25(OH)D or $25(OH)D_3$ for season provides an assessment of the individuals' average status (i.e. across seasons). It would therefore be expected that where associations are real, they may be stronger or the same with seasonal adjustment. Thus, the attenuation of the association of triglycerides with seasonal adjustment, and a lack of previous evidence for the association, suggests that the association not adjusted for season is a chance finding.

We previously reported positive associations of 25(OH)D_2 with inflammatory markers (CRP and IL-6) in the cross-sectional study of ALSPAC children.[13] In contrast to those findings, a lack of prospective association between childhood 25(OH)D_2 and CRP measured in adolescence suggests that childhood 25(OH)D_2 concentration is unlikely to be an important determinant of chronic inflammation levels. It therefore seems possible that the associations previously reported arose by chance.

Study strengths and limitations

To our knowledge, this is the first study to examine prospective associations of childhood 25(OH)D concentration with a broad range of cardiovascular risk factors in adolescence. It is also the first prospective study to compare the two analogues of 25(OH)D in relation to these outcomes, which is important in the consideration of appropriate supplementation, should further evidence support causality. Moreover, our analyses were conducted on a non-select general population.

A limitation of our study is attrition in participant numbers. However, this is unlikely to have biased results, as we cannot think of a reason why associations between 25(OH)D status and cardiovascular risk factors would differ between included and excluded participants. Analogues of 25(OH)D were assessed using single measures, and so regression dilution of associations could have occurred, meaning that the associations presented here may be weaker than any true association. Total 25(OH)D concentrations have been shown to correlate over time,[35] and our use of a single measurement is consistent with previous studies in adults and children of similar associations.[4,7,8,14]

Conclusion and implications

Based on results from studies in adults, there have been some calls for policies to recommend higher levels of safe sun exposure, greater use of vitamin D supplementation and/or increased fortification of foods with the vitamin in order to prevent cardiovascular disease.[12,36,37] In contrast, others have suggested guidelines should not currently be changed for the purposes of reducing cardiovascular risk because existing evidence remains inconsistent and insufficient.[38] Thus, further prospective studies and randomised controlled trials investigating this are necessary. Current guidelines about the fortification of foods with vitamin D analogues and the strength/type of vitamin D supplements vary between countries, but where these are recommended it may not be specified whether practices should be based on the use of vitamin D_2 or D_3.[39] The findings from this prospective study – that childhood 25(OH)D_3 is associated with higher levels of HDL-C and lower insulin in adolescence, and that 25(OH)D_2 is not associated with any risk factors – suggest that future randomised controlled trials assessing the effectiveness of vitamin D supplementation for reducing cardiovascular risk factors should usefully compare the benefits of vitamin D_3 versus D_2 supplementation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Baseline and follow-up characteristics of participants included in the analyses, and of those excluded because of missing data on one or more variable (mean and standard deviation (SD) unless otherwise stated)

