

Chapter 2

The impact of medication on vitamin D status in older individuals



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ABSTRACT

Objective

Vitamin D deficiency and polypharmacy are common in the elderly. However, knowledge on the associations between the use of specific medicines and serum 25-hydroxyvitamin D (25(OH)D) is limited. The aim of this study was to (better) define the associations between the use of specific medicines and serum 25(OH)D.

Methods

Two different cohorts (1995/1996 and 2002/2003) from the Longitudinal Aging Study Amsterdam (LASA) were used for cross-sectional analyses. LASA is based on an age and sex-stratified random sample of the Dutch older population. Study participants were aged 65-88 years in the first cohort (N = 1301) and 55-65 years in the second cohort (N = 736). Serum 25(OH)D of users of several groups of medicines were compared with levels of non-users using multiple linear regression analysis.

Results

Of all participants, 75.4% (first cohort) and 61.1% (second cohort) were using at least one medicine. In both cohorts, the number of medicines was associated with lower serum 25(OH)D. In the first cohort, after adjustment for confounding, users of any kind of medicine, loop diuretics and inhaled corticosteroids (only men) had respectively 4.4 nmol/l ($P < 0.01$), 4.7 nmol/l ($P = 0.04$), and 7.3 nmol/l ($P = 0.02$) lower serum 25(OH)D than non-users. In the second cohort, the use of oral antidiabetics, calcium-channel blockers, and angiotensin-converting enzyme inhibitors was associated with respectively 7.4 nmol/l ($P = 0.04$), 7.7 nmol/l ($P = 0.01$), and 7.6 nmol/l ($P < 0.01$) lower serum 25(OH)D.

Conclusions

These data show that users of several medicines have lower serum 25(OH)D than non-users. Vitamin D supplementation may be considered in patients with chronic use of medicines.

INTRODUCTION

Vitamin D deficiency is common in older individuals. Depending on country and used definition, the prevalence of vitamin D deficiency in the older Western population ranges from 0 up to 90% [1]. Low serum 25-hydroxyvitamin D (25(OH)D) in the elderly is caused by a less efficient vitamin D production in the skin, low sunshine exposure, and low dietary intake [1;2].

Older individuals often suffer from chronic diseases [3], prompting the frequent use of medication. Previous research, performed in the United States, demonstrated that 23% of women and 19% of men took five or more prescription medicines. In addition, rates of use were increasing with advancing age [4]. In the Netherlands, individuals of 75 years and older use five times as much medication as the average Dutch person [5].

As stated above, low serum 25(OH)D as well as polypharmacy are common in older individuals. To our knowledge, information about the influence of medicines on serum 25(OH)D is limited. For example, some statins [6-8] are found to elevate serum 25(OH)D, whereas anti-epileptic drugs lower serum 25(OH)D [9;10]. Further knowledge about associations is warranted, because of the large influence of vitamin D in several physiological processes [1] and the possibility to prescribe vitamin D supplements in case of a lowering effect.

The aim of this study was to determine whether frequently used medicines are associated with serum 25(OH)D in older individuals. The analyses were performed with data derived from the Longitudinal Aging Study Amsterdam (LASA), an ongoing cohort study of Dutch older individuals.

SUBJECTS AND METHODS

Study participants

Data for this study were collected in the ongoing LASA. LASA is a prospective Dutch cohort study of older individuals, aged 55-85 years at the start in 1992. The sampling and data collection procedures and nonresponse data are described elsewhere in detail [11;12]. Briefly, a random sample of men and women, stratified by age, sex and expected five-year mortality rate, was drawn from population registers from eleven municipalities (in three geographical regions) in the Netherlands. At baseline 3107 subjects aged 55-85 years were enrolled, and in 2002 an additional cohort was recruited which consisted of 1002 subjects aged 55-65 years. The study was approved by the Medical Ethics Committee of the VU University Medical Center and all participants gave informed consent.

For the present study, data from the second measurement cycle of the first cohort (1995/1996) and from the first measurement cycle of the second cohort (2002/2003) were used. In the first cohort, persons who participated in the medical interview in 1995/1996, born in or before 1930 (aged 65 years and older as of January 1, 1996), were selected (n=1509). In 1352 of these persons, blood samples were drawn and serum 25(OH)D could be determined in 1320 samples. After exclusion due to missing values for potential confounders (n=19), the study sample consisted of 1301 subjects.

