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Predictors and correlates of serum 25-hydroxyvitamin D concentrations in young women: results from the Safe-D study

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Abstract

Vitamin D deficiency is a global public health concern. Studies of serum 25-hydroxyvitamin D (25(OH)D) determinants in young women are limited and few include objective covariates. Our aims were to define the prevalence of vitamin D deficiency and examine serum 25(OH)D correlates in an exploratory study of women aged 16–25 years. We studied 348 healthy females living in Victoria, Australia, recruited through Facebook. Data collected included serum 25(OH)D assayed by liquid chromatography-tandem MS, relevant serum biochemistry, soft tissue composition by dual-energy X-ray absorptiometry, skin melanin density, Fitzpatrick skin type, sun exposure using UV dosimeters and lifestyle factors. Mean serum 25(OH)D was 68 (SD 27) nmol/l and 26 % were vitamin D deficient (25(OH)D <50 nmol/l). The final model explained 56 % of 25(OH)D variance. Serum sex hormone-binding globulin levels, creatinine levels, sun exposure measured by UV dosimeters, a positive attitude towards sun tanning, typically spending >2 h in the sun in summer daily, holidaying in the most recent summer period, serum Fe levels, height and multivitamin use were positively associated with 25(OH)D. Fat mass and a blood draw in any season except summer was inversely associated with 25(OH)D. Vitamin D deficiency is common in young women. Factors such as hormonal contraception, sun exposure and sun-related attitudes, as well as dietary supplement use are essential to consider when assessing vitamin D status. Further investigation into methods to safely optimise vitamin D status and to improve understanding of the impact of vitamin D status on long-term health outcomes is required.

Key words: 25-Hydroxyvitamin D: MS/MS: Young women

Vitamin D is a precursor of a system of hormones that assists in the active absorption of Ca, thereby facilitating skeletal mineralisation. Exposure to UV radiation (UVR) from the sun increases vitamin D synthesis in the skin, which is the source of approximately 90 % of circulating vitamin D in the body[(1)]. Few foods naturally contain vitamin D. Thus, only small amounts of vitamin D are usually obtained through diet. Vitamin D is metabolised in the liver to form 25-hydroxyvitamin D (25(OH)D), the major circulating metabolite. Serum 25(OH)D is then converted in the kidney into the highly active metabolite 1,25-dihydroxyvitamin D (1,25D). Serum 25(OH)D levels are used to determine vitamin D status as this biomarker reflects both dietary intake (food, supplements) and endogenously synthesised vitamin D. Serum 25(OH)D has a much longer half-life than 1,25D, implying that it is the more stable metabolite. Circulating 25(OH)D levels are almost 1000-fold more than 1,25D, making 25(OH)D the major circulating substrate. The liver has a high capacity for 25-hydroxylation, which is loosely regulated compared with the production of 1,25D in the kidneys[(2)]. Therefore, vitamin D nutritional status is better reflected by the more available substrate, 25(OH)D.

Adolescence and young adulthood are critical times in a young woman's life as independent behaviours and lifestyle choices are established[(3)]. These choices made as an emerging adult lay the foundation for future health trajectories not only for individuals but also for their future partners and families[(4)]. Vitamin D deficiency (VDD) is more common in females than males[(5)]. In adults, inadequate 25(OH)D impacts adversely on

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; COC, combined oral contraceptive pill; IQR, interquartile range; PTH, parathyroid hormone; SEIFA, Socio-Economic Indexes for Areas; SHBG, sex hormone-binding globulin; VDD, vitamin D deficiency.

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musculoskeletal health (e.g. osteoporosis, secondary hyperparathyroidism, osteomalacia). Observational studies suggest that VDD during pregnancy may also be a risk factor for a number of reproductive health outcomes. Therefore, serum 25(OH)D concentrations of >50 nmol/l are recommended by the WHO, National Institutes of Health and the Royal Australia New Zealand College of Obstetricians and Gynaecologists[6]. Clinical, behavioural and lifestyle factors associated with vitamin D status in young females of child-bearing age require further attention as previous studies have focused largely on elderly populations, where the risk factors for and consequences of chronically low vitamin D levels are better established. A better understanding of the determinants of vitamin D status and addressing VDD in young women are likely to improve their overall well-being, productivity and long-term health outcomes, as well as the health of their potential future offspring.

Serum 25(OH)D has been most commonly been found to correlate with season, personal sun exposure, obesity and demographic factors such as age, country of birth or socio-economic status[5,7]. Less commonly, concentrations have been associated with cardiometabolic markers such as lipids and markers of insulin resistance[8], creatinine levels[9] and medication use including hormonal contraception[10], which may be interlinked with changes in reproductive hormones.

Studies assessing vitamin D status in young Australian adults have previously been limited to clinical populations (e.g. oncology, psychiatry) or have focused on associations with a specific health outcome, such as CVD risk. Despite large sample sizes, other studies have had relatively small sample sizes across the late adolescent and young adult age range[5,11–13]. Therefore, the first objective of this study was to examine the prevalence of VDD, defined as serum 25(OH)D <50 nmol/l, in a community sample of young women aged 16–25 years. The second aim was to explore the clinical, demographic and lifestyle determinants of vitamin D status in this under-represented demographic.

