RESEARCH

100 YEARS OF VITAMIN D

Combined hormonal contraceptives and vitamin D metabolism in adolescent girls

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Abstract

Objective: Combined hormonal contraceptive (CHC) use has been associated with higher total 25-hydroxyvitamin D (25(OH)D) levels. Here, we investigate the relation between CHC use and vitamin D metabolism to elucidate its clinical interpretation.
Methods: The cross-sectional Fit Futures 1 included 1038 adolescents. Here, a subgroup of 182 girls with available 25(OH)D, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), 24,25-dihydroxyvitamin D ($24,25(OH)_2D$), vitamin D-binding protein (DBP) and measured free 25(OH)D levels, in addition to parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23), was investigated. Vitamin D metabolites were compared between girls using (CHC+) and not using CHC (CHC−). Further, the predictability of CHC on 25(OH)D levels was assessed in a multiple regression model including lifestyle factors. The ratios $1,25(OH)_2D/25(OH)D$ and $24,25(OH)_2D/25(OH)D$ (vitamin D metabolite ratio (VMR)) in relation to 25(OH)D were presented in scatterplots.
Results: CHC+ (n = 64; 35% of the girls) had higher 25(OH)D levels (mean ± S.D., 60.3 ± 22.2) nmol/L) than CHC- (n = 118; 41.8 ± 19.3 nmol/L), P-values <0.01. The differences in 25(OH)D levels between CHC+ and CHC− were attenuated but remained significant after the adjustment of lifestyle factors. CHC+ also had higher levels of $1,25(OH)_2D$, $24,25(OH)_2D$, DBP and calcium than CHC−, whereas $1,25(OH)_2D/25(OH)D$, PTH, FGF23 and albumin were significantly lower. Free 25(OH)D and VMR did not statistically differ, and both ratios appeared similar in relation to 25(OH)D, irrespective of CHC status.
Conclusion: This confirms a clinical impact of CHC on vitamin D levels in adolescents. Our observations are likely due to an increased DBP-concentration, whereas the free 25(OH)D appears unaltered.

Key Words

- vitamin D
- adolescence
- combined hormonal contraceptive
- metabolite ratio
- free 25-hydroxyvitamin D
- impact of vitamin D binding protein

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Introduction

In the adult population, hormonal contraceptive (HC) use has been associated with increased total 25-hydroxyvitamin D (25(OH)D) levels (1, 2, 3), but few studies have assessed this in adolescents (4). As HC use in this age group is quite common in some countries, including Norway (5), it is important to understand how such use affects vitamin D metabolism, and how to interpret 25(OH)D measurements in this population. The effect of HC use on 25(OH)D levels is so far not accounted for in existing guidelines (6, 7, 8, 9).

In contrary to 25(OH)D (free and protein bound), free 25(OH)D has not differed according to HC use in the adult population (3, 10). It has been raised concern that the vitamin D status in HC users might be over-estimated, as many cells utilize free 25(OH)D (10). As such, free 25(OH)D could represent a more useful marker of vitamin D status in a HC using population.

Also, an increase in vitamin D-binding protein (DBP) in users of combined hormonal contraceptives (CHC), which includes an estrogenic component, has been consistently reported (3, 11). Several tissues, including the parathyroid gland, can utilize DBP-bound 25(OH)D, possibly modifying the significance of free 25(OH)D levels (12).

To evaluate the vitamin D status, 25(OH)D is the preferred choice (13). It encompasses skin production as well as oral intake, is easily measured and has a relatively long half-life compared to the other vitamin D metabolites (13, 14). However, to correctly interpret 25(OH)D levels in CHC users, other metabolites in the vitamin D pathway might be useful. Also, $1,25(OH)_2D$/25(OH)D and vitamin D metabolite ratio defined by $24,25(OH)_2D$/25(OH)D (VMR) have been suggested as alternatives to 25(OH)D measurements (15, 16).

The aim of this study was therefore to compare 25(OH)D, $1,25(OH)_2D$, $24,25(OH)_2D$, DBP, free 25(OH)D, $1,25(OH)_2D$/25(OH)D, VMR, parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23), according to CHC use in adolescents. Further, we wanted to investigate if CHC use was an independent predictor of 25(OH)D levels when accounting for other lifestyle factors. Finally, we wanted to explore whether the ratios $1,25(OH)_2D$/25(OH)D and VMR in relation to 25(OH)D differed by CHC use, presented in scatterplots.

Methods

Study population

Fit Futures is a population-based, longitudinal study of the transition from adolescence to adulthood ongoing in Tromsø and Balsfjord municipality in North Norway (69°N). Fit Futures 1 was performed at The Research Unit at the University Hospital in North Norway, from September 2010 to April 2011, with 1038 attendees (girls $n = 508$) in their first year of upper secondary school (92.9% attendance rate), >95% were of Caucasian ethnicity. All participants gave their written consent prior to the study; for those under 16 years old, additional consent from a parent/proxy was required. The Regional Committee for Medical and Health Research Ethics North Norway and The Norwegian Data Protection Authority approved the study (2011/1684/REC North). Further study characteristics are accessible elsewhere (17).

We included a subsample of 182 girls from Fit Futures 1, whose stored serum samples were analyzed for $1,25(OH)_2D$, $24,25(OH)_2D$, DBP and FGF23 in 2016, free 25(OH)D in 2020, in addition to the 25(OH)D and PTH measured in 2012/2011. All participants <19 years with 25(OH)D levels ≥70 nmol/L or <20 nmol/L were included. In addition, a random sample of 25 individuals from each 10 nmol/L groups (20–29, 30–39, 40–49, 50–59 and 60–69 nmol/L) was selected (Table 1). If the selected participant lacked available serum ($n = 1$), the participant was excluded and not replaced. The selection process was designed to cover the whole range of 25(OH)D levels within available financial frames. $25(OH)D_2$ was measured simultaneously, but not detected; hence, vitamin D will refer to $25(OH)D_3$ (17).