In the second cohort, 919 subjects completed the medical interview. Blood samples were drawn from 747 persons, and serum 25(OH)D could be determined in 739 persons. After exclusion due to missing values for potential confounders or medication use (n = 3), the study sample consisted of 736 subjects.

Serum 25(OH)D

Morning blood samples were obtained in 1995/1996 and in 2002/2003. Subjects were only allowed to take tea and toast, but no dairy products. The samples were centrifuged and stored at -20 °C until determination. For the samples from 1995/1996 serum 25(OH)D was measured in 1997/1998, and for the samples from 2002/2003 measurement took place in 2009. A competitive protein binding assay was used in 1997/1998 (Nichols Diagnostics, San Juan Capistrano, CA, USA) and a radioimmunoassay in 2009 (Diasorin, Stillwater, Minnesota, USA). The interassay coefficients of variation were 10% for both methods. The Nichols and Diasorin assay were compared by measuring 117 samples (41 LASA participants, and 76 patient samples, measured for clinical purposes) with both methods (range from <5 - 123 nmol/l). This cross-calibration showed that levels of 25, 50, and 75 nmol/l measured with the Nichols device equaled 26.0, 48.2, and 70.4 nmol/l, respectively, when measured with the Diasorin-device. The correlation coefficient was $r = 0.94$. For this study, original non-calibrated values were used. All analyses were performed in the Endocrine Laboratory of the VU University Medical Center.

Medication use

Medication use was assessed during the medical interview. Participants were asked to show their medication containers to the interviewers. The medication names were recoded into ATC-codes using the ATC index from the World Health Organization. These ATC-codes were used to create groups of users of specific groups of medicines [13].

Potential effect modifier

Gender was examined as a potential effect modifier. This was done because gender is known to be important in pharmacokinetics and pharmacodynamics due to differences in, for example, hepatic and renal processes [14].

Potential confounders

The following potential confounders were included in the statistical analyses: age, gender, number of chronic diseases, body mass index (BMI), smoking, alcohol use, education level, season of blood collection, albumin, estimate of renal function (modification of diet in renal diseases, MDRD), and physical activity.

The number of chronic diseases was obtained by self-report, using questions on seven major diseases: chronic obstructive pulmonary disease, cardiac disease, peripheral arterial disease, diabetes mellitus, stroke, cancer, and rheumatoid arthritis/osteoarthritis. BMI was calculated as body weight in kilograms divided by height in square meters and subsequently it was categorized into three groups: underweight ($\text{BMI} < 20 \text{ kg/m}^2$), normal weight ($20 \text{ kg/m}^2 \leq \text{BMI} < 25 \text{ kg/m}^2$) and overweight ($\text{BMI} \geq 25 \text{ kg/m}^2$). Body weight was measured without clothes and shoes using a calibrated balance scale. Body height was measured using a stadiometer. Smoking (never, former and, current smoker) and alcohol consumption (non, light, moderate and (very) excessive drinker) were based on self-report. Classification of alcohol use was based on the number of days per week alcohol was consumed and the number of drinks per time [15].

Education level was converted into years of education, and subsequently it was dichotomized into two categories: low level (≤ 9 years) and high level (> 9 years). Serum albumin was measured using a photometric assay in three different laboratories (one in each region). MDRD was calculated from serum creatinine (measured using Hitachi 747 analyzer), using the following formula: $186(\text{creatinine}(\mu\text{mol/l})/88.4)^{-1.154} \times \text{age}(\text{years})^{-0.203}$ ($\times 0.742$ if woman) [16]. Season of blood collection was dichotomized into summer (April-September) and winter (October-March). Physical activity was assessed using the LASA Physical Activity Questionnaire (LAPAQ), a validated interviewer-administered questionnaire about the duration and frequency of activities during the past two weeks [17].

