Sun exposure behavior, seasonal vitamin D deficiency and relationship to bone health in adolescents

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Abstract

Context: Vitamin D is essential for bone health in adolescence, where there is rapid bone mineral content accrual. As cutaneous sun-exposure provides vitamin D, there is no recommended oral intake for UK adolescents.

Objective: Assess seasonal vitamin D status and its contributors in white Caucasian adolescents, and examine bone health in those found deficient.

Design: Prospective cohort study.

Setting: Six schools in Greater Manchester, UK.

Participants: 131 adolescents, 12–15 years.

Intervention(s): Seasonal assessment of circulating 25-hydroxyvitamin D (25OHD), personal sun-exposure and dietary vitamin D. Adolescents deficient (25OHD <10 ng/mL/25 nmol/L) in ≥one season underwent dual-energy X-ray absorptiometry (lumbar spine, femoral neck), with bone mineral apparent density (BMAD) correction for size, and peripheral quantitative computed tomography (distal radius) for volumetric (v)BMD.

Main Outcome Measure: Serum 25OHD; BMD.

Results: Mean 25OHD was highest in September: 24.1 (SD 6.9) ng/mL and lowest in January: 15.5 (5.9) ng/mL. Over the year, 16% were deficient in ≥one season and 79% insufficient (25OHD <20 ng/mL/50 nmol/L) including 28% in September. Dietary vitamin D was low year-round while personal sun-exposure was seasonal and predominantly across the school week. Holidays accounted for 17% variation in peak 25OHD ($p<0.001$). Nineteen adolescents underwent bone assessment, which showed low femoral neck BMAD versus matched reference data ($p=0.0002$), 3 with $Z\leq -2.0$ distal radius trabecular vBMD.

Conclusions: Sun-exposure levels failed to provide adequate vitamin D, ~one-quarter adolescents insufficient even at summer-peak. Seasonal vitamin D deficiency was prevalent and those affected had low BMD. Recommendations on vitamin D acquisition are indicated in this age-group.

Introduction

Vitamin D regulates calcium absorption and bone mineralization and is essential for bone health. Vitamin D deficiency, a circulating 25-hydroxyvitamin D (25OHD) concentration of <10 ng/mL (25 nmol/L) (1, 2), can cause rickets in children and osteomalacia in adolescents and adults. Higher levels are desirable, with <20 ng/mL (50 nmol/L) regarded insufficient by several authorities (3–5) due to association with sub-optimal musculoskeletal health (6). Maintaining vitamin D status is particularly important during adolescence, a critical time for bone development, with ~one-third of adult peak bone mineral content (BMC) accrued during the pubertal growth spurt (7, 8).

The major source of vitamin D is cutaneous synthesis initiated through sunlight's ultraviolet B (UVB), with usually only low amounts obtained through diet. Previtamin D, formed by UVB-conversion of 7-dehydrocholesterol, undergoes heat isomerization to vitamin D then hepatic hydroxylation to 25OHD, the main circulating form and accepted indicator of vitamin D status. A further, renal, hydroxylation produces the active hormone 1,25-dihydroxyvitamin D ($1,25(OH)_2D$). At northerly latitudes, negligible UVB in winter sunlight means vitamin D must be made and stored during summer months (9).

Current UK national guidance on vitamin D acquisition assumes those aged 4–64 years gain their vitamin D requirements from sunlight alone, thus there is no recommended nutrient intake (1). Short, regular exposures to summer sunlight containing the requisite UVB are considered to avoid vitamin D deficiency (10). However, excessive UV radiation (UVR) exposure is the principal external cause of most skin cancers, with childhood sunburn a risk factor for later development of melanoma (11). Thus, recommendations to protect children include limiting sun-exposure between 11am–3pm, wearing covering clothing and sunscreen (12). Meanwhile, substantial proportions of the global population, including the UK, are reported to have low vitamin D status (9, 13), and rickets has returned as a clinical concern (14).

The cross-sectional National Diet and Nutrition Survey (NDNS) of the UK white population indicated that in 1997–1998, 10% blood samples of 11–14 year olds had 25OHD <10 ng/mL and 40% <20 ng/mL, all seasons combined (15). Adolescents appeared at greater risk of low vitamin D status than younger children, with amount of outdoor exercise a contributor (16). A more recent mixed-ethnicity

study found 73% of 51 female adolescents (37 non-white) had 25OHD <12 ng/mL (17). These studies indicate the need for more detailed, longitudinal appraisal of vitamin D status and contributory factors in adolescents. The aim of this study was to determine vitamin D status throughout the year, influence of personal sun-exposure and dietary vitamin D intake in white adolescents, and to examine bone health parameters in those found vitamin D deficient in one or more seasons.

