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Vitamin D status and predictors of serum 25-hydroxyvitamin D concentrations in Western Australian adolescents

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Running title: Vitamin D status in adolescents

Keywords: vitamin D, 25-hydroxyvitamin D, Raine Study, adolescents
Abstract

Despite the importance of skeletal growth during adolescence, there is limited research reporting vitamin D status and its predictors in adolescents. Using prospective data from the Western Australian Pregnancy Cohort (Raine) Study, we investigated vitamin D status and predictors of serum 25-hydroxyvitamin D (25(OH)D) concentrations in adolescents. Serum 25(OH)D concentrations were measured in the same participants at 14 and 17 years (n=1045 at both time points). The percentage of adolescents with serum 25(OH)D concentrations <50, 50-74.9 and ≥75 nmol/L was reported year-round and by month of blood collection. We examined predictors of serum 25(OH)D concentrations using a general linear mixed model, including sex, race, month of blood collection, physical activity, body mass index, family income, calcium and vitamin D intakes (n=919 at 14 years; n=570 at 17 years). At 14 years, 31% of adolescents had serum 25(OH)D concentrations between 50-74.9 nmol/L and a further 4% had concentrations <50 nmol/L. At 17 years, 40% of adolescents had serum 25(OH)D concentrations between 50-74.9 nmol/L and 12% had concentrations <50 nmol/L. Caucasian ethnicity, being sampled at the end of summer, exercising more, having a lower body mass index, a higher calcium intake and a higher family income were significantly associated with higher serum 25(OH)D concentrations. The proportion of adolescents with serum 25(OH)D concentrations <50 nmol/L was low in this Western Australian cohort. There is a need for international consensus on defining adequate vitamin D status in order to determine whether strategies to increase vitamin D status in adolescents are warranted.
Introduction

The role of vitamin D in promoting bone growth and maintenance is well established\(^{(1)}\), while growing evidence associates vitamin D with non-skeletal conditions, such as cancer and cardiovascular disease\(^{(2)}\). The serum 25-hydroxyvitamin D (25(OH)D) thresholds that signal vitamin D deficiency and sufficiency remain controversial. The Institute of Medicine defines deficiency as serum 25(OH)D concentrations below 30 nmol/L\(^{(3)}\), while guidelines from the Endocrine Society suggest that vitamin D deficiency be defined as concentrations below 50 nmol/L and concentrations should be at least 75 nmol/L to maximise the effect of vitamin D on calcium, bone and muscle metabolism\(^{(4)}\). Serum 25(OH)D concentrations well below 50 nmol/L have been reported in populations worldwide, including the United Kingdom (UK)\(^{(5)}\), Ireland\(^{(6)}\), the United States (US)\(^{(7)}\), Canada\(^{(8)}\), Australia\(^{(9)}\), Asia and the Middle East\(^{(10)}\). In adolescents, Looker et al.\(^{(11)}\) reported that 29% and 34% of boys and girls aged 14-18 years participating in the US 2001-2006 National Health and Nutrition Examination Survey (NHANES) had 25(OH)D concentrations below 50 nmol/L. In four European countries (Denmark, Finland, Ireland and Poland), 92% of girls aged 11-13 years had 25(OH)D concentrations below 50 nmol/L at the end of winter\(^{(12)}\). Mean 25(OH)D concentrations were below 50 nmol/L in children aged 11-18 years and adults aged 19-64 years participating in the UK’s National Diet and Nutrition Survey rolling programme (2008-2009)\(^{(5)}\). In a national sample of Australian adults aged over 25 years, the prevalence of vitamin D deficiency (defined as serum 25(OH)D concentrations < 50 nmol/L) was 31%, while 73% of participants had serum 25(OH)D concentrations below 75 nmol/L\(^{(9)}\).

The major source of vitamin D for humans is exposure to sunlight and a number of factors affect the cutaneous production of vitamin D including latitude, season, race, time spent outdoors, sunscreen, sun-protective clothing and age. Other factors affecting vitamin D status may include obesity and vitamin D intake. Daly and colleagues recently examined the prevalence of vitamin D deficiency and its determinants in Australian adults aged 25 years and older: advancing age, race, latitude, season, lack of physical activity, obesity and education were found to be independent predictors of vitamin D deficiency\(^{(9)}\). However, similar data in Australian adolescents have not been reported. Adequate vitamin D status may be important in adolescence in order to optimise calcium absorption for skeletal growth. Therefore, understanding the predictors of vitamin D status is essential for developing public health strategies to address vitamin D deficiency. The aims of this study were to report vitamin D status, and to examine predictors of serum 25(OH)D concentrations, in a population-based cohort of adolescents aged 14 and 17 years in Perth, Western Australia (latitude 32° S).
Methods

Study design and population

The study population comprised adolescents who participated in the 14 and 17 year follow-ups of the Western Australian Pregnancy Cohort (Raine) Study. Raine Study methodology has been described previously\(^1\). In brief, 2900 pregnant women were recruited into the Raine Study from the public antenatal clinic at King Edward Memorial Hospital or surrounding private clinics in Perth, Western Australia, between May 1989 and November 1991. A total of 2868 children underwent serial assessment at birth and at regular intervals. Data collection at the 14 and 17 year follow-ups occurred between May 2003-June 2006 and July 2006-June 2009, respectively. Recruitment and all follow-ups were approved by the ethics committees of King Edward Memorial Hospital for Women and the Princess Margaret Hospital for Children, Perth, Western Australia. Informed and written consent was obtained from the participant and/or their primary caregiver at each follow-up.