Statistical analysis

Analyses were performed if the user group consisted of $\geq 5\%$ of the study sample. Multiple linear regression analysis was used to examine differences in serum 25(OH)D between medication users and non-users. Assumptions of linear regression analysis were tested by normal probability plots and histograms. To test for interaction with gender, P -value < 0.1 was considered as statistically significant. When considered significant, further analyses were performed in subgroups. To check for confounding, all potential confounders were added one by one to the univariable model. Parameters which gave a change in regression coefficient (unstandardized Beta (B)) of more than 10% were added to the model. After this procedure, we created three models with the most relevant confounders. The first model adjusted for age and gender. The second model additionally adjusted for chronic diseases. The third model further adjusted for BMI, MDRD, albumin, smoking, alcohol use and season of blood collection. For all models $P < 0.05$ was considered as significant. To further demonstrate differences in age, an additional analysis was performed in subgroups in the first cohort (divided by the median age, 75 years). Because we did not have data on vitamin D supplementation, sensitivity analyses were performed using the fully adjusted models, in which we added multi-vitamin use as an additional confounder in the first cohort. In the second cohort, data on multi-vitamin use was not available. Physical activity was added separately to the third model to determine whether it was a mediator or a confounder. All analyses were performed using SPSS version 15.0 for windows (SPSS Inc., Chigaco, IL, USA).

RESULTS

In the first cohort (1995/1996) a total of 1301 participants (634 men, 667 women) and in the second cohort (2002/2003) 737 participants (338 men, 399 women) were included in the analyses. Table 1 shows the characteristics of the study population. Owing to inclusion criteria with regard to age (65+ in first cohort; 55-65 years in second cohort) participants of the first cohort were much older than participants of the second cohort (mean age 75.5 (SD 6.6) versus 60.0 (SD 2.9) years). The mean 25(OH)D was 53.4 (SD 24.1) nmol/l in the first cohort and 56.7 (SD 20.7) nmol/l in the second cohort. In the two cohorts, 11.1% (first cohort) and 3.4% (second cohort) of the participants had serum 25(OH)D below 25 nmol/l, 48.3% (first cohort) and 40.5% (second cohort) had serum 25(OH)D below 50 nmol/l. Serum 25(OH)D values cannot be compared directly, because different assays were used. Of all participants, 25.1% (first cohort) and 15.4% (second cohort) used four or more medicines.

Table 1. Characteristics of the whole study sample.

	1995/1996	2002/2003
N	1301 (100)	736 (100)
Gender		
male	634 (48.7)	338 (45.9)
female	667 (51.3)	398 (54.1)
Age (years)	75.5 (6.6)	60 (2.9)
25-hydroxyvitamin D (nmol/l)	53.4 (24.1)	56.7 (20.7)
Albumin (g/l)	41.9 (4.0)	40.9 (3.0)
MDRD (ml/min/1.73m ²)	65.0 (13.9)	69.7 (13.0)
BMI (kg/m ²)		
< 20 (underweight)	51 (3.9)	13 (1.8)
20-25 (healthy weight)	399 (30.7)	215 (29.2)
> 25 (overweight)	851 (65.4)	508 (69.0)
Smoker status		
Never	465 (35.7)	177 (24.0)
Former	598 (46.0)	359 (48.8)
Current	238 (18.3)	200 (27.2)
Alcohol consumption		
Non-drinker	318 (24.4)	59 (8.0)
Light drinker	653 (50.2)	341 (46.3)
Moderate drinker	251 (19.3)	257 (34.9)
(Very) excessive drinker	79 (6.1)	79 (10.7)
Level of education		
Low (\leq 9 years)	797 (61.3)	320 (43.5)
High (> 9 years)	504 (38.7)	416 (56.5)
Season of blood collection		
Winter	721 (55.4)	650 (88.3)
Summer	580 (44.6)	86 (11.7)
Chronic diseases*	1.0 [0-2]	1.0 [0-1]
Number of medicines		
none	320 (24.6)	286 (38.9)
1-3	654 (50.3)	336 (45.7)
4-6	266 (20.4)	85 (11.5)
> 6	61 (4.7)	29 (3.9)
Physical activity (min/day)	149.2 (97.4)	167.5 (105.6)

Values are mean (SD), number (percentage) or median [interquartile range]. * Chronic diseases from seven majors: chronic obstructive pulmonary disease, cardiac disease, peripheral arterial disease, stroke, diabetes mellitus, rheumatoid arthritis/osteoarthritis and cancer.