Materials and Methods

Study design and subjects

A single-centre, prospective cohort study in healthy, ambulant white Caucasian (sun-reactive skin type I–IV) (18) adolescents, 12–15 years. History of photosensitivity was an exclusion criterion. Subjects were recruited through five fee-paying schools and one free (state) school in Greater Manchester, UK. In January, April, June and September 2011, blood samples were taken for 25OHD, personal sun-exposure and ambient UVB levels were evaluated, and dietary vitamin D estimated. Adolescents with vitamin D deficiency underwent bone assessment between June–September 2012. Ethical approval was obtained (North Manchester Research Ethics Committee, 10/H1008/58) and volunteers gave written informed consent. The study was performed according to Declaration of Helsinki principles.

Serum 25OHD and biochemical analyses

Serum was stored at -20^{o}C until study completion. Concentrations of 25OHD were determined by liquid chromatography tandem mass spectrometry (LC-MS/MS) using an ABSciex 5500 tandem mass spectrometer (Warrington, UK) and the Chromsystems (Munich, Germany) 25OHD kit for LC-MS/MS, following the manufacturers' instructions (intra- and inter-assay CV 3.7% and 4.8% respectively). The laboratory is accredited by Clinical Pathology Accreditation UK (No. 0865) and certified proficient by the Vitamin D Quality Assurance Scheme (DEQAS). Serum PTH was measured in January and September using the OCTEIA immunoenzymometric assay following the manufacturer's instructions (Immunodiagnostic Systems Ltd, Boldon, U.K.) with sensitivity 0.06

pmol/L (intra- and inter-assay CV 4% and 6% respectively). Baseline serum biochemistry included calcium, renal and liver function.

Personal and ambient UVR, time outdoors and dietary vitamin D assessment

Adolescents wore polysulphone film badge UVR dosimeters (9), one week in each season, on outer clothing of upper chest/anterior shoulder, with one badge for weekdays and one for weekend. Dosimeters recorded total erythemal dose as a proxy for UVR exposure effective for vitamin D synthesis. Badges were included in analyses unless unworn (information from subject; absent pin-hole in badge-holder). Ambient UVR was recorded during badge-wearing weeks using a GUV-541 filter radiometer (Biospherical Instruments Inc.; positioned at latitude 53.47°N, longitude -0.23W, altitude 76m) based at the University of Manchester. This instrument supplies a one minute average of erythemal irradiance, and runs continuously as part of an atmospheric monitoring programme (19) providing data for the European UV Database and the World Ozone and Ultraviolet Radiation Data Centre of the World Meteorological Organization. Adolescents concurrently completed sun-exposure diaries detailing time outdoors (in 15 minute blocks), weather conditions, clothing, dedicated sunscreen-product and SPF-containing face-cream use (9), and oral vitamin D intake was estimated through daily dietary logs recording: vitamin D supplements; vitamin D-fortified foods; oily fish, butter, margarine and other spreads; milk; eggs; cheese; red meat (20). Diary days were excluded from analyses if not completed.

Bone assessments

Dual-energy X-ray absorptiometry (DXA) scans of lumbar spine (LS) and proximal femur were performed using an Hologic QDR 45000 Discovery scanner (Hologic, Bedford, MA USA), software version 12.6.1, fast array mode (21). Measurements of BMC, bone mineral density (BMD) and bone area (BA) were made in the LS L1-L4 and femoral neck (FN). Bone mineral apparent density (BMAD) was calculated as described for LS (22) and FN (23). Peripheral quantitative computed tomography (pQCT) of non-dominant radius was performed by XCT-2000 scanner (Stratec, Pforzheim, Germany), software version 5.5d (24). Trabecular and total (cortical + trabecular)

volumetric BMD (vBMD) were measured at the 4% distal site. Data were compared to sex- and age-matched published reference data for Manchester white Caucasian adolescents for DXA (n=442, 239 male) (21) and pQCT (n=629, 380 male) (24). Results are expressed as SD from the age-matched mean (Z score) (25, 26), with Z score < -2.0 defined as reduced BMAD or vBMD.

