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Associations between calcium and vitamin D supplement use as well as their serum concentrations and subclinical cardiovascular disease phenotypes

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ABSTRACT

Background: Supplementation of calcium (Ca) and vitamin D for the prevention of osteoporosis is frequently found in Western countries. Recent re-analyses of clinical trials observed a higher risk of myocardial infarction and stroke in subjects taking Ca (+vitamin D) supplements, although the underlying mechanisms are not clear.
Objective: Thus, we analyzed the associations between Ca and vitamin D supplementation as well as serum concentrations of Ca and 25-hydroxyvitamin D (25(OH)D) and subclinical cardiovascular disease (CVD) phenotypes, namely intima-media thickness, ankle-brachial-index (ABI), intermittent claudication, and atrial fibrillation (AF).
Design: Data of 1601 participants aged 50–81 years of the population-based cross-sectional Cooperative Health Research in the Region of Augsburg (KORA) F4 study in Germany were analyzed. Logistic and linear regression models were used to estimate odds ratios (OR) (95% confidence intervals (CI)) and β-estimates (p-values), respectively.
Results: Regular Ca supplementation showed a significant positive association with the presence of AF after multivariable adjustment (OR = 3.89; 95% CI 1.28–11.81). Higher serum 25(OH)D concentrations were independently associated with a lower prevalence of asymptomatic peripheral arterial disease as

Abbreviations: ABI, ankle-brachial-index; AF, atrial fibrillation; Ca, Calcium; CI, confidence interval; CRP, C-reactive protein; CV, coefficient of variation; CVD, cardiovascular disease; GFR, glomerular filtration rate; IDOM, instrument for database-supported online registration of medication; IMT, intima-media thickness; KORA, Cooperative Health Research in the Region of Augsburg; OR, odds ratio; r_s, Spearman's rank correlation coefficient; TC/HDL, total cholesterol/high-density lipoprotein cholesterol.

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assessed by ABI measurements (β = 0.007; p = 0.01). No other significant associations between supplementation or serum concentrations of Ca or vitamin D and CVD phenotypes were identified.
Conclusions: Although based on few cases the finding of a significant higher prevalence of AF in Ca supplement users hints at one possible mechanism that may contribute to an increased risk of myocardial infarction and stroke. The observed association between serum 25(OH)D and ABI supports results from other studies.

1. Introduction

The positive effect of calcium (Ca) and vitamin D supplementation on bone health has been confirmed at different doses in many randomized controlled trials worldwide [1–3]. Therefore, use of supplements containing Ca or Ca in combination with vitamin D, is frequently recommended for prevention of osteoporosis, especially in postmenopausal women [4].

In addition, non-skeletal beneficial effects were observed for Ca and vitamin D supplementation and high serum concentrations of 25-hydroxyvitamin D (25(OH)D), including a potential protection against cardiovascular diseases (CVD) [5]. However, recent meta-analyses and a re-evaluation of the Women's Health Initiative study showed a potentially higher risk of myocardial infarction and stroke in subjects taking Ca supplements [4,6,7]. These analyses are controversially discussed [8–11]. Due to the high frequency of Ca supplementation in the population, even a small increase in CVD risk would be meaningful. However, the biological mechanisms behind such an effect are not clear yet.

Thus, we investigated the association between Ca and vitamin D supplementation and subclinical (intermediate) CVD phenotypes, namely intima-media thickness (IMT), ankle-brachial-index (ABI), and intermittent claudication in a cross-sectional population-based study. IMT, as an intermediate marker of atherosclerosis, and ABI, as an indicator for (asymptomatic) peripheral arterial disease, have already been described as useful predictors of coronary events and stroke [12–15]. Intermittent claudication is representative of symptomatic peripheral arterial disease. Since a potential relationship of Ca supplementation on myocardial infarction may also be mediated by atrial fibrillation (AF) and as AF is associated with ischemic stroke [16], AF was chosen as a further outcome.

Moreover, the relationship between serum concentrations of Ca and 25(OH)D, an accepted biomarker of vitamin D status, and the named CVD phenotypes were analyzed.

2. Subjects and methods

2.1. Study population

The present analyses are based on data of the Cooperative Health Research in the Region of Augsburg (KORA) F4 study (conducted between 2006 and 2008), the seven-year follow-up of the KORA S4 study. KORA is a research platform performing population-based surveys and subsequent follow-ups in the city of Augsburg and its two surrounding counties in Southern Germany. It has been extensively described elsewhere [17]. The study was approved by the ethics committee of the Bavarian Medical Association. Written informed consent was obtained from each participant in accordance with institutional requirements and the Declaration of Helsinki Principles.

The entire F4 survey sample comprised 3080 participants aged 32–81 years (1486 men, 1594 women). In the present analyses only subjects aged 50 years and older were included (n = 1997), because the number of subclinical CVD phenotypes and supplement users was extremely low in younger participants. Participants with prevalent myocardial infarction, stroke or cancer were excluded (n = 346). Furthermore, missing (n = 15) and implausible (n = 4) exposure data led to exclusions. Moreover participants who used supplements only as needed, i.e. not regularly (vitamin D: n = 16, Ca: n = 36), were excluded. Finally, the analytic sample comprised 1601 subjects aged 50–81 years.