Questionnaire

The participants filled out a questionnaire regarding various health aspects. To categorize participants into

Table 1 Selection process of subsample from Fit Futures 1. Girls available for primary (original) analysis (≤18 years and available serum sample): $n = 415$.

25(OH)D, nmol/L	Original sample	Subsample
<10	0	0
10–20	22	22
20–30	78	25
30–40	96	25
40–50	77	25
50–60	62	25
60–70	44	24
70-80	19	19
80–90	14	14
90–100	1	1
100–110	2	2
Total	415	182

25(OH)D, 25-hydroxyvitamin D.

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using (CHC+) or not using (CHC–) CHC, questions regarding contraceptive use were utilized. Images of HC products including brand names, were shown to aid participants recalling the name of their contraceptive. Sexual maturation was assessed by using age of menarche to divide the participants into early (<12.5 years), intermediate (12.5–14 years) and late (>14 years) sexual maturation. Age was measured in years.

In addition, we included the following variables regarding lifestyle and skin type: smoking and snuff habits, intake of fatty fish, cod liver and roe 'mølje', semi-skimmed milk (fortified with 0.4 µg vitamin D per 100 g), cod liver oil, vitamin/mineral supplements, screen time in weekends, physical activity, skin type according to sun sensitivity, sunbathing holiday and use of solarium.

For use in statistical analyses, some categorical answer options had to be merged due to low numbers of respondents: 'Do you smoke'? (No/never, sometimes, daily); alternatives 'sometimes' and 'daily' were merged into 'sometimes or daily', 'How often do you usually eat fat fish (e.g. salmon, trout, mackerel and herring)' (Rarely/never, one to three times per month, one to three times per week, four to six times per week, every day); alternatives 'one to three times per week', 'four to six times per week' and 'every day' were merged into 'weekly fish', 'How often do you usually eat cod liver and roe (mølje)?' (Rarely/never, one to three times per year, four to five times per year, six to nine times per year, 10 or more times per year); alternatives 'four to five times per year', 'six to nine times per year', 'ten or more times per year' were merged into 'four or more times per year', 'How often do you usually drink extra semi-skimmed milk?' (Rarely/never, one to six glasses per week, one glass per day, two to three glasses per day, four or more glasses per day); alternatives 'two to three glasses per day' and 'four or more glasses per day' were merged into 'more than one glass per day', 'How does your skin react to sunbathing in the summer?' (Always red never brown, almost always red sometimes brown, almost always brown sometimes red, always brown); alternatives 'always red, never brown' and 'almost always red, sometimes brown' were merged into 'mostly red'.

Laboratory analyses

Non-fasting blood samples were collected at the study site in 2010/2011 and stored in the Biobank at the UiT The Arctic University of Norway, at –70°C. Samples from the entire cohort (n = 890) were analyzed for 25(OH)D at the Hormone Laboratory, Haukeland University Hospital, Bergen, Norway by high-pressure liquid chromatography mass spectroscopy (LC-MS/MS), total analytical variation (CVa) was 10%. A selection of the samples was reanalyzed for 25(OH)D at University College Cork, Cork, Ireland, by LC-MS/MS, as a part of the International Vitamin D Standardization Program (VDSP). Further, a statistical regression equation was applied to the remaining 25(OH)D measurements to achieve standardized serum 25(OH)D values according to VDSP protocol, R^2 between the original 25(OH)D measurements and the standardized 25(OH)D measurements was 0.98 (18). The standardized values were applied here.

$1,25(OH)_2D$ was analyzed by enzyme immunoassay (CVa 14% at 55 pmol/L and 82 pmol/L; 13% at 152 pmol/L), $24,25(OH)_2D$ by LC-MS/MS (no coefficient of variation (CV) available) and DBP by RIA, (CVa 10% at 3.2 µmol/L and 6.2 µmol/L), using polyclonal antibodies. These analyses were performed at the Hormone Laboratory at Oslo University Hospital, Oslo, Norway, which participates in Vitamin D external quality assessment for $1,25(OH)_2D$ measurements. The lower limit of detection of $24,25(OH)_2D$ was 20 nmol/L, hence all participants with values under this limit were allocated the value 0 (n = 27).

Serum free 25(OH)D was analyzed by a competitive ELISA assay, cat. no. KAPF 1991, DIAsource ImmunoAssays SA (Louvain-la-Neuve, Belgium) DIAsource – Vitamin D (diasource-diagnostics.com), accessed on February 8, 2022. The assay had an intermediate precision of ≤ 6.3% (CV) as tested at four concentrations ranging from 14.5 to 70.1 pmol/L. FGF23 (intra CV 9%, inter CV 26%), was analyzed by immunoassay at OLINK® Proteomics, Uppsala, Sweden, and reported in Normalized Protein eXpression (NPX) values (log2 scale). Serum PTH, calcium and albumin were analyzed at the Department of Laboratory Medicine, University Hospital of North Norway, Tromsø, Norway. PTH was analyzed by an electrochemiluminescence immunoassay using an automated clinical chemistry analyser, Cobas® 6000, Roche Diagnostics. The laboratory used Quality Management from Tieto Enator (Helsinki, Finland) (CVa of 6.4, 3.7 and 4.6% at 1.9, 3.4 and 10.1 pmol/L, respectively) for PTH measurements. Calcium was analyzed by photometry using an automated clinical chemistry analyser, Cobas® 8000, Roche Diagnostics . CVa was 1.35% at 2.37 mmol/L. Likewise, albumin was analyzed by the Cobas® 8000, CVa was 1.4% at 45.0 g/L.

Physical measurements

Anthropometric measures in light clothing and without shoes included height measured to the nearest cm and

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weight to the nearest hectogram. BMI was calculated by weight (kg)/height $(m)^2$.

