Nutrition&Metabolism

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Serum 25-hydroxyvitamin D₃ and body composition in an elderly cohort from Germany: a cross-sectional study

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Abstract

Background: Emerging evidence indicates that there is an association between vitamin D and obesity. The aim of this study was to investigate whether the level of serum 25-hydroxyvitamin D₃ [25(OH)D₃] in the elderly is influenced by parameters of anthropometry and body composition independent of potential confounding lifestyle factors and the level of serum intact parathyroid hormone (iPTH).

Methods: Cross-sectional data of 131 independently living participants (90 women, 41 men; aged 66–96 years) of the longitudinal study on nutrition and health status in senior citizens of Giessen, Germany were analysed. Concentrations of 25(OH)D₃ and iPTH were ascertained by an electrochemiluminescence immunoassay. Body composition was measured by a bioelectrical impedance analysis. We performed univariate and multiple regression analyses to examine the influence of body composition on 25(OH)D₃ with adjustments for age, iPTH and lifestyle factors.

Results: In univariate regression analyses, 25(OH)D₃ was associated with body mass index (BMI), hip circumference and total body fat (TBF) in women, but not in men. Using multiple regression analyses, TBF was shown to be a negative predictor of 25(OH)D₃ levels in women even after controlling for age, lifestyle and iPTH ($\beta = -0.247$; $P = 0.016$), whereas the associations between BMI, hip circumference and 25(OH)D₃ lost statistical significance after adjusting for iPTH. In men, 25(OH)D₃ was not affected by anthropometric or body composition variables.

Conclusions: The results indicate that 25(OH)D₃ levels are affected by TBF, especially in elderly women, independent of lifestyle factors and iPTH.

Keywords: 25-Hydroxyvitamin D₃, Body composition, Fat mass, Elderly

Background

There is emerging evidence that, in addition to its well-established role in the regulation of calcium homeostasis and bone metabolism, vitamin D has multiple functions in human health. Accumulating epidemiological data indicate that a low vitamin D status is linked to a variety of chronic diseases that are associated with aging, including cancer, autoimmune diseases, hypertension and diabetes mellitus [1-3]. Elderly people are especially at risk for a vitamin D deficiency because of age-related declines in the endogenous vitamin D synthesis, sun exposure and dietary intake [4]. It is a moot point whether adiposity, which is increasingly prevalent in people with advanced age [5], may also negatively impact the vitamin D status in the elderly. Numerous studies have linked low 25-hydroxyvitamin D levels [25(OH)D], the commonly used indicator of the vitamin D status [6], with obesity [7-21], whereas other researchers have failed to confirm this observation [22-24]. There are some limitations of these previous studies. First, these studies often included only women, vitamin D-deficient individuals, ambulatory patients, morbidly obese subjects or subjects of young or middle age, and the interpretation of these results is limited, especially in reference to elderly individuals. Second, the majority of these studies relied on body mass index (BMI) without a further

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ascertainment of total body fat (TBF). Third, some of these studies did not control for potential confounders, such as age, nutrient intake, use of vitamin D supplements, daily sun exposure, physical activity and smoking, which may be associated with both vitamin D status and adiposity. In this context, intact parathyroid hormone (iPTH) is also expected to be a relevant confounder or even the causal factor of the association between TBF and 25(OH)D [25], and previous studies often concentrated exclusively on 25(OH)D or iPTH without considering the interaction between both. Fourth, anthropometric and body composition variables were frequently included simultaneously in the multiple regression model. Consequently, these results must be interpreted with caution.