2.2. Assessment and definition of subclinical CVD phenotypes

Common carotid artery IMT was measured non-invasively using high resolution B-mode ultrasound techniques. The mean far-wall IMT was determined 1 cm below the bifurcation using the average of three measurements of frozen images from both the left and right common carotid artery [18]. Elevated IMT was defined according to Stein et al. as greater than or equal to the sample's sex- and age-specific (5-year age groups) 75th percentile [19]. Accordingly, 5-year age groups were chosen for categorization (footnote 1 in Table 1).

The ABI was measured according to a standardized protocol by trained and certified examiners as described recently [20]. Systolic blood pressure was obtained with a stethoscope and a blood pressure cuff on the right arm, and with a Doppler device (HNE Healthcare) on both ankles (posterior tibial artery). Generally, two measurements were performed and the mean of these was used for further calculations. The ABI was calculated as mean systolic blood pressure of that ankle side which was lowest divided by the mean of the two brachial systolic blood pressure measurements of the right arm. As recommended by the American Heart Association, a value of 0.9 was chosen as cut-point [21] indicating asymptomatic peripheral arterial disease.

Intermittent claudication was surveyed by a standardized interview based on the Edinburgh Claudication Questionnaire [22].

Study participants received 12-lead resting electrocardiograms, applying a standardized protocol after a 10 min rest in a supine position. A trained cardiologist assessed all electrocardiograms. AF was defined visually from 10-s-electrocardiogram.

2.3. Assessment of Ca and vitamin D supplementation

During a standardized interview, all participants were asked about their intake of prescribed and over-the-counter medications and vitamin or mineral supplements within the prior seven days, using the standardized software IDOM (instrument for database-supported online registration of medication) [23]. After the exact assessment of the brand name, subjects stated, if they took the supplements regularly or as needed (irregularly). If the intake was on a regular basis, participants were additionally asked about the dose per time interval (e.g. daily, weekly).

2.4. Analysis of serum Ca and 25(OH)D concentrations

In KORA F4 blood samples were collected in a fasting state (at least 8 h overnight fasting), while the participants were sitting. Ca

Table 1
Characteristics of the participants in the KORA F4 study aged 50–81 years.

