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

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## 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 the predictors of serum 25(OH)D concentrations, including sex, race, month of blood collection, physical activity, BMI, family income, and Ca and vitamin D intakes (*n* 919 at 14 years; *n* 570 at 17 years), using a general linear mixed model. At 14 years, 31 % of adolescents had serum 25(OH)D concentrations between 50 and 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 and 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 BMI, a higher Ca 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.

**Key words: Vitamin D: 25-Hydroxyvitamin D: Raine Study: Adolescents**

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 CVD[2]. Serum 25-hydroxyvitamin D (25(OH)D) thresholds that signal vitamin D deficiency and sufficiency remain to be controversial. The Institute of Medicine defines deficiency as serum 25(OH)D concentrations <30 nmol/l[3], while guidelines from the Endocrine Society suggest that vitamin D deficiency be defined as concentrations <50 nmol/l and concentrations should be at least 75 nmol/l to maximise the effect of vitamin D on Ca, bone and muscle metabolism[4]. Serum 25(OH)D concentrations well below 50 nmol/l have been reported in populations worldwide, including the UK[5], Ireland[6], the USA[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 <50 nmol/l. In four European countries (Denmark, Finland, Ireland and Poland), 92 % of girls aged 11–13 years had 25(OH)D concentrations <50 nmol/l at the end of winter[12]. Mean 25(OH)D concentrations were <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 <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 *et al.*[9] recently examined the prevalence of vitamin D deficiency and its determinants in

**Abbreviations:** 25(OH)D, 25-hydroxyvitamin D; LC–MS/MS, liquid chromatography–tandem MS; NHANES, National Health and Nutrition Examination Survey.

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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. However, similar data in Australian adolescents have not been reported. Adequate vitamin D status may be important in adolescence in order to optimise Ca 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 the present study were to report vitamin D status, and to examine the 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[13]. 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-hydroxyvitamin 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°C until analysis. Serum 25(OH)D concentrations at 14 years were measured by enzyme immunoassay (Immunodiagnostic Systems Limited). 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 MS (LC–MS/MS; RDDT), according to published methodology[14]. 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 twelve 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). There was good agreement between the enzyme immunoassay and LC–MS/MS as shown in the Bland–Altman plot (Fig. S1, available online)[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. 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/week, 1–3 times/week and < 1 time/week).

A self-reported, semi-quantitative FFQ developed by the Commonwealth Scientific and Industrial Research Organisation in Adelaide, Australia[17] was used to assess Ca 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 12 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 $\chi^2$ tests. Baseline characteristics – including sex, age, race, month of blood collection, physical activity, BMI, and median daily vitamin D and Ca 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. To examine predictors of serum 25(OH)D concentrations, we performed a single general linear mixed model combining the 14- and 17-year data. Pearson's $\chi^2$ 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; physical activity (≥4 times/week, 1–3 times/week and <1 time/week). Vitamin D intake from food and supplements (μg/d), Ca intake from food and supplements (g/d) 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, Ca intake, vitamin D intake and BMI were investigated along with interactions between sex and physical activity, Ca intake, vitamin D intake and BMI. Analyses were performed using IBM SPSS Statistics Release version 19.9.9.1 (IBM SPSS, Inc.) and StataCorp 2011 Stata Statistical Software, Release 12 (StataCorp LP). Statistical significance was defined as two-tailed $P < 0{\cdot}05$.

## Results

### Characteristics of the participants

A total of 1045 adolescents provided a blood sample for the analysis of serum 25(OH)D concentrations at both follow-ups (Fig. 1). Complete data – including physical activity, BMI, Ca 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 present 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 the 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 the participants were physically inactive (exercising <1 time/week), increasing to 21% at 17 years. The mean BMI at 14 years was 21 kg/m$^2$, increasing to 23 kg/m$^2$ 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 Ca intake from food and supplements was 1107 mg at 14 years, decreasing to 1018 mg at 17 years.