Methods

Study design and population

Part A of the Safe-D study was a cross-sectional study of vitamin D and related health in females aged 16–25 years at the time of recruitment, living in Victoria, Australia (latitude 34–39°S). A detailed description of the study methodology has been reported elsewhere[14]. In brief, participants were recruited through the online social networking site, Facebook. Individuals were recruited into the study if they were able to provide verbal and written consent, and complete all three components of the study: an online questionnaire, wearing an UV dosimeter for 14 consecutive days and a study site visit, including phlebotomy. Pregnant or breast-feeding women were excluded from the study.

Ethics

The study was approved by the Melbourne Health Human Research Ethics Committee, Melbourne Health, Victoria, Australia (project no. 2013·007). The study was carried out in accordance with the National Statement on Ethical Conduct in Research Involving Humans (2007) produced by the National Health and Medical Research Council of Australia (NHMRC). The study was supported by NHMRC project grant APP1049065.

Online questionnaire

Participants were emailed links to an extensive, online questionnaire[14]. Demographic, health and lifestyle information collected included date of birth, country of birth, location of residence, education level, hormonal contraception including combined oral contraceptive pill (COC) use, self-reported Fitzpatrick skin type that categorises an individual's skin type by their response to sun exposure (e.g. skin type I represents skin that always burns, rarely tans and is pale white, whereas skin type VI never burns and is deeply pigmented)[15], sun behaviours (exposure, sun-protection measures and tanning preference), physical activity using a modified Active Australia Survey[16], smoking status, vitamin D supplementation and multivitamin use. Daily alcohol consumption and Ca intake were sourced from the Cancer Council Victoria FFQ, which collects data on usual eating habits in the past 12 months[17]. A sun tanning attitude score was calculated from a range of statements relating to tanning behaviour[18]. The statements included the following: (1) I feel more healthy with a suntan, (2) a suntan makes me feel more attractive to others, (3) this coming summer I intend to sunbathe regularly to get a suntan, (4) most of my friends think that a suntan is a good thing, (5) a suntan makes me feel better about myself, (6) most of my close family think that a suntan is a good thing and (7) a suntan protects you against melanoma and other skin cancers. The higher the score the more likely the participant felt positively about sun tanning. A score for the use of sun-protection measures was calculated from responses to how often a participant: (1) sought shade between 11.00 and 16.00 hours, (2) covered their head, (3) wore clothing to protect their skin from the sun, (4) wore sunglasses and (5) used sunscreen on skin exposed to the sun. These questions were adapted from the Cancer Council Australia SunSmart recommendations 'Slip Slop Slap Seek Slide'[19]. A higher sun-protection score indicated that a participant was less likely to regularly use sun-protection measures.

Sun exposure

Personal, real-time UVR exposure was measured using UV dosimeters developed at the National Institute of Water and Atmospheric Research in New Zealand (Scienterra). Dosimeters were set up and calibrated at the Australian Radiation Protection and Nuclear Safety Agency (ARPANSA). Data from the UV dosimeter were downloaded and analysed to calculate the average standard erythemal dose (SED) for each participant for the previous fortnight (1 SED = 100 J/m^2).

Biochemical measures

Participants were instructed to fast overnight for a minimum of 8 h before their allocated site visit. Site visits were conducted in the morning between 08.00 and 11.00 hours at the Royal

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Melbourne Hospital, Parkville, Australia. Blood samples were processed by the Melbourne Health Shared Pathology Service. Serum biochemistry was measured using an Abbott ARCHITECT c16000 integrated system (Abbott Diagnostics) in real time. Parathyroid hormone (PTH) was measured using an Abbott ARCHITECT i2000 SR immunoassay connected to a FlexLab track (Abbott Diagnostics). The CV for PTH was 4·7 % at 2·90 pmol/l.

A serum aliquot was stored at −80°C for 25(OH)D analysis ($25(OH)D_3$ plus $25(OH)D_2$), which was measured using liquid chromatography-tandem MS (LC-MS/MS) at VivoPharm Laboratories. The D_2 metabolite had a detection limit of 4·91 nmol/l, whereas for $25(OH)D_3$ it was 6·71 nmol/l. Tri-Level Vitamin D metabolite Quality Control samples from UTAK Laboratories (PM Separations) were used for quality control in each assay run. The CV for $25(OH)D_3$ was 2·0 % at 24·74 nmol/l, 1·6 % at 72·72 nmol/l and 1·4 % at 163·33 nmol/l. The CV for $25(OH)D_2$ was 4·9 % at 21·35 nmol/l, 2·5 % at 63·48 nmol/l and 2·5 % at 152·47 nmol/l.

Physical measurements

Height and weight were measured using standard procedures, from which BMI (BMI) was calculated (kg/m^2) and categorised according to WHO criteria. Cutaneous melanin density was measured at the upper inner arm, hand and cheek using a CM-2500d Konica Minolta portable spectrophotometer (Konica Minolta) coupled with a skin analysis program (CM-SA; Konica Minolta Sensing Inc.)[20].

Dual-energy X-ray absorptiometry

Dual-energy X-ray absorptiometry (DXA; QDR 4500 A densitometer; Hologic Inc.) was used to quantify body fat as a percentage of body weight, fat mass and lean mass. Scans were analysed using QDR software version 9.1D.