characteristic	total	elevated IMT [1]	reduced ABI [2]	intermittent claudication	atrial fibrillation
	N = 1601 (100.0 %)	n = 360 (25.4%) missings: 182	n = 64 (4.4 %) missings: 157	n = 42 (2.6 %) missings: 2	n = 20 (1.3 %) missings: 26
			n (%)		
sex					
male	758 (47.35)	170 (47.22)	40 (62.50)	23 (54.76)	9 (45.00)
female	843 (52.65)	190 (52.78)	24 (37.50)	19 (45.24)	11 (55.00)
physical activity					
active	881 (55.13)	191 (53.20)	20 (31.75)	20 (47.62)	9 (45.00)
inactive	717 (44.87)	168 (46.80)	43 (68.25)	22 (52.38)	11 (55.00)
smoking status					
current smoker	209 (13.08)	48 (13.37)	16 (25.40)	7 (16.67)	0 (0.00)
former smoker	628 (39.30)	140 (39.00)	33 (52.38)	24 (57.14)	10 (50.00)
never smoked	761 (47.62)	171 (47.63)	14 (22.22)	11 (26.19)	10 (50.00)
alcohol intake					
high (men: ≥ 40 g/day, woman: ≥ 20 g/day)	310 (19.40)	60 (16.71)	9 (14.29)	6 (14.29)	1 (5.00)
medium (men: < 40 g/day, woman: < 20 g/day)	761 (47.62)	130 (36.21)	28 (44.44)	23 (54.76)	10 (50.00)
low (0 g/day)	628 (39.30)	169 (47.08)	26 (41.27)	13 (30.95)	9 (45.00)
education level (school + apprenticeship/studies)					
low (< 12 years)	1023 (63.98)	233 (64.72)	50 (78.13)	35 (83.33)	14 (70.00)
high (≥ 12 years)	576 (36.02)	127 (35.28)	14 (21.88)	7 (16.67)	6 (30.00)
actual hypertension					
yes	773 (48.40)	196 (54.60)	46 (71.88)	28 (66.67)	14 (70.00)
no	824 (51.60)	163 (45.04)	18 (28.13)	14 (33.33)	6 (30.00)
diabetes					
yes	236 (15.05)	48 (13.71)	23 (36.51)	15 (35.71)	5 (25.00)
prediabetes [3]	348 (22.19)	76 (21.71)	15 (23.81)	15 (35.71)	3 (15.00)
no	984 (62.76)	226 (64.57)	25 (39.68)	12 (28.57)	12 (60.00)
parental myocardial infarction					
yes	469 (30.22)	112 (31.82)	21 (33.87)	8 (20.51)	4 (20.00)
no	841 (54.19)	179 (50.85)	29 (46.77)	17 (43.59)	14 (70.00)
'don't know'	242 (15.59)	61 (17.33)	12 (19.35)	14 (35.90)	2 (10.00)
vitamin D supplement use					
yes, regularly	149 (9.31)	35 (9.72)	4 (6.25)	5 (11.90)	4 (20.00)
no	1452 (90.69)	325 (90.28)	60 (93.75)	37 (88.10)	16 (80.00)
calcium supplement use					
yes, regularly	177 (11.06)	42 (11.67)	6 (9.38)	5 (11.90)	6 (30.00)
no	1424 (88.94)	318 (88.33)	58 (90.63)	37 (88.10)	14 (70.00)
serum 25(OH)D (quartiles) [4]					
> 0 nM, ≤ 25.01 nM	399 (24.92)	82 (22.78)	29 (45.31)	19 (45.24)	11 (55.00)
> 25.01 nM, ≤ 34.74 nM	401 (25.05)	89 (24.72)	12 (18.75)	7 (16.67)	1 (5.00)
> 34.74 nM, ≤ 46.42 nM	400 (24.98)	104 (28.89)	10 (15.63)	7 (16.67)	2 (10.00)
> 46.42 nM	401 (25.05)	85 (23.61)	13 (20.31)	9 (21.43)	6 (30.00)
serum calcium (quartiles)					
> 0 mM, ≤ 2.33 mM	437 (27.30)	93 (25.83)	15 (23.44)	14 (33.33)	7 (35.00)
> 2.33 mM, ≤ 2.40 mM	462 (28.86)	110 (30.56)	17 (26.56)	9 (21.43)	8 (40.00)
> 2.40 mM, ≤ 2.45 mM	323 (20.17)	56 (15.56)	13 (20.31)	9 (21.43)	4 (20.00)
> 2.45 mM	379 (23.67)	101 (28.06)	19 (29.69)	10 (23.81)	1 (5.00)
glucocorticoid use					
yes	31 (1.94)	5 (1.39)	2 (3.13)	0 (0.00)	16 (80.00)
no	1570 (98.06)	355(98.61)	62 (96.88)	42 (100.00)	4 (20.00)
vitamin K antagonist use					
yes	34 (2.13)	9 (2.51)	2 (3.17)	3 (7.14)	13 (65.00)
no	1566 (97.88)	350 (97.49)	61 (96.83)	39 (92.86)	7 (35.00)
osteoporosis					
yes	74 (5.94)	17 (5.94)	2 (6.90)	4 (15,38)	0 (0.00)
no	989 (79.37)	235 (82.17)	23 (79.31)	19 (73.08)	8 (100.00)
'don't know'	183 (14.69)	34 (11.89)	4 (13.79)	3 (11.54)	0 (0.00)
			mean ± SD		
age [years]	63.30 ± 8.56	63.17 ± 8.45	69.05 ± 8.96	66.40 ± 8.47	71.55 ± 8.02
body-mass-index [kg/m²]	28.36 ± 4.74	29.06 ± 4.64	29.46 ± 4.72	29.85 ± 5.00	30.80 ± 6.34
TC/HDL ratio	4.18 ± 1.17	4.40 ± 1.18	4.58 ± 1.24	4.48 ± 1.40	4.05 ± 1.12
glomerular filtration rate [ml/min]	84.88 ± 15.93	85.19 ± 15.01	74.32 ± 18.81	79.77 ± 18.94	69.43 ± 13.62
vitamin D supplement dose of regular users	11.29 ± 10.35	11.06 ± 7.18	14.59 ± 16.02	16.00 ± 8.22	15.00 ± 13.54
calcium supplement dose of regular users [mg/d]	449.61 ± 343.28	477.79 ± 371.16	313.28 ± 189.74	654.00 ± 439.98	493.67 ± 150.73
serum 25(OH)D [nM]	37.05 ± 18.48	35.89 ± 18.12	32.88 ± 20.56	33.29 ± 20.38	33.94 ± 26.83
deseasonalized serum 25(OH)D [nM] [4]	36.99 ± 16.54	37.42 ± 16.11	31.94 ± 19.16	33.03 ± 17.65	35.78 ± 27.12
serum calcium [mM]	2.40 ± 0.11	2.41 ± 0.11	2.41 ± 0.12	2.42 ± 0.13	2.36 ± 0.07
serum testosterone [nM]	16.20 ± 14.42	16.14 ± 14.42	18.88 ± 12.94	18.44 ± 15.02	16.67 ± 16.32
			median [25th percentile; 75th percentile]		
C-reactive protein [mg/l]	13.40 [6.60; 28.80]	16.60 [7.50; 31.00]	36.20 [17.30; 62.65]	22.20 [11.00; 42.80]	17.05 [11.40; 36.90]

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; ABI, ankle-brachial index; IMT, intima-media thickness; SD, standard deviation, TC/HDL, total cholesterol/high-density lipoprotein cholesterol ratio

1: elevated IMT is defined as sex- and age-specific IMT ≥ 75th percentile.
IMT values in millimeter for the 75th percentile:

	50-54 y	55-59 y	60-64 y	65-69 y	70-74 y	75-81 y
male	0.882	0.949	1,011	1,062	1,070	1,086
female	0.839	0.917	0.954	0.988	1,033	1,042