	Excluded participants		Study sample (*n* = 2470)	
	***n* missing**	**Mean (SD) or %**	**Mean (SD) or %**	***p*** *
Baseline characteristics				
Age at 25(OH)D sampling, years	2121	9.8 (1.3)	10.0 (0.9)	<0.001
% Male		45.2	49.5	0.001
Clinic of 25(OH) D sampling, %:	2121			
7-year follow-up		16.9	4.0	
9-year follow-up		66.2	79.6	
11-year follow-up		16.9	16.4	
Socioeconomic position, %	1626			0.01
I/II		61.6	65.6	
III (non-manual work)		24.6	22.4	
III (manual work)		9.4	8.7	
IV/V		4.4	3.4	
BMI (kg/m^2)	1989	17.6 (2.9)	17.8 (2.9)	0.10
Waist circ., cm	2100	62.1 (8.1)	63.3 (8.2)	<0.001
Recent vigorous physical activity, %[a]	1569			0.30
None		0.9	0.5	
Less than once		4.0	3.6	
1–3 times a week		50.3	49.0	
4–6 times a week		29.1	31.3	
Daily		15.8	15.6	
Daily energy intake, kcal[a]	1743	1693.6 (307.0)	1730.9 (307.0)	<0.001
Pubertal stage at 25(OH)D sampling, %[a]	1531			0.13
I/II		95.7	97.3	
III		3.5	2.2	
IV/V		0.8	0.5	
25(OH)D_2, nmol/l[b]	2121	3.5 (1.2, 7.0)	3.2 (1.2, 6.5)	0.11
25(OH)D_3, nmol/l	2121	58.3 (23.4)	56.9 (20.2)	0.01
PTH, pmol/l	2115	4.8 (2.2)	4.9 (2.1)	0.01
Calcium, mmol/l	2112	2.37 (0.11)	2.38 (0.11)	0.71
Phosphate, mmol/l	2095	1.54 (0.16)	1.54 (0.16)	0.33
Measures at follow-up				
BMI (kg/m^2)	2061	21.4 (3.4)	21.5 (3.7)	0.18
Waist circ., cm	1670	76.7 (9.2)	76.7 (8.6)	0.96
Systolic blood pressure, mmHg	1862	122.7 (10.9)	123.6 (10.7)	0.01
Diastolic blood pressure, mmHg	1862	68.3 (9.1)	66.9 (8.4)	<0.001

	Excluded participants		Study sample (*n* = 2470)	
	***n* missing**	**Mean (SD) or %**	**Mean (SD) or %**	***p*** *
Triglycerides, mmol/l[b]	699	0.77 (0.60, 1.01)	0.74 (0.59, 0.97)	0.03
LDL-C, mmol/l	699	2.13 (0.6)	2.07 (0.5)	0.001
HDL-C, mmol/l	699	1.27 (0.28)	1.28 (0.29)	0.83
Glucose, mmol/l	699	5.2 (0.5)	5.2 (0.4)	0.52
Insulin, IU/l[b]	699	9.4 (6.9, 13.5)	8.9 (6.7, 11.9)	<0.001
CRP, mg/l[b]	699	0.41 (0.23, 1.05)	0.38 (0.22, 0.85)	0.02

[a] Data on physical activity, daily energy intake and pubertal status were available for 1724 participants of our study sample;

[b] Medians (with interquartile ranges) are shown because of skewed distribution;

* *p* based on two-tailed *t*-test or chi-square test for differences between groups;

25(OH)D_2: 25-hydroxyvitamin D_2; 25(OH)D_3: 25-hydroxyvitamin D_3; CI: confidence interval; CRP: C-reactive protein; DBP: diastolic blood pressure; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; SBP: systolic blood pressure.

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Table 2

Associations between 25(OH)D_2 measured during childhood (at mean age 7.4, 9.8 or 11.7 years) and cardiovascular risk factors measured at mean age 15.5 years (*n* = 2470)

	Model 1[a]			Model 2[b]			Model 3[c]		
	Mean difference per doubling of 25(OH)D_2	95% CI	*p*	Mean difference per doubling of 25(OH)D_2	95% CI	*p*	Mean difference per doubling of 25(OH)D_2	95% CI	*p*
SBP, mmHg	−0.22	(−0.72, 0.30)	0.40	−0.14	(−0.63, 0.37)	0.59	−0.12	(−0.61, 0.39)	0.65
DBP, mmHg	−0.09	(−0.57, 0.38)	0.69	−0.08	(−0.56, 0.39)	0.72	−0.10	(−0.59, 0.37)	0.68
Triglycerides, % change*	−0.97	(−2.76, 1.13)	0.33	−0.66	(−2.48, 1.34)	0.51	−0.72	(−2.66, 1.29)	0.47
LDL-C, mmol/l	0.00	(−0.02, 0.03)	0.75	0.01	(−0.02, 0.03)	0.63	0.01	(−0.02, 0.03)	0.68
HDL-C, mmol/l	0.00	(−0.02, 0.01)	0.61	−0.01	(−0.02, 0.01)	0.37	−0.01	(−0.02, 0.01)	0.27
Glucose, mmol/l	0.00	(−0.02, 0.01)	0.63	0.00	(−0.02, 0.01)	0.65	0.00	(−0.02, 0.01)	0.70
Insulin, % change*	0.64	(−1.73, 3.09)	0.60	1.33	(−0.88, 3.49)	0.24	1.17	(−1.11, 3.41)	0.31
CRP, % change*	−2.64	(−8.05, 3.10)	0.36	−1.34	(−6.48, 4.04)	0.63	−1.34	(−6.44, 4.32)	0.63