Statistical analysis

Participants were excluded from the analysis if they had not completed the medical history section of the questionnaire, had abnormal pathology results, were previously diagnosed with relevant medical conditions, had undergone relevant surgery, were taking medication/s that may affect 25(OH)D levels or had a diagnosis of osteoporosis before commencing the study. VDD was defined as serum 25(OH)D level <50 nmol/l according to the Australian and New Zealand Bone and Mineral Society (ANZBMS) position statements[21,22]. This cut-off is supported by the Endocrine Society of Australia, Osteoporosis Australia and The National Academy of Medicine (formerly the Institute of Medicine). The 2005 ANZBMS position statements further categorises deficiency as mild (25(OH)D 25–50 nmol/l), moderate (12·5–25 nmol/l) or severe (<12·5 nmol/l)[21].

Each of the following factors was categorised as follows: country of birth as Australia or elsewhere; education as high school only or further education; location of residence as urban or regional; season as summer (December–February), autumn (March–May), winter (June–August) or spring (September–November); BMI category as underweight (<18·5 kg/m^2), normal (18·5–24·9 kg/m^2), overweight (25–29·9 kg/m^2) and obese (>30 kg/m^2); Fitzpatrick skin type as type I–IV and V–VI; COC use as yes/no; physical activity as minimal-low (0–599 metabolic equivalent of task (MET)-min) or moderate-high levels (600 + MET-min); daily alcohol consumption as 0, 1–14, 15–29 or ≥30 g; smoking as current smoker or ex-/non-smoker; vitamin D supplementation in the last 2 weeks as yes/no; use of a multivitamin in the last 2 weeks as yes/no; Ca intake as above or below 1000 mg/d; use of SPF30 + sunscreen use as yes/no; took a holiday in the most recent summer period as yes/no; and reported spending >2 h in the sun on a typical day in summer or winter as yes/no. The Socio-Economic Indexes for Areas (SEIFA) percentile was used to determine socio-economic status[23].

Scatterplot smoothing (Lowess) curves were used to examine the relationships between serum 25(OH)D and continuous variables. Continuous variables were checked for normality using the Shapiro–Wilk test. Pearson's correlation was used to test associations between 25(OH)D levels and continuous variables that were normally distributed (Spearman's correlation was used for data that were not normally distributed). Either Student's *t* test or an ANOVA was used to examine associations between serum 25(OH)D and categorical variables. A multivariate linear regression model was used to explore associations between serum 25(OH)D and relevant variables. Participants with data missing for a particular variable were excluded from analysis where that variable was required in analysis. A *P* value of <0·05 was considered statistically significant. All statistical analysis was performed using StataSE 13 (StataCorp LP).

Sample size

It was necessary for the sample size for part A of the Safe-D study to provide sufficient eligible participants to recruit adequate numbers for part B of the study, a randomised-controlled trial aiming to assess the effectiveness of an mHealth-based behavioural intervention to improve 25(OH)D levels and related health in young women with 25(OH)D levels ranging from 25 to 75 nmol/l[24]. Sample-size calculations have been reported previously and yielded a recruitment target of 468 participants[14]. This sample size provides 80 % power at a 5 % significance level to detect small-medium effect sizes (Cohen's $d = 0{\cdot}25–0{\cdot}30$) in outcome measures.

Results

In all, 557 participants were recruited into part A of the Safe-D study by November 2015. Serum 25(OH)D concentrations were available for 407 participants. We excluded fifty-nine participants for the following reasons (note: some participants fulfilled multiple exclusion criteria): the participant had not completed the medical history section of the questionnaire (*n* 4), corrected Ca >2·60 mmol/l (*n* 2), thyroid-stimulating hormone <0·35 mIU/l (*n* 4), C-reactive protein >10 mg/l (*n* 31), was previously diagnosed with one of the following conditions: hyperthyroidism (*n* 2), hypothyroidism (*n* 1), cystic fibrosis (*n* 1), coeliac disease (*n* 8), inflammatory spondyloarthritis (*n* 1),

congenital heart defects (*n* 1), anorexia, bulimia or other eating disorders (*n* 7), or malabsorption conditions (*n* 1), had undergone previous surgery potentially affecting relevant outcomes (*n* 3) and the participant was taking specific medications (prednisolone (*n* 3), hydroxychloroquine (*n* 1), phenothiazine (*n* 1) or immunosuppressive drugs (*n* 1)). Two participants were excluded as they were diagnosed with osteoporosis before commencing the study. After exclusions were applied, data for 348 (85 %) participants were included in the analysis. Adequate data for personal sun exposure measured by the UV dosimeters were available for 258 (74 %) participants.

Participant characteristics

Participant characteristics are presented in Table 1. In all, 84 % of the participants were born in Australia. Participants born outside Australia (*n* 55) reported their country of birth as in Europe (31 %), New Zealand (7 %), China, Japan or in Southeast Asia (29 %), in Southern Asia (11 %), America or Canada (9 %), in South America (7 %) or in Africa (5 %). A third of the participants were educated to a high school level only, whereas 14 % were from the lowest socioeconomic status quartile according to SEIFA.