2: reduced ABI is defined as ABI < 0.9
3: prediabetes is defined as impaired fasting glucose and/or impaired glucose tolerance
4: deseasonalized: the residual of each value of the LOESS regression was added to the measured overall mean of serum 25(OH)D

and 25(OH)D concentrations were measured in the serum samples after storage at −80 °C. Total Ca was measured using a photometric o-Cresolphtalein method. The inter-assay coefficients of variation (CVs) were 1.2% at a concentration of 2.45 mM and 1.3% at 3.34 mM 25(OH)D was analyzed using a chemiluminescence immunoassay (LIAISON by DiaSorin) with a specificity of 100% for 25OHD2, 104% for 25OHD3 and a detection limit of 10.0 nM. The inter-assay CVs were 12.9% at a concentration of 18.0 nM and 7.3% at 319.4 nM [24].

2.5. Assessment and definition of covariables

All variables were collected by trained medical staff by standardized interviews, standardized medical examinations or standard laboratory methods, respectively, as described previously elsewhere [25]. The covariables included in the present analysis were sex, age (continuous), body-mass-index (BMI) (continuous), physical activity (active/inactive), smoking (current/former/never), alcohol intake (low/medium/high), education (low/high), actual hypertension (yes/no), diabetes (yes/prediabetes/no), total cholesterol/high-density lipoprotein cholesterol ratio (TC/HDL ratio) (continuous), C-reactive protein (CRP) (continuous), glomerular filtration rate (GFR) (continuous), history of parental myocardial infarction (yes/no/don't know), self-reported history of osteoporosis (yes/no/don't know), serum concentrations of testosterone (continuous), and use of vitamin K antagonist compounds as oral anticoagulants (yes/no), assessed by means of IDOM (see above). The definition of the covariables, especially the categorization, was also outlined previously and is displayed in Table 1 [25].

2.6. Statistical analyses

The serum 25(OH)D concentration depends among others on the participant's sun exposure and therefore on the season. To remove this seasonal effect, a de-seasonalization of serum 25(OH)D was conducted. A locally weighted regression (LOESS) was performed with the date (day/month/year) of blood collection on the 25(OH)D values, according to Cleveland [26]. A LOESS regression fits a regression surface to data through multivariate smoothing [26]. To get the final de-seasonalized values, the residual of each value of the LOESS regression was added to the measured overall mean. Hereby, three values became negative which is biologically impossible. Therefore, these were replaced by the minimum positive value of the de-seasonalized values.

For both supplementation data and serum values, logistic regression analyses were used to estimate age- and sex-adjusted as well as multivariable adjusted odds ratios (OR) of the presence of elevated IMT, reduced ABI, intermittent claudication, and AF, respectively. The exposure variables were categorized as follows: supplement use as “yes, regularly” and “no” and serum levels as quartiles, with the lowest quartile serving as reference category (except for the outcome AF, because the number of cases was too low).

To increase statistical power we used continuous serum levels in further logistic regression analyses with each outcome and in linear regressions (generalized linear models) with the outcomes IMT and ABI. To measure the goodness of fit, we used the coefficient of determination R^2. This is the percentage of the outcome variable variation that is explained by the linear model, ranging from 0 to 1. To assess possible dose–response relationships we included as sub-analyses only regular supplement users in fully adjusted regression models with the daily dose of Ca or vitamin D, respectively, as continuous exposure variable.

The multivariable adjusted models contained sex, age, BMI, physical activity, smoking, alcohol intake, education, actual hypertension, diabetes, TC/HDL ratio, CRP, GFR, and history of parental myocardial infarction as covariables. The models dealing with supplementation data were additionally adjusted for supplementary vitamin D (with exposure Ca) or Ca (with exposure vitamin D), respectively.

Moreover, additional sub-analyses were conducted to examine the relationship between supplementation and serum concentrations: correlation was tested by Spearman's rank correlation coefficient r_s and the difference in serum concentrations between supplement users and non-users was tested by the Wilcoxon-Mann-Whitney-Test. We also tested whether there is a correlation (Cramer's V and Spearman's r_s) or an effect modification (interaction term in regression models) between supplement use or serum levels of Ca or vitamin D, respectively, and the intake of vitamin K antagonists or osteoporosis. In a further sub-analysis we also included testosterone levels as covariable in the fully adjusted model.

All analyses were performed with SAS software version 9.2 (SAS Institute, Inc., Cary, NC, USA).

3. Results

Baseline characteristics of the study subjects are provided in Table 1. Except for elevated IMT the prevalence of other outcomes was low. Elevated IMT and AF were slightly more frequent in women, whereas intermittent claudication and especially reduced ABI were more frequently observed in men.

3.1. Supplementation of Ca and vitamin D

Supplementary Ca was consumed regularly by 11.1% of all participants. Regular Ca intake was highest among subjects with AF (30.0%). On average, the daily dose of all regular Ca users was 450 mg. Among all participants, 9.3% regularly consumed vitamin D. The frequency of regular vitamin D intake was similar in participants with elevated IMT and intermittent claudication, but lower in participants with reduced ABI (6.3%) and much higher in AF cases (20.0%). The daily dose of all regular vitamin D users was on average 11 μg. Most of the regular Ca users simultaneously consumed vitamin D regularly (73.5%) and, vice versa, 87.3% of the constant vitamin D users regularly consumed Ca.