[a] Model 1: adjusted for age and gender;

[b] Model 2: as model 1 plus socioeconomic position, childhood BMI and follow-up BMI;

[c] Model 3: as model 2 plus season-adjusted 25(OH)D_3, PTH, circulating calcium and phosphate;

* These outcomes were log transformed and differences represent a relative per cent change in the outcome per doubling of 25(OH)D_2;

25(OH)D_2: 25-hydroxyvitamin D_2; CI: confidence interval; CRP: C-reactive protein; DBP: diastolic blood pressure; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; SBP: systolic blood pressure.

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Table 3

Associations between 25(OH)D_3 measured during childhood (at mean age 7.4, 9.8 or 11.7 years) and cardiovascular risk factors measured at mean age 15.5 years (*n* = 2470)

	Model 1[a]			Model 2[b]			Model 3[c]		
	Mean difference per doubling of 25(OH)D_3	95% CI	*p*	Mean difference per doubling of 25(OH)D_3	95% CI	*p*	Mean difference per doubling of 25(OH)D_3	95% CI	*p*
SBP, mmHg	0.16	(−0.72, 1.05)	0.73	0.49	(−0.42, 1.35)	0.27	0.47	(−0.45, 1.35)	0.29
DBP, mmHg	−0.11	(−0.90, 0.68)	0.78	−0.05	(−0.85, 0.78)	0.91	−0.06	(−0.87, 0.75)	0.88
Triglycerides, % change*	−1.68	(−4.74, 1.34)	0.30	−0.63	(−3.52, 2.35)	0.68	−0.56	(−3.43, 2.37)	0.72
LDL-C, mmol/l	−0.01	(−0.06, 0.04)	0.76	0.01	(−0.04, 0.05)	0.83	0.00	(−0.05, 0.05)	0.95
HDL-C, mmol/l	0.04	(0.01, 0.06)	0.003	0.03	(0.001, 0.05)	0.03	0.02	(0.001, 0.05)	0.04
Glucose, mmol/l	−0.02	(−0.05, 0.01)	0.23	−0.02	(−0.05, 0.01)	0.33	−0.02	(−0.05, 0.01)	0.27
Insulin, % change*	−6.63	(−10.57, −2.66)	0.002	−4.21	(−8.04, −0.35)	0.04	−4.35	(−8.35, −0.48)	0.03
CRP, % change*	−1.57	(−10.05, 7.90)	0.73	4.65	(−4.32, 14.94)	0.32	3.84	(−5.05, 14.17)	0.41

25(OH)D3: 25-hydroxyvitamin D3; CI: confidence interval; CRP: C-reactive protein; DBP: diastolic blood pressure; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; SBP: systolic blood pressure;

[a]Model 1: adjusted for age and gender;

[b]Model 2: as model 1 plus socioeconomic position, childhood BMI and follow-up BMI;

[c]Model 3: as model 2 plus 25(OH)D2, PTH, and circulating calcium and phosphate;

*These outcomes were log transformed and differences represent a relative per cent change in the outcome per doubling of 25(OH)D3.