Statistical analysis

A sample of n=100 permitted assessment of population SD with adequate precision (27), and gave 80% power (0.05α) to detect correlations >0.3 between 25OHD level and sun-exposure variables. Recruitment of n=125 allowed for 20% drop-out. Post-hoc comparisons were made between males versus females and fee-paying versus state schools using Mann-Whitney test, and within-season weekday versus weekend UVR exposure and time outdoors by Wilcoxon signed-rank test, without adjustment for multiple testing. Multiple linear regression explored associations between late-summer peak 25OHD and demographic and behavioral characteristics; models first assessed how much 25OHD variation demographic factors could explain, following which behavioral factors were added. Sun-exposure, 25OHD and BMI data were log-transformed to linearize relationships and stabilize variance. Proportion of explained variance was quantified by reduction in R^2 as behavioral variables were removed from the model. To reduce 'overfitting', variables were selected based on substantive interest and previous multivariable analyses of other cohorts (9). Analyses were performed on available-case basis and assumed no systematic differences between subjects with complete and missing data, beyond those accounted for by factors included in the model.

Results

The study population comprised 131 adolescents. Three withdrew after the first data collection period and six after the second. Seventy-five adolescents provided blood samples in four seasons, 38 in three, 11 in two and 7 in one season. Dietary log return was 71-88% across seasons (88-97% complete); sun-exposure diary return was 72-91% (84-95% complete); badge return was 61-71% for weekday (93-99% complete i.e. worn 5 days) and 52-76% for weekend (96-100% complete i.e. worn 2 days).

Baseline characteristics are shown in Table 1. Baseline serum biochemistry showed no substantive abnormalities.

Circulating 25OHD levels showed a seasonal cycle (Figure 1A), mean (SD) 25OHD reaching a September peak of 24.1 (6.9) ng/mL where 28% and 3% adolescents had levels <20 and <10 ng/mL, respectively (Figure 1B). Mean 25OHD at January trough was 15.5 (5.9) ng/mL, with 74% and 17% at <20 and <10 ng/mL respectively. In total, 21 adolescents had 25OHD <10 ng/mL: 2 year-round, 2 in three seasons, 6 in two seasons and 11 in one season; only 4 adolescents in this sub-group achieved 25OHD ≥20ng/mL during the year (June/September). For males, mean 25OHD was 23.9 (7.2) and 14.7 (5.5) ng/mL in September and January, and for females 24.3 (6.7) and 16.3 (6.1) ng/mL respectively, with no significant between-sex difference in levels or %subjects <20 or <10 ng/mL. Mean (SD) PTH in January and September was 45 (16) and 41 (16) pg/mL, respectively.

Median personal UVR dose assessed through polysulphone badges followed seasonal ambient UVR, and showed higher exposure was acquired across the school-week than the weekend (Figure 1C). Total UVR dose over seven days correlated with 25OHD in June (Spearman ρ=0.31; P=0.02; n=59) and not in other seasons. Median personal UVR/day was lower on weekend-days than preceding weekdays in all seasons, with males receiving higher UVR doses/day on weekdays in June and September than females (P<0.001; data not shown).

In their diaries, adolescents recorded a similar total time/day outdoors on the weekends and weekdays, such that the school-week contributed more than the weekend to total time/week outdoors (Table 2, Figure 2A, 2B). Overall, the longest periods of time outdoors were in June, the month with most daylight hours and ambient UVB, males spending more time outdoors than females (Table 2). Time recorded outdoors/day between 10:00–15:00h was lower on weekdays, where dictated by the school-day routine, than on weekend-days in all seasons (all P<0.001).

Males exposed less %skin surface area on weekdays than weekends in all seasons, attributable to more covering school uniform, while females exposed similar skin surface area at weekdays and weekends (Table 2). Head garments (comprising hats and caps) were worn infrequently. Remarkably, no adolescents reported wearing dedicated sunscreen products in any season in the UK. No males and few females used SPF-containing face-cream once/more during the reporting periods:

six (6%), nine (9%), ten (11%) and nine (8%) adolescents in June, September, January and April, respectively.

The majority (86%) of adolescents reported a holiday on ≥1 occasion during the study, most frequently at European locations, latitudes 41–50^{o}N, during July-August (Supplemental Table 1). Their mean 25OHD was 24.8 (6.3) and 15.9 (5.7) ng/mL in September and January, respectively, compared with 18.5 (8.9) and 12.7 (6.7) in those not taking a holiday.

Estimated dietary vitamin D was low with no seasonal difference. Median (IQR) vitamin D intake for January, April, June and September was 2.1 (1.3–3.4), 1.9 (1.1–3.3), 1.7 (1.0–2.7) and 1.9 (1.1–2.9) μg/day, respectively. Only 27 adolescents (21%; 5 male) took supplements containing vitamin D in ≥1 season (range 0.02–20 μg/day). Mean 25OHD of this group was 24.6 (6.2) and 17.0 (5.4) ng/mL in September (n=25) and January (n=16) respectively. Only five adolescents (4%; all female) took supplements year-round; their mean 25OHD was 27.5 (6.8) and 17.3 (6.2) ng/mL in September and January, respectively.