Analysis of serum 25(OH)D concentrations

At the 14 and 17 year follow-ups, serum was prepared from venous blood samples taken from an antecubital vein after an overnight fast and stored at \(-80^\circ\text{C}\) until analysis. Serum 25(OH)D concentrations at 14 years were measured by enzyme immunoassay (Immunodiagnostic Systems Ltd, Scottsdale, Arizona, USA). This assay did not differentiate between serum 25(OH)D\(_2\) and 25(OH)D\(_3\). At 17 years, serum 25(OH)D\(_2\) and 25(OH)D\(_3\) concentrations were measured using isotope-dilution liquid chromatography-tandem mass spectrometry (LC-MS/MS) (RDDT, Victoria, Australia), according to published methodology\(^1\). At the 17 year follow-up, only four participants had detectable serum 25(OH)D\(_2\) concentrations, with values of 5.44, 5.76, 7.23 and 8.12 nmol/L. Since the enzyme immunoassay at the 14 year follow-up did not differentiate between serum 25(OH)D\(_2\) and 25(OH)D\(_3\), analyses at both time points were performed on total serum 25(OH)D concentrations.

We randomly selected 12 samples across the entire range (43 – 148 nmol/L) of 25(OH)D values obtained from the enzyme immunoassay at the 14 year follow-up and validated them with the LC-
MS/MS used at the 17 year follow-up (RDDT, Victoria, Australia). There was good agreement between the enzyme immunoassay and LC-MS/MS as shown on the Bland-Altman plot (Supplementary Figure 1)\(^{(15)}\). Since the blood samples were collected year-round, serum 25(OH)D concentrations were described by month of blood collection and vitamin D status was described by season (spring, September-November; summer, December-February; autumn, March-May; winter, June-August). In order to report year-round serum 25(OH)D concentrations and vitamin D status, the seasonal component was removed (deseasonalised) by fitting a sinusoidal model to serum 25(OH)D concentrations incorporating the month the blood sample was taken, according to published methodology\(^{(16)}\).

**Potential predictors of vitamin D status**

Participants were classified as Caucasian if both parents were Caucasian, or as non-Caucasian if at least one parent was of an alternate ethnicity. Participants were weighed to the nearest 100 g using a Wedderburn Digital Chair Scale and height was determined to the nearest 0.1 cm with a Holtain Stadiometer. Body mass index (BMI) was calculated as weight (kg)/height (m\(^2\)). Physical activity was assessed using a self-reported questionnaire based on exercise outside of school hours per week, with exercise defined in three categories as activity causing breathlessness or sweating (≥ 4 times per week, 1-3 times per week and < once per week).

A self-reported, semi-quantitative food frequency questionnaire (FFQ) developed by the Commonwealth Scientific and Industrial Research Organisation in Adelaide, Australia\(^{(17)}\) was used to assess calcium and vitamin D intakes. This 212-item FFQ assesses usual dietary intake over the previous year, collecting information on the frequency of consumption of individual foods, mixed dishes and beverages, along with information on usual serving sizes in relation to a standard serving size in household units. Participants also recorded the type and dose of any dietary supplements consumed over the last twelve months. The composition of these supplements was derived from the product label or directly from the manufacturer to provide a total daily intake from food and supplements. At the 14 year follow-up, the primary caregiver was asked to complete the FFQ in association with the adolescent.

**Statistical analyses**

Characteristics of participants who provided a blood sample at both the 14 and 17 year follow-ups were compared with non-participants from the original cohort. Sex, race, family income during
pregnancy, maternal age at birth, maternal education and maternal pre-pregnancy BMI were compared using Chi-square tests. Baseline characteristics - including sex, age, race, month of blood collection, physical activity, BMI, and median daily vitamin D and calcium intakes from food and supplements – were described for participants who provided a blood sample at both the 14 and 17 year follow-ups. Differences between males and females in deseasonalised serum 25(OH)D concentrations were analysed using independent samples t-tests.

