Do Postmenopausal Women Have Higher Serum 25-Hydroxyvitamin D$_3$ Concentrations Compared to Men or Younger Women?

Martha A Kaeslin*, Cyril A Fuhrer, Roberto Herklotz and Andreas R Huber

Institute of Laboratory Medicine, Cantonal Hospital Aarau, Tellstrasse, 5001 Aarau, Switzerland

Abstract

Background: Vitamin D deficiency is a widely discussed topic in medicine. However, the phenomenon that postmenopausal women have higher 25(OH)D$_3$ concentrations compared to men or younger women is rarely described in the scientific literature. Therefore the objective was to investigate this phenomenon in a representative population between different life periods and gender in Aarau (Switzerland) at the end of wintertime when the lowest 25(OH)D$_3$ concentrations can be observed from seasonal cycling.

Methods: 25(OH)D$_3$ serum concentrations were measured in a volunteer study population (88 women, 26 men; age: 25-82 years) and we compared all 25(OH)D data of patients of the cantonal Hospital of Aarau in the years from 2010 to 2012(2,766 women, 1,532 men; age: 3 month - 99 years) from the end of February to the end of March 2011. Measurements were performed by HPLC and LC-MS/MS routine assays. Further information was obtained from an especially designed questionnaire.

Results: Overall vitamin D deficiency (25(OH)D$_3$<50 nmol/L) was more present in men ($\geq$ 50%) than women (<50%). In the volunteer study population and in the hospital patient population elderly women, especially postmenopausal women, had significantly higher mean 25(OH)D$_3$ concentrations (66.5 and 61.0 nmol/L) compared to men (52.3 and 48.2 nmol/L, P<0.05).

Conclusions: At the end of wintertime postmenopausal women had the highest 25(OH)D$_3$ serum concentrations compared to younger females and any age cohort of men. Therefore increased testing should be performed for younger women and men to prevent vitamin D deficiency especially during wintertime.

Introduction

Vitamin D deficiency is nowadays a widely discussed topic in medicine. The lipophilic vitamin D plays an important role in calcium homeostasis and bone metabolism and in addition seems to decrease the risk of important chronic illnesses such as cancer, infectious and cardiovascular diseases [1-4]. Humans obtain vitamin D mainly from exposure to sunlight (ultraviolet B radiation, UVB), from their diet, and from dietary supplements. In the skin previtamin D$_3$ is formed from 7-dehydrocholesterol by UVB radiation and is further converted to vitamin D$_3$(cholecalciferol). The vitamin D binding protein (VDBP) transports vitamin D, the active hormone and its metabolites in the blood circulation. In the liver vitamin D is converted to 25-hydroxyvitamin D[25(OH)D]. This major circulating form is used to determine the vitamin D status. 25(OH)D is then converted in the kidneys to the biologically active form [1,25(OH)2D].

In this article we discuss measurements of the endogenous 25(OH)D$_3$ in serum which accounts for approximately 95% of the circulating pool. Only in a few cases a relevant concentration of 25(OH)D$_2$ (>5 nmol/l) could be measured influencing the total amount of 25(OH)D. Normally 25(OH)D$_2$ was not detectable or below the lower limit of quantification (LLOQ, 5 nmol/l). The mean concentration of 25(OH)D$_2$ was 3.1 nmol/l. Thus, 25(OH)D$_3$ was used to represent the total amount of 25(OH)D i.e. free 25(OH)D and 25(OH)D bound to VDBP [5]. Routinely, the total amount of 25(OH)D is measured by commercially available 25(OH)D assays including steps for releasing 25(OH)D from VDBP. However, in the case of immunoassays (IA) used to measure 25(OH)D, inaccuracies were observed that are VDBP concentration dependent [6]. This is one aspect for the explanation of

the differences between IA and HPLC and LC-MS/MS methods. Over 99% of circulating vitamin D compounds are protein bound and thus VDBP appears to buffer the free levels of active vitamin D compounds, preventing against vitamin D intoxication. VDBP levels are not regulated by vitamin D and the concentration of free 1,25(OH)2D$_3$, however, remains constant even when VDBP concentrations change [7,8]. Experiments with VDBP-null mice hypothesise that VDBP influences total circulating levels of 1,25(OH)2D$_3$ but does not directly modulate the bioactive levels of the hormone *in vivo* [9].