Logistic regression analyses showed a statistically significant relationship between regular Ca supplementation and the prevalence of AF after multivariable adjustment (OR = 3.89; 95% CI 1.28–11.81) (Table 2). No further significant associations with the other outcomes were observed in the logistic regression analyses, neither for Ca nor for vitamin D supplementation. The sub-analyses among regular Ca or vitamin D users, respectively, did not show a significant linear dose–response relationship between Ca or vitamin D supplement dose and AF or the other outcomes (data not shown).

3.2. Serum Ca and 25(OH)D concentrations

The mean serum Ca concentration of all participants was 2.40 ± 0.11 mM; the respective values of subjects with AF were lower (2.36 ± 0.07 mM) (Table 1). The mean de-seasonalized serum 25(OH)D concentration of all participants was 37.0 ± 16.5 nM. The average concentration was lower in all outcome groups, except for participants with increased IMT (Table 1).

In fully adjusted logistic regression analyses, neither serum Ca nor 25(OH)D concentrations were significantly associated with any of the four subclinical CVD phenotypes (Table 3). Only the association between the third quartile of serum Ca concentration and IMT became significant in comparison to the first quartile after multivariable adjustment, while the results observed for the other

Table 2
Association between calcium or vitamin D supplementation and intima-media thickness, ankle-brachial index, intermittent claudication and atrial fibrillation, given as OR with 95% CI.

Exposure variable	Outcome							
	Elevated IMT[a]		Reduced ABI (<0.9)		Intermittent claudication		Atrial fibrillation	
	n (elevated IMT[a]/total)	*n (elevated IMT[a]/total)*	*n (ABI < 0.9/total)*	*n (ABI < 0.9/total)*	*n (claudication/total)*	*n (claudication/total)*	*n (AF/total)*	*n (AF/total)*
	Minimally adjusted[b] OR [95% CI]	Fully adjusted[c] OR [95% CI]	Minimally adjusted[b] OR [95% CI]	Fully adjusted[c] OR [95% CI]	Minimally adjusted[b] OR [95% CI]	Fully adjusted[c] OR [95% CI]	Minimally adjusted[b] OR [95% CI]	Fully adjusted[c] OR [95% CI]
Supplementary CALCIUM								
	318/1271	*303/1206*	*58/1278*	*54/1217*	*37/1422*	*34/1347*	*14/1402*	*13/1331*
No	1	1	1	1	1	1	1	1
	42/148	*36/135*	*6/166*	*6/154*	*5/177*	*5/162*	*6/173*	*6/160*
Yes, regularly	1.186	1.373	0.762	1.108	1.035	0.816	2.783	3.890
	[0.81; 1.75]	[0.85; 2.22]	[0.32; 1.83]	[0.38; 3.24]	[0.39; 2.72]	[0.28; 2.37]	[1.01; 7.70]	[1.28; 11.81]
Supplementary VITAMIN D								
	325/1289	*311/1225*	*60/1301*	*56/1241*	*37/1450*	*34/1374*	*16/1426*	*15/1356*
No	1	1	1	1	1	1	1	1
	35/130	*28/116*	*4/143*	*4/130*	*5/149*	*5/135*	*4/149*	*4/135*
Yes, regularly	1.088	0.752	0.540	0.909	1.266	0.704	1.661	1.130
	[0.72; 1.65]	[0.38; 1.51]	[0.19; 1.54]	[0.19; 4.47]	[0.48; 3.36]	[0.13; 3.80]	[0.52; 5.29]	[0.20; 6.38]

Abbreviations: ABI, ankle-brachial index; AF, atrial fibrillation; CI, confidence interval; IMT, intima-media thickness; OR, odds ratio.
[a] Elevated IMT is defined as sex- and age-specific IMT ≥75th percentile.
[b] Adjusted for sex and age.
[c] Adjusted for sex, age, body-mass-index, physical activity, smoking, alcohol intake, education, actual hypertension, diabetes, TC/HDL ratio, C-reactive protein, glomerular filtration rate, parental myocardial infarction, supplementary vitamin D (with exposure Calcium)/Calcium (with exposure vitamin D).

quartiles or the continuous variable showed no association.

Linear regression models showed a significant positive association between serum 25(OH)D concentrations and ABI values ($\beta = 0.007$; $p = 0.010$) (Table 4). In other words, with increasing serum 25(OH)D concentrations also the ABI values increased and therefore the occurrence of asymptomatic peripheral arterial disease (ABI<0.9) decreased.

3.3. Additional sub-analyses

There was a modest correlation between vitamin D supplementation (daily dose, continuous) and serum 25(OH)D concentration (in vitamin D supplement users: $r_s = 0.32$, $p < 0.001$). Moreover, the 25(OH)D concentrations of persons with regular vitamin D use (median: 46.03 nM) were significantly higher than the concentrations of vitamin D in non-users (median: 33.83 nM). No significant correlation was found between Ca supplement use and serum Ca concentrations; accordingly, there was no significant difference in Ca concentrations between persons with regular Ca supplementation and participants who did not supplement Ca (median of both: 2.39 mM).