Adolescents at private school had lower 25OHD levels than those at state school (mean 21.8 versus 24.6 ng/mL in June; P=0.02). Further exploration revealed their lower UVR dose/day than state school adolescents on weekdays in April, June and September (P=0.02 to <0.001), no difference at weekends, and that they spent less time/day outdoors on weekdays in these periods (median 72, 83, 69 versus 150, 162, 158 minutes, respectively; all P<0.001).

Multiple linear regression showed demographic factors explained very little (R^2=0.07) of variation in September 25OHD, no individual factor being a significant predictor (Table 3). Addition of behavioral factors substantially increased the variation in September 25OHD explained by the model (R^2=0.33; Table 3). Taking a holiday at any time during the year explained 17% variation, accounting for approximately half the predictive value of the model.

Bone densitometry data for the 19 vitamin D deficient adolescents (10 male) completing bone assessments are expressed as SD (Z) scores, calculated using Manchester ethnicity-specific, sex- and age-matched reference data for DXA (21) and pQCT (24) (Figure 3). The group's mean Z score of -0.8 for FN BMAD was significantly lower than the reference population (P=0.0002). Mean LS

BMAD Z score was -0.2 and LS BMAD was categorized as normal in all but one adolescent whose measurement was reduced (Z score ≤ -2.0) while the FN BMAD Z score was ≥ -2.0 in all adolescents. The distal radius mean vBMD Z score of -0.4 for trabecular bone was the same as for total bone. Three males had reduced (Z score ≤ -2.0) trabecular vBMD, one of whom also had reduced total vBMD (and reduced LS BMAD).

Discussion

We have addressed a knowledge gap regarding longitudinal vitamin D status and personal sun-exposure levels in adolescence. A clear seasonal pattern was seen in circulating 25OHD in white adolescents at mid-UK latitude (equivalent to Edmonton, Canada; Hamburg, Germany), with highest mean concentration (24.1 ng/mL) at end-summer, followed by early summer, reflecting the higher personal UVR exposure observed in summer and spring, respectively (Figure 1A, 1C). A key finding was the remarkably high prevalence of vitamin D deficiency in this white cohort, 16% adolescents demonstrating 25OHD <10 ng/mL in at least one season. Moreover, 28% and 74% adolescents failed to reach the desirable 20 ng/mL level at the general summer-peak and winter-trough, respectively. Previous UK studies reporting 10% (15) and 2.9% (27) of white adolescents with 25OHD <10 ng/mL examined 25OHD on a single occasion. Our study highlights the value of repeated, seasonal, measures, and shows a greater than anticipated potential for sub-optimal bone health revealed in this age-group.

We found dietary vitamin D did not contribute to seasonal vitamin D status change, median intake across seasons being low and constant at 1.7–2.1 μg/day. This low intake is consistent with the 1.7 μg/day reported for a similar age-group in Northern Ireland, UK (28) and the lower-end of the range reported in Europe (29). This is lower than the 5 μg/day recommended by the World Health Organization (30) and the 2.7–4.2 μg/day across seasons found in white adults who were studied under identical protocols to the current study, in Greater Manchester (9). Vitamin D supplement use was low and irregular with very few adolescents taking them in all seasons, although a gender difference was noted, with 22/27 taking supplements being female.

Personal sun-exposure levels assessed through both measurement of polysulphone badge UVR dose and diaries recording time outdoors, highlighted the greater contribution of the school-week relative to the weekend (Figures 1C, 2A). A pattern of higher badge UVR dose/day was seen according to season, and also on weekdays compared with weekend-days in all seasons. Weekend dose showed a greater variability than weekday, reflecting school-day regularity. Adolescents' diaries indicated that the higher badge UVR dose/day on weekdays compared to weekend-days was not due to longer recorded time spent outdoors (total time or 10:00–15:00h). Future studies could explore whether this relates to greater use of shaded outdoor space on weekends, such as town centres with tall buildings. Compliance with badge-wearing may also contribute, through enhanced supervision on school days. Previous data on personal sun-exposure of adolescents at northerly latitudes are sparse, with one available study in the UK; this assessed 14–15 year olds (n=86) across April–July 1994 and revealed their lower exposure levels relative to younger children (31, 32). Median times outdoors for adolescents were 99 and 90 minutes/day on weekdays and weekends, thus by comparison with our data (Table 2) there is no indication that overall time spent outdoors by UK adolescents has fallen in the past 20 years. A Danish study performed in 3 summers, 1999 to 2001, indicated that children and older teenagers spent less time outdoors (approximately 40-50 minutes/day; again found to be higher in the younger than the older group) than the adolescents in our study; however assessment methods differed and prevailing weather conditions may have contributed (32, 33).