Beside vitamin D$_3$ vitamin D$_2$ (ergocalciferol) plays an important role in food supplementation. It is manufactured through UV irradiation of ergosterol from yeast [1-4]. The impact of vitamin D supplementation in food to prevent osteoporosis and other illnesses therefore seems likely.

Although vitamin D deficiency is widely discussed, there are still discrepancies in the proposed cut-off. This cut-off seems to be between 50 nmol/l and 75 nmol/l, depending on actual research findings and the according national guidelines. In Switzerland and the United States

*Corresponding author: Martha A Kaeslin, PhD, Institute of Laboratory Medicine, Cantonal Hospital Aarau, Tellstrasse, 5001 Aarau, Switzerland, Tel: +41 62 838 52 90; Fax: +41 62 838 53 99; Email: martha.kaeslinmeyer@ksa.ch

of America a serum concentrations of 25(OH)D of less than 50 nmol/l (<20 ng/ml) is considered as an indicator of vitamin D deficiency. Severe vitamin D deficiency is marked by a threshold of less than 25 nmol/l (<10 ng/ml) [10,11]. The reference value in Swiss diagnostic laboratories is 50 and 175 nmol/l, respectively until the age of 50 thereafter 63 and 175 nmol/l [8].

In this article we describe two settings of comparing 25(OH)D serum levels in various ages of women and men resident around Aarau (Switzerland) with the geographic coordinates 47° 23′ 40″ N, 8° 2′ 42.01″ E. The study cohort consists of volunteers with blood samplings from March 2011. A questionnaire especially designed for this study group collected information on the period of life they were in, weight, height, hair colour, skin taint, daily sun exposure and vitamin D supplementation and estrogen therapy at the time point of taking the blood sample. This group is referred to as study group. 114 apparently healthy volunteers (88 women, 26 men; age: 21-86 years) were members of this group (Table 1). In the second group (Table 2) which is referred to as clinical group, we compared all 25(OH)D data of patients of the cantonal Hospital of Aarau in the years from 2010 to 2012 (2,766 women, 1,532 men; age: 3 month-99 years).

Materials and Methods

Serum samples were drawn (BD Vacutainer® Z) from the participants during February and March 2011. All samples were handled identically. After centrifugation, serum was aliquoted and frozen at -20°C until analysis.

All 25(OH)D measurements were performed in the centre of laboratory medicine at the cantonal hospital of Aarau by using HPLC and LC-MS/MS routine assays according to manufacturers' instructions. The 25-OH-Vitamin D3/D2 Reagent Kit from Bio-Rad Laboratories GmbH was used for HPLC analysis (Agilent 1100 Series). For LC-MS/MS analysis the CE-marked MSMS Vitamin D Kit from PerkinElmer (AB SciexQTrap® 5500 and DionexUltiMate 3000, Waters Acquity UPLC® BEH C18 1.7 μ, 2.1×50 mm Column with VanGuard™ Pre-Column, 2.1×5 mm Column) was used. Both methods showed a good correlation concerning 25(OH)D3, which was determined by using a Quality Control. But only the LC-MS/MS method was able to distinguish between 25(OH)D$_2$ and 25(OH)D$_3$. Measurements in the study group were only performed withLC-MS/MS whereas the dataset for the clinical group was collected from both LC-MS/MS and HPLC measurements. In the study group 25-OH-Vitamin D$_3$ results are separated from 25-OH-Vitamin D$_2$ results which are listed separately.