Altogether 11.9% of the Ca supplement users reported to suffer from osteoporosis. There was a weak correlation between them ($r_s = 0.10$) and their interaction term in the logistic regression with AF was not significant, so osteoporosis was not an effect modifier of Ca supplementation. Equally, the association between Ca use and AF was not altered by testosterone (data not shown).

Vitamin K antagonists were ingested by 65.0% of the participants with AF (i.e. 38.2% of all vitamin K antagonist users) and by 4.0% of the Ca supplement users. There were no significant correlations between the use of vitamin K antagonists and the use of Ca (Cramer' $V = 0.045$; $p = 0.074$), or vitamin D supplements (Cramer' $V = 0.027$; $p = 0.27$), or serum 25(OH)D ($r_s = 0.014$; $p = 0.564$). The correlation between vitamin K antagonists and serum Ca was extremely weak ($r_s = 0.056$; $p = 0.024$). In logistic regression analysis with AF as outcome, the interaction term between vitamin K antagonists and Ca supplementation was not significant.

4. Discussion

In the present analyses based on data from the population-based KORA F4 study, we observed that regular Ca supplementation was significantly associated with a higher prevalence of AF. No other significant relationship between Ca or vitamin D supplementation and subclinical CVD phenotypes existed.

Furthermore, in the linear regression analysis higher serum 25(OH)D concentrations were found to be significantly associated with a lower prevalence of asymptomatic peripheral arterial disease even after multivariable adjustment. Serum Ca concentrations were not significantly related to any of the subclinical CVD phenotypes.

4.1. Supplementation of Ca and vitamin D

The current literature provides no data on the association between Ca and vitamin D supplementation and ABI, intermittent claudication or AF. In a randomized placebo-controlled intervention study in postmenopausal women no significant differences in the change of IMT between the vitamin D group, the vitamin K1 group, and the placebo group were observed [27], which is in agreement with our null finding.

Results of Bolland et al. [6,7] and Reid et al. [4], as well as of a recent observational study [28], suggested a higher risk of myocardial infarction or stroke with Ca supplement use.

Our finding of a significant positive association between regular Ca supplementation and AF provides support for a possible mediating mechanism of how Ca supplementation could lead to increased risk of myocardial infarction and ischemic stroke that has not attracted much attention so far.

Normally, the serum Ca concentration is quite constant, because it is tightly regulated due to Ca uptake and release by bone surfaces [29]. However, it is known that taking Ca supplements leads to increases of serum Ca concentrations, reaching high-normal to high values few hours after administration [30], while after ingestion of normal and even high amounts of dietary Ca, no reactive increase in Ca serum concentrations has been described [31]. Arrhythmias as a

Table 3
Association between serum calcium or 25(OH)D levels and intima-media thickness, ankle-brachial index, intermittent claudication and atrial fibrillation, given as OR with 95% CI.

Exposure variable			Outcome							
			Elevated IMT[a]		Reduced ABI (<0.9)		Intermittent claudication		Atrial fibrillation	
			n (elevated IMT[a]/ total)	*n (elevated IMT[a]/total)*	*n (ABI < 0.9/total)*	*n (ABI < 0.9/ total)*	*n (claudication/ total)*	*n (claudication/ total)*	*n (AF/total)*	*n (AF/total)*
			Minimally adjusted[b] OR [95% CI]	Fully adjusted[c] OR [95% CI]	Minimally adjusted[b] OR [95% CI]	Fully adjusted[c] OR [95% CI]	Minimally adjusted[b] OR [95% CI]	Fully adjusted[c] OR [95% CI]	Minimally adjusted[b] OR [95% CI]	Fully adjusted[c] OR [95% CI]
Serum CALCIUM	Categorical[d]		*93/368*	*91/349*	*15/385*	*13/366*	*14/436*	*12/410*		
		Q1	1	1	1	1	1	1	–	–
			110/407	*100/379*	*17/419*	*15/393*	*9/461*	*9/431*		
		Q2	1.094	1.039	1.039	1.194	0.598	0.618	–	–
			[0.79; 1.51]	[0.74; 1.46]	[0.51; 2.13]	[0.52; 2.77]	[0.26; 1.40]	[0.25; 1.54]		
			56/286	*52/275*	*13/291*	*13/281*	*9/323*	*8/309*		
		Q3	0.721	0.630	1.288	1.624	0.897	0.827	–	–
			[0.50; 1.05]	[0.42; 0.94]	[0.60; 2.78]	[0.68; 3.88]	[0.38; 2.11]	[0.32; 2.14]		
			101/358	*96/338*	*19/349*	*19/331*	*10/379*	*10/359*		
		Q4	1.163	1.120	1.639	2.383	0.855	0.744	–	–
			[0.84; 1.62]	[0.79; 1.59]	[0.81; 3.32]	[1.06; 5.38]	[0.37; 1.96]	[0.30; 1.83]		
	Continuous	Per 0.1 mM	*360/1419*	*339/1341*	*19/349*	*60/1371*	*42/1599*	*39/1509*	*20/1575*	*19/1491*
			1.016	0.998	1.639	1.215	1.129	1.083	0.697	0.743
			[0.92; 1.13]	[0.90; 1.11]	[0.81; 3.32]	[0.98; 1.51]	[0.88; 1.45]	[0.84; 1.40]	[0.44; 1.10]	[0.51; 1.09]
Serum 25(OH)D	Categorical[e]		*82/348*	*80/332*	*29/362*	*27/347*	*19/399*	*17/379*		
		Q1	1	1	1	1	1	1	–	–
			89/354	*86/337*	*12/356*	*12/340*	*7/401*	*7/380*		
		Q2	1.090	1.157	0.417	0.631	0.365	0.420	–	–
			[0.77; 1.54]	[0.80; 1.67]	[0.21; 0.84]	[0.30; 1.35]	[0.15; 0.88]	[0.16; 1.07]		
			104/361	*95/339*	*10/362*	*9/343*	*7/398*	*7/374*		
		Q3	1.317	1.418	0.337	0.463	0.371	0.452	–	–
			[0.94; 1.85]	[0.98; 2.05]	[0.16; 0.71]	[0.20; 1.10]	[0.15; 0.90]	[0.18; 1.17]		
			85/356	*78/333*	*13/364*	*12/341*	*9/401*	*8/376*		
		Q4	1.021	1.173	0.439	0.747	0.473	0.619	–	–
			[0.72; 1.45]	[0.80; 1.72]	[0.22; 0.87]	[0.34; 1.65]	[0.21; 1.10]	[0.24; 1.59]		
	Continuous	Per 10 nM	*360/1419*	*339/1341*	*64/1444*	*60/1371*	*42/1599*	*39/1509*	*20/1575*	*19/1491*
			1.016	1.055	0.805	0.916	0.854	0.904	1.014	1.032
			[0.95; 1.09]	[0.97; 1.14]	[0.68; 0.96]	[0.75; 1.11]	[0.67; 1.05]	[0.71; 1.15]	[0.78; 1.32]	[0.82; 1.30]