Serum 25(OH)D concentrations were normally distributed. In order to examine predictors of serum 25(OH)D concentrations, we performed a single general linear mixed model combining 14 and 17 year data. Pearson’s Chi-square test was used to determine any differences in sex and race between those with complete data who were included in the final model and those with missing data who were excluded from the final model. The following categorical variables were included as potential predictors: sex, time (14 year follow-up/17 year follow-up), race (Caucasian/non-Caucasian), month of blood collection and physical activity (≥ 4 times per week, 1-3 times per week and once per week or less). Vitamin D intake from food and supplements (µg/day), calcium intake from food and supplements (g/day) and BMI were included as continuous variables, after confirming a linear relationship with serum 25(OH)D concentrations. Interactions between time and sex, physical activity, calcium intake, vitamin D intake and BMI were investigated along with interactions between sex and physical activity, calcium intake, vitamin D intake and BMI. Analyses were performed using IBM SPSS Statistics Release Version 19.9.9.1 (IBM SPSS Inc., 2010, Chicago, IL) and StataCorp 2011 Stata Statistical Software: Release 12 (College Station, TX: StataCorp LP). Statistical significance was defined as two-tailed \( p < 0.05 \).

Results

Characteristics of participants

A total of 1045 adolescents provided a blood sample for analysis of serum 25(OH)D concentrations at both follow-ups (Figure 1). Complete data - including physical activity, BMI, calcium and vitamin D intakes, and family income - were available for 919 adolescents at the 14 year follow-up and 570 adolescents at the 17 year follow-up. Compared with those from the original cohort who did not participant in the current study (\( n = 1823 \)), participants completing both the 14 and 17 year follow-ups (\( n = 1045 \)) were more likely to be Caucasian, to come from families with a higher income during pregnancy and to have mothers with a higher age, higher education and healthier BMI (Table 1).
Approximately 51% of participants who provided a blood sample at both the 14 and 17 year follow-ups were male and 85% were Caucasian (Table 2). At 14 years, 10% of participants were physically inactive (exercising once per week or less), increasing to 21% at 17 years. The mean BMI at 14 years was 21 kg/m², increasing to 23 kg/m² at 17 years. Median daily vitamin D intake from food and supplements at 14 years was 2.0 µg, decreasing to 1.6 µg at 17 years. Median daily calcium intake from food and supplements was 1107 mg at 14 years, decreasing to 1018 mg at 17 years.

**Serum 25(OH)D concentrations and vitamin D status**

At 14 years, mean deseasonalised serum 25(OH)D concentrations were 86 nmol/L, with levels in males significantly higher than in females (Table 2). At 17 years, mean deseasonalised serum 25(OH)D concentrations decreased to 75 nmol/L and there was no significant difference between males and females. A total of 31% of adolescents at the 14 year follow-up had serum 25(OH)D concentrations between 50-74.9 nmol/L and a further 4% had concentrations below 50 nmol/L (Table 2). At 17 years, the percentage of adolescents with concentrations between 50-74.9 nmol/L and below 50 nmol/L increased to 40% and 12%, respectively. Mean serum 25(OH)D concentrations were highest at the end of summer (Figure 2). At the 14 year follow-up, 10% of adolescents in winter and 4% in spring had serum 25(OH)D concentrations below 50 nmol/L, increasing to 28% and 21%, respectively, in the 17 year follow-up (Table 3, Supplementary Figure 2).

**Predictors of serum 25-hydroxyvitamin D concentrations**

There were no significant differences in sex or race between those with complete data who were included in the final model and those with missing data who were excluded from the final model. Caucasian ethnicity, being sampled at the end of summer, exercising more, having a lower BMI, a higher calcium intake and a higher family income were significantly associated with higher serum 25(OH)D concentrations (Table 4). Vitamin D intakes from food and supplements were not significantly associated with serum 25(OH)D concentrations. There was a significant interaction between sex and time. At the 14 year follow-up, serum 25(OH)D concentrations were significantly higher in males ($p = 0.026$); at the 17 year follow-up, there was no significant difference in serum 25(OH)D concentrations between males and females ($p = 0.194$).

**Discussion**
The percentage of adolescents with serum 25(OH)D concentrations below 50 nmol/L was substantially lower in this Western Australian cohort compared with global reports in similar age groups. In a nationally representative sample of 4–18 year olds in Great Britain (n = 1102), 35% of participants had concentrations below 50 nmol/L\(^{(18)}\). Similarly, in the 2001-2006 NHANES, 29% and 34% of 14-18 year old boys and girls (n = 3801), respectively, had concentrations below 50 nmol/L\(^{(10)}\). In a cross-sectional study of 12 and 15 year olds in Northern Ireland (n = 1015), 36% of 12-19 year olds (n = 231) participating in the Canadian Health Measures Survey (CHMS) Cycle 1 (2007-2009) had concentrations below 50 nmol/L\(^{(8)}\). Perth and surrounding regions have a mean of 8.8 daily hours of sunshine\(^{(20)}\), encouraging an outdoors lifestyle. Higher sun exposure may partly explain the higher serum 25(OH)D concentrations in this adolescent cohort compared with other populations. Furthermore, the Raine cohort is predominantly Caucasian, which may contribute to the higher serum 25(OH)D concentrations compared with reports from the UK, US and Canada. However, despite the low percentage of adolescents with serum 25(OH)D concentrations below 50 nmol/L in our cohort compared with international populations, the percentage of adolescents in this cohort with concentrations between 50-74.9 nmol/L was substantial.