Overall, studies in which the associations of anthropometry and body composition with 25(OH)D were examined in predominantly non-obese, well-functioning elderly women and men are scarce. Therefore, and in view of the inconsistent results and the above mentioned limitations of previous studies, the primary objective of our study was to analyse whether anthropometric characteristics and body composition contribute to the 25-hydroxyvitamin D_3 [25(OH)D_3] status in non-institutionalised, primarily non-obese elderly women and men. In this context, potential confounders of this association, such as age, sun exposure, physical activity, vitamin D and calcium intake, smoking history, alcohol consumption and iPTH, were considered. In this way, we could scrutinise whether anthropometric and body composition variables are independent predictors for 25(OH)D_3 levels in the elderly or whether age, lifestyle or iPTH may account for the effects of body composition on the 25(OH)D_3 status.

Methods
Subjects
Subjects were participants of the GISELA study, a prospective cohort study in which the nutrition and health status of senior citizens from Giessen (50°35′North), Germany, have been observed since 1994. All investigations took place in the Institute of Nutritional Science in Giessen from July to October. For enrolment, subjects had to be at least 60 years of age and able to visit the institute without assistance. A written informed consent was obtained from each participant. The study protocol was approved by the Ethical Committee of the Faculty of Medicine at the Justus-Liebig-University, Giessen.

The present investigation reports cross-sectional data from the GISELA study obtained in 2008. Subjects with incomplete data were excluded. Those individuals who took diuretics, had undergone a hormone replacement therapy or suffered from chronic kidney disease or oedema were also excluded from the analysis. Eight people were characterised as outliers due to their serum

25(OH)D_3 and iPTH measurements as well as the residuals of the regression analyses and were therefore not included. Of the 275 elderly people who took part in the follow-up in 2008, data from 90 women and 41 men were included in the following analysis.

Anthropometric data and body composition
Body weight was determined by a calibrated digital scale (Seca, Vogel & Halke, Hamburg, Germany) to the nearest 0.1 kg in light clothes without shoes. Based on the weight of the remaining clothes, 0.5 to 1.0 kg was subtracted from the measured weight. Body height was recorded standing upright without shoes via a telescopic rod integrated in the scale to the nearest 0.5 cm. The BMI categories were established such that subjects with a BMI < 25.0 kg/m² were classified as normal weight, whereas participants with a BMI ≥ 25.0 kg/m² were defined as overweight to obese. The waist-to-hip ratio (WHR) and waist circumference (WC) were used as markers for body fat distribution. Abdominal obesity was defined according to the WHO [26] as a WHR > 0.85 for women and > 1.0 for men or a WC ≥ 88 cm for women and ≥ 102 cm for men. The WC and hip circumference (HC) were determined in an upright position by a tape measure to the nearest 1.0 cm.

Body composition was recorded by a single-frequency (50 kHz) bioelectrical impedance analyser (Akern-RJL BIA 101/S, Data Input, Frankfurt, Germany) according to the instructions of the manufacturer and the predictive formula from Roubenoff et al. [27].

Laboratory measurements
Blood samples for serum 25(OH)D_3 and iPTH were collected between 7:00 a.m. and 11:00 a.m. after an overnight fast and serum aliquots were stored at −70°C until further analysis. Both 25(OH)D_3 and iPTH were measured by a direct electrochemiluminescence immunoassay (ECLIA, Roche Diagnostics, Mannheim, Germany) [28,29]. Importantly, the ECLIA specifically detects the 25(OH)D_3 concentration. We defined 25(OH)D_3 < 25.0 nmol/L as vitamin D-deficient, 25.0–49.9 nmol/L as insufficient and ≥ 50.0 nmol/L as adequate. In addition, we accounted for the ongoing debate concerning the use of higher cut-off values by means of an accessory cut-off value of ≥ 75.0 nmol/L [1,30].

Lifestyle factors
A three-day estimated dietary record, which was developed and validated for the GISELA study, functions to determine the nutritional intake of each subject [31]. Smoking behaviour, current daily time spent outdoors, physical activity pattern and further data, such as age, diseases, medications and vitamin D supplement intake, were collected using self-administered questionnaires. The current time spent outdoors (min/d) was used as an

indicator for sun exposure. The smoking behaviour (never-smokers vs. current and ex-smokers) and the use of vitamin D supplements (no/yes) were coded as dichotomous variables. The physical activity level (PAL) of each participant was assessed as described elsewhere [32].

