Risk Factors for Variation in 25-Hydroxyvitamin D_3 and D_2 Concentrations and Vitamin D Deficiency in Children

Anna-Maija Tolppanen, Abigail Fraser, William D. Fraser, and Debbie A. Lawlor

Medical Research Council Centre for Causal Analyses in Translational Epidemiology (A.-M.T., A.F., D.A.L.), Department of Social and Community Medicine, University of Bristol, Bristol BS8 2BN, United Kingdom; and Norwich Medical School (W.D.F.), University of East Anglia, Norwich NR4 7TJ, United Kingdom

Context: Vitamin D status is believed to be best indicated by serum 25-hydroxyvitamin D [25(OH)D; consisting of 25(OH)D_3 and 25(OH)D_2] that are obtained from different sources. Suboptimal vitamin D status is common and associated with adverse health outcomes.

Objectives: The objectives were to report the prevalence and risk factors of vitamin D deficiency and determine associations of characteristics that have been shown to relate to total 25(OH)D with 25(OH)D_3 and 25(OH)D_2 concentrations.

Design and Setting: The Avon Longitudinal Study of Parents and Children is a population-based contemporary birth cohort (children born in 1991–1992) from southwest England.

Participants and Outcome Measures: Seven thousand five hundred sixty children with serum 25(OH)D_3 and 25(OH)D_2 concentrations measured at the mean age of 9.9 yr participated in the study.

Results: Vitamin D deficiency [total 25(OH)D concentration $<$20 ng/ml] was common (29%). The main risk factors were winter season, less time spent outdoors, low socioeconomic position, nonwhite ethnicity, older age, more advanced puberty stage, and female gender. Although there were some common risk factors for lower 25(OH)D_3 and 25(OH)D_2 concentrations (age, gender, puberty stage, body mass index, physical activity, household income, maternal education), several characteristics were associated with 25(OH)D_3 only (ethnicity, vitamin D intake, time spent outdoors, and UVB protection score) and others with 25(OH)D_2 only (protein and carbohydrate intake, parent's social class, and housing tenure).

Conclusions: Vitamin D deficiency was common in this contemporary U.K. cohort. Despite some overlap, there are differences in potential confounding structures for associations of 25(OH)D_3 and 25(OH)D_2 with health outcomes. These should be accounted for in future studies. ***(J Clin Endocrinol Metab* 97: 1202–1210, 2012)**

The increasing number of observational studies suggesting that inadequate vitamin D status is associated with a wide range of adverse health outcomes (1, 2) has resulted in calls for changes to public health guidance regarding extreme protection against UVB exposure (3). However, it remains unclear whether these associations are causal or the extent to which they might be explained by residual confounding (4). Randomized controlled trials have failed to provide convincing evidence that vitamin D supplementation reduces, for example, cardiovascular risk (5), but the trials have been criticized for using inadequate doses or using vitamin D_2, which is less potent at

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Abbreviations: BMI, Body mass index; 25(OH)D, 25-hydroxyvitamin D; 25(OH)D_3, 25(OH)D synthesized from vitamin D_3 obtained mainly from UVB-induced synthesis in skin but also from animal food sources and supplements; 25(OH)D_2, 25(OH)D synthesized from vitamin D_2 from plant sources and supplements.

increasing serum 25-hydroxyvitamin D [25(OH)D] concentrations than vitamin D_3 (6). With this background it becomes ever more important to identify characteristics that allow best adjustment for potential confounders in observational studies and help to assess whether differences in associations between these two forms of 25(OH)D are consistent with different confounding structures between the two.

Serum 25(OH)D concentrations reflect both the synthesis in skin and dietary intake (7). Circulating 25(OH)D consists mainly of $25(OH)D_3$ (synthesized from vitamin D_3 obtained mainly from UVB induced synthesis in skin but also from animal food sources and supplements) and to lesser extent of $25(OH)D_2$ (synthesized from vitamin D_2 from plant sources and supplements) (8). Due to different sources, the confounding structures for these two analytes may be different, but all previous studies on vitamin D status have used total 25(OH)D concentrations.

The aims of this study were: 1) to report the prevalence of deficient and insufficient concentrations of total 25(OH)D concentrations in a contemporary cohort of U.K. children who have been influenced by health promotion messages to limit UVB exposure and determine risk factors for vitamin D deficiency; (1) to determine the relative contributions of $25(OH)D_3$ and $25(OH)D_2$ to total 25(OH)D in these children; and 3) to determine the associations of risk factors that have been previously shown to relate to total 25(OH)D with $25(OH)D_3$ and $25(OH)D_2$ separately and thereby clarify whether these two are likely to be influenced by different confounders in their associations with health outcomes.

Materials and Methods

Population

The Avon Longitudinal Study of Parents and Children is a population-based birth cohort from southwest England. The cohort consisted of 14,062 live births from 14,541 enrolled pregnant women who were expected to give birth between April 1, 1991, and December 31, 1992 (9). From age 7 yr, all children were invited for an annual assessment of physical and psychological development. Parents gave informed consent at enrolment and at each follow-up clinic assessment. Ethical approval was obtained from the Avon Longitudinal Study of Parents and Children Law and Ethics Research Committee and local National Health Service research ethics committee. Children from single and twin births were included in this study. Altogether 7560 children had data on serum $25(OH)D_3$ and $25(OH)D_2$ concentration, respectively. The degree of missing data for risk factors varied and the final number in vitamin D deficiency analyses was 4393 and between 5628 and 7555 in univariable analyses with $25(OH)D_3$ or $25(OH)D_2$ as outcome (Fig. 1).