Recently, Daly and colleagues reported the vitamin D status in a national sample of Australian adults (n = 11,247) from each of the six states and the Northern Territory\(^{(9)}\). They reported that approximately 60% of participants aged 25-34 years had serum 25(OH)D concentrations below 75 nmol/L, which is similar to 17 year olds in our cohort. The authors suggested a number of potential factors that may have contributed to the relatively high level of vitamin D insufficiency (as defined by the authors as < 75 nmol/L) in adults across Australia, including sun-consciousness (including sun avoidance, use of sunscreen or protective clothing), low levels of physical activity, inadequate vitamin D intake and the increasing prevalence of obesity. In 272 healthy Tasmanian adults (< 60 years old), 49% had year-round serum 25(OH)D concentrations below 50 nmol/L\(^{(21)}\). Compared with the current study, this higher prevalence of participants with serum 25(OH)D concentrations below 50 nmol/L may reflect both the difference in latitude between Hobart and Perth (42° S and 32° S, respectively) and the older age of the participants.

In our study, season was a strong predictor of serum 25(OH)D concentrations, with the percentage of adolescents with serum 25(OH)D concentrations ≥ 75 nmol/L approximately two-fold higher in summer compared to winter. Similar seasonal differences in vitamin D levels have been reported previously in Australian adults\(^{(9,21)}\) and in European populations\(^{(12,22,23)}\). It is well known that
latitude and season affect cutaneous vitamin D production. At latitudes above 37°, winter sunlight is not of sufficient intensity to promote cutaneous production of vitamin D, and very little vitamin D synthesis occurs at lower latitudes in the morning or late afternoon during winter months\(^{24}\). In addition, vitamin D synthesis is reduced in cloudy conditions compared with clear sky\(^{25}\). Despite the relatively low latitude of Perth (32° S), we saw a substantial increase in those with serum 25(OH)D concentrations < 75 nmol/L during winter and spring, which may be a product of low sunlight intensity, less time spent outdoors and increased cloud cover. Physical activity is often used as a proxy for the amount of time spent outdoors and, therefore, of sunlight exposure. We identified physical activity as a significant predictor of serum 25(OH)D concentrations in adolescents - a finding that has also been reported in Australian adults\(^{9}\) and children in Great Britain\(^{18}\).

Higher BMI was associated with lower serum 25(OH)D concentrations in this population of adolescents. The relationship between obesity and vitamin D deficiency remains ambiguous. Recent evidence suggests that the inverse association between body fat and 25(OH)D levels is related to the dilution of vitamin D in the large fat mass of obese patients\(^{26}\). It is also possible that obesity results in lower vitamin D levels due to decreased sun exposure from a sedentary indoor lifestyle\(^{27}\). Obesity has been reported as a determinant of vitamin D status in Australian adults\(^{9}\) and international populations from the US\(^{7}\), the UK\(^{18}\) and Ireland\(^{19}\). Since the prevalence of obesity is increasing in Australia, the assessment and treatment of low vitamin D levels in these at-risk individuals may be warranted.

In this cohort, vitamin D intake from food and supplements was not associated with serum 25(OH)D concentrations, which is similar to findings in a Tasmanian cohort study of 8 year olds (\(n = 201\))\(^{28}\). Vitamin D occurs naturally in fish, meat, egg yolk and mushrooms, many of which are consumed episodically and contain relatively small amounts of vitamin D\(^{29}\). It is generally recognised that diet alone is insufficient to maintain adequate vitamin D status\(^{30}\). Rather, we found that calcium intake was associated with higher serum 25(OH)D concentrations. It has been shown that higher calcium intake reduces circulating concentrations of calcitriol, which subsequently raises serum 25(OH)D concentrations\(^{31}\).

In this adolescent cohort, males had significantly higher serum 25(OH)D concentrations than females at the 14 year follow-up. Lower vitamin D status in girls has also been reported in children and adolescents in Northern Ireland\(^{19}\), the US\(^{11}\) and New Zealand\(^{32}\). It is not clear why vitamin D status differs between boys and girls. Hill and colleagues\(^{19}\) found that girls had a lower vitamin D
intake than boys, while Rockell and colleagues\(^{32}\) noted that physical activity was higher in boys than girls. However, in our study, serum 25(OH)D concentrations were higher in males than females at 14 years after adjusting for confounding factors, including physical activity and vitamin D intake. Therefore, the observed sex differences may be due to a confounder that was not included in the model or may be related to outdoor activity that was not captured by our measurement of physical activity. Serum 25(OH)D concentrations decreased more in males than females between 14 and 17 years, and there was no significant difference between the sexes at the 17 year follow-up. Overall, serum 25(OH)D concentrations were lower at 17 years than 14 years. Increasing vitamin D deficiency has also been observed in children and adolescents in the US, where risk of deficiency increased significantly with age until 30 years in males and 18 years in females\(^{11}\).