Statistical analysis

The characteristics of the study subjects were expressed as medians and the 5th–95th percentiles due to non-normally distributed data. Because of significant sex differences in the amount of TBF, fat distribution and lifestyle factors, we performed sex-stratified analyses. According to the sample size, a normal distribution was tested by the Shapiro-Wilk test for men and by the Kolmogorov-Smirnov test with the Lilliefors correction for women. Descriptive characteristics were compared between groups via the Mann-Whitney U test for continuous variables and via the χ^2 test or, alternatively, Fisher's exact test for categorical variables.

Whenever subjects with an adequate vitamin D status were compared with vitamin D-insufficient individuals, we used two different $25(OH)D_3$ cut-off values (≥ 50 nmol/L and ≥ 75 nmol/L). The study cohort was split by BMI into two groups to compare normal-weight subjects with overweight to obese participants for the prevalence of vitamin D insufficiency by means of the Fisher's exact test. In addition, we divided the cohort into subjects with abdominal obesity and participants without an abdominal fat distribution, as defined by the WHR and WC, and analysed whether differences concerning the prevalence of vitamin D insufficiency exist. Furthermore, subjects with an adequate vitamin D status were compared with vitamin D-insufficient individuals with regard to parameters of anthropometry and body composition by using the Mann-Whitney U test.

We examined univariate associations of $25(OH)D_3$ with iPTH and parameters of lifestyle, anthropometry and body composition by simple regression analyses. Serum iPTH was regarded as a covariate because iPTH can promote the turnover of circulating $25(OH)D_3$ by inducing the formation of 1,25-dihydroxyvitamin D_3 [33]. On the basis of non-normally distributed residuals, the dependent variable $25(OH)D_3$ was logarithmically transformed ($\log_{10}$) to obtain a normal distribution of residuals.

Finally, we performed sex-stratified hierarchical multiple regression analyses. In detail, we created three models with different levels of adjustment. In each of the three models, the log $25(OH)D_3$ was considered as the dependent variable and either the WC, HC, WHR, BMI or % TBF was included as a predictor variable. The fat-free mass (FFM), body weight and absolute TBF were not tested either because of their limited or somewhat

lower explanatory power compared with the other anthropometric variables or the % TBF, and therefore were not expected to provide any additional information. Model 1 represents each association of the anthropometric and body composition variables with the log 25 $(OH)D_3$ adjusted for age, while model 2 additionally adjusts for lifestyle factors (PAL, sun exposure, smoking, use of vitamin D supplements, alcohol consumption, vitamin D and calcium intake). Model 3 comprises all covariates of model 2 and additionally considers iPTH as a confounding variable. Statistical analyses were done using the SPSS 18.0 statistical package for Windows (SPSS Inc., Chicago, USA). The significance level was set at $P < 0.05$. All tests were two-tailed.

Results

Characteristics of the study subjects

The characteristics of the subjects are presented in Table 1. None of the subjects had a serious vitamin D deficiency, but 25.6 % of the women and 19.5 % of the men had 25 $(OH)D_3$ levels < 50 nmol/L. Levels ≥ 75 nmol/L were observed in 18.9 % of the females and 22.0 % of the males.