Table 2. Unadjusted mean serum 25-hydroxyvitamin D (25(OH)D) for users and non-users of different medication groups

Medication group	1995/1996; N = 1307			2002/2003; N = 737		
	Serum 25(OH)D (nmol/l)		N (users)	Serum 25(OH)D (nmol/l)		N (users)
	users	non-users		users	non-users	
Any kind	981	51.4 (24.0)	451	55.8 (20.5)	451	54.3 (16.6)
total	467	-	205	56.3 (21.8)	205	60.9 (21.8)
men	514	-	246	-	18	-
women	78	-	9	-	9	-
Coumarin derivatives	55	58.9 (24.9)	58.0 (24.4)	-	-	-
total	23	35.5 (21.1)	49.4 (22.8)	-	-	-
men	64	52.1 (26.5)	53.4 (24.0)	-	-	-
women	36	-	-	51.6 (17.2)	54	57.1 (20.9)
H ₂ -blockers	52	-	-	48.2 (19.4)	36	57.1 (20.7)
Proton pump inhibitors	58	-	-	52.7 (18.7)	95	57.3 (20.9)
Oral antidiabetics	85	48.8 (23.7)	53.7 (24.1)	-	4	-
Statins	115	49.8 (23.2)	53.7 (24.2)	-	18	-
Digoxin	137	53.4 (26.6)	53.4 (23.8)	48.5 (18.0)	51	57.3 (20.8)
Nitrates	144	51.1 (23.4)	53.6 (24.2)	49.1 (18.7)	75	57.6 (20.8)
Ca-channel blockers	161	49.4 (24.0)	53.9 (24.1)	53.9 (23.4)	64	57.0 (20.4)
ACE-inhibitors	106	43.9 (22.4)	54.2 (24.1)	-	7	-
Thiazide diuretics	120	46.5 (22.7)	54.1 (24.1)	-	14	-
Loop diuretics	208	53.6 (23.2)	53.3 (24.3)	54.3 (20.7)	99	57.1 (20.7)
Potassium sparing diuretics	355	50.8 (23.6)	54.3 (24.2)	-	135	-
Beta-blocking agents	170	-	-	56.3 (22.5)	70	54.9 (18.2)
NSAID's ¹	185	-	-	53.3 (21.5)	65	59.0 (22.0)
Platelet aggregation inhibitors	240	50.4 (23.2)	54.0 (24.3)	54.3 (19.5)	77	57.0 (20.8)
Benzodiazepines	183	46.3 (23.4)	54.5 (24.0)	52.7 (22.7)	54	57.1 (20.5)
Paracetamol	70	-	-	-	19	-
Inhaled corticosteroids	18	43.5 (25.7)	58.5 (24.3)	-	5	-
total	52	44.2 (25.5)	49.3 (22.6)	-	14	-
men	80	-	-	54.9 (20.4)	37	56.8 (20.7)
women	55	52.0 (24.6)	58.7 (24.4)	-	17	-
total	25	53.4 (27.3)	48.7 (22.7)	-	20	-

¹ Non-steroidal anti-inflammatory drugs. Analysis was performed in subgroups (stratified by sex) if p-value for interaction term was < 0.1.

Table 2 shows unadjusted mean values of serum 25(OH)D for users and non-users of several medication groups. Not all medication groups were analyzed in both groups, because we only performed an analysis when the user group consisted of $\geq 5\%$ of the study population and because medication use differed between the two cohorts due to differences in date of measurement, and in age of the participants. In general, the use of medicines was associated with lower serum 25(OH)D in comparison with non-use. Comparing the two cohorts, a higher use in proton pump inhibitors and statins and a lower use in digoxin, nitrates, coumarin derivatives, diuretics, benzodiazepines, and H₂-blockers were demonstrated in the second cohort compared with the first cohort.

Table 3 presents the results of linear regression analyses of the association between the use of several types of medication and serum 25(OH)D for the first cohort. A significant interaction with gender was found for coumarin derivatives, paracetamol, and inhaled corticosteroids. Using any medicine was associated with a lower serum 25(OH)D in the fully adjusted model. In the analyses stratified for age, we only found a significant association in the oldest group (75 years and older; data not shown). For coumarin derivatives, only in women was a significant association (models 1 and 2) with serum 25(OH)D observed, while in model 3 the association became borderline significant. In the first and third models, loop diuretics were significantly associated with lower serum 25(OH)D. Only in men, the use of inhaled corticosteroids and paracetamol (in models 1 and 2) was associated with lower serum 25(OH)D.

Table 4 presents the results of linear regression analyses of the association between the use of several types of medication and serum 25(OH)D for the second cohort. A significant interaction for gender was found for the use of any kind of medicine and non-steroidal anti-inflammatory drug (NSAID). A significant association between the use of any medicine and lower serum 25(OH)D was found, but only for women in the first model. In the second model the association became borderline significant. A borderline significant association between the use of proton pump inhibitors and lower serum 25(OH)D was observed in the fully adjusted model. The associations between oral antidiabetics, calcium-channel blockers, and ACE inhibitors and lower serum 25(OH)D were significant in the fully adjusted model. We did not observe any significant relationship between statin use and serum 25(OH)D.