### Serum 25-hydroxyvitamin D concentrations and vitamin D status

At 14 years, mean deseasonalised serum 25(OH)D concentration was 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 and 74·9 nmol/l and a further 4% had concentrations <50 nmol/l (Table 2). At 17 years, the percentage of adolescents with

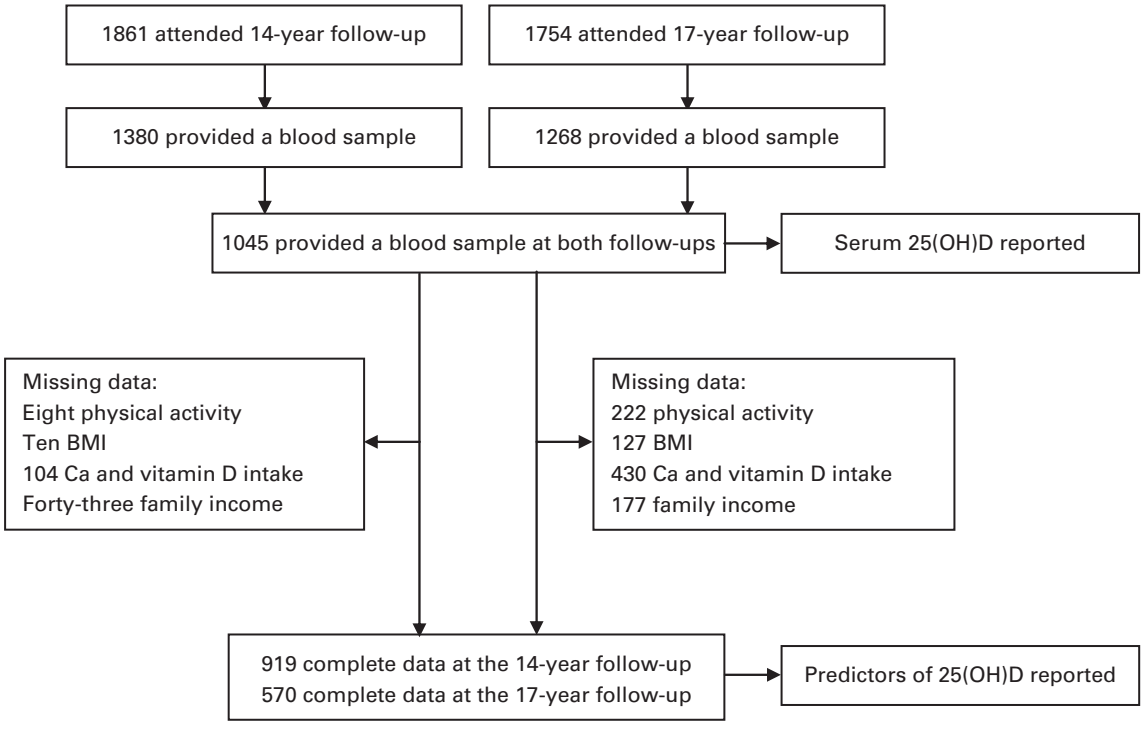


**Fig. 1.** Flow diagram of adolescents attending the 14- and 17-year follow-ups. 25(OH)D, 25-hydroxyvitamin D.

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

| | Participants | | Non-participants | | |
|---|---|---|---|---|---|
| | *n* | % | *n* | % | *P* |
| Sex | | | | | 0·650 |
| Male | 536 | 51·3 | 919 | 50·4 | |
| Female | 509 | 48·7 | 904 | 49·6 | |
| Race | | | | | 0·013 |
| Caucasian | 887 | 84·3 | 1481 | 81·2 | |
| Non-Caucasian | 158 | 15·7 | 342 | 18·8 | |
| Family income per year during pregnancy | | | | | <0·001 |
| <$7000 | 58 | 5·6 | 171 | 9·4 | |
| $7000–$11 999 | 67 | 6·4 | 176 | 9·7 | |
| $12 000–$23 999 | 222 | 21·2 | 461 | 25·3 | |
| $24 000–$35 999 | 270 | 25·8 | 378 | 20·7 | |
| ≥$36 000 | 363 | 34·7 | 472 | 25·9 | |
| Maternal age at birth (years) | | | | | <0·001 |
| <20 | 58 | 5·6 | 220 | 12·1 | |
| 20–24 | 181 | 17·3 | 426 | 23·4 | |
| 25–29 | 293 | 28·0 | 561 | 30·8 | |
| 30–34 | 317 | 30·3 | 409 | 22·4 | |
| 35–39 | 156 | 14·9 | 144 | 7·9 | |
| ≥40 | 36 | 3·4 | 27 | 1·5 | |
| Maternal education since school | | | | | <0·001 |
| None | 447 | 42·8 | 1001 | 54·9 | |
| Trade certificate or apprenticeship | 76 | 7·3 | 159 | 8·7 | |
| Professional registration (non-degree) | 114 | 10·9 | 132 | 7·2 | |
| College diploma or degree | 194 | 18·6 | 254 | 13·9 | |
| University degree | 140 | 13·4 | 140 | 7·7 | |
| Other | 52 | 5·0 | 95 | 5·2 | |
| Maternal pre-pregnancy BMI (kg/m$^2$) | | | | | 0·038 |
| Underweight (<18·5 kg/m$^2$) | 99 | 9·5 | 216 | 11·8 | |