Statistics

All statistical calculations were performed using IBM SPSS statistics version 25 (IBM Corp. Released 2017. IBM SPSS statistics for Windows, Version 25.0.: IBM Corp.) Normality of the data was judged by visual inspection of histogram and Q-Q plot. Homoscedasticity was assessed by residual plot and linearity by scatterplot. All tests were two-tailed, and a *P*-value <0.05 was considered statistically significant.

There was one extreme outlier (92.4 pmol/L) present in the free 25(OH)D measurements, which was removed from the analysis. Due to many participants with undetectable $24,25(OH)_2D$ concentrations, we reported this metabolite as a percentage of participants with undetectable $24,25(OH)_2D$ values in each category.

25(OH)D levels were categorized consistent with international guidelines: <30, 30–50, 50–75 and >75 nmol/L (6, 7). ANOVA was used to calculate means and to assess weighted linear trends across vitamin D categories.

Vitamin D metabolites were compared between CHC+ and CHC- by chi-square test for categorical variables and *t*-tests for continuous variables. Similarly, lifestyle and biological variables included in previous analyses in the complete Fit Futures cohort (17), were compared between CHC+ and CHC–. Lifestyle variables significantly different between the groups in univariate analyses were further included in multiple regression analyses together with age, BMI and CHC status with 25(OH)D as a dependent variable.

A Lowess curve was fitted to the observations of $1,25(OH)_2D$/25(OH)D and VMR in relation to 25(OH)D levels in a scatterplot, stratified by CHC status.

Results

Fit Futures 1 included 415 eligible girls, where 182 met the selection criteria for this study (Table 1). The median age was 16 years with a mean ± S.D. BMI of 22.3 ± 4.0 kg/m^2. Mean ± S.D. 25(OH)D was 48.3 ± 22.1 nmol/L with corresponding PTH at 3.9 ± 1.3 pmol/L. Of the participants, 64 (35%) were CHC+. Table 2 demonstrates the baseline characteristics and biological variables across 25(OH)D categories. CHC+ were overrepresented in the highest categories, constituting 74.1% of the participants with 25(OH)D >75 nmol/L. There were significant linear increasing levels of $1,25(OH)_2D$, $24,25(OH)_2D$, DBP, free 25(OH)D and decreasing levels of PTH across the 25(OH)D categories (Table 2). In participants with 25(OH)D levels <30 nmol/L, 44.7% of $24,25(OH)_2D$ levels were undetectable, while all participants with 25(OH)D >75 nmol/L had detectable $24,25(OH)_2D$ values (Table 2).

In univariate models, CHC+ were more likely to use snuff, vitamin/mineral supplements, and solarium and less likely to eat fatty fish (Table 3). Further, the differences in 25(OH)D levels between CHC+ and CHC– were significant (60.3 ± 22.2 and 41.8 ± 19.3 nmol/L, respectively, $P < 0.01$). CHC+ also had higher levels of $1,25(OH)_2D$,

Table 2 Results from ANOVA analysis: baseline characteristics, vitamin D metabolites, PTH, FGF23 and calcium according to categories of 25(OH)D levels.

25(OH)D, nmol/L	<30	30–50	50-75	>75	*P*-value for linear trend
Baseline characteristics					
Number of persons (*n*)	44	48	53	26	n.a
CHC+ (%)	17.0	28.0	37.9	74.1	<0.001
BMI (kg/m^2)	22.5 ± 4.7	23.2 ± 4.7	21.8 ± 3.4	21.2 ± 2.2	0.091
Biological variables					
$1,25(OH)_2D$ (pmol/L)	98.2 ± 31.0	119.0 ± 38.4	124.4 ± 33.0	148.4 ± 66.2	<0.001
$24,25(OH)_2D$ (nmol/L)	21.1 ± 21.0	39.6 ± 22.4	62.2 ± 32.5	79.0 ± 36.3	<0.001
$24,25(OH)_2D$ percentage below limit of detection	44.7%	10.0%	1.7%	0%	na
DBP (µmol/L)	3.5 ± 0.7	3.6 ± 0.7	4.0 ± 0.9	4.6 ± 1.3	<0.001
Free 25(OH)D (pg/mL)	5.6 ± 3.9	7.2 ± 3.7	8.6 ± 4.9	8.5 ± 3.5	<0.001
PTH (pmol/L)	4.3 ± 1.5	4.0 ± 1.5	3.6 ± 0.9	3.5 ± 1.1	0.004
FGF23, NPX values	2.8 ± 0.9	2.8 ± 1.0	2.5 ± 0.6	2.6 ± 0.7	0.073
Calcium (mmol/L)	2.30 ± 0.1	2.30 ± 0.1	2.31 ± 0.1	2.30 ± 0.1	0.834
Albumin (g/L)	45.0 ± 2.7	44.0 ± 2.4	45.0 ± 2.3	43.6 ± 3.0	0.191

CHC+, combined hormonal contraceptive users; DBP, vitamin D-binding protein; free 25(OH)D , measured free 25(OH)D; FGF23, fibroblast growth factor; PTH, parathyroid hormone; $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; $24,25(OH)_2D$, 24,25-dihydroxyvitamin D.

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Table 3 Results from Pearson's chi-square test of categorical variables and CHC use.