Since total sun-exposure received during the school-week is greater than at weekends, school activity timetabling and sun protection policy can have major impact on vitamin D status in adolescents. Interestingly, in our cohort state school adolescents demonstrated higher UVR doses and longer time outdoors on weekdays than private school adolescents, which may reflect differences in school activities/conditions. Overall, males spent more time outdoors than females, although their weekday uniform covered a higher skin surface area (Table 2), and a similar vitamin D status was seen. Approximately one-third of the observed variation in peak (September) 25OHD was accounted for with the factors included in the multivariable regression model. Holiday-taking dominated as a predictor of peak level, accounting for 17% of the variation in the sample. We estimate that a participant taking a holiday had 1.8x the peak 25OHD, on average, of a participant who did not. In

contrast, the school day routine and uniform of the adolescents during weekdays produced an overall low variation in sun-exposure behavior in this UK sample, which may explain why these parameters weren't explanatory in the model.

Subclinical vitamin D deficiency may adversely affect bone mineralization in adolescents, due to high percentage BMC accrual at this life-stage (8). Peak bone mass is also considered an important determinant of osteoporotic fracture risk in later life (7). Female adolescents with low 25OHD (≤16 or ≤18.5 ng/mL) are reported to have lower distal radius areal BMD than those with higher 25OHD (>16 or 29.6 ng/mL respectively) (6, 34), with no difference for males (6). Our wider assessment of bone health in seasonal vitamin D deficiency (25OHD <10 ng/mL) contributes evidence that bone mineralization is sub-optimal in low vitamin D status adolescents. We found a small proportion of these deficient adolescents had clinically significantly reduced BMD (either BMAD or vBMD; Z score < -2.0), while the sub-group as a whole had significantly lower FN BMAD scores than the Manchester reference population for this age-group (Figure 3). If a pattern of seasonal vitamin D deficiency is maintained through adolescence children may fail to accrue their potential peak bone mass, with implications for future bone health. A meta-analysis of 6 randomized trials of vitamin D supplementation conducted in Finland, Denmark, China and Lebanon, in mainly female, mixed-ethnicity 10–17 year olds, concluded that while supplementation of those with 25OHD <14 ng/mL could give clinically useful BMD improvement, this requires confirmation (35).

Strengths of this study include pan-seasonal assessment of 25OHD, personal UVR dose, time outdoors and diet, providing a more comprehensive picture of behavior/lifestyle and vitamin D status than single-point assessments, and distinguishing seasonal from the less prevalent sustained vitamin D deficiency. A further strength is our follow-up investigation of bone health parameters in the deficient sub-set, using robust bone densitometry and interpretative methods. For LS and FN, BMAD was calculated, as the standard measure of DXA (areal BMD) can cause underestimates in children. BMAD adjusts for bone thickness by calculating volumetric density and is a more appropriate measurement in this age-group (22, 23). Moreover, Z scores were calculated by comparison to large local reference datasets for DXA and pQCT, avoiding incorrect classification of individuals as having low bone density for their age (21, 24). Limitations include behavioral assessments in 1-week snap-

shots per season for reasons of compliance, sparse detail on outdoor activities due to overall intensity of assessments, and absent bone densitometry data for the whole cohort. The *P*-values presented in this manuscript are nominal owing to the large number of comparisons available.

In conclusion, a high percentage of this UK white adolescent sample had serum 25OHD falling below the 20 ng/mL sufficiency level and even into deficiency (<10 ng/mL). Seasonally deficient adolescents had significantly lowered FN BMAD compared with local reference data. Taking holidays was a significant predictor of 25OHD, while vitamin D supplement use was scarce. As UK current sun-exposure patterns do not provide an adequate source of vitamin D, amendments are required to recommendations on vitamin D acquisition in this age-group. While wider skin surface area exposure to sunlight might safely increase vitamin D status, oral vitamin D supplements may be beneficial during this critical time for bone development.

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Table 1. Characteristics of study participants (n=131).	
Age (years)	13.5 (0.8)
BMI (kg/m^2)*	21.1 (3.2)
Sex	
Male	51 (39)
Female	80 (61)
Skin type	
I	15 (12)
II	38 (29)
III	62 (47)
IV	16 (12)

Data are mean (SD) or number (percentage). *n=116.