| Healthy weight (18·5–24·9 kg/m$^2$) | 724 | 69·3 | 1170 | 64·2 | |
| Overweight (25–29·9 kg/m$^2$) | 110 | 10·5 | 203 | 11·1 | |
| Obese (≥30 kg/m$^2$) | 63 | 6·0 | 115 | 6·3 | |

concentrations between 50 and 74·9 nmol/l and <50 nmol/l increased to 40 and 12 %, respectively. Mean serum 25(OH)D concentrations were highest at the end of summer (Fig. 2). At the 14-year follow-up, 10 % of adolescents in winter and 4 % in spring had serum 25(OH)D concentrations <50 nmol/l, increasing to 28 and 21 %, respectively, in the 17-year follow-up (Table 3; Fig. S2, available online).

### *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 Ca 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 <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- to 18-year-olds in Great Britain (*n* 1102), 35 % of participants had concentrations <50 nmol/l[18]. Similarly, in the 2001–2006 NHANES, 29 and 34 % of 14- to 18-year-old boys and girls (*n* 3801), respectively, had concentrations <50 nmol/l[10]. In a cross-sectional study of 12- and 15-year-olds in Northern Ireland (*n* 1015), 36 % of participants had year-round serum 25(OH)D concentrations <50 nmol/l[19], while 26 % of 12- to 19-year-olds (*n* 231) participating in the Canadian Health Measures Survey Cycle 1 (2007–2009) had concentrations <50 nmol/l[8]. Perth and surrounding regions have a mean of 8·8 daily hours of sunshine[20], encouraging an outdoor 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, USA and Canada. However, despite the low percentage of adolescents with serum 25(OH)D concentrations <50 nmol/l in our cohort compared with international

**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)

(Number of participants and percentages; mean values and standard deviations; median values and interquartile ranges (IQR))