Adiposity and the prevalence of vitamin D insufficiency by using two cut-off values

The women with BMIs ≥ 25 kg/m² had a higher prevalence of vitamin D insufficiency compared to the normal-weight women when the cut-off value of 75 nmol/L was applied (69.9 % vs. 30.1 %; $P = 0.012$), but not when the lower value of 50 nmol/L was used ($P = 0.132$). Men showed no differences regarding the prevalence of vitamin D insufficiency when stratified according to BMI, which was independent of the cut-off value that was used (both $P > 0.200$). No differences were found after dividing the cohort into subjects with abdominal obesity and those without, independent of sex (all $P > 0.200$). In women, but not in men, the BMI, WC and HC were higher in subjects with $25(OH)D_3$ levels < 50 nmol/L compared to subjects with $25(OH)D_3$ levels ≥ 50 nmol/L (all $P < 0.05$). When using the cut-off value of 75 nmol/L, the % TBF was significantly higher in both the female and male subjects with $25(OH)D_3$ levels < 75 nmol/L (both $P < 0.05$).

Univariate associations between 25-hydroxyvitamin D_3 and other parameters

Table 2 provides the results of the univariate linear regression analyses. In women, the log $25(OH)D_3$ was associated with body weight, HC, BMI, absolute and % TBF, alcohol consumption, sun exposure, PAL and iPTH. In men, sun exposure, PAL and current or past smoking significantly affected the log $25(OH)D_3$. All other parameters, including age, WC, WHR, absolute FFM and intake of vitamin D, calcium and vitamin D

Table 1 Descriptive characteristics of the study population

Characteristics	Women ($n = 90$) Median ($P_5–P_{95}$)	Men ($n = 41$) Median ($P_5–P_{95}$)	*P*-value [a]
Age (y)	75.5 (69.0–86.5)	76.0 (70.0–84.8)	0.546
Height (cm)	160.0 (150.5–168.7)	174.5 (161.6–183.5)	< 0.0001
Weight (kg)	67.8 (52.9–85.2)	78.5 (63.7–98.0)	< 0.0001
Body mass index (kg/m²)	26.3 (20.8–34.5)	26.4 (22.9–32.3)	0.806
Waist circumference (cm)	90.0 (71.6–108.4)	100.0 (84.4–113.8)	< 0.0001
Hip circumference (cm)	106.0 (91.6–122.5)	104.0 (95.2–116.8)	0.170
Waist-to-hip ratio	0.85 (0.76–0.93)	0.96 (0.86–1.06)	< 0.0001
Fat-free mass (kg)	39.5 (34.5–45.1)	55.3 (48.6–62.6)	< 0.0001
Total body fat (kg)	28.1 (17.2–41.6)	24.2 (14.4–36.0)	0.001
Total body fat (%)	41.9 (32.4–50.1)	29.0 (21.5–37.9)	< 0.0001
25(OH)D$_3$ (nmol/L)	59.4 (39.9–90.6)	67.5 (39.7–88.9)	0.096
iPTH (pmol/L)	4.5 (2.3–7.9)	4.1 (2.1–8.3)	0.360
Vitamin D intake (µg/d)	2.3 (0.3–10.1)	3.5 (1.0–11.3)	0.078
Calcium intake (g/d)	1.0 (0.5–1.8)	1.0 (0.6–1.6)	0.953
Alcohol intake (g/d)	2.3 (0.0–16.1)	5.1 (0.0–30.0)	0.018
Sun exposure (min/d)	120.0 (40.0–360.0)	150.0 (22.5–396.0)	0.130
Physical activity level	1.7 (1.5–2.0)	1.7 (1.4–1.9)	0.254
Current or ex-smokers, n (%)	20 (22.2)	29 (70.7)	< 0.0001
Vitamin D supplement users, n (%)	15 (16.7)	2 (4.9)	0.091

Abbreviations: 25(OH)D$_3$, 25-hydroxyvitamin D$_3$; iPTH, intact parathyroid hormone.
[a] Tests of significance between the sexes were based on the Mann-Whitney *U* test for continuous variables and the χ^2 test for categorical variables.

supplements, were not associated with the log 25(OH)
D$_3$. The linear relationships of the log 25(OH)D$_3$ with
HC, BMI and % TBF in women are illustrated in
Figure 1.