Vitamin D status and parathyroid hormone

The prevalence of VDD in the Safe-D cohort was 26·2 %. Less than 1 % of the participants had severe deficiency (25(OH)D <12·5 nmol/l), 5·5 % had moderate deficiency (12·5–29·9 nmol/l) and 20·4 % had mild deficiency (30–49·9 nmol/l). In all, ten participants (2·9 %) had 25(OH)D ≥125 nmol/l. The mean serum 25(OH)D was 68 (SD 27) nmol/l. A total of thirty-one samples (9 %) had detectable 25(OH)D_2 with a median level of 6 (interquartile range (IQR) 5, 7) nmol/l (Table 1). The median PTH concentration was 6 (IQR 4, 7) pmol/l. Serum 25(OH)D showed seasonal variations (see Fig. 1 and Table 3). The prevalence of VDD was 8 % in summer, 25 % in autumn, 37 % in winter and 21 % in spring ($P<0·001$).

Serum 25(OH)D was negatively correlated with PTH ($\rho=-0·31$, $P<0·001$; Table 2). The median PTH levels were 7·6 (IQR 5·8–9·4) pmol/l in participants with serum 25(OH)D <12·5 nmol/l, 6·2 (IQR 5·1–7·8) pmol/l with 25(OH)D 25–49·9 nmol/l, 5·5 (IQR 4·4–7·1) pmol/l with 25(OH)D 50–74·9 nmol/l and 4·7 (IQR 3·9–5·9) pmol/l with 25(OH)D >75 nmol/l ($P<0·001$).

Association between 25-hydroxyvitamin D and demographic variables

A negative association was found between serum 25(OH)D levels and chronological age (Table 2). Serum 25(OH)D and SEIFA percentile were positively associated (Table 2).

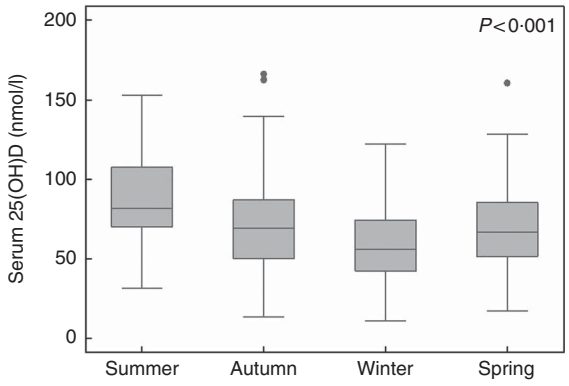

Fig. 1. Box plot of seasonal variations in 25-hydroxyvitamin D (25(OH)D) levels.

Table 1. Characteristics of Safe-D participants (*n* 348)
(Mean values and standard deviations; medians and interquartile ranges (IQR))

Characteristics	Mean	SD	Median	IQR	Range
Age (years)	21·9	2·8			16·3–26·4
25(OH)D					
25(OH)D_2 (nmol/l)*			6	5, 7	5–12
25(OH)D_3 (nmol/l)	65	27			9–164
Total 25(OH)D (nmol/l)	68	27			11–166
PTH (pmol/l)			6	4, 7	2–19
Body fat (%)	31·2	6·2			16·1–50·2
BMI (kg/m^2)	23·5	4·1			16·7–42·4
Melanin density index					
Upper, inner arm			0·69	0·55, 0·85	0·32–2·16
Hand			0·88	0·72, 1·09	0·37–2·20
Facial cheek			0·93	0·81, 1·04	0·52–2·11
Fitzpatrick skin type†	3	1			1–5
Daily personal sun exposure in previous 2 weeks (SED)			1·89	1·09, 3·47	0·09–16·56
Sun tanning attitude score‡	22	8			7–42
Sun-protection score§	13	3			5–23
Physical activity (MET-min)			1140	520, 2085	0–9015
Alcohol consumption (g/d)			3·0	0·5, 9·3	0–80·6
Ca intake (mg/d)	811	316			89–2864

25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone; SED, standard erythemal dose; MET, metabolic equivalent.
* 25(OH)D_2 detected in thirty-one samples (9 %). The remaining samples were below the assay limit of detection.
† The minimum possible Fitzpatrick skin type is 1, whereas the maximum possible is 6.
‡ The minimum possible sun tanning attitude score is 7, whereas the maximum possible score is 42.
§ The minimum possible sun-protection score is 5, whereas the maximum possible score is 25.

Table 2. Univariate associations between serum 25-hydroxyvitamin D and continuous variables in young women

Variable	*r**	*ρ*†	*P*
Demographic variables			
Age (years)	−0·13		0·014
SEIFA (%)	0·13		0·015
Clinical variables			
Melanin density of the hand		0·12	0·027
Height (m)	0·17		0·002
BMI (kg/m^2)	−0·14		0·008
Fat mass (kg)		−0·15	0·004
Body fat (%)		−0·16	0·003
Lifestyle variables			
Daily personal sun exposure (SED)		0·37	<0·001
Sun tanning attitude score	0·22		0·001
Reported number of times sunburnt in the previous 12 months	0·27		<0·001
Biomarkers			
PTH (pmol/l)		−0·31	<0·001
Creatinine (μmol/l)	0·34		<0·001
eGFR (ml/min/1·73 m^2)	−0·30		<0·001
Hb (g/l)	0·12		0·033
Corrected serum Ca (mmol/l)‡	0·18		<0·001
Prolactin (μmol/l)		−0·16	0·003
SHBG (nmol/l)		0·34	<0·001
Fe (μg/l)	0·18		0·002
Transferrin (μmol/l)	0·18		0·002
TIBC (μg/l)	0·18		0·002

SEIFA, Socio-Economic Indexes for Areas; SED, standard erythemal dose; PTH, parathyroid hormone; eGFR, estimated glomerular filtration rate; SHBG, sex hormone-binding globulin; TIBC, total Fe-binding capacity.
* Calculated using Pearson's correlation.
† Calculated using Spearman's correlation.
‡ Serum Ca corrected for albumin. Ca + ((40−albumin) × 0·02).