Measurement of serum $25(OH)D_3$ and $25(OH)D_2$

Serum $25(OH)D_3$ and $25(OH)D_2$ were assayed on nonfasting blood samples collected at mean age 9.9 yr for the majority of participants. If no samples were available from the 9.9 yr assessment, samples from mean age 11.8 yr or, secondly, mean age 7.6 yr were used. The mean age at sample collection in the whole study sample was 9.8 yr (SD 1.1). After collection, samples were immediately spun, frozen, and stored at −80 C. Assays were performed after a maximum of 12 yr in storage with no previous freeze-thaw cycles. The $25(OH)D_3$ and $25(OH)D_2$ concentrations were measured with HPLC tandem mass spectrometry using internal standard in a laboratory meeting the performance target set by the Vitamin D External Quality Assessment Scheme Advisory Panel for 25(OH)D assays. Interassay coefficients of variation for the assay were less than 10% across a working range of 1–250 ng/ml for both $25(OH)D_3$ and $25(OH)D_2$.

Risk factors

We considered gender, seasonality, ethnicity (white/nonwhite), socioeconomic position, UVB exposure, body mass index (BMI), waist circumference, health status, vigorous physical activity, pubertal stage, total energy intake and dietary intake of macronutrients, vitamin D, and calcium to be potentially important risk factors for $25(OH)D_3$ and $25(OH)D_2$ because of their known associations with total 25(OH)D concentrations. Seasons were defined as winter (reference category; December, January, and February), spring (March, April, and May), summer (June, July, and August), and autumn (September, October, and November).

Data on ethnicity, household income, housing tenure, maternal education, and head of household occupational social class were obtained from parent-completed questionnaires. Household disposable income was averaged over two observations obtained when the child was 33 and 47 months old. This measure also accounted for the family size and composition and the estimated housing benefits and was expressed in 1995 prices for comparability among the cohort members.

Time spent outdoors during the summer months on school days, school weekends, and holidays was reported as none, 1 h/d, 1–2 h/d, and 3 or more h/d in parent-completed questionnaires at the mean age of 8.5 yr. Responses were coded as follows: none, 0; 1′, 1; 1–2′, 1.5; and 3′, 5. Average hours spent outdoors per summer day (June 1 through August 31) were calculated using term dates from Bristol City Council's Education Committee term dates for 2001–2002 (summer term June 1 through July 23, holidays July 24 through August 31). Information on protection from UVB exposure (use of sunblock, covering clothing or hat, and avoidance of midday sun) were obtained from the same questionnaires A summary variable for UVB protection score was derived by scoring the responses to questions on use of sunblock, covering clothing or hat, and avoidance of midday sun as always, 3, usually, 2 sometimes, 1, and never, 0, and summing them. This gives a single variable with a range from zero to 12, with zero indicating less meticulous protection from UVB.

BMI was calculated from height and weight measured at the same time as blood samples were taken. Weight and height were measured in light clothing and without shoes. Weight was measured to the nearest 0.1kg using Tanita scales. Height was measured to the nearest 0.1 cm using a Harpenden stadiometer. BMI was categorized to underweight (corresponding to BMI <18.5 kg/m^2 at age 18 yr), normal (corresponding to BMI 18.6–25.0 at