All calculations were performed using IBM SPSS Statistics (Version 20) and Excel 2003. Comparisons of continuous variables were performed with t-test. One-tailed and two-tailed P values were used for calculating statistical significance; a value of P<0.05 was taken to be significant. The Kolmogorov-Smirnov test was used to investigate the normal distribution of the data.

Results

General overview of the study group

114 apparently healthy volunteers (88 women, 26 men; age: 21-86 years), who gave their written consent to participate in the study, were acquired. Blood sampling took place from the end of February to the end of March 2011 and a questionnaire especially designed for this study group collected information on the period of life they were in, weight, height, hair colour, skin taint, daily sun exposure, vitamin D

supplementation and estrogenic therapy at the time point of taking the blood sample. This group is referred to as study group (Table 1).

In two cases the 25(OH)D$_3$ level did not represent the total amount of 25(OH)D. 25(OH)D$_2$ concentrations of 28.3 and 31.4 nmol/l were measured in the blood serum of these participants which most likely resulted from the declared intake of vitamin D supplementation. Usually 25(OH)D$_2$ was not detectable or below the lower limit of quantification (LLOQ, 5 nmol/l). The mean concentration of 25(OH)D$_2$ was 3.1 nmol/l(range 0-31.4).

Assuming that a concentration of 75 nmol/l (50 nmol/l) or greater indicates a sufficient vitamin D status [10], 65.8% (38.6%) of all participants demonstrated a vitamin D insufficiency (or deficiency). 60.2% (35.2%) of all women and 84.6% (50.0%) of all men had 25(OH)D$_3$ concentrations below 75 nmol/l (50 nmol/l). The mean of all participants was 63.3 nmol/l while women had a significantly higher mean concentration of 25(OH)D$_3$ (mean: 66.5 nmol/l) compared to men (mean: 52.3 nmol/l, P=0.023) (Table 1).

25(OH)D$_3$ serum levels compared between age and gender

To compare age dependent 25(OH)D$_3$ serum levels within women in the study group. Participants were divided into three different groups according to their developmental stage declared in the questionnaire into pre-, meno- and postmenopausal status. The same procedure was applied to men into age related groups. These age depending groups were divided into three classes preandropausal (22-46 years), andropausal(47-67 years) and postandropausal (67-83 years) (Table 3).

Correlating the according vitamin D$_3$ serum concentration in the study group, significantly higher mean concentrations of 25(OH)D$_3$ were observed in post- and menopausal women compared to premenopausal women (means: 77.2 and 69.1 vs.47.0 nmol/l, P=0.003 and P=0.004; Table 3) while no significant difference was found between pre-, post- and andropausal men (means: 45.4 vs. 55.1 vs.

Variable	Women (n=88)	Men (n=26)
Age [a] (mean ± SD)	57.4 ± 4.3	62.8 ± 14.0
Weigt [kg] (mean ± SD)	61.0 ± 9.5	73.9 ± 8.6
Height [m] (mean ± SD)	1.64 ± 0.06	1.75 ± 0.07
BMI [kg/m²] (mean ± SD)	22.8 ± 3.5	24.2 ± 1.8
Using vitamin D supplements [N]	22	5
25(OH)D$_3$ [nmol/l] (mean ± SD)	66.5 ± 33.4	52.3 ± 24.8
	P=0.023	
Percentage <50 nmol/l	35.20%	50.00%
Percentage <75 nmol/l	60.20%	84.60%

Table 1: Characteristics of the study group. Women have a significantly higher mean 25(OH)D$_3$ concentration compared to men (P=0.023).

| Variable		Women	Men
Characteristics of clinical group		(n=2'766)	(n=1'532)
Age(years)		0-99	0-98
25(OH)D$_3$	[nmol/l] (mean ± SD)	69.6 ± 34.8	62.4 ± 30.2
		P<0.00001	
Percentage of			
25(OH)D$_3$	<50 nmol/l	34.30%	40.50%
Percentage of			
25(OH)D$_3$	<75 nmol/l	61.70%	68.6%

Table 2: Mean and standard deviation of 25(OH) D$_3$ serum concentration for men and women in the clinical group. 34.3% women and 40.5% men had 25(OH)D$_3$ concentrations below 50 nmol/l. 61.7% women and 68.6% men had 25(OH)D$_3$ concentrations below 75 nmol/l. Women have a significantly higher mean 25(OH)D$_3$ concentration (mean: 69.6 nmol/l) compared to men (mean: 62.4 nmol/l, P<0.00001).