Participants born outside of Australia had, on average, 26 nmol/l lower 25(OH)D compared with Australian-born participants (Table 3). Participants with university or further education had, on average, 7 nmol/l lower 25(OH)D levels compared with those with high school education only (Table 3). No association was found between 25(OH)D levels and location of residence (urban *v.* rural; $P = 0·095$; data not shown).

Association between 25-hydroxyvitamin D and sun exposure

A summary of sun exposure-related variables is presented in Table 1. In all, 7 % of the participants had been sunburnt >5 times in the previous 12 months. In summer, 55 % of the participants reported spending >2 h in the sun on a typical day, whereas in winter 20 % reported spending >2 h in the sun daily. A total of 62 % of the participants with adequate 25(OH)D levels reported spending >2 h in the sun on a typical day in summer compared with 37 % with VDD ($P < 0·001$).

Serum 25(OH)D was positively associated with daily personal exposure measured by UV dosimetry ($R^2 = 0·08$; see online Supplementary Fig. S2), the sun tanning attitude score and reported number of times sunburnt in the previous 12 months (Table 2). In addition, serum 25(OH)D levels were higher in participants who reported spending >2 h in the sun in summer on a typical day and in those who took a holiday in the most recent summer period (see Table 3). Serum 25(OH)D was not associated with spending >2 h in the sun in winter ($P = 0·098$), the sun-protection score ($P = 0·067$) or the use of a sunscreen with SPF30+ or higher ($P = 0·416$; data not shown).

Table 3. Univariate associations between serum 25-hydroxyvitamin D (25(OH)D) and categorical variables in young women (Mean values and standard deviations)

		25(OH)D (nmol/l)		
Variable	*n*	Mean	SD	*P**
Demographic variables				
Country of birth				<0·001
Australia	293	70	27	
Outside Australia	55	56	27	
Education				0·021
High school only	114	73	26	
University or further education	229	66	27	
Clinical variables				
Season of blood draw				<0·001
Summer	52	87	27	
Autumn	68	72	32	
Winter	139	58	2	
Spring	89	69	25	
BMI category				<0·001
Underweight	18	54	23	
Normal	231	72	28	
Overweight	73	63	23	
Obese	26	55	19	
Fitzpatrick skin type				0·016
I–IV	328	69	27	
V–VI	16	52	29	
COC use				<0·001
Yes	145	79	29	
No	184	60	24	
Lifestyle variables				
Reported spending >2 h in the sun in summer daily				<0·001
Yes	191	72	27	
No	157	63	27	
Took a holiday in the recent summer period				<0·001
Yes	234	72	28	
No	104	59	21	
Physical activity levels				0·009
Minimal-to-low	99	62	24	
Moderate-to-high	246	70	28	
Alcohol consumption (g)				0·018
0	50	65	28	
1–14	246	66	25	
15–29	32	81	28	
≥30	12	75	31	
Multivitamin use in the previous week				0·002
Yes	58	78	31	
No	289	66	26	
Vitamin D supplement use in the previous week				0·066
Yes	27	77	26	
No	320	67	27	

COC, combined oral contraceptive pill.
* Differences between groups were analysed using Student's *t* test. If data were grouped into more than two groups ANOVA was used.

Association between 25-hydroxyvitamin D and other lifestyle variables

Serum 25(OH)D levels were, on average, 8 nmol/l higher in participants who reported moderate-to-high physical activity levels compared with those who reported minimal-to-low activity levels (see Table 2). Serum 25(OH)D levels were

Table 4. Stepwise regression model assessing a number of potential correlates of 25-hydroxyvitamin D in young women* (Regression coefficients (β) and 95 % confidence intervals)

Factors	Unadjusted β†	Adj β†	95 % CI	*P*	R^2
SHBG (nmol/l)	0·11	0·07	0·04, 0·10	<0·001	
Creatinine (μmol/l)	1·38	0·93	0·44, 1·42	<0·001	
Personal sun exposure in previous 14 days (SED)	25·86	10·60	1·29, 19·91	0·026	
Holiday taken in most recent summer period	13·52	9·15	2·17, 16·13	0·011	
Season (summer reference)					
Autumn	−14·19	−17·99	−27·61, −8·37	<0·001	
Winter	−17·21	−31·83	−40·64, −23·02	<0·001	0·56
Spring	−17·47	−21·55	−32·18, −10·92	<0·001	
Fat mass (kg)	−0·55	−0·60	−1·01, −0·18	0·005	
Multivitamin use	12·16	12·96	4·62, 21·30	0·003	
Reported spending >2 h in the sun in summer on a typical day	9·41	7·84	1·59, 14·09	0·014	
Fe (μmol/l)	0·64	0·39	0·01, 0·77	0·043	
Height (cm)	0·70	0·62	0·12, 1·12	0·016	
Sun tanning attitude score	0·73	0·40	0·04, 0·77	0·032	

SHBG, sex hormone-binding globulin, COC, combined oral contraceptive pill; SED, standard erythemal dose.