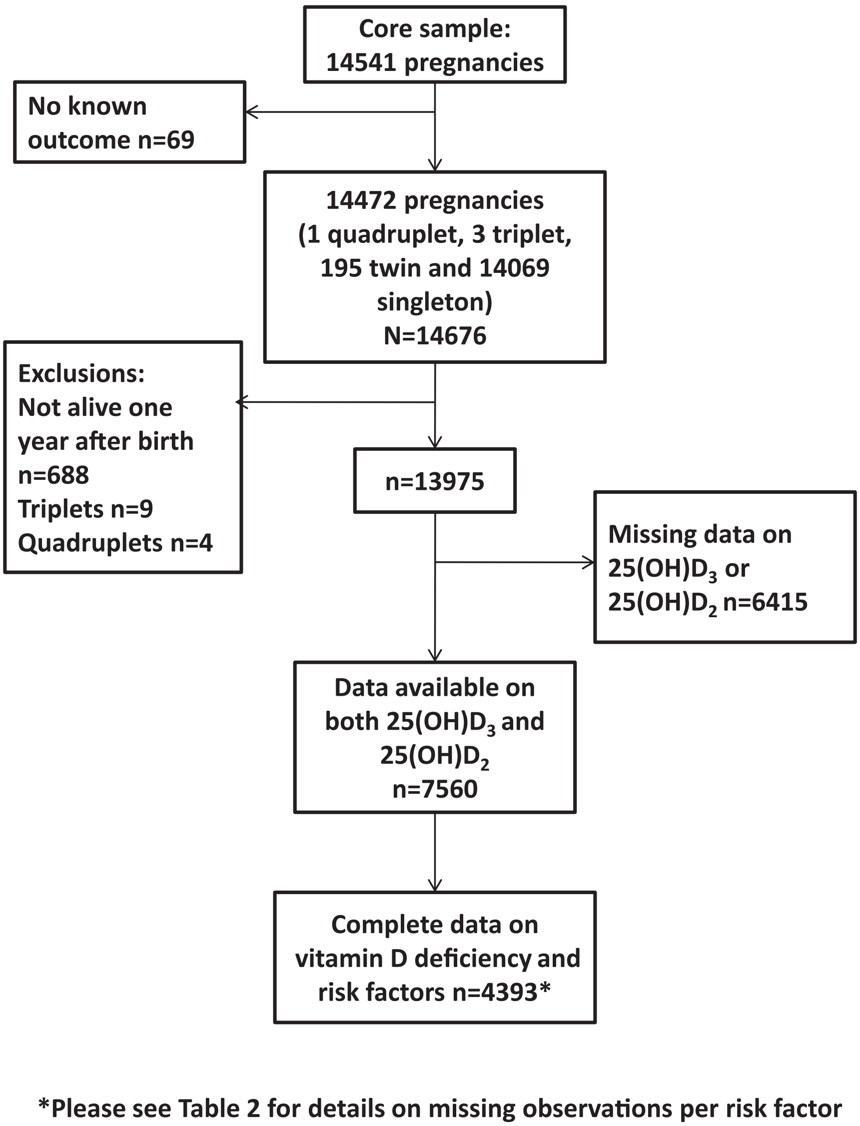

FIG. 1. Flow of participants.

age 18 yr), overweight (corresponding to BMI 25.1–30 at age 18 yr), and obese (corresponding to BMI $<$30 kg/m^2 at age 18 yr) using international age-and gender-specific cutoff points according to Cole *et al.* (10, 11). Waist was measured to the nearest 1 mm at the midpoint between the lower ribs and the pelvic bone with a flexible tape and the measurement closest to the time of phlebotomy was used in the analyses.

Health status during the last year or month was obtained from questionnaires completed by the main caregiver when the children were on average 6.8, 7.6, 8.6, 10.7, and 11.7 yr old by the following questions: 'How would you assess the health of your child in the past month?' and 'How would you assess the health of your child in the past year?' and with the following answer categories: very healthy, no problems/ healthy but a few minor problems, sometimes quite ill, and almost always unwell. Due to low frequencies, the two latter categories were combined in the analyses. Physical activity and puberty stage were assessed when children were 8.1, 9.5, 10.7, and 11.7 yr old. Parents answered questions that asked the average number of times that their children had participated in vigorous physical activity (including indoor and outdoor activities such as running, gymnastics, or swimming) in the past month, choosing from the following categories: none, less than once a week, one to three times per week, four to six times a week, or daily. To combine low-frequency categories, data were categorized as less than four times per week, four to six times per week, or daily. Puberty stage was assessed by parental report using Tanner staging (12) of breast development and pubic hair. This involves providing parents with standard pictures of different stages of breast and pubic hair development (developed by Tanner). Higher numbers indicate more advanced sexual maturation. We used data from the health, physical activity, and Tanner questionnaire closest to the time of phlebotomy for the exposures for each child.

Food frequency questionnaire (described in detail in Ref. 13) was used to calculate an approximate daily nutrient intake for each child at age 7 and 9 yr. Macronutrient, vitamin D, and calcium intakes were adjusted for total energy intake using the nutrient density model (14). Nutritional data closest to the time of phlebotomy for the exposures for each child were used in the analyses. The macronutrient, vitamin D and calcium intakes were log transformed.

Statistical analyses

Statistical analyses were conducted with Stata 11.2 (Stata Corp LP, College Station, TX). We present median (interquartile range) of total 25(OH)D, 25(OH)D_3, and 25(OH)D_2 and the number (percentage) of participants with vitamin D insufficiency [total 25(OH)D below 30 ng/ml, deficiency [total 25(OH)D below 20 ng/ml], and severe deficiency (total 25(OH)D below 10 ng/ml] (8, 15) and the relative contribution of 25(OH)D_3 to total 25(OH)D. We also graphed the percentage contribution against total 25(OH)D (25(OH)D_3/ total 25(OH)D100) for the whole cohort to examine whether the contribution of 25(OH)D_3 varied by total concentration.

Uni- and multivariable associations with vitamin D deficiency were analyzed with logistic regression, and the associations between potential risk factors and 25(OH)D_3, and 25(OH)D_2 concentrations were examined with univariable linear regression. To include all participants on whom 25(OH)D_2 was assayed, those with a value below the detectable limit of the assay (0.5 ng/ml) were given a value of 0.5 ng/ml and indicated using a binary covariable in all regression models (n = 2775, 36.7%). Log-transformed 25(OH)D_3 and 25(OH)D_2 concentrations were used on their SD scale (z-scores) in all linear regression models so that the magnitude of regression coefficients between models was directly comparable. The coefficients can be interpreted as 1 SD increase/decrease in 25(OH)D_3 or 25(OH)D_2 per 1 unit or category change in risk factor.