| | 14-year follow-up | | 17-year follow-up | |
|---|---|---|---|---|
| | *n* | % | *n* | % |
| Sex | | | | |
| Male | 536 | 51·3 | 536 | 51·3 |
| Female | 509 | 48·7 | 509 | 48·7 |
| Race | | | | |
| Caucasian | 887 | 84·9 | 887 | 84·9 |
| Non-Caucasian | 158 | 15·1 | 158 | 15·1 |
| Age (years) | 1045 | | 810 | |
| Mean | 14·1 | | 17·1 | |
| SD | 0·2 | | 0·3 | |
| Deseasonalised 25(OH)D (nmol/l) | | | | |
| Total | 1045 | | 1045 | |
| Mean | 86 | | 75 | |
| SD | 27 | | 24 | |
| Males | 536 | | 536 | |
| Mean | 90* | | 75 | |
| SD | 27 | | 25 | |
| Females | 509 | | 509 | |
| Mean | 83 | | 76 | |
| SD | 26 | | 24 | |
| Year-round vitamin D status (nmol/l) | | | | |
| ≥75 | 671 | 64·2 | 496 | 47·5 |
| 50–74·9 | 328 | 31·4 | 421 | 40·3 |
| <50 | 46 | 4·4 | 128 | 12·2 |
| BMI (kg/m$^2$) | 1039 | | 918 | |
| Mean | 21·4 | | 23·0 | |
| SD | 4·3 | | 4·5 | |
| Month of blood collection | | | | |
| Jan | 37 | 3·5 | 75 | 7·2 |
| Feb | 75 | 7·2 | 97 | 9·3 |
| Mar | 109 | 10·4 | 108 | 10·3 |
| Apr | 97 | 9·3 | 57 | 5·5 |
| May | 94 | 9·0 | 79 | 7·6 |
| Jun | 84 | 8·0 | 64 | 6·1 |
| Jul | 87 | 8·3 | 93 | 8·9 |
| Aug | 97 | 9·3 | 82 | 7·8 |
| Sep | 97 | 9·3 | 101 | 9·7 |
| Oct | 122 | 11·7 | 131 | 12·5 |
| Nov | 84 | 8·0 | 80 | 7·7 |
| Dec | 62 | 5·9 | 78 | 7·5 |
| Physical activity | | | | |
| ≥4 times/week | 349 | 33·7 | 204 | 24·8 |
| 1–3 times/week | 585 | 56·4 | 445 | 54·1 |
| <1 time/week | 103 | 9·9 | 174 | 21·1 |
| Family income | | | | |
| ≤$40 000/year | 291 | 29·0 | 156 | 18·0 |
| $40 001–$78 000/year | 368 | 36·7 | 268 | 30·9 |
| >$78 000/year | 343 | 34·2 | 444 | 51·2 |
| Vitamin D intake (μg/d) | 941 | | 665 | |
| Median | 2·0 | | 1·6 | |
| IQR | 1·2–3·1 | | 0·8–3·0 | |
| Ca intake (mg/d) | 941 | | 665 | |
| Median | 1107 | | 1018 | |
| IQR | 797–1470 | | 695–1423 | |

*Mean value was significantly different from that of females ($P<0·05$).

populations, the percentage of adolescents in this cohort with concentrations between 50 and 74·9 nmol/l was substantial.

Recently, Daly *et al.*[9] 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. They reported that approximately 60 % of participants aged 25–34 years had serum 25(OH)D concentrations <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 <50 nmol/l[21]. Compared with the present study, this higher prevalence of participants with serum 25(OH)D concentrations <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 the present 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 2-fold higher in summer compared with 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),

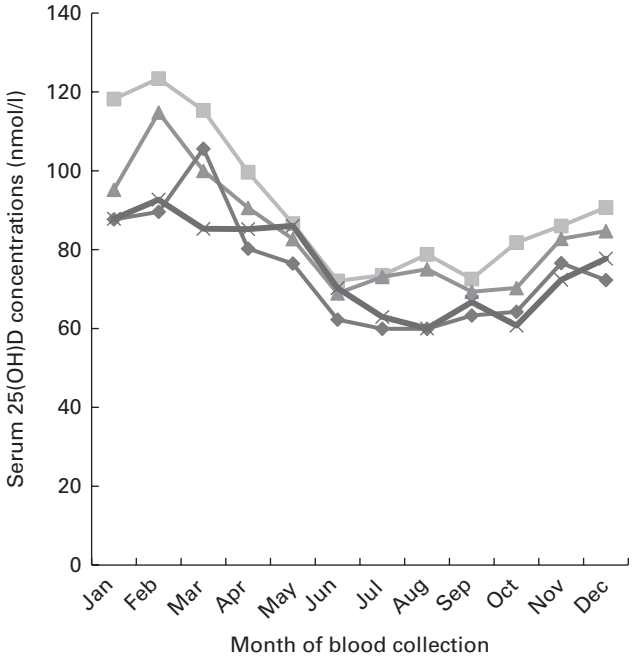


**Fig. 2.** Serum 25-hydroxyvitamin D (25(OH)D) concentrations of participants providing a blood sample at both follow-ups (*n* 1045), stratified by month of blood collection (spring, September–November; summer, December–February; autumn, March–May; winter, June–August). —■—, Male (14 years); —▲—, female (14 years); —◆—, male (17 years); —✕—, female (17 years).