Table 2. Time spent outdoors and surface area exposed in each season

	Minutes per day outdoors – Weekdays								
	Male			Female			All subjects		
	n	06:00–20:00	10:00–15:00	n	06:00–20:00	10:00–15:00	n	06:00–20:00	10:00–15:00
Jan	41	87 (65–143)	36 (20–59)	50	57 (33–105)**	12 (2–33)***	91	72 (45–117)	21 (9–45) †††
Apr	39	105 (63–168)	45 (18–81)	71	90 (54–144)	33 (15–66)	110	92 (59–156)	36 (15–70) †††
Jun	35	120 (87–177)	54 (24–75)	61	90 (60–153)*	39 (14–78)	96	113 (64–164) †	47 (18–77) †††
Sep	34	95 (65–150)	36 (12–63)	60	74 (40–125)	17 (6–60)	94	84 (56–135)	24 (9–60) †††
	Minutes per day outdoors – Weekend days								
	Male			Female			All subjects		
	n	06:00–20:00	10:00–15:00	n	06:00–20:00	10:00–15:00	n	06:00–20:00	10:00–15:00
Jan	40	86 (34–143)	64 (8–96)	48	60 (30–88)	30 (8–60)	88	68 (30–135)	42 (8–83)
Apr	38	131 (53–219)	83 (26–145)	68	90 (47–163)	38 (15–88)	106	109 (53–182)	53 (15–105)
Jun	34	131 (71–219)	75 (30–109)	58	101 (51–189)	53 (21–107)	92	120 (54–195)	60 (23–105)
Sep	34	158 (68–227)	79 (21–160)	60	71 (38–146)*	38 (0–73)*	94	83 (38–184)	45 (13–107)
	Percentage skin surface area exposed								
	Weekdays				Weekends				
	n	Male	n	Female	N	Male	n	Female	
Jan	40	8 (8–9)	49	10 (8–17) ‡‡‡	38	8 (8–14) ¶	46	11 (8–16)	
Apr	39	8 (8–12)	71	13 (8–17) ‡‡	39	14 (10–23) ¶¶¶	68	11 (8–16)	
Jun	35	12 (8–14)	61	16 (9–17) ‡‡	34	14 (10–19) ¶¶¶	59	14 (11–19)	
Sep	34	8 (8–13)	61	10 (8–16) ‡	34	14 (8–19) ¶¶¶	61	12 (8–17)	

Data are median (IQR). *p<0.05 **p<0.01 ***p<0.001 compared to males in the same month; †p<0.05 †††p<0.001 compared to weekend-days in the same month. ‡p<0.05 ‡‡p<0.01 ‡‡‡p<0.001 compared to males in the same month; ¶p<0.05 ¶¶¶p<0.001 compared to weekdays in the same month.

Table 3. Multifactorial regressions of September 25OHD on demographic and behavioral factors

	Estimate (95% CI)*	***P***	**R^2 change†**
Demographic factors only (n=111; R^2=0.07)			
Age (years)	1.03 (0.95–1.07)	0.52	
Sex		0.35	
Female	1.00		
Male	0.94 (0.82–1.07)		
Log(BMI)	0.71 (0.45–1.12)	0.14	
Skin type		0.51	
I	1.00		
II	1.18 (0.94–1.47)	0.15	
III	1.16 (0.93–1.44)	0.19	
IV	1.13 (0.87–1.45)	0.36	
Demographic and behavioral factors (n=88; R^2=0.33)			
Age, years	1.03 (0.94–1.12)	0.54	
Sex		0.76	
Female	1.00		
Male	1.03 (0.85–1.24)		
Log(BMI)	0.71 (0.43–1.17)	0.18	
Skin type		0.15	
I	1.00		
II	1.23 (0.96–1.57)	0.10	
III	1.04 (0.82–1.32)	0.73	
IV	1.09 (0.83–1.45)	0.52	
Dietary vitamin D intake (μg/day)	1.05 (0.97–1.14)	0.21	0.01
Vitamin D supplement intake (μg/day)	1.03 (0.99–1.07)	0.12	0.02
Time outdoors (h/day, weekend)	0.99 (0.93–1.05)	0.67	0.00
Time outdoors (h/day, weekdays)	1.03 (0.93–1.12)	0.60	0.00
UVR dose (Log[SED/day], weekend)	1.02 (0.98–1.07)	0.37	0.00
UVR dose (Log[SED/day], weekdays)	1.01 (0.92–1.09)	0.89	0.00
Surface area exposed (Log[%/day], weekend)	1.03 (0.77–1.38)	0.84	0.00
Surface area exposed (Log[%/day], weekdays)	1.26 (0.94–1.68)	0.12	0.02
Holiday taken		<0.001	0.17
No	1.00		
Yes	1.80 (1.38–2.35)		
Constant intercept term	38.52 (4.66–318.60)	<0.001	

* denotes relative change in 25OHD as the factor changes.
† change in the proportion of variance explained by the model when the behavioral factor is removed.