Because a key focus here with respect to characteristics related to 25(OH)D_3 and 25(OH)D_2 is establishing potential confound-

TABLE 1. Participant characteristics

	n	Mean (interquartile range)	Range
Age (yr)	7552	9.8 (9.6, 10.3)	7.3, 13.6
BMI (kg/m^2)	7544	16.1 (15.1, 17.6)	11.9, 38.0
Serum total 25(OH)D	7560	24.3 (19.2, 30.1)	3.3, 103.2
Serum 25(OH)D_3	7560	22.3 (17.3, 28.2)	2, 93.5
Serum 25(OH)D_2	7560	1.4 (0.5, 2.7)	0.5, 66.9
Category		**n (%)**	
Ethnicity			
White		7123 (95.4)	
Nonwhite		342 (4.6)	
Gender			
Boy		3811 (50.4)	
Girl		3744 (49.6)	

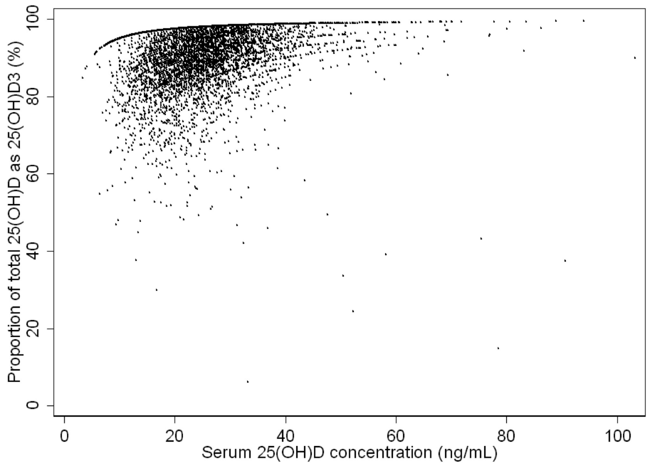

FIG. 2. Proportion of total 25(OH)D as 25(OH)D_3.

ing factors for future studies exploring associations of 25(OH)D_3 and 25(OH)D_2 with a wide range of outcomes we focus on univariable associations. This provides a more complete list of characteristics associated with each form of 25(OH)D so that adjustment for all likely confounders can be considered in future studies. In examining associations with vitamin D deficiency in this cohort, we were more interested in potential modifiable risk factors for this condition and in which a key focus might be to prevent deficiency, and therefore, for this outcome, we present both univariable and multivariable analyses in the main paper.

Results

Table 1 shows the characteristics of the study participants. The median (interquartile range) for 25(OH)D_3, 25 (OH)D_2, and total 25 (OH)D were 22.3 (17.2–28.2), 1.4 (0.5–2.7), and 24.3 (19.2–30.1) ng/ml, respectively. The mean (SD) for 25(OH)D_3, 25 (OH)D_2, and total 25 (OH)D were 23.4 (8.6), 1.9 (2.2), and 25.3 (8.7) ng/ml, respectively. The similarity of average concentrations of 25(OH)D_3 and total 25(OH)D illustrate that 25(OH)D_3 accounts for most of the total 25(OH)D in these children. On average, 94.4% (median, range 6.0–99.5%) of total 25(OH)D was in 25(OH)D_3 form. The contribution of 25(OH)D_3 to total 25(OH)D was constant across total 25(OH)D concentration (Fig. 2), with 95% of children having at least 75% of their 25(OH)D in 25(OH)D_3 form. Altogether 124 children (1.6%) had total 25(OH)D concentration less than 10 ng/ml, 2158 children (29%) were vitamin D deficient [total 25(OH)D concentration less than 20 ng/ml], and 5631 (75%) were insufficient [total 25(OH)D concentration less than 30 ng/ml]. 25(OH)D_2 contributed over 50% of the total 25(OH)D concentrations in only 20 individuals (0.3%), but if levels of deficiency were based only on 25(OH)D_3, the prevalence of deficiency would be increased to 38%, suggesting 25(OH)D_2 was still relevant to reaching nondeficient concentrations in some children.

Table 2 shows uni- and multivariable associations of risk factors with vitamin D deficiency. Vitamin D deficiency was more common during winter months than other seasons. In the univariable analyses younger age, male gender, white ethnicity, lower BMI or waist, higher levels of vigorous physical activity, lower Tanner stage, higher household income, owned housing, time spent outdoors during summer, and more meticulous protection from UVB were associated with lower odds of vitamin D deficiency. Maternal education had a weak U-shaped association with vitamin D deficiency. In multivariable analysis (adjusted for all other risk factors excluding categorized BMI and waist circumference because continuous BMI was included in the model), winter season, higher age, female gender, nonwhite ethnicity, lower household income, rental housing, time spent outdoors during summer, and higher Tanner stage were associated with vitamin D deficiency, whereas the associations of BMI, maternal education, physical activity, and UVB protection were attenuated toward the null, possibly because gender was associated with physical activity, UVB protection, and BMI. Boys were more likely to spend more time outdoors, be less meticulous about UVB protection, and have lower BMI. They also had less advanced puberty stage.