* The following variables were entered into a single stepwise elimination regression model: age (years), Socio-Economic Indexes for Areas percentile, creatinine (μmol/l), estimated glomerular filtration rate (ml/min/1·73 m^2), corrected Ca (mmol/l), prolactin (μmol/l), SHBG (nmol/l), Hb (g/l), Fe (μmol/l), height (m), fat mass (kg), melanin density index of the hand, personal sun exposure measured using UV dosimeters (SED), sun tanning attitude score, reported number of times sunburnt in previous 12 months, country of birth, education, season, Fitzpatrick skin type V–VI, COC use, reported spending >2 h in the sun in summer, reported going on holidays in the most recent summer period, physical activity, alcohol consumption and multivitamin use.

† Estimates are given as nmol/l per unit of covariate.

lower in those who reported drinking <15 g of alcohol daily or abstained from drinking (Table 3). Participants who had reported taking a multivitamin in the previous week had on average 12 nmol/l higher 25(OH)D levels than those who did not (Table 3). A trend towards higher 25(OH)D with vitamin D supplementation was observed, but did not reach statistical significance. Serum 25(OH)D levels were not associated with dietary Ca ($P=0·172$) or energy intake ($P=0·722$) as continuous variables, nor were 25(OH)D levels associated with current smoking status ($P=0·464$; data not shown).

Association between 25-hydroxyvitamin D and biomarkers

Serum 25(OH)D was positively associated with serum creatinine ($R^2=0·11$), Ca corrected for albumin, sex hormone-binding globulin (SHBG; $R^2=0·15$), Fe, transferrin and TIBC values (Table 2). The positive association between 25(OH)D and creatinine remained significant after adjustment for lean mass and height ($\beta=1·4$; 95 % CI 0·99, 1·84, $P<0·001$). A positive trend was observed between 25(OH)D and transferrin saturation ($P=0·090$; data not shown). Serum 25(OH)D was inversely associated with estimated glomerular filtration rate and prolactin (Table 2). No association was observed between 25(OH)D and the following analytes: C-reactive protein ($\rho=0·05$, $P=0·357$), thyroid stimulating hormone (r 0·06, $P=0·289$), ferritin ($\rho=0·09$, $P=0·103$), luteinising hormone ($\rho=-0·05$, $P=0·547$), follicle-stimulating hormone (r −0·07, $P=0·401$), oestradiol ($\rho=-0·06$, $P=0·466$), progesterone ($\rho=0·14$, $P=0·076$), testosterone (r 0·08, $P=0·307$) and dehydroepiandrosterone sulphate (r −0·03, $P=0·289$).

Association between 25-hydroxyvitamin D and clinical variables

Serum 25(OH)D was positively associated with height, whereas 25(OH)D was negatively associated with percent body fat, BMI and fat mass (Table 2). Serum 25(OH)D levels were significantly lower in those who were categorised as either underweight or obese (Table 3). Serum 25(OH)D levels were on average 19 nmol/l higher in COC users compared with non-users ($R^2=0·12$; Table 3). Serum 25(OH)D was not associated with body weight (r −0·10, $P=0·215$), but tended to be positively associated with lean mass ($\rho=0·10$, $P=0·057$).

Serum 25(OH)D was positively associated with melanin density of the hand (Table 2). No association was observed between 25(OH)D and melanin density of the upper, inner arm ($\rho=-0·01$, $P=0·905$) or facial cheek ($\rho=-0·02$, $P=0·654$). A trend towards an association was observed between 25(OH)D levels and Fitzpatrick skin type ($P=0·077$). Serum 25(OH)D levels were, on average, 17 nmol/l lower in participants with Fitzpatrick skin type V–VI compared with skin types I–IV (Table 3).

Stepwise regression model

Factors found to be significantly associated with 25(OH)D in univariate analyses were included in a stepwise elimination regression model (Table 4). Serum SHBG, creatinine, daily sun exposure measured by UV dosimetry, a holiday taken in the most recent summer period, multivitamin use, spending >2 h daily in the sun in summer, Fe concentrations, height and the sun tanning attitude score were independently associated with higher serum 25(OH)D levels. Factors independently associated with lower serum 25(OH)D were season (autumn, winter or spring compared with summer) and fat mass. The final model explained 56 % of the variation in serum 25(OH)D.

Discussion

The Safe-D study is the first to evaluate vitamin D status in young women recruited through Facebook advertising, a novel, non-traditional method of recruitment. The prevalence of VDD in 16–25-year-old females was 26 %. The following variables were

found to be positively associated with serum 25(OH)D (Table 4): SHBG levels, creatinine levels, personal sun exposure in the previous 2 weeks, holiday taken in the most recent summer period, blood drawn in the summer season, multivitamin use, reporting spending more than 2 h in the sun in summer on a typical day, Fe levels, body height and having a positive attitude towards sun tanning. Fat mass was negatively correlated with serum 25(OH)D. Our final model was able to explain more than 50 % of the variation in serum 25(OH)D concentrations in a community sample of 348 healthy young women. By contrast, Kimlin *et al.*[7] were able to explain 40 % of the variance in 25(OH)D levels in 1002 Australians aged 18–75 years, living across 24° of latitude. We found that 26 % of the young women studied were vitamin D deficient, which is in close agreement with the current literature that has reported prevalence rates of 21–27 %[5,25]. A US report found that 25(OH)D levels were significantly lower in young adults compared with adults aged older than 60 years of age[26]. Collectively, these findings suggest that VDD may be as common in adolescents and young adults as in older populations, who are usually considered the most at risk for VDD. The lower prevalence of VDD in older adults may be, in part, due to the higher vitamin D supplement use in older adults. Current Australian Bureau of Statistics (ABS) data suggest that 20 % of adults aged >75 years have VDD compared with 31 % in 18–34 year olds. However, 14 % of older adults use vitamin D supplements compared with <3 % in 18–34 year olds[27]. Nonetheless, these results support the need for future vitamin D research, including supplementation trials, in this currently understudied demographic.