Categorical variables	CHC+	CHC−	Pearson's chi-square, *P*-value
Lifestyle variables	*n* (%)	*n* (%)	
Smoking status			
Never	46 (73.0)	93 (80.9)	0.226
Sometimes or daily	17 (27.0)	22 (19.1)	
Snuff use			
Never	24 (38.1)	86 (74.1)	<0.001
Sometimes	16 (25.4)	14 (12.1)	
Daily	23 (36.5)	16 (13.8)	
Fatty fish intake			
Rarely/never	13 (20.6)	19 (16.4)	0.010
One to three times per month	42 (66.7)	58 (50.0)	
Weekly fish	8 (12.7)	39 (33.6)	
Cod liver and roe 'Mølje' intake			
Rarely/never	42 (66.7)	70 (60.3)	0.706
1–3 times per year	15 (23.8)	33 (28.4)	
4 or more times per year	6 (9.5)	13 (11.2)	
Extra semi-skimmed milk intake			
Rarely/never	32 (50.8)	44 (37.9)	0.202
1–6 glasses per week	15 (23.8)	29 (25.0)	
1 glass per day	8 (12.7)	29 (25.0)	
>1 glass per day	8 (12.7)	14 (12.1)	
Cod liver oil intake			
No	31 (49.2)	57 (49.6)	0.636
Sometimes	22 (34.9)	34 (29.6)	
Daily	10 (15.9)	24 (20.9)	
Vitamin/mineral supplements intake			
No	12 (19.0)	46 (39.7)	0.016
Sometimes	33 (52.4)	49 (42.2)	
Daily	18 (28.6)	21 (18.1)	
Screen time weekends			
0–1.5 h	11 (17.5)	18 (15.5)	0.584
2–3 h	19 (30.2)	47 (40.5)	
4–6 h	24 (38.1)	36 (31.0)	
>7 h	9 (14.3)	15 (12.9)	
Physical activity in leisure time			
Sedentary	9 (14.3)	12 (10.3)	0.827
≥4 h a week	26 (41.3)	49 (41.9)	
Recreational sports	16 (25.4)	35 (29.9)	
Hard training	12 (19.0)	21 (17.9)	
Sunbathing holiday last 2 months			
No	55 (87.3)	107 (92.2)	0.282
Yes	8 (12.7)	9 (7.8)	
Solarium use last 4 weeks			
No	26 (41.3)	84 (72.4)	<0.001
Yes	37 (58.7)	32 (27.6)	
Biological variables			
Puberty status			
Early	28 (45.9)	40 (34.5)	0.186
Intermediate	18 (29.5)	50 (43.1)	
Late	15 (24.6)	26 (22.4)	
Skin type according to sun sensitivity			
Mostly red	11 (17.7)	23 (20.0)	0.774
Almost always brown, sometimes red	39 (62.9)	66 (57.4)	
Always brown, never red	12 (19.4)	26 (22.6)	

CHC+, combined hormonal contraceptive users; CHC−, combined hormonal contraceptive non-users.

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Table 4 Results from *t*-tests on continuous variables by CHC use. Mean values ± S.D. adjusted for BMI and age.

	CHC+	CHC–	*t*-Test, *P*-value
Number of persons (*n*)	64	117	
25(OH)D (nmol/L)	60.3 ± 22.2	41.8 ± 19.3	<0.001
$1,25(OH)_2D$ (pmol/L)	128.1 ± 48.2	115.2 ± 39.9	0.042
$24,25(OH)_2D$ (nmol/L)	61.9 ± 37.6	40.4 ± 30.2	<0.001
DBP (µmol/L)	4.5 ± 1.0	3.4 ± 0.7	<0.001
Free 25(OH)D (pg/mL)	7.7 ± 4.6	7.3 ± 4.1	0.603
$1,25(OH)_2D/25(OH)D$	2.4 ± 1.2	3.4 ± 1.9	<0.001
VMR	1.1 ± 0.6	1.0 ± 0.7	0.374
PTH (pmol/L)	3.6 ± 1.1	4.1 ± 1.4	0.035
FGF23, NPX values	2.5 ± 0.8	2.8 ± 0.8	0.023
Calcium (mmol/L)	2.30 ± 0.1	2.27 ± 0.1	0.005
Albumin (g/L)	43.2 ± 2.6	45.1 ± 2.4	<0.001

CHC+, combined hormonal contraceptive users; DBP, vitamin D-binding protein; free 25(OH)D , measured free 25(OH)D; FGF23, fibroblast growth factor; PTH, parathyroid hormone; VMR, $24,25(OH)_2D/25(OH)D$ vitamin D metabolite ratio; 2325(OH)D, 25-hydroxyvitamin D; $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; $24,25(OH)_2D$, 24,25-dihydroxyvitamin D.

$24,25(OH)_2D$, DBP and calcium than CHC–, whereas $1,25(OH)_2D/25(OH)D$, PTH, FGF23 and albumin were significantly lower. There were no differences in free 25(OH)D and VMR levels (Table 4).

The multiple regression model included the categorical variables that differed according to CHC use in univariate models (snuff habits, intake of fatty fish, vitamin/mineral supplement intake and solarium use), in addition to age, BMI and CHC status. Adjustment for these variables attenuated, but did not remove, the differences in 25(OH)D levels between CHC+ and CHC–. Solarium and CHC use significantly predicted an increase in 25(OH)D levels by unstandardized B (95% CI) of 20.7 (15.0–26.4) and 9.7 (3.5–16.0) nmol/L, respectively. BMI, age, snuff habits, intake of fatty fish and vitamin/mineral supplement intake did not contribute significantly to the model. The model explained 37.6% of the variation in 25(OH)D levels (Table 5).

By visualizing $1,25(OH)_2D/25(OH)D$ and VMR in relation to 25(OH)D in scatterplots, there was no clear differences in the patterns of observations according to CHC status (Figs 1A, B and 2A, B).

Discussion

This study reports a marked significant difference in 25(OH)D levels according to CHC use among adolescent girls, the difference remained, but was attenuated after adjustment for lifestyle variables. The higher 25(OH)D levels were congruent with higher $1,25(OH)_2D$, $24,25(OH)_2D$, DBP and calcium while $1,25(OH)_2D/25(OH)D$, PTH, FGF23 and albumin were lower. Free 25(OH)D and VMR did not differ, also there were no differences in the patterns of $1,25(OH)_2D/25(OH)D$ and VMR in relation to 25(OH)D levels, stratified by CHC use.