Adjusted associations of 25-hydroxyvitamin D$_3$ with anthropometric and body composition variables

The results of the multiple regression analyses with different levels of adjustment are shown in Table 3. Due to the observed collinearity of the anthropometric and body composition variables (data not shown), these variables were added as potential predictor variables in separate models by replacing each other. No associations existed between the log 25(OH)D$_3$ and WC or WHR in either sex. In men, the BMI, HC and % TBF were not associated with the log 25(OH)D$_3$, independent of the level of adjustment. In contrast, the % TBF emerged as a negative predictor for the log 25(OH)D$_3$ in women both before and after an additional adjustment for iPTH, whereas the BMI and HC were not significantly associated with the log 25(OH)D$_3$ after a full adjustment that included iPTH. In women, the fully adjusted model 3, with % TBF as a predictor variable, explained 27.4 % of the variation in 25(OH)D$_3$ levels and the % TBF accounted for 4.9 % of the variation in this regard.

Besides the % TBF, iPTH ($ß = -0.345$; $P < 0.001$) and sun exposure ($ß = 0.239$; $P = 0.018$) were independent determinants of the log 25(OH)D$_3$ in women, while alcohol intake showed a borderline significant association ($ß = 0.188$; $P = 0.052$). In men, only smoking ($ß = -0.438$; $P = 0.005$) had an independent impact on the log 25(OH)D$_3$. When we created multiple regression models including only those variables as confounders that exhibited a significant association with the log 25(OH)D$_3$ in the univariate analyses, so that iPTH levels, alcohol intake, sun exposure and PAL were included in the female model and sun exposure, PAL and smoking behaviour in the male model, this yielded equal results (data not shown).

Discussion

To our knowledge, this is the first study that examines the associations of anthropometric and body composition variables with the vitamin D status of predominantly non-obese, non-vitamin D-deficient, well-functioning elderly women and men from Germany while also considering the potentially confounding effects of age, lifestyle factors and iPTH.

In contrast to other studies of elderly people [15,23,34], none of the GISELA subjects had a severe vitamin D

Table 2 Univariate linear regression analyses between the log-transformed 25-hydroxyvitamin D$_3$ and other parameters

Characteristics	log 25(OH)D$_3$			
	Women (*n* = 90)		Men (*n* = 41)	
	ß	*P*-value	ß	*P*-value
iPTH (pmol/L)	−0.379	< 0.001	−0.212	0.182
Age (y)	−0.107	0.315	−0.200	0.209
Weight (kg)	−0.240	0.023	0.103	0.521
Body mass index (kg/m²)	−0.261	0.013	−0.040	0.806
Waist circumference (cm)	−0.181	0.088	−0.126	0.434
Hip circumference (cm)	−0.235	0.026	−0.039	0.809
Waist-to-hip ratio	−0.029	0.789	−0.140	0.382
Fat-free mass (kg)	−0.137	0.199	0.268	0.090
Total body fat (kg)	−0.261	0.013	−0.012	0.939
Total body fat (%)	−0.283	0.007	−0.088	0.584
Vitamin D intake (μg/d)	−0.029	0.788	−0.092	0.568
Calcium intake (g/d)	−0.049	0.645	−0.015	0.925
Alcohol intake (g/d)	0.211	0.046	0.100	0.533
Sun exposure (min/d)	0.301	0.004	0.370	0.017
Physical activity level	0.212	0.044	0.442	0.004
Current or past smoking [a]	0.007	0.946	−0.520	< 0.001
Vitamin D supplement use [a]	0.134	0.207	0.172	0.283

Abbreviations: log 25(OH)D$_3$, log-transformed 25-hydroxyvitamin D$_3$; ß, standardised coefficient; iPTH, intact parathyroid hormone.
[a] Dummy variable (no/yes).

deficiency. Moreover, while in our investigation fewer than 25 % of the subjects had 25(OH)D$_3$ levels < 50 nmol/L, the prevalence of vitamin D insufficiency in the general German population aged 65–79 years exceeds 50 %, even during the summer [34].