Table 3 shows the univariable associations between potential risk factors and 25(OH)D_3 and25(OH)D_2 concentrations. Both 25(OH)D_3 and 25(OH)D_2 concentrations had seasonal variation. 25(OH)D_3 concentrations were higher during spring, summer, and autumn, whereas 25(OH)D_2 concentrations peaked during winter months. Female gender, age, waist circumference, BMI, more advanced puberty stage, and higher maternal education were inversely associated with 25(OH)D_3 and25(OH)D_2 concentrations. Household income was positively associated with 25(OH)D_3 and negatively with 25(OH)D_2 concentrations. Children who engaged in vigorous physical ac-

TABLE 2. Uni- and multivariable association of risk factors with vitamin D deficiency [total 25(OH)D < 20 ng/ml] (n = 4393)

Exposure	Category	n	Univariable odds ratio (95% CI)	Multivariable odds ratio (95% CI)
Season	Winter	959	Reference (1.00)	Reference (1.00)
	Spring	1235	0.57 (0.48, 0.68)	0.53 (0.45, 0.64)
	Summer	1226	0.09 (0.07, 0.11)	0.08 (0.06, 0.10)
	Autumn	973	0.26 (0.21, 0.32)	0.23 (0.18, 0.28)
Age (months)		2141	1.02 (1.01, 1.02)	1.02 (1.01, 1.03)
Gender	Girl		Reference (1.00)	Reference (1.00)
	Boy	2252	0.79 (0.69, 0.91)	0.86 (0.73, 1.00)
Ethnicity	White	4251	Reference (1.00)	Reference (1.00)
	Nonwhite	142	2.07 (1.48, 2.91)	2.26 (1.53, 3.33)
BMI (kg/m^2)		4393	1.04 (1.01, 1.07)	1.00 (0.97, 1.04)
Categorized BMI[a]	Underweight	413	1.05 (0.83, 1.32)	N.A
	Normal	3549	Reference (1.00)	
	Overweight	515	1.34 (1.10, 1.64)	
	Obese	116	1.49 (1.01, 2.20)	
Waist circumference (cm)		4393	1.02 (1.01, 1.03)	N.A
Parent's assessment of child's health status during last year	Very healthy, no problems	2757	Reference (1.00)	Reference (1.00)
	Healthy, minor problems	1578	0.98 (0.86, 1.13)	1.00 (0.84, 1.19)
	Sometimes quite ill/almost always unwell	58	0.93 (0.51, 1.68)	1.23 (0.61, 2.48)
Parent's assessment of child's health status during last month	Very healthy, no problems	3328	Reference (1.00)	Reference (1.00)
	Healthy, minor problems	1035	0.92 (0.79, 1.08)	0.84 (0.69, 1.03)
	Sometimes quite ill/Almost always unwell	30	0.66 (0.27, 1.61)	0.79 (0.30, 2.10)
Tanner stage	1	2892	Reference (1.00)	Reference (1.00)
	2	1092	1.29 (1.10, 1.50)	1.23 (1.03, 1.47)
	3–5	409	1.82 (1.46, 2.26)	1.50 (1.15, 1.97)
Frequency of vigorous physical activity	<4 h/wk	2423	Reference (1.00)	Reference (1.00)
	4–6 h/wk	1283	0.82 (0.70, 0.95)	0.89 (0.75, 1.05)
	Daily	687	0.71 (0.58, 0.86)	0.82 (0.66, 1.03)
UVB protection score		4393	0.88 (0.82, 0.94)	0.98 (0.95, 1.01)
Average hours per day spent outdoors during summer		4393	0.89 (0.84, 0.95)	0.89 (0.82, 0.95)
Total energy intake (kJ, log transformed)		4393	0.86 (0.65, 1.12)	0.89 (0.66, 1.21)
Carbohydrate intake (g, log transformed)[b]		4393	1.42 (0.59, 3.43)	0.22 (0.00, 28.42)
Fat intake (g, log transformed)[b]		4393	0.88 (0.47, 1.63)	0.32 (0.01, 8.13)
Protein intake (g, log transformed)[b]		4393	0.68 (0.40, 1.15)	0.56 (0.12, 2.52)
Vitamin D intake (mg, log transformed)[b]		4393	0.97 (0.79, 1.19)	0.97 (0.76, 1.23)
Calcium intake (mg, log transformed)[b]		4393	0.89 (0.63, 1.25)	0.89 (0.59, 1.34)
Equalized average household income (log transformed)		4393	0.76 (0.66, 0.88)	0.75 (0.62, 0.91)
Housing	Own/mortgaged	2892	Reference (1.00)	Reference (1.00)
	Rented from council/housing association	1092	1.53 (1.20, 1.95)	1.60 (1.18, 2.16)
	Rented from private landlord/other	409	1.23 (0.94, 1.59)	1.13 (0.84, 1.53)
Mother's highest educational qualification	None/Certificate of Secondary Education	431	Reference (1.00)	Reference (1.00)
	Vocational	336	0.74 (0.54, 1.01)	0.78 (0.55, 1.11)
	O level	1558	0.69 (0.55, 0.87)	0.72 (0.55, 0.93)
	A level	1228	0.79 (0.62, 1.00)	0.93 (0.70, 1.24)
	Degree	840	0.84 (0.65, 1.07)	0.94 (0.68, 1.29)
Head of household occupational social class	I	771	Reference (1.00)	Reference (1.00)
	Ii	2030	0.86 (0.72, 1.04)	0.84 (0.67, 1.04)
	iii nonmanual	1032	0.86 (0.70, 1.06)	0.83 (0.64, 1.08)
	iii manual	409	1.04 (0.80, 1.36)	0.86 (0.62, 1.20)
	iv/v	151	1.00 (0.68, 1.46)	0.76 (0.48, 1.20)

Multivariable analyses are adjusted for all other risk factors (categorical BMI and waist circumference are excluded from multivariable model). CI, Confidence interval; NA, not available.