It is important to note that the thresholds for vitamin D deficiency are controversial. The Clinical Guidelines Subcommittee of the Endocrine Society defines vitamin D deficiency as serum 25(OH)D concentrations below 50 nmol/L\(^4\), whereas the Institute of Medicine defines deficiency as below 30 nmol/L, stating that practically all persons are sufficient at concentrations of 50 nmol/L and above\(^3\). Substituting 50 nmol/L for 30 nmol/L to define deficiency has a major impact on the prevalence estimates of deficiency, and there is a pressing need for consensus in defining 25(OH)D thresholds for vitamin D deficiency, insufficiency and sufficiency.

Furthermore, there is substantial variation in the analytical techniques used to measure circulating 25(OH)D concentrations. Differences in methodology have led to significant variation in 25(OH)D measurement depending on the laboratory and assay used, confounding the diagnosis of vitamin D deficiency. A cross-calibration of the 25(OH)D assays of five laboratories showed that the mean 25(OH)D concentration was 80% higher using CPBA compared with HPLC, while RIA gave intermediate values\(^{33}\). A further study involving six laboratories found markedly different results in 25(OH)D yields and authors concluded that a diagnosis of low or normal vitamin D status in an individual is dependent on the laboratory used\(^{34}\). LC-MS/MS is now considered a definitive method of quantifying 25(OH)D concentrations\(^{35}\) and the Vitamin D Standardization Program (VDSP) has developed protocols for standardising the measurement of 25(OH)D using LC-MS/MS\(^{36}\). A recent evaluation of the VDSP protocols found that the comparison between serum 25(OH)D concentrations measured by enzyme immunoassay and reanalysed using LC-MS/MS was not linear, but involved two linear relations, one for concentrations below 46.6 nmol/L and the other for concentrations greater than or equal to this cut-off\(^{37}\).
A strength of our study was the longitudinal nature of the data, allowing us to investigate differences in predictors of serum 25(OH)D concentrations over time. A further strength was access to comprehensive data that may influence serum 25(OH)D concentrations, including BMI, physical activity, family income and dietary intakes of calcium and vitamin D from food and supplements. A limitation of our study was the loss to follow-up. Participants included in the current study were more likely to be from families with higher socioeconomic status compared with those in the original cohort. However, the original Raine cohort slightly over-represented socially disadvantaged families, and children of socially disadvantaged families were less likely to remain in the Raine Study after the third year. Therefore, the remaining cohort is more representative of the general Western Australian population than the original cohort\(^{38,39}\).

Our study has shown that, among adolescents living at latitude 32° S in Perth, Western Australia, the percentage with serum 25(OH)D concentrations below 50 nmol/L is low compared with international populations. However, the percentage of adolescents with serum 25(OH)D concentrations below 75 nmol/L is substantial, particularly during winter months. Given that the thresholds of vitamin D insufficiency and deficiency are controversial, there is a need for international consensus on defining adequate vitamin D status in order to determine whether strategies to increase vitamin D status in adolescents are warranted.
Acknowledgements

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Conflict of interest None

Authorship

LJ Black wrote the manuscript, conducted statistical analysis, contributed to interpretation of data and critical revision of the manuscript for important intellectual content; S Burrows contributed to statistical analysis and interpretation of data; P Jacoby contributed to statistical analysis and interpretation of data; WH Oddy contributed to acquisition of data and critical revision of the manuscript for important intellectual content and was responsible for funding of this study; LJ Beilin contributed to study concept and design, acquisition of data, analysis and interpretation of data and critical revision of the manuscript for important intellectual content; W Chan She Ping-Delfos was responsible for funding the assay of 17 year serum 25(OH)D concentrations and contributed to critical revision of the manuscript for important intellectual content; C Marshall contributed to interpretation of data and critical revision of the manuscript for important intellectual content; PH Hart and PG Holt contributed to acquisition of data and critical revision of the manuscript for important intellectual content; TA Mori contributed to study concept and design, acquisition of data, analysis and interpretation of data, and critical revision of the manuscript for important intellectual content.
References


Table 1. Characteristics of participants providing a blood sample at both follow-ups (14 and 17 years) (n = 1045) vs non-participants from the original cohort (n = 1823)