Differences in 25(OH)D levels according to estrogen use in women, both postmenopausal and in childbearing age (>18 years) is well described in previous publications (1, 2, 3, 19). However, studies on 25(OH)D levels in the CHC using adolescent population are scarce. In this study of adolescent girls, the difference between CHC+ and CHC– in 25(OH)D levels was profound, constituting almost 20 nmol/L in univariate analyses, consistent with findings in 16–25 years old Australian girls (4). Of note, differences in solarium use explained half the difference in our population.

Increased DBP and 25(OH)D levels in CHC users have been previously reported (3, 20), and is considered due to the estrogen component in CHC stimulating the hepatic production of DBP, as is the case for many binding globulins (21, 22). As approximately 90% of 25(OH)D is bound to DBP, this carrier protein clearly has an important role in maintaining 25(OH)D levels (23, 24). This point was further demonstrated in a patient lacking DBP, with almost

Table 5 Results from multivariate linear regression analysis: predictors for serum 25(OH)D levels.

Independent variables	Unstandardized B (CI)	Standardized beta	*P*-value
BMI (kg/m^2)	−0.4 (−1.0 to 0.3)	-0.07	0.286
Age (years)	1.3 (−3.8 to 6.5)	0.03	0.608
Snuff use (no/never, sometimes, daily)	3.0 (−0.4 to 6.5)	0.11	0.086
Intake of fatty fish (rarely/never, one to three times per month, weekly fish)	−0.6 (−4.7 to 3.6)	-0.02	0.789
Vitamin/mineral supplement (no, sometimes, daily)	2.1 (−1.7 to 5.8)	0.07	0.273
Solarium use last 4 weeks (no/yes)	20.7 (15.0 to 26.4)	0.46	<0.001
CHC use (no/yes)	9.7 (3.5 to 16.0)	0.21	0.003
R^2	0.376		

CHC+, combined hormonal contraceptive users; CHC–, combined hormonal contraceptive non-users; 25(OH)D, 25-hydroxyvitamin D.

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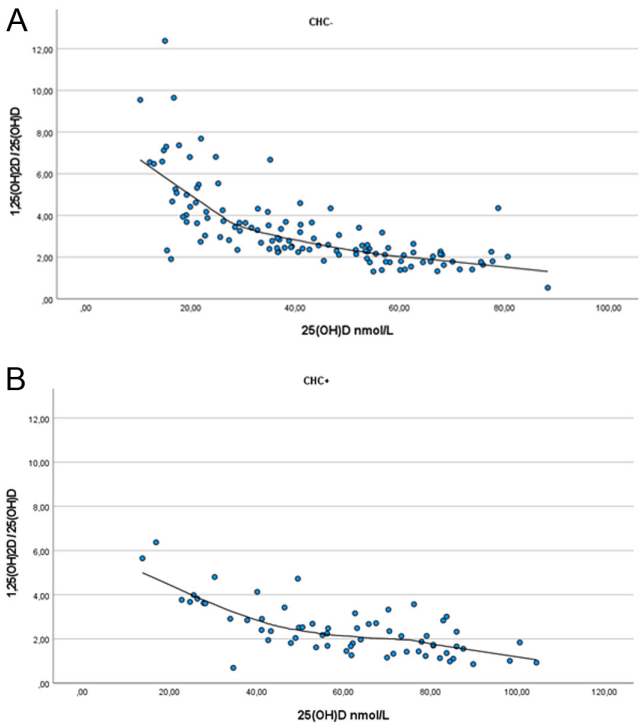

Figure 1
(A) Scatterplot of 1,25(OH)2D/25(OH)D in relation to 25(OH)D in CHC–. (B) Scatterplot of 1,25(OH)2D/25(OH)D in relation to 25(OH)D in CHC+.

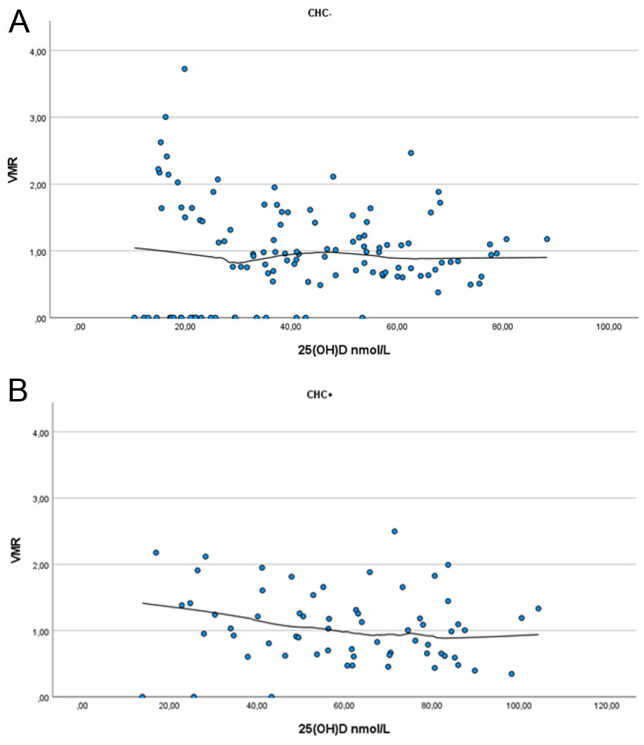

Figure 2
(A) Scatterplot of VMR in relation to 25(OH)D in CHC–. (B) Scatterplot of VMR in relation to 25(OH)D in CHC+.

undetectable 25(OH)D levels (25). In addition, progestins may affect DBP levels (20), also in line with other globulins (21). DBP concentration and binding affinity have genetic variation, with different ethnic distributions (23); however, as the majority of our study participants are of Caucasian ethnicity, we do not believe this will largely impact our findings. Previous studies have reported lower albumin levels in women taking exogenous estrogen, in line with our findings (26, 27).