We found no association between 25(OH)D and education levels or socioeconomic status in the final model, suggesting that they are not strong correlates of 25(OH)D in this demographic. In univariate analyses, 25(OH)D was positively associated with socioeconomic status, whereas 25(OH)D concentrations were lower in participants receiving further education. It should be noted that there was no association between education levels and SEIFA percentile in the current study. The SEIFA index used in this study is calculated from a number of socioeconomic status variables including, but not limited to, education, assets, income, debt, occupation and housing details. The education aspect of the SEIFA index looks at the proportion of individuals with an education of year 11 (approximately 16–17 years old) or lower. Due to the age range studied and cohort demographics, participants in the Safe-D study in current education would be in late high school (year 11–12) or in tertiary/further education. The discrepancy between the education measures is likely the reason for the contradictory direction of the association between socioeconomic status and 25(OH)D, as well as between 25(OH)D and education level.

As expected, serum 25(OH)D was inversely associated with PTH. Serum 25(OH)D was positively associated with serum creatinine in the final model, a marker of renal function, which has been previously reported[9,28]. Vieth *et al.*[9] found the same association in participants <51 years old ($P<0{\cdot}001$), but not in participants older than 70 years of age. Treatment with calcitriol has been shown to cause an increase in creatinine, but the mechanism is unclear[29]. The lack of association between 25(OH)D and age in the final model is likely due to the narrow age range studied. The Safe-D participants were primarily Caucasian and Australian-born, which might explain the lack of an observed association between 25(OH)D and country of birth in the final model (Table 4), despite the significant association observed in univariate analyses.

Serum 25(OH)D showed a seasonal variation, which has been consistently demonstrated and is predominantly due to increased ambient UVR in summer, thereby facilitating cutaneous vitamin D synthesis[30–33]. Although season plays an important role in influencing vitamin D status, behavioural factors also contribute[12]. We demonstrated that daily sun exposure, a higher sun tanning attitude score, spending >2 h daily in the sun in summer and taking a holiday in the summer period, as well as height were positively associated with 25(OH)D in the final model. The association between 25(OH)D and height has been described previously in young American women aged 16–22 years[34]. It correlates with a larger body surface area and therefore a greater potential capacity to synthesise vitamin D. In addition to increasing vitamin D synthesis, UVR exposure is the cause of 95–99 % of skin cancers. The association between 25(OH)D and spending >2 h in the sun is particularly striking as this amount of sun exposure would be considered excessive and likely increase the risk of skin cancer significantly. Melanoma remains the most common cancer among 15–24-year-old Australians; therefore, it is crucial that efforts are made to achieve a balance between safe sun exposure, to minimise the risk of skin cancer, and sufficient sunlight exposure to achieve adequate vitamin D status[35,36].

Only 9 % of samples tested had detectable 25(OH)D_2, suggesting that plant-based dietary sources of supplemental forms of vitamin D_2 contribute very little to circulating 25(OH)D in young Australians. In contrast, a US population-based study found that individuals with a vitamin D intake of >5 μg/d had significantly higher 25(OH)D levels[26]. This observation suggests that a possible approach to improve vitamin D levels might be to adopt more active food-fortification strategies. Alternatively, increasing total vitamin D intake with supplementation is a strategy that has been demonstrated to improve 25(OH)D levels[22]. In Australia, multivitamins are the most commonly used dietary supplement and contain about 5–10 μg (200–400 IU) vitamin D[37]. We demonstrated that participants who reported taking a multivitamin had approximately 12 nmol/l higher 25(OH)D levels in the final model. Multivitamins have been shown to be safe to use; however, data on the benefits of multivitamin use in the general population are limited[38,39]. It is likely that we did not find a significant association between 25(OH)D and vitamin D supplement use due to the low proportion of use (approximately 8 %) and data were limited to the previous 2 weeks, rather than the previous month, for example. In Australia, most vitamin D supplements contain 25 μg (1000 IU) vitamin D. Randomised-controlled trials would provide an opportunity to gain insights into the potential benefits of a multivitamin or vitamin D supplementation on vitamin D status and health outcomes in young women[24].

The association between 25(OH)D and Fe levels is consistent with previous literature, suggesting that low 25(OH)D levels are associated with an increased risk of anaemia[40,41].

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Haeme-bound Fe is used in the hydroxylation of vitamin D metabolites by the cytochrome P450 enzymes, providing a probable link between vitamin D and Fe metabolism[42]. Other proposed links between vitamin D and Fe status include vitamin D modulating inflammation, stimulating erythropoiesis, changes in hepcidin levels and associations with fibroblast growth factor 23[43,44].Vitamin D supplementation trials may be able to resolve whether increasing 25(OH)D is beneficial to Fe status in young women.