<table>
<thead>
<tr>
<th>Description</th>
<th>Participants</th>
<th>Non-participants</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td>0.650</td>
</tr>
<tr>
<td>Male</td>
<td>536 (51.3)</td>
<td>919 (50.4)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>509 (48.7)</td>
<td>904 (49.6)</td>
<td></td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td>0.013</td>
</tr>
<tr>
<td>Caucasian</td>
<td>887 (84.3)</td>
<td>1481 (81.2)</td>
<td></td>
</tr>
<tr>
<td>Non-Caucasian</td>
<td>158 (15.7)</td>
<td>342 (18.8)</td>
<td></td>
</tr>
<tr>
<td>Family income per year during pregnancy</td>
<td></td>
<td></td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>&lt; $7,000</td>
<td>58 (5.6)</td>
<td>171 (9.4)</td>
<td></td>
</tr>
<tr>
<td>$7,000-$11,999</td>
<td>67 (6.4)</td>
<td>176 (9.7)</td>
<td></td>
</tr>
<tr>
<td>$12,000-$23,999</td>
<td>222 (21.2)</td>
<td>461 (25.3)</td>
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<tr>
<td>$24,000-$35,999</td>
<td>270 (25.8)</td>
<td>378 (20.7)</td>
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<tr>
<td>≥ $36,000</td>
<td>363 (34.7)</td>
<td>472 (25.9)</td>
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<tr>
<td>Maternal age at birth</td>
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<tr>
<td>&lt; 20 years</td>
<td>58 (5.6)</td>
<td>220 (12.1)</td>
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<tr>
<td>20-24 years</td>
<td>181 (17.3)</td>
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<td>25-29 years</td>
<td>293 (28.0)</td>
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<td>30-34 years</td>
<td>317 (30.3)</td>
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<tr>
<td>35-39 years</td>
<td>156 (14.9)</td>
<td>144 (7.9)</td>
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<tr>
<td>≥ 40 years</td>
<td>36 (3.4)</td>
<td>27 (1.5)</td>
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<tr>
<td>Maternal education since school</td>
<td></td>
<td></td>
<td>&lt; 0.001</td>
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<tr>
<td>None</td>
<td>447 (42.8)</td>
<td>1001 (54.9)</td>
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<tr>
<td>Trade certificate or apprenticeship</td>
<td>76 (7.3)</td>
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<tr>
<td>Professional registration (non-degree)</td>
<td>114 (10.9)</td>
<td>132 (7.2)</td>
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<tr>
<td>College diploma or degree</td>
<td>194 (18.6)</td>
<td>254 (13.9)</td>
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<tr>
<td>University degree</td>
<td>140 (13.4)</td>
<td>140 (7.7)</td>
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<tr>
<td>Other</td>
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<td>95 (5.2)</td>
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<td>Maternal pre-pregnancy body mass index</td>
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<tr>
<td>Underweight (&lt; 18.5)</td>
<td>99 (9.5)</td>
<td>216 (11.8)</td>
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<td>Healthy weight (18.5-24.9)</td>
<td>724 (69.3)</td>
<td>1170 (64.2)</td>
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<tr>
<td>Overweight (25-29.9)</td>
<td>110 (10.5)</td>
<td>203 (11.1)</td>
<td></td>
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<tr>
<td>Obese (≥ 30)</td>
<td>63 (6.0)</td>
<td>115 (6.3)</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Characteristics of the Raine Study participants for whom serum 25-hydroxyvitamin D (25(OH)D) concentrations were available at both follow-ups (14 and 17 years) \((n = 1045)\)

<table>
<thead>
<tr>
<th></th>
<th>14 year follow-up</th>
<th>17 year follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>536</td>
<td>536</td>
</tr>
<tr>
<td>Female</td>
<td>509</td>
<td>509</td>
</tr>
<tr>
<td><strong>Race (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>887</td>
<td>887</td>
</tr>
<tr>
<td>Non-Caucasian</td>
<td>158</td>
<td>158</td>
</tr>
<tr>
<td><strong>Age [years, mean (SD)]</strong></td>
<td>1045</td>
<td>1045</td>
</tr>
<tr>
<td></td>
<td>14.1 (0.2)</td>
<td>17.1 (0.3)</td>
</tr>
<tr>
<td><strong>Deseasonalised 25(OH)D [nmol/L, mean (SD)]</strong></td>
<td>1045</td>
<td>1045</td>
</tr>
<tr>
<td>Total</td>
<td>86 (27)</td>
<td>75 (24)</td>
</tr>
<tr>
<td>Males</td>
<td>90 (27)*</td>
<td>75 (25)</td>
</tr>
<tr>
<td>Females</td>
<td>83 (26)</td>
<td>76 (24)</td>
</tr>
<tr>
<td><strong>Year-round vitamin D status (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 75 nmol/L</td>
<td>671</td>
<td>496</td>
</tr>
<tr>
<td>50-74.9 nmol/L</td>
<td>328</td>
<td>421</td>
</tr>
<tr>
<td>&lt; 50 nmol/L</td>
<td>46</td>
<td>128</td>
</tr>
<tr>
<td><strong>Body mass index [kg/m², mean (SD)]</strong></td>
<td>1039</td>
<td>918</td>
</tr>
<tr>
<td></td>
<td>21.4 (4.3)</td>
<td>23.0 (4.5)</td>
</tr>
<tr>
<td><strong>Month of blood collection (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>January</td>
<td>37</td>
<td>75</td>
</tr>
<tr>
<td>February</td>
<td>75</td>
<td>97</td>
</tr>
<tr>
<td>March</td>
<td>109</td>
<td>108</td>
</tr>
<tr>
<td>April</td>
<td>97</td>
<td>57</td>
</tr>
<tr>
<td>May</td>
<td>94</td>
<td>79</td>
</tr>
<tr>
<td>June</td>
<td>84</td>
<td>64</td>
</tr>
<tr>
<td>July</td>
<td>87</td>
<td>93</td>
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<td>August</td>
<td>97</td>
<td>82</td>
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<td>September</td>
<td>97</td>
<td>101</td>
</tr>
<tr>
<td>October</td>
<td>122</td>
<td>131</td>
</tr>
<tr>
<td>November</td>
<td>84</td>
<td>80</td>
</tr>
<tr>
<td>December</td>
<td>62</td>
<td>78</td>
</tr>
<tr>
<td><strong>Physical activity (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 4 times per week</td>
<td>349</td>
<td>204</td>
</tr>
<tr>
<td>1-3 times per week</td>
<td>585</td>
<td>445</td>
</tr>
<tr>
<td>&lt; once per week</td>
<td>103</td>
<td>174</td>
</tr>
<tr>
<td><strong>Family income (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ $40,000 per year</td>
<td>291</td>
<td>156</td>
</tr>
<tr>
<td>$40,001-78,000 per year</td>
<td>368</td>
<td>268</td>
</tr>
<tr>
<td>&gt; $78,000 per year</td>
<td>343</td>
<td>444</td>
</tr>
<tr>
<td><strong>Vitamin D intake [µg/day, median (IQR)]</strong></td>
<td>941</td>
<td>665</td>
</tr>
<tr>
<td></td>
<td>2.0 (1.9)</td>
<td>1.6 (2.2)</td>
</tr>
<tr>
<td><strong>Calcium intake [mg/day, median (IQR)]</strong></td>
<td>941</td>
<td>665</td>
</tr>
<tr>
<td></td>
<td>1107 (673)</td>
<td>1018 (728)</td>
</tr>
</tbody>
</table>