Figure 1 presents the relation between number of medicines and serum 25(OH)D. In both cohorts the number of medicines was negatively associated with serum 25(OH)D (first cohort: $B = -0.7$, $P < 0.01$, second cohort: $B = -1.1$, $P < 0.01$), after adjustment for confounding.

The addition of multi-vitamin use to the models did not materially change the results. After the addition of physical activity, only the B of the use of oral antidiabetics with 25(OH)D in the second cohort changed from -7.3 to -6.9 ($P = 0.06$; data not shown).

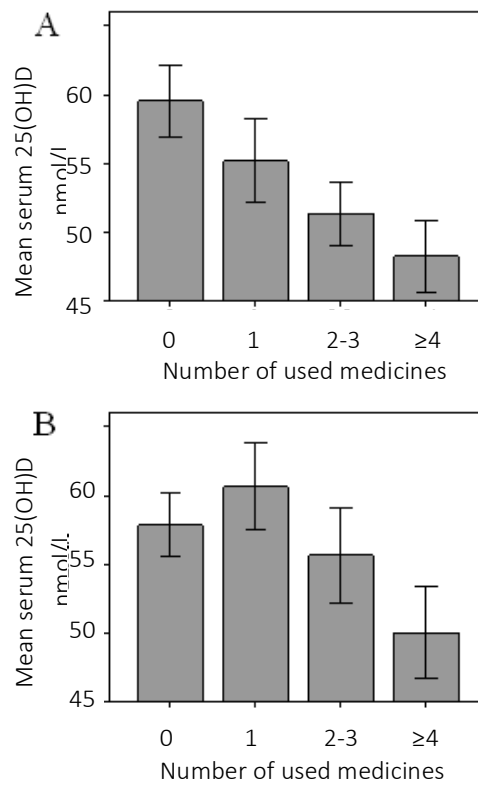


Figure 1. Mean serum 25(OH)D (in nmol/L), with 95% confidence intervals, plotted against number of used medicines. Users of medicines are divided into three groups according to tertiles. A: First cohort (1995/1996), B: Second cohort (2002/2003).

Table 3. Results of multiple linear regression analysis of associations between medication use and serum 25(OH)D in the first cohort (1995/1996). Data are expressed as regression coefficient (B) (95% confidence intervals)

	Model 1 ¹		Model 2 ²		Model 3 ³	
	B (95% CI)	P-value	B (95% CI)	P-value	B (95% CI)	P-value
Any kind	-4.4 (-7.2; -1.5)	<0.01	-3.5 (-6.5; -0.5)	0.02	-4.2 (-7.0; -1.3)	<0.01
Coumarin derivatives						
men	-0.3 (-6.7; 6.0)	0.92	1.5 (-5.1; 8.3)	0.65	1.6 (-4.8; 8.1)	0.62
women	-12.2 (-21.0; -3.4)	<0.01	-10.5 (-19.5; -1.4)	0.02	-8.6 (-17.3; 0.1)	0.05
H ₂ -blockers	-0.6 (-6.1; 5.0)	0.83	0.0 (-5.5; 5.6)	0.99	0.3 (-5.0; 5.6)	0.90
Digoxin	-0.1 (-5.1; 4.8)	0.96	1.3 (-3.8; 6.3)	0.62	0.5 (-4.3; 5.3)	0.84
Nitrates	1.0 (-5.3; 3.3)	0.66	0.4 (-3.9; 4.9)	0.84	0.1 (-4.1; 4.3)	0.96
Ca-channel blockers	2.6 (-1.3; 6.6)	0.19	3.8 (-0.2; 7.8)	0.07	2.9 (-0.9; 6.8)	0.13
ACE-inhibitors	-2.6 (-6.4; 1.2)	0.19	-1.7 (-5.6; 2.1)	0.38	-2.7 (-6.4; 1.0)	0.15
Thiazide diuretics	-1.1 (-4.8; 2.6)	0.57	-1.0 (-4.6; 2.7)	0.61	-0.7 (-4.2; 2.8)	0.67
Loop diuretics	-4.5 (-8.9; 0.0)	0.05	-3.5 (-8.0; 1.0)	0.13	-4.7 (-9.0; -0.3)	0.04
Potassium Sparring diuretics	-2.3 (-6.5; 1.9)	0.29	-2.0 (-6.2; 2.2)	0.35	-2.0 (-6.0; 2.0)	0.32
Beta-blocking agents	0.0 (-3.3; 3.3)	0.99	0.6 (-2.7; 3.9)	0.71	0.4 (-2.8; 3.6)	0.80
NSAID's ⁴	-1.0 (-3.7; 1.7)	0.46	-0.2 (-3.0; 2.5)	0.87	-0.7 (-3.3; 2.0)	0.62
Platelet aggregation blockers	-1.2 (-4.3; 2.0)	0.47	-0.3 (-3.5; 2.9)	0.87	-1.1 (-4.2; 1.9)	0.48
Benzodiazepines	-3.7 (-7.2; -0.1)	0.04	-3.0 (-6.6; 0.5)	0.09	-2.8 (-6.2; 0.6)	0.10
Paracetamol	-11.7 (-22.5; -1.0)	0.03	-10.8 (-21.6; 0.0)	0.05	-7.4 (-17.4; 3.0)	0.16
Inhaled corticosteroids						
men	-2.0 (-8.1; 4.0)	0.51	-1.1 (-7.2; 5.0)	0.73	0.2 (-5.7; 6.1)	0.95
women	-6.5 (-12.8; -0.2)	0.05	-5.5 (-12.0; 1.1)	0.10	-7.3 (-13.7; -1.0)	0.02
	2.7 (-5.8; 11.2)	0.53	4.1 (-4.4; 12.7)	0.34	4.8 (-3.4; 13.0)	0.25