Previous studies have reported a higher prevalence of vitamin D insufficiency in obese subjects compared to lean individuals [9,35]. In our study, the prevalence of vitamin D insufficiency was 39.8 % lower in normal-weight women than in women with BMIs ≥ 25 kg/m² when using the cut-off value of 75 nmol/L. When our study cohort was stratified by 25(OH)D$_3$ status, individuals with an adequate vitamin D status (≥ 75 nmol/L) had a lower % TBF than subjects with 25(OH)D$_3$ levels < 75 nmol/L. This suggests that a higher % TBF may have limited the increase in 25(OH)D$_3$ levels over the threshold of 75 nmol/L or, alternatively, a sufficient vitamin D status may have protected against an increasing TBF.

At present there is a great debate on the 25(OH)D$_3$ target thresholds as regards multiple health outcomes and hence guidelines for dietary intakes. While the Institute of Medicine (IoM) suggests 50 nmol/L as the target value for dietary reference intakes of vitamin D [36], others favour 75 nmol/L or even higher values as sufficiency threshold [30]. We observed distinct differences in the % TBF of subjects with vitamin D insufficiency only when using 75 nmol/L as cut-off level, whereas anthropometric variables already differed between subjects with 25(OH)D$_3$ levels ≥ 50 nmol/L and those with < 50 nmol/L. As the multiple regression analyses revealed that the % TBF, but none of the anthropometric variables, is a key determinant of 25(OH)D$_3$ levels, this might favour the view that the threshold of 75 nmol/L reflects a more adequate cut off-value than 50 nmol/L. However, the observed linear relationship between % TBF and 25(OH)D$_3$ indicates that a defined threshold concentration of 25(OH)D$_3$ with regard to adiposity may not exist in our study population of non-vitamin D-deficient elderly individuals. Consequently, the requirements for vitamin D of individuals to achieve or exceed a defined cut-off level may depend on their % TBF, which may be highly variable even at a given BMI.

In our univariate analyses, the anthropometric and body composition variables had an impact on 25(OH)D$_3$ only in women, which may be attributed to the small sample size of men and to the lower % TBF in men and
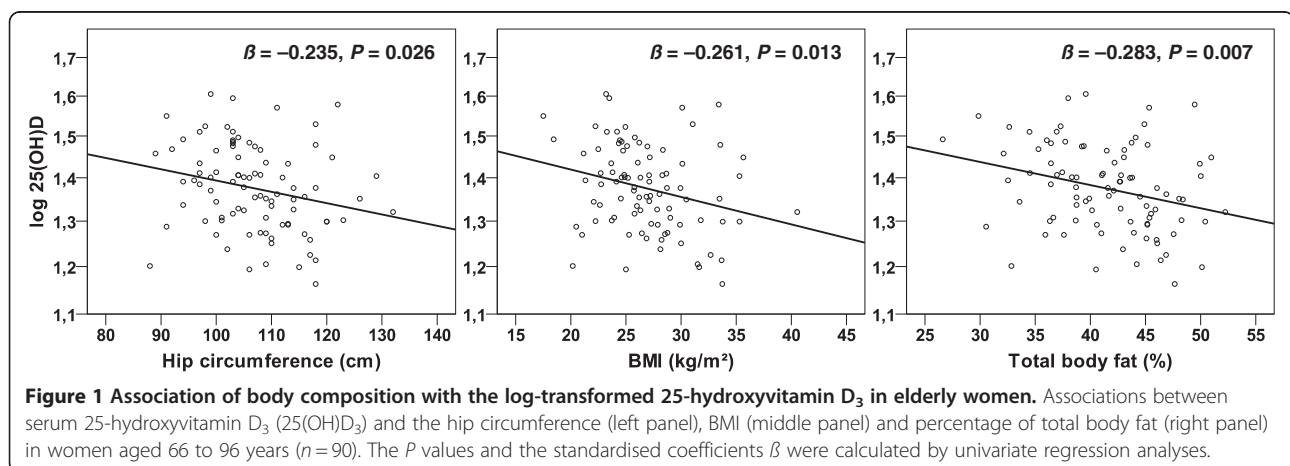