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; ABI, ankle-brachial index; CI, confidence interval; IMT, intima-media thickness; OR, odds ratio.
[a] Elevated IMT is defined as sex- and age-specific IMT ≥75th percentile.
[b] Adjusted for sex and age.
[c] Adjusted for sex, age, body-mass-index, physical activity, smoking, alcohol intake, education, actual hypertension, diabetes, TC/HDL ratio, C-reactive protein, glomerular filtration rate, parental myocardial infarction.
[d] Categorized in quartiles: Q1 ≤ 2.33 mM; 2.33 mM > Q2 ≤ 2.40 mM; 2.40 mM > Q3 ≤ 2.45 mM; Q4 > 2.45 mM.
[e] Categorized in quartiles: Q1 ≤ 25.01 nM; 25.01 nM > Q2 ≤ 34.74 nM; 34.74 nM > Q3 ≤ 46.42 nM; Q4 > 46.42 nM.

Table 4
Association between serum calcium or 25-(OH)D concentrations respectively and intima-media thickness or ankle-brachial index, as analyzed in linear regression models.

Exposure variable	Outcome							
	Intima-media thickness [mm]				Ankle-brachial index			
	Minimally adjusted[a]		Fully adjusted[b]		Minimally adjusted[a]		Fully adjusted[b]	
	β-estimate [95% CI]	R^2	β-estimate [95% CI]	R^2	β-estimate [95% CI]	R^2	β-estimate [95% CI]	R^2
Serum CALCIUM [per 0.1 mM]	−0.001 [−0.006; 0.004]	0.23	−0.002 [−0.007; 0.003]	0.27	−0.007 [−0.014; −0.0003]	0.03	−0.006 [−0.013; 0.0004]	0.11
Serum 25(OH)D [per 1 nM]	0.001 [−0.003; 0.004]	0.23	0.004 [<−0.001; 0.007]	0.27	0.008 [0.003; 0.013]	0.03	0.007 [0.002; 0.011]	0.12

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; CI, confidence interval; R^2, coefficient of determination.
[a] Adjusted for sex and age.
[b] Adjusted for sex, age, body-mass-index, physical activity, smoking, alcohol intake, education, actual hypertension, diabetes, TC/HDL ratio, C-reactive protein, glomerular filtration rate, parental myocardial infarction.

consequence of hypercalcemia are described [32], although it is unclear whether high values in form of repeated Ca peaks are a sufficient condition. The regulation of Ca homeostasis is controlled by a number of factors, such as parathyroid hormones, calcitonin and sex hormones [33]. In general, steroid hormones prevent bone loss and thus, the 'deficiency' of estrogen and testosterone in older adults increases bone resorption [34]. On the other hand, medications such as glucocorticoids have the overall effect of decreasing serum Ca and the most significant effect of long-term glucocorticoid excess on Ca and bone metabolism is osteoporosis [34]. However, our findings were not biased by the presence of osteoporosis (prevalence only 5.9%) as we could show in our subanalyses. Additionally we could exclude that testosterone levels altered our result of a significant association between Ca supplementation and the prevalence of AF.