SD, standard deviation; IQR, inter-quartile range; *Significantly different from females at \(p < 0.05\)
Table 3. Vitamin D status at the 14 and 17 year follow-ups (n = 1045), stratified by sex and season of blood collection.

<table>
<thead>
<tr>
<th></th>
<th>14 year follow-up</th>
<th>17 year follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1045</td>
<td></td>
</tr>
<tr>
<td>≥ 75 nmol/L</td>
<td>671</td>
<td>64.2</td>
</tr>
<tr>
<td>50-74.9 nmol/L</td>
<td>328</td>
<td>31.4</td>
</tr>
<tr>
<td>&lt; 50 nmol/L</td>
<td>46</td>
<td>4.4</td>
</tr>
<tr>
<td><strong>Males</strong></td>
<td>536</td>
<td></td>
</tr>
<tr>
<td>≥ 75 nmol/L</td>
<td>375</td>
<td>70.0</td>
</tr>
<tr>
<td>50-74.9 nmol/L</td>
<td>141</td>
<td>26.3</td>
</tr>
<tr>
<td>&lt; 50 nmol/L</td>
<td>20</td>
<td>3.7</td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td>509</td>
<td></td>
</tr>
<tr>
<td>≥ 75 nmol/L</td>
<td>209</td>
<td>58.2</td>
</tr>
<tr>
<td>50-74.9 nmol/L</td>
<td>187</td>
<td>47.7</td>
</tr>
<tr>
<td>&lt; 50 nmol/L</td>
<td>20</td>
<td>5.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Year-round¹</th>
<th>Spring</th>
<th>Summer</th>
<th>Autumn</th>
<th>Winter</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>1045</td>
<td>303</td>
<td>174</td>
<td>300</td>
<td>268</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td>64.2</td>
<td>46.9</td>
<td>78.7</td>
<td>74.0</td>
</tr>
<tr>
<td>n</td>
<td></td>
<td>142</td>
<td>48.8</td>
<td>37</td>
<td>21.3</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td>4.4</td>
<td>13</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>n</td>
<td></td>
<td>46</td>
<td>11</td>
<td>67</td>
<td>22</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td>671</td>
<td>81</td>
<td>71</td>
<td>222</td>
</tr>
<tr>
<td>n</td>
<td></td>
<td>141</td>
<td>68</td>
<td>16</td>
<td>28</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td>328</td>
<td>44.4</td>
<td>18.4</td>
<td>20.1</td>
</tr>
<tr>
<td>n</td>
<td></td>
<td>20</td>
<td>2.6</td>
<td>0</td>
<td>1.4</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td>46</td>
<td>13</td>
<td>0</td>
<td>67</td>
</tr>
</tbody>
</table>

¹Deseasonalised serum 25(OH)D concentrations

17 year follow-up

Spring, September-November; summer, December-February; autumn, March-May; winter, June-August
Table 4. General linear mixed model of predictors of serum 25(OH)D concentrations at the 14 and 17 year follow-ups