Figure 1 Association of body composition with the log-transformed 25-hydroxyvitamin D$_3$ in elderly women. Associations between serum 25-hydroxyvitamin D$_3$ (25(OH)D$_3$) and the hip circumference (left panel), BMI (middle panel) and percentage of total body fat (right panel) in women aged 66 to 96 years (*n* = 90). The *P* values and the standardised coefficients ß were calculated by univariate regression analyses.

Table 3 Multiple regression analyses between 25-hydroxyvitamin D₃ and the respective anthropometric or body composition parameter [a]

	Women (n = 90)			Men (n = 41)		
	log 25(OH)D₃			log 25(OH)D₃		
	B	ß	P-value	B	ß	P-value
WC (cm)						
Model 1	−0.002	−0.189	0.075	−0.001	−0.146	0.362
Model 2	−0.002	−0.176	0.096	−0.001	−0.097	0.489
Model 3 [b]	−0.001	−0.155	0.115	−0.001	−0.071	0.632
HC (cm)						
Model 1	−0.003	−0.260	0.015	−0.001	−0.043	0.786
Model 2	−0.002	−0.202	0.058	−0.001	−0.042	0.769
Model 3 [b]	−0.002	−0.141	0.161	−0.0004	−0.026	0.855
WHR						
Model 1	−0.035	−0.018	0.869	−0.252	−0.168	0.293
Model 2	−0.137	−0.070	0.522	−0.157	−0.105	0.462
Model 3 [b]	−0.251	−0.128	0.208	−0.118	−0.079	0.602
BMI (kg/m²)						
Model 1	−0.007	−0.285	0.007	−0.003	−0.084	0.608
Model 2	−0.006	−0.240	0.026	−0.002	−0.060	0.678
Model 3 [b]	−0.005	−0.186	0.065	−0.001	−0.037	0.803
TBF (%)						
Model 1	−0.006	−0.315	0.003	−0.002	−0.104	0.517
Model 2	−0.005	−0.284	0.010	−0.001	−0.031	0.827
Model 3 [b]	−0.005	−0.247	0.016	−0.0003	−0.014	0.920

Abbreviations: WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio; BMI, body mass index; TBF, total body fat.
[a] Multiple regression analyses using the log-transformed 25-hydroxyvitamin D₃ as the dependent variable and the respective anthropometric and body composition parameters as the predictor variables. The results of the regression analyses are expressed in terms of B (the unstandardised coefficient), ß (standardised coefficient), and the adjusted coefficient of determination (R^2) for the final model 3. Model 1: association adjusted for age (y). Model 2: model 1 with additional adjustments for physical activity level, sun exposure (min/d), vitamin D intake (μg/d), calcium intake (g/d), alcohol consumption (g/d), smoking (never-smokers vs. current or ex-smokers) and use of vitamin D supplements (no/yes). Model 3: model 2 controlled for intact parathyroid hormone (pmol/L).
[b] Adjusted coefficient of determination (R^2) for the respective model 3 (including the respective anthropometric and body composition variable and all covariates) for women (WC = 0.243; HC = 0.238; WHR = 0.235; BMI = 0.252; and % TBF = 0.274) and for men (WC = 0.275; HC = 0.270; WHR = 0.276; BMI = 0.271; and % TBF = 0.270).

the unequal fat distribution between the female and male subjects. Of the anthropometric and body composition variables examined in our study, the % TBF was the strongest predictor of the 25(OH)D₃ status in the women. An inverse association between the concentration of 25(OH)D and % TBF was also reported in a study of 410 women aged 20–80 years [8] and in a study of 112 postmenopausal women [18]; however, no association of 25(OH)D levels with BMI was found in either

study. In contrast, 25(OH)D levels were negatively affected by the BMI, but not by the % TBF, in a study of only overweight and obese middle-aged subjects [37]. Other authors [20] reported an inverse association of 25 (OH)D levels with the % TBF in elderly people, which was stronger in women and stronger than the associations of 25(OH)D levels with the anthropometric variables. Our results support the conclusion that the % TBF represents a more potent predictor of the 25(OH)D₃ status compared with BMI or other anthropometric variables.