We demonstrated that serum 25(OH)D levels were inversely associated with fat mass. The relationship between reduced 25(OH)D and obesity has consistently been reported and is commonly explained by increased 25(OH)D storage in adipose tissue as vitamin D metabolites are lipophilic, thereby reducing circulating concentrations[5,8,45,46]. Other mechanisms for reduced 25(OH)D levels with higher fat mass may be more specific to personal choices that reduce personal sun exposure, such as reduced outdoors activity or covering up, limiting vitamin D synthesis. The increasing prevalence of obesity in young women is likely to continue to contribute towards the prevalence of VDD[47].

Serum 25(OH)D was positively associated with SHBG levels, which has been demonstrated to increase with COC oestrogen dose[48]. In addition, COC use was associated with 19 nmol/l higher 25(OH)D levels in univariate analysis, which is in agreement with previous literature[10,49,50]. Harmon *et al.*[10] recently found that COC use was associated with a 20 % increase in 25(OH)D levels in 1662 African American women aged 23–34 years. Some studies have suggested that vitamin D binding protein (DBP) levels increase with COC use, varying the proportion of free and protein-bound 25(OH)D[49,50]. Alternatively, increased DBP binding may protect 25(OH)D from 24-hydroxylation, thereby increasing circulating 25(OH)D[49,50]. The use of COC should be taken into account when interpreting 25(OH)D results and a review of vitamin D status might be considered when a young woman ceases COC use. The latter is particularly important for women planning to conceive, where ceasing COC use may further exacerbate VDD and affect pregnancy outcomes adversely.

The Safe-D study has a number of methodological strengths. We successfully recruited a broadly representative sample of young women and were able to produce a data set of generally healthy young women through extensive health data collection[51]. In addition, LC-MS/MS was employed by National Association of Testing Authorities, Australia, accredited laboratory to assay serum 25(OH)D as this method has the highest sensitivity and specificity compared with other 25(OH)D assays and is therefore often considered the current 'gold standard' for 25(OH)D measurement[52–54]. The objective measurement of sun exposure through UV dosimeters has previously been shown to be a feasible and more accurate measure of time spent outdoors compared with self-reported data[55]. Finally, the study could explain 56 % of the variability in 25(OH)D levels by assessing a wide range of clinical, behavioural and lifestyle factors associated with 25(OH)D and indicated that SHBG levels, creatinine levels, sun exposure, holiday in the most recent summer period, season, fat mass, multivitamin use, Fe levels, height and attitudes towards sun tanning were the major sources of vitamin D status in young women.

Our study is not without limitations. A number of countries use a cut-off of <30 nmol/l to define VDD. Due to the small proportion of participants with serum 25(OH)D <30 nmol/l (20/348; 6 %), it is difficult to make conclusive judgements about associations between health outcomes within this concentration range. Although the cohort was broadly representative of young Victorian women[51], there were some slight differences. Participants were primarily Australian born (84 *v.* 78 %), had a higher education level (67 *v.* 43 %) and more resided in urban areas (91 *v.* 79 %) compared with the ABS Census of Population and Housing 2011 data in 16–25-year-old Victorian females. The underrepresentation of overseas-born individuals and those from regional areas was likely due to language/cultural barriers and difficulties travelling to the study site centre in Melbourne, respectively. The greater proportion of higher educated participants is common in volunteer samples in research and may also be contributed to by the close vicinity of a number of universities to the study site centre. The Victorian Population Health Survey 2011–2012 found that 14·6 % of 18–24-year-old Victorian women were current smokers and 21·9 % were insufficiently active, which are similar to our results (9·5 and 28·7 %, respectively)[56]. In terms of body composition, 18·2 % of Victorians were considered overweight or obese in the 2011–2012 survey, which was lower than our data (28·5 %), which could potentially reflect population trends in obesity levels since the precious census[47]. Nonetheless, slight differences in participant demographics are not necessarily biologically significant. Due to loss, damage or inaccurate use of UV dosimeters, the number with eligible UV data was reduced, thus reducing analytical power. We measured a large number of possible 25(OH)D correlates, which may lead to identifying significant correlations by chance alone. By using a stepwise elimination regression model in our final model we could present the variables that were the strongest statistical determinants of 25(OH)D. This is also why weaker correlates may have been significant correlated with 25(OH)D in univariate analyses alone as confounding variables may have been eliminated from the final model. Finally, due to the study's cross-sectional nature, causation cannot be inferred; however, we are examining these relationships in part B of the Safe-D study[24].

In conclusion, VDD was found to be as prevalent in young women as reported previously in older Australian adults. We were able to explain over half of the variation in serum 25(OH)D levels using sun exposure-related, biochemical and anthropometric variables. A better understanding of the factors influencing vitamin D status may help better identify individuals at increased risk of VDD. Our findings support the need for further vitamin D research specifically in young women and the need to address modifiable risk factors for VDD such as low sun exposure and obesity. The feasibility of safely improving vitamin D levels through lifestyle interventions in young women requires further attention in this currently understudied demographic.

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J. D. W. conceived the study. E. T. C. participated in the design of the study, establishment of the study methods, data collection and drafted the manuscript. J. D. W., S. M. G., A. G. and N. J. R. are study investigators and were involved in study design, study coordination and helped draft the manuscript. A. G. is the study statistician and was involved in sample size and power calculations and also advised on the statistical analysis of the data. All authors read, contributed to and approved the final manuscript.

The authors declare that there are no conflicts of interest.

Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114517002021

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