[a] For details, see references (10, 11).

[b] Adjusted for energy density.

TABLE 3. Univariable association of risk factors with standardized serum 25(OH)D_3 (unadjusted for season) and serum 25(OH)D_2 concentrations (all children included, n = 5628–7555)

Confounder	Category	n	Serum 25(OH)D_3 SD change per unit/category increase[a]	Serum 25(OH)D_2 SD change per unit/category increase[a]
Season	Winter	1624	Reference	Reference
	Spring	2210	0.17 (0.07, 0.27)	0.14 (0.10, 0.17)
	Summer	2039	0.99 (0.90, 1.09)	−0.20 (−0.24, −0.16)
	Autumn	1678	0.53 (0.42, 0.63)	−0.14 (−0.18, −0.10)
Age (months)		7552	−0.07 (−0.07, −0.07)	−0.01 (−0.01, −0.01)
Gender	Girl	3744	Reference	Reference
	Boy	3811	0.21 (0.14, 0.28)	0.03 (0.00, 0.06)
Ethnicity	White	7123	Reference	Reference
	Nonwhite	342	−0.58 (−0.74, −0.41)	0.02 (-0.05, 0.08)
BMI (kg/m^2)		7544	−0.05 (−0.06, −0.04)	−0.02 (−0.03, −0.01)
Categorised BMI[b]	Underweight	754	0.10 (−0.02, 0.22)	0.03 (−0.01, 0.08)
	Normal	5639	Reference	Reference
	Overweight	916	−0.12 (−0.22, −0.01)	−0.05 (−0.09, 0.00)
	Obese	233	−0.19 (−0.40, 0.01)	−0.07 (−0.15, 0.01)
Waist circumference (cm)		7544	−0.03 (−0.03, −0.02)	−0.01 (−0.01, −0.01)
Parent's assessment of child's health status during last year	Very healthy, no problems	4414	Reference	Reference
	Healthy, minor problems	2600	−0.02 (−0.09, 0.05)	−0.01 (−0.04, 0.02)
	Sometimes quite ill/almost always unwell	105	−0.01 (−0.31, 0.28)	0.01 (−0.11, 0.12)
Parent's assessment of child's health status during last month	Very healthy, no problems	5352	Reference	Reference
	Healthy, minor problems	1705	−0.07 (−0.15, 0.02)	−0.01 (−0.04, 0.03)
	Sometimes quite ill/almost always unwell	60	0.12 (−0.26, 0.51)	0.06 (−0.10, 0.21)
Tanner stage	1	3593	Reference	Reference
	2	1347	−0.24 (−0.33, −0.15)	−0.04 (−0.08, 0.00)
	3–5	688	−0.48 (−0.60, −0.36)	−0.10 (−0.15, −0.05)
Frequency of vigorous physical activity	<4 h/wk	3557	Reference	Reference
	4–6 h/wk	1869	0.02 (−0.06, 0.10)	−0.05 (−0.08, −0.01)
	Daily	1081	0.12 (0.02, 0.22)	0.00 (−0.04, 0.04)
UVB protection score		5902	0.02 (0.00, 0.04)	0.00 (0.00, 0.01)
Average hours per day spent outdoors during summer		5886	0.05 (0.02, 0.09)	0.00 (−0.01, 0.02)
Total energy intake (kJ, log transformed)		6564	0.11 (−0.03, 0.26)	−0.02 (−0.08, 0.04)
Carbohydrate intake (g, log transformed)[c]		6564	0.06 (−0.42, 0.54)	0.19 (0.00, 0.39)
Fat intake (g, log transformed)[c]		6564	−0.13 (−0.48, 0.21)	−0.07 (−0.21, 0.07)
Protein intake (g, log transformed)[c]		6564	0.19 (−0.09, 0.47)	−0.12 (−0.23, 0.00)
Vitamin D intake (mg, log transformed)[c]		6564	0.12 (0.01, 0.23)	0.02 (−0.03, 0.06)
Calcium intake (mg, log transformed)[c]		6564	−.06 (−0.24, 0.12)	−0.04 (−0.11, 0.04)
Equalised average household income (log transformed)		6164	0.11 (0.03, 0.19)	−0.07 (−0.11, −0.04)
Housing	Own/mortgaged	5616	Reference	Reference
	Rented from council/housing association	698	0.01 (−0.11, 0.13)	0.07 (0.03, 0.12)
	Rented from private landlord/other	566	0.06 (−0.07, 0.19)	0.05 (0.00, 0.10)
Mother's highest educational qualification	None/Certificate of Secondary Education	981	Reference	Reference
	Vocational	579	0.02 (−0.13, 0.18)	−0.06 (−0.12, 0.00)
	O level	2400	−0.02 (−0.14, 0.09)	−0.07 (−0.11, −0.03)
	A level	1770	−0.13 (−0.25, −0.01)	−0.11 (−0.15, −0.06)
	Degree	1106	−0.10 (−0.23, 0.03)	−0.12 (−0.17, −0.07)
Head of household occupational social class	i	1014	Reference	Reference
	ii	2901	−0.01 (−0.12, 0.10)	0.02 (−0.03, 0.06)
	iii nonmanual	1614	0.03 (−0.09, 0.15)	0.02 (−0.03, 0.07)
	iii manual	714	0.03 (−0.11, 0.18)	0.11 (0.05, 0.17)
	iv/v	277	−0.02 (−0.23, 0.18)	0.06 (−0.02, 0.14)

[a] These are the mean differences in 25(OH)D on a SD scale per category or unit of conounder. For example, the value of 0.99 for summer in the 25(OH)D_3 column indicates that on average 25(OH)D_3 levels are 0.99 of a SD higher in summer than in winter.