Vitamin K antagonist compounds as oral anticoagulants are commonly used for treatment of AF and other heart diseases. In our work, 65% of the participants with AF used such agents. Since

vitamin K is essential for the production of gamma-carboxyglutamic acid (Gla) proteins and other Ca binding proteins, treatment with vitamin K antagonists may cause a disarrangement of Ca metabolism with a decrease of Ca deposition and an alteration of Ca channels subsequently enhancing osteopenia/osteoporosis [35], resulting in treatment with Ca and vitamin D supplements. In our study population only a quarter of the persons treated with vitamin K antagonists used Ca supplements and only 14.7% used vitamin D supplements. Accordingly, there were no significant correlations between medication with vitamin K antagonists and the use of Ca or vitamin D supplements.

Further studies are needed to elucidate the underlying mechanisms responsible for our findings.

4.2. Serum concentrations of 25(OH)D and Ca

Published results on 25(OH)D concentrations and the presence of an increased IMT are inconsistent. Two out of three cross-sectional population-based studies found no association with common carotid IMT [36,37]. Only in the Northern Manhattan study, an inverse relationship between serum 25(OH)D concentrations and IMT was observed [38].

To the best of our knowledge no studies on intermittent claudication are available. Some prior studies examined the association between serum 25(OH)D and ABI or peripheral arterial disease, respectively. A population-based American survey confirms our results by reporting that low serum 25(OH)D concentrations are associated with a higher prevalence of peripheral arterial disease [39]. Similarly, one study in peripheral arterial disease patients [40] and one case-control study in men with peripheral arterial disease versus healthy men [41] showed that lower concentrations of 25(OH)D were associated with decreased ABI. Only one community-based Korean study found no significant association between serum 25(OH)D and ABI [37].

In contrast to 25(OH)D levels, supplementary vitamin D did not show a significant relationship with ABI in our analysis, although we were able to show a significant correlation between vitamin D supplementation and 25(OH)D levels, which gives reassurance in the validity of the vitamin D supplementation variable. We conclude that the results of the serum analysis are different due to other factors that determine the body's vitamin D status, especially the ultraviolet light exposure.

Concerning serum 25(OH)D and AF, findings are inconsistent. Our null finding is in accordance with the result of a population-based follow-up study of the Framingham Heart Study [42]. In contrast, a case-control study revealed a significant relationship between lower 25(OH)D and nonvalvular AF, but not valvular AF [43].

Carelli and coworkers examined the relationship between serum Ca concentrations and IMT in a cross-sectional study [38]. As in our study, they observed no significant associations. Other studies with slightly different atherosclerotic lesion measures, such as carotid artery plaque thickness or abdominal aortic calcification, showed a positive association with serum Ca concentrations [44,45].

To the best of our knowledge, scientific publications on the association between serum Ca and intermittent claudication or AF or ABI in population-based studies are not available.

The significant relationship between Ca supplementation and AF is not confirmed by our respective serum analysis. Reid and colleagues hypothesized that it might be rather peak serum Ca concentrations (after Ca supplementation) than mean concentrations which drive potential adverse cardiovascular effects [11]. However, the blood samples of the KORA F4 participants were drawn after overnight fasting. Thus, serum Ca concentrations as measured in our study do not reflect the situation few hours after supplement intake. Accordingly, serum Ca concentrations in our study do not correlate with the use of Ca supplements.

4.3. Strengths and limitations of the analyses

As in any cross-sectional study, the established associations do not necessarily indicate cause-effect relationships. Furthermore, the present study has low numbers of participants with intermittent claudication and AF and is therefore lacking statistical power. The goodness of fit of the linear regression models was not high and the β-coefficients, i.e., the change in outcome, were small, thus the clinical significance of the results remains unclear. R^2; does not clearly indicate an increased goodness of fit of the fully adjusted model compared to the minimally adjusted one, as R^2 increases as the number of variables increases.

We dealt with total serum Ca concentrations, though free (ionized) Ca would have been more informative since it is the biologically active form of Ca. Furthermore, no levels of estrogen hormones and calcitonin were available and the information on the presence of osteoporosis was based on the participants' self-report.

A major strength of the present work is the analysis of both supplementation data and serum concentrations of Ca as well as vitamin D and several subclinical CVD phenotypes. To the best of our knowledge we are among the first to examine the association between Ca or vitamin D supplementation and ABI, intermittent claudication, and AF in a population-based study. The advantage of using subclinical CVD phenotypes as outcome instead of myocardial infarction or stroke, is that reverse causation can be neglected.

5. Conclusion

In conclusion, although based on few cases, the present analyses showed that Ca supplementation was significantly positively associated with the occurrence of AF. This result might be an explanatory approach to how Ca supplementation could lead to increased risk of myocardial infarction and ischemic stroke, as AF is associated with both. Moreover, higher serum 25(OH)D concentrations were associated with a lower presence of asymptomatic peripheral arterial disease. However, due to the cross-sectional study design, the results have to be carefully interpreted and confirmed in prospective studies of appropriate size.

Conflict of interest

The authors declare no conflict of interest.

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