¹ Adjusted for age and gender (if no stratification for gender).

² Adjusted for age, gender (if no stratification for gender), and number of chronic diseases.

³ Adjusted for age, gender (if no stratification for gender), number of chronic diseases, BMI, MDRD, albumin, smoking, alcohol use, and season of blood collection.

⁴ Non-steroidal anti-inflammatory drugs

Table 4. Results of multiple linear regression analysis of associations between medication use and serum 25(OH)D in the second cohort (2002/2003)

	Model 1 ¹		Model 2 ²		Model 3 ³		
	B (95% CI)	P-value	B (95% CI)	P-value	B (95% CI)	P-value	
Any kind	men	1.2 (-3.0; 5.5)	0.56	2.9 (-1.6; 7.5)	0.21	3.0 (-1.5; 7.5)	0.20
	women	-4.4 (-8.9; 0.0)	0.05	-4.4 (-9.0; 0.3)	0.07	-3.4 (-8.1; 1.2)	0.14
Proton pump inhibitors		-5.6 (-11.3; 0.1)	0.06	-5.2 (-11.0; 0.5)	0.07	-5.4 (-11.1; 0.2)	0.06
Oral antidiabetics		-8.6 (-15.5; -1.7)	0.02	-7.7 (-14.8; -0.5)	0.04	-7.3 (-14.4; -0.3)	0.04
Platelet aggregation inhibitors		-1.7 (-6.6; 3.3)	0.51	-0.3 (-5.6; 5.0)	0.91	-0.7 (-6.0; 4.4)	0.77
Statins		-4.2 (-8.7; 0.3)	0.07	-3.4 (-8.1; 1.4)	0.17	-3.4 (-8.0; 1.3)	0.16
Ca-channel blockers		-7.8 (-13.8; -1.9)	0.01	-7.2 (-13.2; -1.1)	0.02	-7.7 (-13.6; -1.7)	0.01
ACE-inhibitors		-7.8 (-12.7; -2.8)	<0.01	-7.4 (-12.4; -2.3)	<0.01	-7.6 (-12.5; -2.7)	<0.01
Thiazide diuretics		-3.2 (-8.5; 2.1)	0.23	-2.8 (-8.2; 2.5)	0.30	-3.8 (-9.0; 1.5)	0.16
Beta-blocking agents		-2.5 (-6.9; 1.8)	0.26	-2.1 (-6.5; 2.4)	0.36	-2.0 (-6.4; 2.4)	0.37
NSAID's ⁴	men	1.3 (-3.8; 6.3)	0.62	3.9 (-1.8; 9.5)	0.18	3.1 (-2.5; 8.7)	0.27
	women	-5.5 (-11.3; 0.3)	0.06	-5.3 (-11.3; 0.7)	0.08	-4.0 (-9.9; 1.9)	0.19
Benzodiazepines		-5.2 (-11.1; 0.6)	0.08	-4.7 (-10.6; 1.2)	0.12	-2.4 (-8.2; 3.4)	0.42
Inhaled corticosteroids		-1.8 (-8.6; 5.0)	0.61	-0.5 (-7.5; 6.6)	0.89	-0.5 (-7.4; 6.4)	0.89

Data are expressed as regression coefficient (B) (95 % confidence intervals)

¹ Adjusted for age and gender (if no stratification for gender).