[b] For details, see references (10, 11).

[c] Adjusted for energy density.

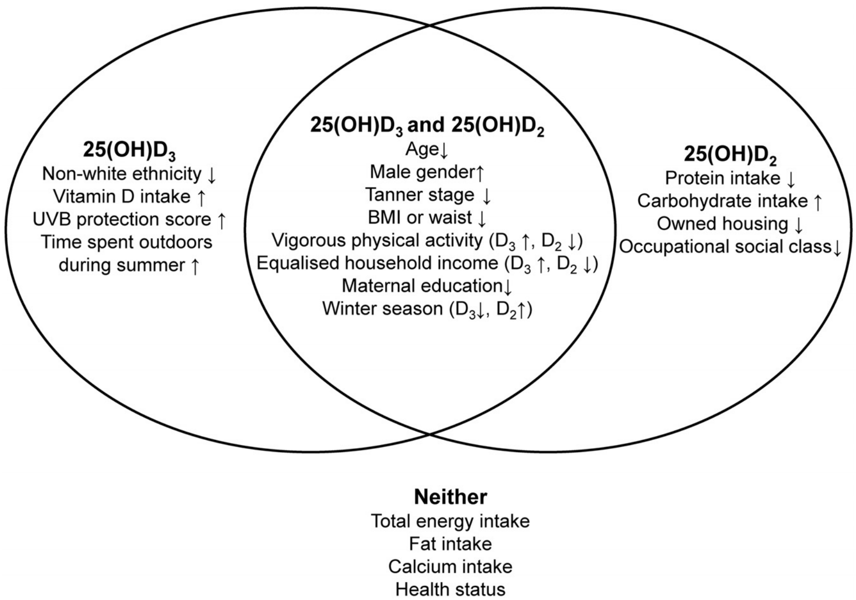

FIG. 3. Summary of characteristics associated with serum 25(OH)D_3 and/or 25(OH)D_2 concentrations in univariable analyses.

tivity more often had higher 25(OH)D_3 concentrations and tended to have lower 25(OH)D_2 concentrations.

Children who were nonwhite, had lower dietary intake of vitamin D, spent less time outdoors during summer, or were less meticulously protected from UVB had lower 25(OH)D_3 concentrations, but these variables were not associated with 25(OH)D_2. Higher carbohydrate intake and lower protein intake, rental housing, and lower head of household occupational social class were associated with higher 25(OH)D_2 concentrations only. Parent-assessed health status, total energy intake, calcium intake, and fat intake were not associated with 25(OH)D_3 or 25(OH)D_2. The results of univariable analyses are summarized in Fig. 3.

Discussion

In this contemporary U.K. population of mainly white 7–11 yr olds, 29% were vitamin D deficient and 75% had insufficient concentrations of total 25(OH)D. These prevalence estimates are consistent with recent studies of other children in Northern Hemisphere countries, largely conducted in Northern America and using the same cutoffs (16–18) and highlight the worrying trend of inadequate vitamin D status in children since successful initiatives to reduce UVB exposure (19). Suboptimal vitamin D status is classified into two categories: vitamin D deficiency as a severe form and vitamin D insufficiency as a mild form. Although there is no consensus on optimal total 25(OH)D concentration, vitamin D deficiency is defined by most experts as less than 20 ng/ml and insufficiency as less than 30 ng/ml (8, 15) with threshold values based on optimal bone health (20).

Most of the total 25(OH)D in these children consisted of 25(OH)D_3 and the relative contribution of 25(OH)D_3, and hence 25(OH)D_2, to total 25(OH)D was relatively constant across the range of total 25(OH)D concentrations in this cohort, although there was more variation in relative contribution among children with lower total 25(OH)D concentrations. These findings suggest that even in those with very high concentrations of total 25(OH)D, this is not being achieved through dietary intake (including supplementation) of 25(OH)D_2. Despite 25(OH)D_3 being the major contributor to total 25(OH)D, 25(OH)D_2 made an important contribution to total 25(OH)D concentrations in some children, and understanding how risk factors relate differently to these two forms of 25(OH)D is likely to be important to observational studies examining their associations with outcomes and comparing whether they relate differently to bone and other health related outcomes.