Age class	Women 25(OH)D$_3$ (mean ± SD)	Vitamin D supplementation [%]		Period of life	Men 25(OH)D$_3$ (mean ± SD)
premenopausal	47.0 ± 28.0	10.5	0	preandropausal	45.4 ± 10.0
	P=0.004	*P* =0.463		(22-46 years old)	*P*=0.276
Menopausal	69.1 ± 29.8	26.7	18.2	andropausal	55.1 ± 26.2
	P=0.165	*P* =0.080		(47-67 years old)	*P*=0.360
postmenopausal	77.2 ± 38.2	33.3	25	postandropausal (68-83 years old)	51.4 ± 27.2
pre- *vs.* postmenopausal	*P*=0.003	*P*=0.022		pre- *vs.* postandropausal	*P* =0.372

Table 3: Women and men of the study group were divided into three age classes. Women were divided into premenopausal, menopausal and postmenopausal. Men were divided depending on their age into preandropausal (22-46 years), andropausal (47-67 years) and postandropausal (68-83 years) groups depending on age. 25(OH)D$_3$ serum levels are shown in mean with SD. Vitamin D supplementation is shown in percentage. Men preandropausal (N=4), andropausal men (N=10), men postandropausal (N=12), women premenopausal (N=18), women menopausal (N=48), women postmenopausal (N=22).

Women	25(OH)D$_3$[nmol/l] mean ± SD		Men
N=88	P = 0.263		N=26
Using	**101.6 ± 33.0**	**81.1 ± 22.5**	**Using**
vitamin D supplements	*P*=0.032	*P*=0.001	vitamin D supplements
Not using	**65.1 ± 35.5**	**39.3 ± 15.2**	**Not using**
	P=0.026		

Table 4: Comparison of 25(OH)D$_3$ serum concentrations of men and women in the study group using and not using vitamin D supplements. 25(OH)D$_3$ serum concentrations are presented in mean ± SD. While no significant difference was observed comparing women and men using vitamin D supplements. Significantly higher 25(OH)D$_3$ concentrations were observed by comparing women to men who not using vitamin D supplements (*P* =0.026).
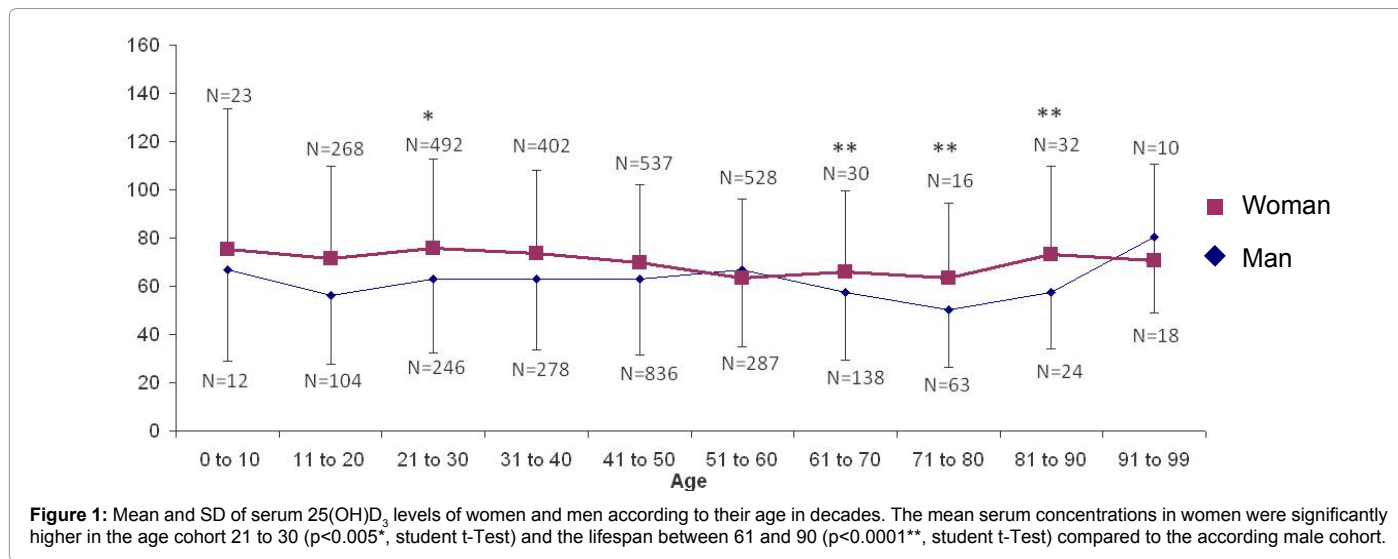
Figure 1: Mean and SD of serum 25(OH)D$_3$ levels of women and men according to their age in decades. The mean serum concentrations in women were significantly higher in the age cohort 21 to 30 (p<0.005*, student t-Test) and the lifespan between 61 and 90 (p<0.0001**, student t-Test) compared to the according male cohort.