² Adjusted for age, gender (if no stratification for gender), and number of chronic diseases.

³ Adjusted for age, gender (if no stratification for gender), number of chronic diseases, BMI, MDRD, albumin, smoking, alcohol use, and season of blood collection.

⁴ Non-steroidal anti-inflammatory drug

DISCUSSION

In this study, we examined associations between specific medicines and serum 25(OH)D. In the first cohort, aged 65 years and older, associations with lower serum 25(OH)D were observed in individuals who used any medication, loop diuretics or inhaled corticosteroids (only in men). In the second cohort, aged 55-65 years, associations with lower serum 25(OH)D were found in individuals using oral antidiabetics, calcium channel blockers or ACE-inhibitors. To our knowledge, these associations have not been investigated before. The addition of physical activity to the models did not change the results significantly. This indicates that physical activity is neither a relevant confounder nor a mediator in the relationship between medication use and vitamin D status. We question the possible causes of lower serum 25(OH)D in the user group, but due to study design no inference can be made about causality. However, three causes have to be considered. First, it could be the medicines themselves. Second, chronic diseases leading to medication use could contribute to lower levels (confounding by indication). And finally, a (chronic) disease might be a consequence of vitamin D deficiency.

In the first cohort, the use of any medication was associated with lower serum 25(OH)D. When analyzing in subgroups (divided by the median age, 75 years), the association was only significant in the oldest group. This is in accordance with the results of the second cohort, which consisted of younger persons and in which we found no association. Also the number of medicines used was associated with lower serum 25(OH)D. To our knowledge, these associations were demonstrated for the first time. It is impossible to speculate on the cause of this relationship, because of the diversity of medicines used. Medication use may be considered as a manifestation of frailty in older individuals, which Lang et al. also suggest [18].

Our findings regarding the association of loop diuretic use and lower serum 25(OH)D are in accordance with previous research of Rejnmark et al. [19], although the significant association that they observed disappeared after adjustment for confounding. Adjustment for confounding was slightly different compared with our study. For example, no correction for chronic diseases was performed in that study, on the other hand the use of vitamin D supplements was taken into account [19]. Unfortunately, over-the-counter vitamin D use was not available in our study. The sensitivity analysis which added multivitamin use, however, did not change our results. A randomized clinical trial showed an increase in serum PTH and serum 1,25(OH)₂D after 7 days of loop diuretic use compared with placebo [20]. Loop diuretics increase renal calcium excretion, causing secondary hyperparathyroidism. Hyperparathyroidism causes an increased hydroxylation of 25(OH)D into 1,25(OH)₂D, which results in higher levels of 1,25(OH)₂D [19;20]. The increased conversion might partly explain the decreased level of 25(OH)D within the user population.

In addition, an increased catabolism of serum 25(OH)D stimulated by higher levels of 1,25(OH)₂D could contribute to a lower serum 25(OH)D [21]. In the present study we did not measure 1,25(OH)₂D. In the second cohort, loop diuretic use was much lower and the association with 25(OH)D could not be studied.

Previous research found no effect of inhaled glucocorticosteroids on serum 25(OH)D [22]. The findings in our second cohort are in line with this research. However, in the first cohort we did observe a significant association with lower serum 25(OH)D in men. Vitamin D deficiency is highly prevalent in chronic obstructive pulmonary disease (for which inhaled corticosteroids are mainly used), and serum levels correlate with severity of this disease [23;24]. Confounding by indication may indeed contribute to the association between inhaled corticosteroids use and lower serum 25(OH)D.

Vitamin D deficiency is associated with increased insulin resistance and decreased insulin secretion [25;26]. In our second study cohort, mean serum 25(OH)D of users of oral antidiabetics was 7.3 nmol/l lower compared with non-users. Although addition of physical activity to the model lead to a non-significant P value, the absolute difference in 25(OH)D levels did not alter materially between users and non-users of oral antidiabetics and thus the clinical relevance will be comparable. Whether this difference is caused by the medication or by the diabetes should be the subject of further study.