Consistent with previous studies (18, 21–28), season, age, gender, ethnicity, puberty stage, socioeconomic position (household income, rental housing), and time spent outdoors during summer were most strongly associated with vitamin D deficiency. A majority of these are not modifiable risk factors, so they may be best used in targeting high risk groups (*i.e.* children with low income families, more advanced puberty stage, and nonwhite ethnicity) when designing potential trials or interventions. Although some modifiable risk factors, including higher BMI and lower levels of vigorous physical activity were associated with vitamin D deficiency in univariable analysis, the associations were attenuated toward the null after adjusting for other risk factors. The only modifiable risk factor that remained associated with vitamin D deficiency after adjustments was time spent outdoors. This is not surprising because synthesis in skin normally contributes the majority of 25(OH)D concentrations by synthesizing the precursor of 25(OH)D_3 (8) and suggests that controlled UVB exposure is a cheap and easy way to improve vitamin D status. We found no association between UVB protection and vitamin D deficiency in multivariable analyses, which is against concerns that protection from UVB increases the risk of vitamin D deficiency (29) and consistent with results from previous randomized trials showing that sunscreen use was not associated with vitamin D deficiency (30). In fact, the results of univariable analyses showed that children who were more protected from UVB (including avoidance of midday sun, use of sunscreen, and covering clothing) had lower risk of vitamin D deficiency, possibly because the children who spent more time outdoors during summer months or were from higher occupational social class were also more carefully protected from UVB in this cohort. We found no association between general health and vitamin D deficiency, but this likely reflects that our study is of a general population of children with very few parents reporting that their child had poor health.

We found that different risk factors were associated with 25(OH)D_3 and 25(OH)D_2 concentrations. Although there was some overlap, some associations were in opposite directions (*e.g.* season, household income). Modifiable risk factors for lower circulating concentrations of 25(OH)D_3 and 25(OH)D_2 that were identified in this study, namely BMI, physical activity, diet, outdoor exposure, and socioeconomic position, are associated with multiple health outcomes, such as bone health, cardiovascular diseases, mental health, cognitive function, asthma, and atopy (31–33). Our findings suggest that season, age, gender, puberty stage, BMI, physical activity, and at least some indicators of socioeconomic position (maternal education and household income) should be considered as confounders in future studies on any form of 25(OH)D, depending on their association with study outcome. Because 25(OH)D_3 normally constitutes the majority of total 25(OH)D, risk factors that were associated with 25(OH)D_3 only (ethnicity, outdoor exposure, vitamin D intake, and UVB protection) should also be included when the exposure is total 25(OH)D or 25(OH)D_3. If the exposure of interest is 25(OH)D_2, additional adjustment for other indices of socioeconomic position and dietary intake of proteins and carbohydrates may be useful. Although the association with protein and carbohydrate intake remained after multivariable adjustments, we do not claim that these macronutrients are sources of vitamin D_2 but rather associated with vitamin D_2 intake. This may be through other characteristics for which we were unable to control.

Because 25(OH)D_3 constituted the vast majority of serum total 25(OH)D, our findings with this form of 25(OH)D are consistent with those of previous research showing strong associations between winter season, nonwhite ethnicity, female gender, higher age, higher BMI, lower socioeconomic position, vitamin D intake, time spent outdoors, vigorous physical activity and low UVB exposure, and lower serum total 25(OH)D concentrations (18, 21–28). Different seasonal patterns of 25(OH)D_3 and 25(OH)D_2 in our study may reflect higher intake of foods or supplements containing D_2 during winter months. Interestingly, the recent Canadian Health Measures Survey did not show any seasonal variation in total 25(OH)D concentrations, suggesting that the seasonal effect in that population was blunted by supplement use (17).

Despite the large sample size and sensitive methodology for assaying 25(OH)D_3 and 25(OH)D_2 separately, our study has some weaknesses that need to be considered. Similar to other prospective cohort studies, there was loss to follow-up and those who have attended follow-up clinics tend to be from higher socioeconomic groups than those originally recruited to the study (9). This may mean that our estimate of deficiency is an underestimate of this in the general population of U.K. children of this age, but the associations we have observed would be biased if only these differed in those who were lost to follow-up. At the time that data were collected for this study, it was mandatory for margarine to be supplemented with vitamin D in the United Kingdom; other food products, for example many cereals, were (and continue to be so) also supplemented. However, there was no legislation as to which form of vitamin D should be used in these food supplementations. We are also unable to determine whether any vitamins used were vitamin D_2 or D_3. Therefore, we cannot specifically determine the extent to which any supplementation as directly affect each form of vitamin D.

Conclusions

Vitamin D deficiency was common in this contemporary U.K. cohort. Less time spent outdoors, lower socioeconomic status, more advanced puberty stage, nonwhite ethnicity, and female gender were most strongly associated with vitamin D deficiency after controlling for other risk factors. In most of the children, 25(OH)D_3 contributed majority of the total 25(OH)D, but 25(OH)D_2 made an important contribution to total 25(OH)D concentrations in some children. Current advancements in technology enable the separate measurement of 25(OH)D_3 and 25(OH)D_2. Sources for these two forms are different, and our study showed that despite some overlap, there are differences in potential confounding structures for association studies of 25(OH)D_3 and 25(OH)D_2. Future studies should consider these differences when assessing associations of vitamin D status with health outcomes.

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Address all correspondence and requests for reprints to: Debbie A. Lawlor, Medical Research Council, Centre for Causal Analysis in Translational Epidemiology, School of Social and Community Medicine, University of Bristol, Oakfield House, 15-23 Oakfield Grove, Clifton, Bristol BS8 2BN, United Kingdom. E-mail: d.a.lawlor@bristol.ac.uk.

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