51.4 nmol/l, P=0.276, P=0.360 and P=0.372; Table 3). This observation was also confirmed by comparing the data of postandropausal men and postmenopausal women not using vitamin D supplements. While no significant difference was found between postandropausal men and postmenopausal women who were using vitamin D supplements (means: 81.1 vs. 101.6 nmol/l, P=0.263, Table 4) postmenopausal women had a significantly higher mean concentration of 25(OH)D$_3$ compared to postandropausal men who were not using vitamin D supplements (65.1 vs. 39.3 nmol/l, P = 0.026; Table 4). So with or without vitamin D supplementation postmenopausal women showed clearly higher mean 25(OH)D$_3$ concentrations compared to postandropausal men (Tables 3 and 4).

To harden our hypothesis, that women after menopause have higher 25(OH)D$_3$ levels than men, we looked at all the 25(OH)D$_3$ measurements from the years 2010 to 2012 made at the centre of laboratory medicine in the cantonal hospital in Aarau.

This group of participants is referred to as clinical group. The clinical group was divided into groups depending on their age according to the study. Additionally we compared cohorts between men and women divided into ten year decades (Figure 1). We show that during childhood (from 0 to 10 years) the serum levels were higher than in any other cohort, the data between boys (N=18) and girls (N=10) (mean: 72.7 vs. 72.8 nmol/l) was comparable and showed no statistical difference. But the number of measurements for childhood seems to be too small to give any further conclusions about 25(OH)D$_3$ concentrations compared to other periods of life. With the beginning of adolescence at the assumed age of 12, the 25(OH)D$_3$ concentration decreases in both men (N=24) and women (N=32) (mean: 57.8 vs.69.0 nmol/l). In men (N=377) the reduction of 25(OH)D$_3$ with the beginning of adolescence in the so called preandropausal stage sinks more dramatically than in women (N=776)(mean: 55.3 vs. 60.5 nmol/l). With the beginning of the andropause 25(OH)D$_3$ rises again within the age of 40 and 50 years to 59.8 nmol/l (N=685) and after that seems at around 60 nmol/L. Women show another trend. 25(OH)D$_3$ drops slightly with the beginning of adolescence until the premenopausal stage (N=776, mean:

Age class (in years)	Women [a] 25(OH)D (mean ± SD) (interquartil)	Numbers		Age class(in years)	Men [a] 25(OH)D (mean ± SD) (interquartil)
premenopausal	64.8 ± 32.9	776	377	preandropausal	60.2 ± 28.7
(22-46)	(11-296)			(22-46)	(12-149)
	$P=0.002$	$P< 0.010$		$P=0.05$	
menopausal	69.3 ± 32.7	1064	685	andropausal	63.4 ± 30.5
(47-67)	(11-214)			(47–67)	(14-108)
	$P<0.001$	$P <0.0001$		$P=0.20$	
postmenopausal	74.2 ± 38.0	912	448	postandropausal	61.84 ± 30.7
(68-83)	(12-290)			(68-83)	(10-270)

Table 5: 25(Oh)D3 comparison between age cohorts in the clinical group. Premenopause (12-46 years), menopause (47-67 years) and the postmenopause (68-99 years).

60.5) and rises with the beginning of the menopause (N=1'064, mean: 65.0 nmol/l) to a peak in the postmenopausal stage (N=912, mean: 69.4 nmol/l). In general, women have higher 25(OH)D₃ levels than man. The statistical significance calculated with student t-Test revealed a p<0.05 between premenopausal women and preandropausal men and a p<0.0001 between postmenopausal women and postandropausal men.

Discussion and Conclusions

Postmenopausal women have the highest 25(OH)D₃ serum levels compared to younger females and any age cohort of men except in childhood. This interesting finding was already described within a cohort of women from 1988 until 1994 [12] but was not compared to men. A possible explanation is an increasing personal interest of elderly women in health issues resulting in more outdoor activities and an increased vitamin D supplementation. In our study group 33.3% of all postmenopausal women compared to only 26.7% of all menopausal and to only 10.5% of all premenopausal women were taking vitamin D supplements. Similar results were observed in the male study group where only 25.0% of all postandropausal men were taking vitamin D supplements (Table 3). Over all less vitamin D supplementation was found in men. However, lifestyle issues may not be the only explanation, because this trend was also detectable when individuals taking vitaminD supplementation were excluded from the calculations. As cholesterol is the precursor of the vitamin D metabolism, this could be an important factor which should be further investigated. The higher serum levels of cholesterol in older women compared to older men [13], could play a role in vitamin D production. Because the increasing amount of cholesterol during aging could also elevate the "starting material" for the synthesis of 25(OH)D₃. The trend shown in the study group could be verified by looking at a broad range of patients from the years 2010 until 2012.

Interestingly the trend of 25(OH)D₃ serum levels of men and women correlates negatively with the production of sexual steroid hormones. This could also be a considerable explanation for the higher levels of 25(OH)D₃ in elder women. To proof if there is a correlation between the production of steroid hormones and vitamin D in the serum, we measured Aldosteron, 11-Deoxycortisol, DHEAS, Corticosteron, Androstendion, Testosteron, 17-OH-Progesteron, DHEA, Progesteron, Estradiol, SHBG, FSH and LH in the serum of the study group and correlated the results with 25(OH)D (data not shown). Unfortunately no significant direct correlation between steroid hormones and 25(OH)D was found. Even though we could not find a direct significant correlation between other steroids and 25(OH)D, we postulate that there is a correlation between age, steroid production and cholesterol content. Regarding the fact, that hormone production and metabolism interact always in a very sophisticated way.

Taken together, the results of both settings showed that the mean concentration of 25 (OH)D₃ in women after childhood is significantly higher than in men (p<0.05, Student T-test). Surprisingly, the serum levels of post- and menopausal women of the clinical group were even higher than in premenopausal women except childhood (p<0.0001, Student t-Test). Our results stay in contrast to the common estimation, that elderly women have a lower blood concentration of 25(OH)D compared to younger women. Therefore our results imply that the discussion about vitamin D should include adapted reference values, more precise knowledge of the interaction between sexual hormones, cholesterol, vitamin D and calcium homeostasis and nevertheless supplementation of Vitamin D even before menopause (Table 5).

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