Calcium channel blocker use resulted in 7.7 nmol/l lower serum 25(OH)D compared with non-use. The mechanism at work might be found in the metabolism of vitamin D. Precursors of 25(OH)D are formed in the skin under influence of u.v. light and some nutrients also contain these precursors [1;26]. These precursors are hydroxylated in the liver into 25(OH)D by 25-hydroxylases of cytochrome P450 isoenzymes, like CYP27A1, CYP2R1, CYP3A4 and CYPJ3. As calcium channel blockers act as an inhibitor of CYP3A4, the formation of 25(OH)D may decrease, resulting in lower serum 25(OH)D [26-28].

ACE inhibitor use resulted in 7.6 nmol/l lower serum 25(OH)D compared with non-use. Indications for ACE-inhibitor-use are heart failure and hypertension in patients with metabolic syndrome and diabetes [29;30]. Hypertension may be associated with lower serum 25(OH)D [31]. However, in LASA no associations between blood pressure and hypertension and serum 25(OH)D were found [32]. Some other epidemiological studies showed associations between low serum 25(OH)D and several cardiovascular outcomes, but evidence for a causal relationship is lacking. The results of a systematic review and meta-analysis were equivocal [33]. However, mechanistic explanations are available [34]. Studies with vitamin D receptor-null mice concluded that absence of effect of 1,25(OH)₂D is involved in the development of hypertension. Serum 1,25(OH)₂D inhibits the production of renin, which is involved in the regulation of electrolyte, volume and blood pressure homeostasis [34]. Despite these mechanistic explanations, a causal relationship between

vitamin D status and hypertension has not been confirmed in clinical trials so far [33]. Whether the observed difference in serum 25(OH)D was caused by the medication or by hypertension and cardiovascular disease or by coincidence should be the subject of further research.

Proton pump inhibitor use was associated with 5.4 nmol/l lower serum 25(OH)D, although it should be considered as borderline significant. The first cohort only contained few users and thus, analyses could not be performed. Proton pump inhibitors are associated with increased risk of osteoporotic fractures [35]. However, information about the relationship with serum 25(OH)D is limited. Recently published research showed no change in serum 25(OH)D after omeprazole therapy, but this study sample consisted of healthy young people and they used omeprazole for only 30 days [36].

Definite conclusions regarding potential relationships between use of coumarin derivatives and paracetamol and serum 25(OH)D cannot be made on the base of our study, because only a very small part of the study population used these drugs.

Previous studies reported different results about the association between statin use and serum 25(OH)D. Rosuvastatin [6;8] and atorvastatin [7] were found to increase serum levels, whereas fluvastatin and simvastatin did not change these levels significantly [6;37]. We observed no significant association between statin use and serum 25(OH)D. In the first cohort we performed the same analysis, despite the user group was smaller than 5% of the population. We did not observe an association (data not shown). Most of our participants used simvastatin (93% in first cohort and 51% in second cohort). Thus, a change in serum 25(OH)D was not expected.

Differences in used medicines are mainly due to different time points between the two cohorts (1995/1996 versus 2002/2003) and to age differences (mean age 75 years versus 60 years). Prescription of medicines changed a little in the period between the cohorts. Age differences may contribute to differences in effects of several medicines. It is likely that older persons will react differently to medication.

The main limitation of this study is that we could not exclude confounding by indication. Pragmatically, we adjusted for the number of chronic diseases. Furthermore, it was not possible to consider vitamin D intake, because information about over-the-counter vitamin D use or diet was not assessed. A sensitivity analysis however, in which multi-vitamin use was added to the models, showed no differences in observed associations. We also realize that we could not investigate the association for one specific medicine separately, because most participants did not use only one kind of medicine. The LASA population is a representative sample of the Dutch older population and this also pertains to the use of medicines. Lastly, although we tested many medication groups, we do not think the results represent a type 1 error, because the significant P values were low. The

strengths of our study include the population-based design, the large study population with a wide age range, and accuracy in reporting medication use.

Our study results show interesting associations between several types of medicines and serum 25(OH)D. Nevertheless, further studies are necessary to confirm our findings and to explore potential underlying mechanisms. Additionally, an increasing number of medicines is associated with lower serum 25(OH)D levels. This suggests that vitamin D supplementation should be considered in patients with chronic use of more than one medication.

Conflict of interest statement

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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