**Table 3.** Vitamin D status at the 14- and 17-year follow-ups (*n* 1045), stratified by sex and season of blood collection*
(Number of participants and percentages)

| | Year-round† | | Spring | | Summer | | Autumn | | Winter | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *n* | % | *n* | % | *n* | % | *n* | % | *n* | % |
| 14-year follow-up | | | | | | | | | | |
| Total | 1045 | | 303 | | 174 | | 300 | | 268 | |
| ≥75 nmol/l | 671 | 64·2 | 142 | 46·9 | 137 | 78·7 | 222 | 74·0 | 117 | 43·7 |
| 50–74·9 nmol/l | 328 | 31·4 | 148 | 48·8 | 37 | 21·3 | 74 | 24·7 | 125 | 46·6 |
| <50 nmol/l | 46 | 4·4 | 13 | 4·3 | 0 | 0·0 | 4 | 1·3 | 26 | 9·7 |
| Males | 536 | | 153 | | 87 | | 139 | | 157 | |
| ≥75 nmol/l | 375 | 70·0 | 81 | 52·9 | 71 | 81·6 | 109 | 78·4 | 69 | 43·9 |
| 50–74·9 nmol/l | 141 | 26·3 | 68 | 44·4 | 16 | 18·4 | 28 | 20·1 | 77 | 49·0 |
| <50 nmol/l | 20 | 3·7 | 4 | 2·6 | 0 | 0·0 | 2 | 1·4 | 11 | 7·0 |
| Females | 509 | | 150 | | 87 | | 161 | | 111 | |
| ≥75 nmol/l | 296 | 58·2 | 61 | 40·7 | 66 | 75·9 | 113 | 70·2 | 48 | 43·2 |
| 50–74·9 nmol/l | 187 | 36·7 | 80 | 53·3 | 21 | 24·1 | 46 | 28·6 | 48 | 43·2 |
| <50 nmol/l | 26 | 5·1 | 9 | 6·0 | 0 | 0·0 | 2 | 1·2 | 15 | 13·5 |
| 17-year follow-up | | | | | | | | | | |
| Total | 1045 | | 312 | | 250 | | 244 | | 239 | |
| ≥75 nmol/l | 496 | 47·5 | 92 | 29·5 | 151 | 60·4 | 164 | 67·2 | 60 | 25·1 |
| 50–74·9 nmol/l | 421 | 40·3 | 154 | 49·4 | 85 | 34·0 | 64 | 26·2 | 112 | 46·9 |
| <50 nmol/l | 128 | 12·2 | 66 | 21·2 | 14 | 5·6 | 16 | 6·6 | 67 | 28·0 |
| Males | 536 | | 158 | | 136 | | 109 | | 133 | |
| ≥75 nmol/l | 253 | 47·2 | 46 | 29·1 | 78 | 57·4 | 72 | 66·1 | 32 | 24·1 |
| 50–74·9 nmol/l | 216 | 40·3 | 77 | 48·7 | 50 | 36·8 | 28 | 25·7 | 60 | 45·1 |
| <50 nmol/l | 67 | 12·5 | 35 | 22·2 | 8 | 5·9 | 9 | 8·3 | 41 | 30·8 |
| Females | 509 | | 154 | | 114 | | 135 | | 106 | |
| ≥75 nmol/l | 243 | 47·7 | 46 | 29·9 | 73 | 64·0 | 92 | 68·1 | 28 | 26·4 |
| 50–74·9 nmol/l | 205 | 40·3 | 77 | 50·0 | 35 | 30·7 | 36 | 26·7 | 52 | 49·1 |
| <50 nmol/l | 61 | 12·0 | 31 | 20·1 | 6 | 5·3 | 7 | 5·2 | 26 | 24·5 |

* Spring, September–November; summer, December–February; autumn, March–May; winter, June–August.
† Deseasonalised serum 25-hydroxyvitamin D concentrations.