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Table 4

Mean differences in cardiovascular risk factors in participants with total 25(OH)D = 50 to 72 nmol/l (*n* = 1104) and those with total 25(OH)D <50 nmol/l (*n* = 736) compared with those with total 25(OH)D ≥72 nmol/l (*n* = 630)

	Model 1			Model 2			Model 3		
	Mean difference in outcomes comparing those with 25(OH)D = 50 to 72 nmol/l with those with 25(OH)D ≥72 nmol/l	Mean difference in outcomes comparing those with 25(OH)D <50 nmol/l with those with 25(OH)D ≥72 nmol/l	*p* †	Mean difference in outcomes comparing those with 25(OH)D = 50 to 2 nmol/l with those with 25(OH)D ≥72 nmol/l	Mean difference in outcomes comparing those with 25(OH)D <50 nmol/l with those with 25(OH)D ≥72 nmol/l	*p* †	Mean difference in outcomes comparing those with 25(OH)D = 50 to 72 nmol/l with those with 25(OH)D ≥72 nmol/l	Mean difference in outcomes comparing those with 25(OH)D <50 nmol/l with those with 25(OH)D ≥72 nmol/l	*p* †
SBP, mmHg	0.62 (−0.39, 1.63)	−0.34 (−1.44, 0.76)	0.48	0.57 (−0.42, 1.55)	−0.62 (−1.69, 0.46)	0.21	0.58 (−0.41, 1.57)	−0.60 (−1.69, 0.49)	0.23
DBP, mmHg	0.68 (−0.15, 1.50)	0.25 (−0.65, 1.14)	0.66	0.67 (−0.16, 1.49)	0.20 (−0.71, 1.10)	0.74	0.65 (−0.18, 1.48)	0.17 (−0.75, 1.08)	0.79
Triglycerides, % change*	1.72 (−1.93, 5.50)	4.67 (0.58, 8.92)	0.02	1.73 (−1.83, 5.41)	4.04 (0.08, 8.16)	0.04	1.65 (−1.92, 5.36)	3.85 (−0.17, 8.04)	0.06
LDL-C, mmol/l	−0.03 (−0.08, 0.02)	0.00 (−0.06, 0.06)	0.93	−0.03 (−0.08, 0.02)	−0.01 (−0.06, 0.05)	0.91	−0.03 (−0.08, 0.02)	0.00 (−0.06, 0.05)	0.98
HDL-C, mmol/l	−0.04 (−0.07, −0.01)	−0.05 (−0.08, −0.02)	0.002	−0.04 (−0.07, −0.01)	−0.04 (−0.07, −0.01)	0.01	−0.04 (−0.07, −0.01)	−0.05 (−0.08, −0.02)	0.002
Glucose, mmol/l	0.01 (−0.02, 0.05)	0.00 (−0.03, 0.04)	0.89	0.02 (−0.02, 0.05)	0.00 (−0.04, 0.04)	0.96	0.02 (−0.02, 0.05)	0.01 (−0.03, 0.05)	0.71
Insulin, % change*	3.16 (−1.39, 7.91)	7.24 (2.10, 12.64)	0.01	2.92 (−1.30, 7.33)	5.54 (0.82, 10.47)	0.02	2.98 (−1.26, 7.41)	5.70 (0.90, 10.73)	0.02
CRP, % change*	0.24 (−9.93, 11.55)	−1.36 (−12.22, 10.85)	0.81	−0.90 (−10.43, 9.65)	−5.42 (−15.31, 5.62)	0.31	−0.62 (−10.23, 10.02)	−4.96 (−15.06, 6.34)	0.36

[a] Model 1: adjusted for age and gender;

[b] Model 2: as model 1 plus socioeconomic position, childhood BMI and follow-up BMI;

[c] Model 3: as model 2 plus 25(OH)D_2, PTH, and circulating calcium and phosphate;

† *p* for trend of difference in risk factors across 25(OH)D concentration groups;

* Results are percentage difference in outcomes compared with the reference group (those with total 25(OH)D ≥72 nmol/l);

25(OH)D: 25-hydroxyvitamin D_3; CI: confidence interval; CRP: C-reactive protein; DBP: diastolic blood pressure; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; SBP: systolic blood pressure.