Figure legends

Figure 1. Seasonal 25-hydroxyvitamin D (25OHD) in adolescents, and personal and ambient UVR levels. (A) Circulating 25OHD levels by subject. Black bars indicate the mean for each month. Red lines indicate the 20 ng/mL and 10 ng/mL cut-off levels for 25OHD. January, n=90; April, n=120; June, n=118; September, n=115. (B) Proportion of subjects according to 25OHD cut-off levels for deficiency (<10 ng/mL), insufficiency (10 to <20 ng/mL) and sufficiency (≥20ng/mL). (C) For personal UVR exposure, black bars indicate the median for each assessment period. Total available ambient UVR recorded over the 7 day monitoring period is shown by black circles. UVR was measured as standard erythema dose (SED) where 1 SED=100 J/m^2 erythemally-effective UVR (~12 minutes midsummer noontime sun-exposure at 53.5^{o}N) (9). January, n=81 (weekend n=76); April, n=103 (n=79 weekend); June, n=80 (n=68 weekend); September, n=85 (n=69 weekend).

Figure 2. Time spent outdoors in each season as recorded in adolescents' diaries. (A) Total time outdoors over the school-week and across weekends, and (B) Mean time outdoors per day on weekdays and weekend-days. Time outdoors was recorded in 15 minute intervals from 06:00 to 20:00. Black bars indicate the median for each assessment period. January, n=91 (weekend n=88); April, n=110 (n=106 weekend); June, n=96 (n=92 weekend); Sep, n=94 (n=94 weekend).

Figure 3. Bone parameter standard deviation (Z) scores for adolescents vitamin D deficient in one or more seasons. Mean and 95% CI of Z scores for bone mineral apparent density (BMAD) measured by dual energy X-ray absorptiometry (DXA) and volumetric (v) BMD measured by peripheral quantitative computed tomography (pQCT). n=19 (ten male), mean (SD) age 13.4 (1.1). Mean femoral neck BMAD Z score was significantly lower than the reference datasets (p=0.0002).

Figure 1.

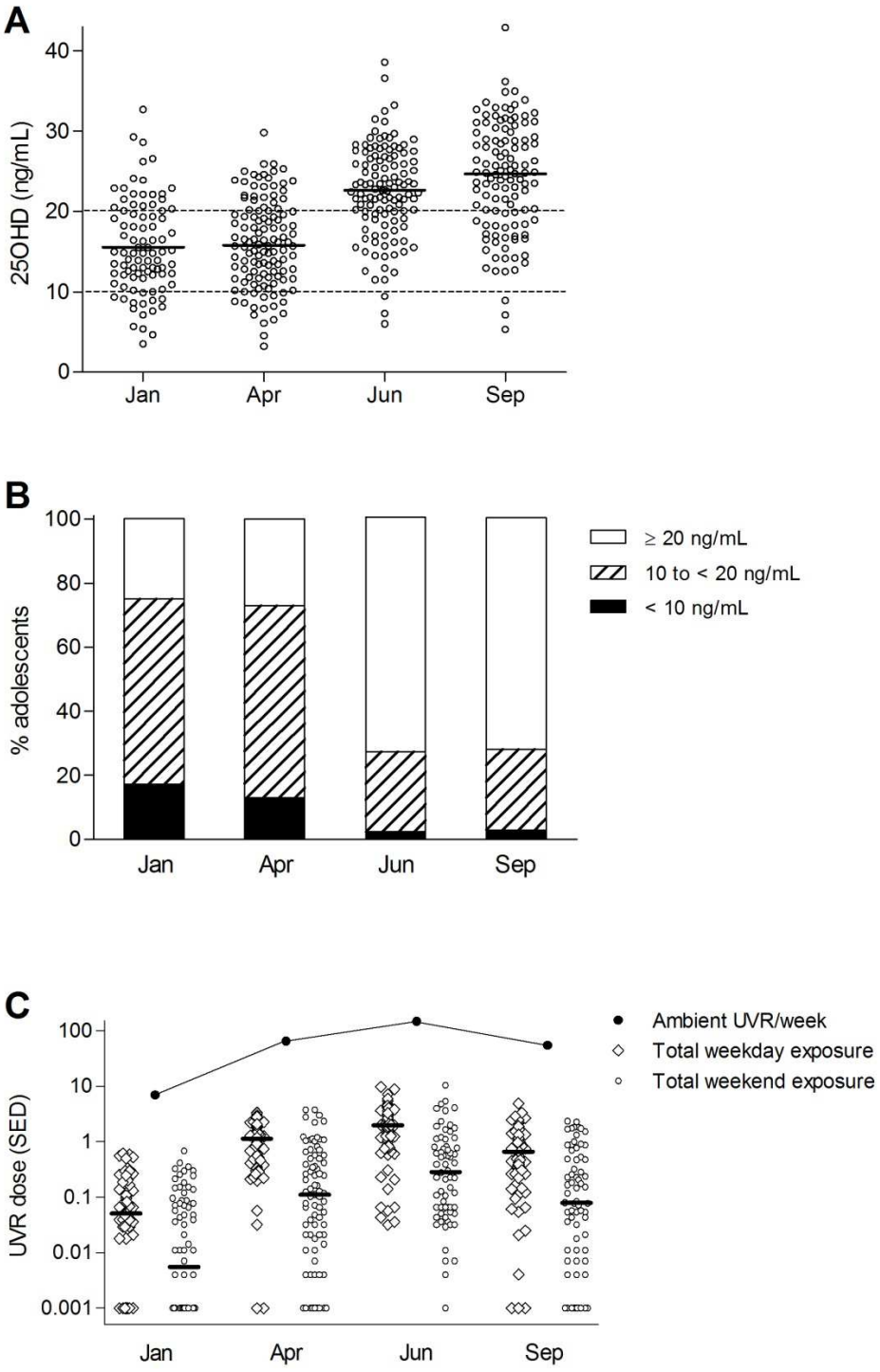
A
25OHD (ng/mL)
0
10
20
30
40
Jan
Apr
Jun
Sep
B
% adolescents
0
20
40
60
80
100
Jan
Apr
Jun
Sep
≥ 20 ng/mL
10 to < 20 ng/mL
< 10 ng/mL
C
UVR dose (SED)
0.001
0.01
0.1
1
10
100
Jan
Apr
Jun
Sep
Ambient UVR/week
Total weekday exposure
Total weekend exposure