There was a linearly increasing trend of free 25(OH)D across 25(OH)D categories. However, free 25(OH)D levels did not differ according to CHC use in line with previous studies in the adult population (3, 10). Due to its bioavailability and proposed independence of DBP levels, free 25(OH)D has been suggested as a marker of vitamin D status, as a replacement for or in addition to 25(OH)D measurements (12, 28). This relates to the free hormone hypothesis, stating that the concentration of free hormone in serum controls the concentration of hormone inside the cell, and thus the biological activity (12, 29). However, the true availability of free hormone to the cell depends on numerous factors, including blood flow, serum concentrations, the type and amount of binding proteins and their dissociation constants, cellular uptake and intracellular elimination (29). One could speculate that an unknown active regulation of any of these steps contributes to maintain a stable free 25(OH)D concentration irrespective of CHC use.

It has been argued that the equal levels of free 25(OH)D could mean an over-estimation of vitamin D status based on 25(OH)D levels in HC users (10). However, DBP-bound 25(OH)D can be utilized by cells which has the membrane-anchored megalin/cubulin-complex, as do the kidneys and the parathyroid gland (30), consistent with the lower PTH and higher calcium levels in the CHC+. In fact, in our population, the difference in free calcium could be expected to be even larger, as albumin was lower in CHC+, however, this was not measured. In addition to the megalin/cubulin-complex, there might be other receptors making DBP-bound 25(OH)D available to cells (12, 31).

The lower PTH and higher calcium could represent clinically relevant differences in 25(OH)D levels according to CHC use, at least in a bone health perspective. This supports previous studies, concluding that 25(OH)D is still the metabolite of choice regarding the effect of vitamin D on bone health (32, 33), although one study reported superior correlations of free 25(OH)D (34).

We investigated possible confounding variables on 25(OH)D levels. In univariate analysis, CHC+ differed in snuff habits, fatty fish intake, vitamin/mineral supplement

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intake and solarium use. These lifestyle habits can influence 25(OH)D levels and was, therefore, included in the multiple regression model together with BMI, age and CHC. Here, solarium and CHC use were significant predictors of higher 25(OH)D levels. This confirms that UV exposure is a strong predictor of 25(OH)D levels in this cohort, as previously published (17).

Both $1,25(OH)_2D$/25(OH)D and VMR have been suggested as alternative markers of vitamin D status due to their close relation to 25(OH)D levels (16, 35). Although the $1,25(OH)_2D$/25(OH)D ratio was higher in CHC- than CHC+, the distribution of the ratio across 25(OH)D levels was similar in both groups. The VMR was not statistically different according to CHC use, and the scatterplots were comparable. These results indicate a similar vitamin D metabolite regulation, irrespective of CHC use.

Although this study was based on a selected young population according to their 25(OH)D levels, the results of this study are relevant for a female adolescent population, at least of Caucasian origin. We had the opportunity to investigate vitamin D metabolism in an adolescent population ranging from deficiency to sufficient vitamin D levels. In addition, the 25(OH)D measurements were standardized according to VDSP. Another strength of the study is that we measured free 25(OH)D, which provides more accurate values than those obtained by calculating levels based on measured 25(OH)D, albumin and DBP (36, 37, 38). Although the use of CHC was self-reported, the information was confirmed through interviews using images to ensure the correct brand names. A limitation is the number of participants. Also, we did not have information regarding the length of CHC use. Last, although we included many variables related to 25(OH)D levels, we cannot exclude unmeasured confounders influencing the effect of CHC on 25(OH)D levels. Further research is needed to establish the long-term effects of CHC use and the effect of cessation of CHC on vitamin D levels, which is important from a clinical point of view.

In conclusion, this study confirms the impact of CHC use on vitamin D levels in an adolescent population, which could be clinically relevant due to corresponding differences in calcium and PTH levels. The 25(OH)D levels in CHC+ therefore seem to represent a valid measure of the vitamin D status in this population.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References

1 Harmon QE, Umbach DM & Baird DD. Use of estrogen-containing contraception is associated with increased concentrations of 25-hydroxy vitamin D. *Journal of Clinical Endocrinology and Metabolism* 2016 **101** 3370–3377. (https://doi.org/10.1210/jc.2016-1658)
2 Harris SS & Dawson-Hughes B. The association of oral contraceptive use with plasma 25-hydroxyvitamin D levels. *Journal of the American College of Nutrition* 1998 **17** 282–284. (https://doi.org/10.1080/07315724.1998.10718760)
3 Moller UK, Streym Sv, Jensen LT, Mosekilde L, Schoenmakers I, Nigdikar S & Rejnmark L. Increased plasma concentrations of vitamin D metabolites and vitamin D binding protein in women using hormonal contraceptives: a cross-sectional study. *Nutrients* 2013 **5** 3470–3480. (https://doi.org/10.3390/nu5093470)
4 Callegari ET, Garland SM, Gorelik A, Reavley NJ & Wark JD. Predictors and correlates of serum 25-hydroxyvitamin D concentrations in young women: results from the Safe-D study. *British Journal of Nutrition* 2017 **118** 263–272. (https://doi.org/10.1017/S0007114517002021)
5 Lindh I, Skjeldestad FE, Gemzell-Danielsson K, Heikinheimo O, Hognert H, Milsom I & Lidegaard Ø. Contraceptive use in the Nordic countries. *Acta Obstetricia et Gynecologica Scandinavica* 2017 **96** 19–28. (https://doi.org/10.1111/aogs.13055)
6 Ross A, Taylor CL, Yaktine AL & Del Valle HB. *Dietary Reference Intakes for Calcium and Vitamin D*. Washington, DC: The National Academies Press 2011. (Committee to Rewiew Dietary Reference Intakes for Vitmain D and Calcium; Food and Nutrition Board; Institute of Medicine). (available at: https://www.nap.edu/catalog/13050/dietary-reference-intakes-for-calcium-and-vitamin-d)
7 Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM & Endocrine Society. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *Journal of Clinical Endocrinology and Metabolism* 2011 **96** 1911–1930. (https://doi.org/10.1210/jc.2011-0385)
8 Meyer H, Brunvand L & Brustad M. *Measures to Ensure a Good Vitamin D Status in the Population. (Report of a Working Group Appointed by the National Nutrition Council. Report IS-1408)*. Oslo: Norwegian Directorate of Health, 2006. (available at: https://docplayer.me/17925723-Tiltak-for-a-sikre-en-god-vitamin-d-status-i-befolkningen.html)
9 Brustad MM, Meyer A & Holvik HK. Vitamin D in Norway: the need to ensure a good vitamin D status. In report of a working group appointed by the National Nutrition Council. Report IS-2772. Oslo: Norwegian Directorate of Health. 2018. (available at: https://www.helsedirektoratet.no/rapporter/vitamin-d-i-norge-behov-for-tiltak-for-a-sikre-god-vitamin-d-status)
10 Pilz S, Obeid R, Schwetz V, Trummer C, Pandis M, Lerchbaum E, Pieber TR, Obermayer-Pietsch B, Wilhelm M, Hahn A, *et al.* Hormonal contraceptive use is associated with higher total but unaltered free 25-hydroxyvitamin D Serum concentrations. *Journal of Clinical Endocrinology and Metabolism* 2018 **103** 2385–2391. (https://doi.org/10.1210/jc.2018-00336)
11 Aarskog D, Aksnes L, Markestad T & Rodland O. Effect of estrogen on vitamin D metabolism in tall girls. *Journal of Clinical Endocrinology and Metabolism* 1983 **57** 1155–1158. (https://doi.org/10.1210/jcem-57-6-1155)