<table>
<thead>
<tr>
<th>Determinant</th>
<th>$\beta$ (95% CI)</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>Ref.</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>-3.6 (-6.8, -0.4)</td>
<td>0.026</td>
</tr>
<tr>
<td>Time</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 year follow-up</td>
<td>Ref.</td>
<td></td>
</tr>
<tr>
<td>17 year follow-up</td>
<td>-11.5 (-14.5, -8.5)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Sex$^*$Time</td>
<td>6.2 (2.2, 10.2)</td>
<td>0.003</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>Ref.</td>
<td></td>
</tr>
<tr>
<td>Non-Caucasian</td>
<td>-15.2 (-19.1, -11.3)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Month</td>
<td></td>
<td></td>
</tr>
<tr>
<td>February</td>
<td>Ref.</td>
<td></td>
</tr>
<tr>
<td>March</td>
<td>-7.3 (-12.8, -1.9)</td>
<td>0.009</td>
</tr>
<tr>
<td>April</td>
<td>-15.3 (-21.2, -9.5)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>May</td>
<td>-24.0 (-29.7, -18.2)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>June</td>
<td>-40.2 (-46.3, -34.2)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>July</td>
<td>-41.5 (-47.3, -35.7)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>August</td>
<td>-38.8 (-44.5, -33.1)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>September</td>
<td>-40.4 (-46.1, -34.7)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>October</td>
<td>-38.5 (-43.8, -33.1)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>November</td>
<td>-25.7 (-31.4, -19.9)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>December</td>
<td>-26.2 (-32.1, -20.4)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>January</td>
<td>-11.8 (-18.2, -5.5)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Physical activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; once per week</td>
<td>Ref.</td>
<td></td>
</tr>
<tr>
<td>1-3 times per week</td>
<td>5.0 (1.4, 8.6)</td>
<td>0.006</td>
</tr>
<tr>
<td>$\geq$ 4 times per week</td>
<td>10.3 (6.3, 14.3)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Body mass index (kg/m$^2$)</td>
<td>-0.9 (-1.3, -0.6)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Calcium intake (g/day)</td>
<td>2.4 (0.4, 4.4)</td>
<td>0.019</td>
</tr>
<tr>
<td>Vitamin D intake (µg/day)</td>
<td>0.0 (-0.1, 0.1)</td>
<td>0.830</td>
</tr>
<tr>
<td>Family income</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\leq$ $40,000</td>
<td>Ref.</td>
<td></td>
</tr>
<tr>
<td>$40,001-78,000</td>
<td>2.2 (-1.1, 5.5)</td>
<td>0.195</td>
</tr>
<tr>
<td>$&gt;78,000</td>
<td>4.6 (1.2, 7.9)</td>
<td>0.009</td>
</tr>
<tr>
<td>Constant</td>
<td>125.6 (115.7, 135.5)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

$^1$Estimated difference in serum 25(OH)D concentrations from the reference category of categorical variables or per unit increase of the continuous variables; $^2$Compared to males, females had significantly lower serum 25(OH)D concentrations at the 14 year follow-up (-3.6; 95% CI -6.8, -0.4; $p = 0.026$), while there was no significant difference between males and females at the 17 year follow-up (2.6; 95% CI -1.3, 6.5; $p = 0.194$; data not shown)
Figure legends

Figure 1. Flow diagram of adolescents attending the 14 and 17 year follow-ups

Figure 2. Serum 25-hydroxyvitamin D (25(OH)D) concentrations of participants providing a blood sample at both follow-ups (n = 1045), stratified by month of blood collection

*Spring, Sep-Nov; Summer, Dec-Feb; Autumn, Mar-May; Winter, Jun-Aug
Figure 1

1861 Attended 14 year follow-up
1754 Attended 17 year follow-up

1380 Provided a blood sample
1268 Provided a blood sample

1045 Provided a blood sample at both follow-ups

Serum 25(OH)D reported

Missing data:
8 Physical activity
10 Body mass index
104 Calcium and vitamin D intake
43 Family income

919 Complete data at the 14 year follow-up
570 Complete data at the 17 year follow-up

Predictors of 25(OH)D reported

Missing data:
222 Physical activity
127 Body mass index
430 Calcium and vitamin D intake
177 Family income
Figure 2

Serum 25(OH)D concentrations (nmol/L)

Month of blood collection*

- Male 14 years
- Female 14 years
- Male 17 years
- Female 17 years
Supplementary Figure 1. Bland-Altman plot showing the agreement between the enzyme immunoassay (14 year follow-up) and LC-MS/MS. The limit of agreement is the mean difference ± two standard deviations.
Supplementary Figure 2. Percentage of participants with serum 25-hydroxyvitamin D concentrations < 50 nmol/L and 50-74.9 nmol/L at the 14 and 17 year follow-ups (n = 1045), stratified by season of blood collection (Spring, September-November; summer, December-February; autumn, March-May; winter, June-August)