Figure 2.

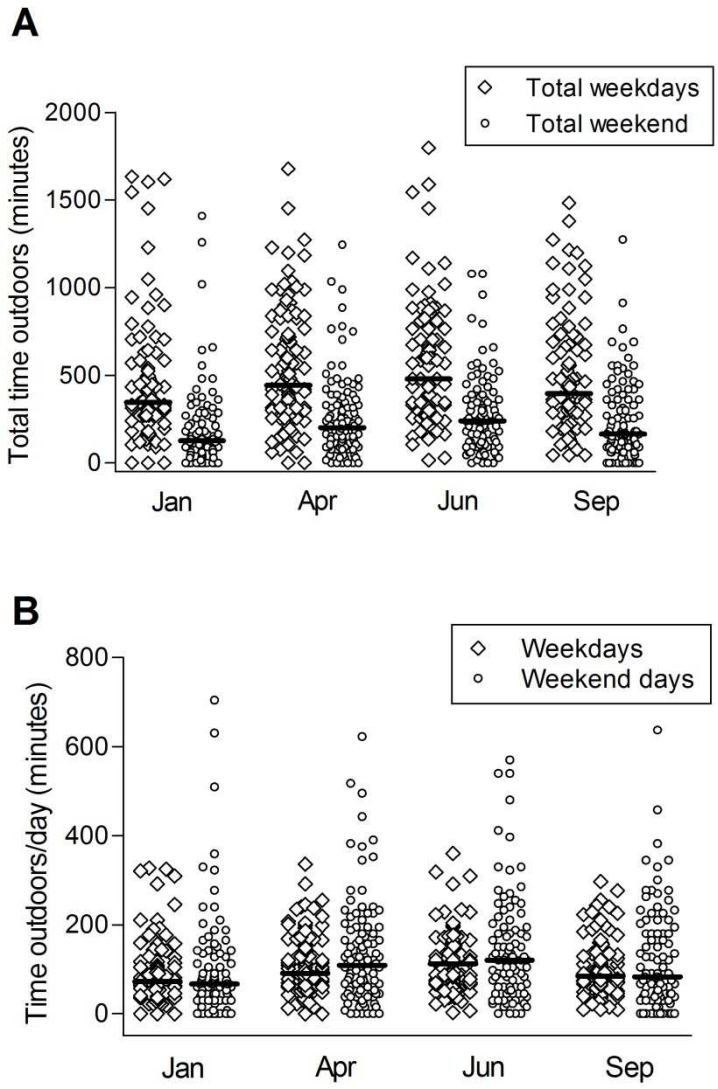
A
Total weekdays
Total weekend
Total time outdoors (minutes)
2000
1500
1000
500
0
Jan
Apr
Jun
Sep
B
Weekdays
Weekend days
Time outdoors/day (minutes)
800
600
400
200
0
Jan
Apr
Jun
Sep

Figure 3.