12 Chun RF, Peercy BE, Orwoll ES, Nielson CM, Adams JS & Hewison M. Vitamin D and DBP: the free hormone hypothesis revisited. *Journal of Steroid Biochemistry and Molecular Biology* 2014 **144** 132–137. (https://doi.org/10.1016/j.jsbmb.2013.09.012)

13 Giustina A, Adler RA, Binkley N, Bollerslev J, Bouillon R, Dawson-Hughes B, Ebeling PR, Feldman D, Formenti AM, Lazaretti-Castro M, *et al.* Consensus statement from 2(nd) International Conference on controversies in vitamin D. *Reviews in Endocrine and Metabolic Disorders* 2020 **21** 89–116. (https://doi.org/10.1007/s11154-019-09532-w)

14 Prentice A, Goldberg GR & Schoenmakers I. Vitamin D across the lifecycle: physiology and biomarkers. *American Journal of Clinical Nutrition* 2008 **88** 500S–506S. (https://doi.org/10.1093/ajcn/88.2.500S)

15 Ginsberg C, Katz R, de Boer IH, Kestenbaum BR, Chonchol M, Shlipak MG, Sarnak MJ, Hoofnagle AN, Rifkin DE, Garimella PS, *et al.* The 24,25 to 25-hydroxyvitamin D ratio and fracture risk in older adults: the cardiovascular health study. *Bone* 2018 **107** 124–130. (https://doi.org/10.1016/j.bone.2017.11.011)

16 Pasquali M, Tartaglione L, Rotondi S, Muci ML, Mandanici G, Farcomeni A, Marangella M & Mazzaferro S. Calcitriol/calcifediol ratio: an indicator of vitamin D hydroxylation efficiency? *BBA Clinical* 2015 **3** 251–256. (https://doi.org/10.1016/j.bbacli.2015.03.004)

17 Oberg J, Jorde R, Almas B, Emaus N & Grimnes G. Vitamin D deficiency and lifestyle risk factors in a Norwegian adolescent population. *Scandinavian Journal of Public Health* 2014 **42** 593–602. (https://doi.org/10.1177/1403494814541593)

18 Cashman KD, Dowling KG, Skrabakova Z, Kiely M, Lamberg-Allardt C, Durazo-Arvizu RA, Sempos CT, Koskinen S, Lundqvist A, Sundvall J, *et al.* Standardizing serum 25-hydroxyvitamin D data from four Nordic population samples using the vitamin D standardization program protocols: shedding new light on vitamin D status in Nordic individuals. *Scandinavian Journal of Clinical and Laboratory Investigation* 2015 **75** 549–561. (https://doi.org/10.3109/00365513.2015.1057898)

19 Bansal N, Katz R, de Boer IH, Kestenbaum B, Siscovick DS, Hoofnagle AN, Tracy R, Laughlin GA, Criqui MH, Budoff MJ, *et al.* Influence of estrogen therapy on calcium, phosphorus, and other regulatory hormones in postmenopausal women: the MESA study. *Journal of Clinical Endocrinology and Metabolism* 2013 **98** 4890–4898. (https://doi.org/10.1210/jc.2013-2286)

20 Stanczyk FZ, Sriprasert I, Danis R, Pandian R, Matharu H, Bender N & Natavio M. Effect of oral contraceptives on total and bioavailable 25-hydroxyvitamin D. *Journal of Steroid Biochemistry and Molecular Biology* 2021 **211** 105879. (https://doi.org/10.1016/j.jsbmb.2021.105879)

21 Wiegratz I, Kutschera E, Lee JH, Moore C, Mellinger U, Winkler UH & Kuhl H. Effect of four different oral contraceptives on various sex hormones and serum-binding globulins. *Contraception* 2003 **67** 25–32. (https://doi.org/10.1016/s0010-7824(02)00436-5)

22 Sitruk-Ware RL, Menard J, Rad M, Burggraaf J, de Kam ML, Tokay BA, Sivin I & Kluft C. Comparison of the impact of vaginal and oral administration of combined hormonal contraceptives on hepatic proteins sensitive to estrogen. *Contraception* 2007 **75** 430–437. (https://doi.org/10.1016/j.contraception.2007.01.027)