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 USA[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 the 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 Ca intake was associated with higher serum 25(OH)D concentrations. It has been shown that higher Ca 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 USA[11] and New Zealand[32]. It is not clear why vitamin D status differs between boys and girls. Hill *et al.*[19] found that girls had a lower vitamin D intake than boys, while Rockell *et al.*[32] noted that physical activity was higher in boys than in girls. However, in the present study, serum 25(OH)D concentrations were higher in males than in 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 in 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

**Table 4.** General linear mixed model of predictors of serum 25-hydroxy-vitamin D (25(OH)D) concentrations at the 14- and 17-year follow-ups ($\beta$-Coefficients and 95 % confidence intervals)

| Determinants | $\beta$ | 95 % CI* | *P* |
|---|---|---|---|
| Sex† | | | |
| Male | Reference | | |
| Female | −3·6 | −6·8, −0·4 | 0·026 |
| Time | | | |
| 14-year follow-up | Reference | | |
| 17-year follow-up | −11·5 | −14·5, −8·5 | <0·001 |
| Sex × time | 6·2 | 2·2, 10·2 | 0·003 |
| Race | | | |
| Caucasian | Reference | | |
| Non-Caucasian | −15·2 | −19·1, −11·3 | <0·001 |
| Month | | | |
| Feb | Reference | | |
| Mar | −7·3 | −12·8, −1·9 | 0·009 |
| Apr | −15·3 | −21·2, −9·5 | <0·001 |
| May | −24·0 | −29·7, −18·2 | <0·001 |
| Jun | −40·2 | −46·3, −34·2 | <0·001 |
| Jul | −41·5 | −47·3, −35·7 | <0·001 |
| Aug | −38·8 | −44·5, −33·1 | <0·001 |
| Sep | −40·4 | −46·1, −34·7 | <0·001 |
| Oct | −38·5 | −43·8, −33·1 | <0·001 |
| Nov | −25·7 | −31·4, −19·9 | <0·001 |
| Dec | −26·2 | −32·1, −20·4 | <0·001 |
| Jan | −11·8 | −18·2, −5·5 | <0·001 |
| Physical activity | | | |
| <1 time/week | Reference | | |
| 1–3 times/week | 5·0 | 1·4, 8·6 | 0·006 |
| ≥4 times/week | 10·3 | 6·3, 14·3 | <0·001 |
| BMI (kg/m$^2$) | −0·9 | −1·3, −0·6 | <0·001 |
| Ca intake (g/d) | 2·4 | 0·4, 4·4 | 0·019 |
| Vitamin D intake (μg/d) | 0·0 | −0·1, 0·1 | 0·830 |
| Family income | | | |
| ≤$40 000 | Reference | | |
| $40 001–$78 000 | 2·2 | −1·1, 5·5 | 0·195 |
| >$78 000 | 4·6 | 1·2, 7·9 | 0·009 |
| Constant | 125·6 | 115·7, 135·5 | <0·001 |

*Estimated difference in serum 25(OH)D concentrations from the reference category of categorical variables or per unit increase of continuous variables.

†Compared with males, females had significantly lower serum 25(OH)D concentrations at the 14-year follow-up ($\beta$ −3·6, 95 % CI −6·8, −0·4; *P*=0·026), while there was no significant difference in serum 25(OH)D concentrations between males and females at the 17-year follow-up ($\beta$ 2·6, 95 % CI −1·3, 6·5; *P*=0·194; data not shown).

than at 14 years. Increasing vitamin D deficiency has also been observed in children and adolescents in the USA, where the 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 <50 nmol/l[4], whereas the Institute of Medicine defines deficiency as <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 a significant variation in the measurement of 25(OH)D concentration 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 a competitive protein-binding assay 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 have 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 has developed protocols for standardising the measurement of 25(OH)D concentration using LC–MS/MS[36]. A recent evaluation of the Vitamin D Standardization Program 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 <46·6 nmol/l and the other for concentrations greater than or equal to this cut-off[37].

A strength of the present study was the longitudinal nature of the data, allowing us to investigate differences in the 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 Ca and vitamin D from food and supplements. A limitation of the present study was the loss to follow-up. Participants included in the present study were more likely to be from families with higher socio-economic 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 3rd year. Therefore, the remaining cohort is more representative of the general Western Australian population than the original cohort[38,39].

The present study has shown that, among adolescents living at latitude 32°S in Perth, Western Australia, the percentage with serum 25(OH)D concentrations <50 nmol/l is low compared with international populations. However, the percentage of adolescents with serum 25(OH)D concentrations <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.

## Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S000711451400186X

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