23 Chun RF, Shieh A, Gottlieb C, Yacoubian V, Wang J, Hewison M & Adams JS. Vitamin D binding protein and the biological activity of vitamin D. *Frontiers in Endocrinology* 2019 **10** 718. (https://doi.org/10.3389/fendo.2019.00718)

24 Bikle DD, Gee E, Halloran B, Kowalski MA, Ryzen E & Haddad JG. Assessment of the free fraction of 25-hydroxyvitamin D in serum and its regulation by albumin and the vitamin D-binding protein. *Journal of Clinical Endocrinology and Metabolism* 1986 **63** 954–959. (https://doi.org/10.1210/jcem-63-4-954)

25 Henderson CM, Fink SL, Bassyouni H, Argiropoulos B, Brown L, Laha TJ, Jackson KJ, Lewkonia R, Ferreira P, Hoofnagle AN, *et al.* Vitamin D-binding protein deficiency and homozygous deletion of the GC Gene. *New England Journal of Medicine* 2019 **380** 1150–1157. (https://doi.org/10.1056/NEJMoa1807841)

26 Cushman M, Meilahn EN, Psaty BM, Kuller LH, Dobs AS & Tracy RP. Hormone replacement therapy, inflammation, and hemostasis in elderly women. *Arteriosclerosis, Thrombosis, and Vascular Biology* 1999 **19** 893–899. (https://doi.org/10.1161/01.atv.19.4.893)

27 Zhang D, Maalouf NM, Adams-Huet B, Moe OW & Sakhaee K. Effects of sex and postmenopausal estrogen use on serum phosphorus levels: a cross-sectional study of the National Health and Nutrition Examination Survey (NHANES) 2003–2006. *American Journal of Kidney Diseases* 2014 **63** 198–205. (https://doi.org/10.1053/j.ajkd.2013.07.012)

28 Bikle DD, Malmstroem S & Schwartz J. Current controversies: are free vitamin metabolite levels a more accurate assessment of vitamin D status than total levels? *Endocrinology and Metabolism Clinics of North America* 2017 **46** 901–918. (https://doi.org/10.1016/j.ecl.2017.07.013)

29 Mendel CM. The free hormone hypothesis. Distinction from the free hormone transport hypothesis. *Journal of Andrology* 1992 **13** 107–116.

30 Lundgren S, Carling T, Hjälm G, Juhlin C, Rastad J, Pihlgren U, Rask L, Akerström G & Hellman P. Tissue distribution of human gp330/megalin, a putative Ca(2+)-sensing protein. *Journal of Histochemistry and Cytochemistry* 1997 **45** 383–392. (https://doi.org/10.1177/002215549704500306)

31 Esteban C, Geuskens M, Ena JM, Mishal Z, Macho A, Torres JM & Uriel J. Receptor-mediated uptake and processing of vitamin D-binding protein in human B-lymphoid cells. *Journal of Biological Chemistry* 1992 **267** 10177–10183. (https://doi.org/10.1016/S0021-9258(19)50216-2)

32 Michaelsson K, Rasmusson A, Wolk A, Byberg L, Mitchell A & Melhus H. The free hormone hypothesis: is free serum 25-hydroxyvitamin D a better marker for bone mineral density in older women? *JBMR Plus* 2018 **2** 367–374. (https://doi.org/10.1002/jbm4.10059)

33 Aloia J, Dhaliwal R, Mikhail M, Shieh A, Stolberg A, Ragolia L, Fazzari M & Abrams SA. Free 25(OH)D and calcium absorption, PTH, and markers of bone turnover. *Journal of Clinical Endocrinology and Metabolism* 2015 **100** 4140–4145. (https://doi.org/10.1210/jc.2015-2548)

34 Chhantyal K, He L, Mo J, Yin M, He T, Chen Y, Yang Y, Zhang L & Rong L. Free vitamin D correlate better with bone mineral density and thoracolumbar junction osteoporotic vertebral fractures than serum vitamin D. *BMC Musculoskeletal Disorders* 2020 **21** 164. (https://doi.org/10.1186/s12891-020-3179-7)

35 Cavalier E, Huyghebaert L, Rousselle O, Bekaert AC, Kovacs S, Vranken L, Peeters S, Le Goff C & Ladang A. Simultaneous measurement of 25(OH)-vitamin D and 24,25(OH)2-vitamin D to define cut-offs for CYP24A1 mutation and vitamin D deficiency in a population of 1200 young subjects. *Clinical Chemistry and Laboratory Medicine* 2020 **58** 197–201. (https://doi.org/10.1515/cclm-2019-0996)

36 Schwartz JB, Lai J, Lizaola B, Kane L, Markova S, Weyland P, Terrault NA, Stotland N & Bikle D. A comparison of measured and calculated free 25(OH) vitamin D levels in clinical populations. *Journal of Clinical Endocrinology and Metabolism* 2014 **99** 1631–1637. (https://doi.org/10.1210/jc.2013-3874)

37 Lopez-Molina M, Santillan C, Murillo M, Valls A, Bosch L, Bel J & Granada ML. Measured free 25-hydroxyvitamin D in healthy children and relationship to total 25-hydroxyvitamin D, calculated free 25-hydroxyvitamin D and vitamin D binding protein. *Clinical Biochemistry* 2018 **61** 23–27. (https://doi.org/10.1016/j.clinbiochem.2018.08.007)

38 Simpson CA, Zhang JH, Vanderschueren D, Fu L, Pennestri TC, Bouillon R, Cole DEC & Carpenter TO. Relationship of total and free 25-hydroxyvitamin D to biomarkers and metabolic indices in healthy children. *Journal of Clinical Endocrinology and Metabolism* 2020 **105** e1631–e1640. (https://doi.org/10.1210/clinem/dgz230)

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