We did not find a relationship of 25(OH)D₃ levels with the WC or WHR, which does not support the hypothesis that the abdominal fat tissue exerts an important effect on the vitamin D status, as others have suggested [12-14,38]. Snijder et al. [20] reported that a greater WC had a negative impact on 25(OH)D levels in both sexes, whereas the WHR was not associated with 25(OH)D levels. Moschonis et al. [18] observed no associations of 25(OH)D levels with either the WC or HC in postmenopausal women. In our study, the negative impact of the HC on 25(OH)D₃ levels indicates a specific role of peripheral or subcutaneous fat tissue, which is in line with the hypothesis that a low 25(OH)D₃ status is a consequence of an increased sequestration of vitamin D in adipose tissue, especially in the subcutaneous fat [39].

At present, it is unclear whether adipose tissue acts as a reservoir for vitamin D by releasing vitamin D into the circulation when required or as a metabolic trap that reduces its bioavailability [6,39]. Moreover, there is uncertainty about the causal character of the association between TBF and 25(OH)D. In this context, it has been hypothesised that a low 25(OH)D status may contribute to obesity by promoting secondary hyperparathyroidism [25,40]. Alternatively, it has been postulated that iPTH may promote weight gain independent of vitamin D by possibly promoting lipogenesis and inhibiting lipolysis [25,40]. We observed an inverse association of 25(OH)D₃ with iPTH in women, as reported by others [7,18,33]. In men, we did not find this association, which may be due to hormonal differences and the sample size. Although the association between the % TBF and 25(OH)D₃ diminished in our study after adjusting for iPTH, the association remained statistically significant. Thus, iPTH does not appear to be responsible for the inverse association of the % TBF with 25(OH)D₃ in our subjects, which is in line with previous studies [18,20]. Contrary to the % TBF, the associations between anthropometric variables and 25(OH)D₃ in women were abolished after adjusting for iPTH, which may be because the BMI does not adequately reflect the % TBF. The marginal effect of iPTH on the association of % TBF with 25(OH)D₃ in our study may possibly due to the relatively high vitamin D status of our participants.

Another suggestion is that differences in lifestyle may contribute to a lower vitamin D status in overweight individuals, such as decreased sun exposure because of clothing habits [41] or low exercise levels [42]. We noticed an association of sun exposure with $25(OH)D_3$, which is consistent with the literature [7,15,18], and an association between PAL and $25(OH)D_3$ in men, as also reported in other studies [10,17,19,35]. A low PAL may increase the TBF, which in turn may decrease the bioavailability of vitamin D. The physically inactive individuals also typically spend less time outdoors than the active individuals, which may lead to a lower cutaneous vitamin D production. While a positive association between the PAL and sun exposure was present in both sexes in our study, a significant association between the PAL and % TBF was only observed in women (data not shown). According to the results of the multiple regression analyses, the amounts of sun exposure and physical activity appear to not be responsible for the inverse association of the % TBF with $25(OH)D_3$, as also suggested by others [12,43]. Moreover, smoking, alcohol or the habitual intake of vitamin D, calcium or vitamin D supplements could not explain the association between the % TBF and vitamin D status in our study. Nevertheless, we noted an unexpected strong and independent negative association between smoking and $25(OH)D_3$ in men. While some previous studies reported a negative association [17,44,45], other researchers failed to confirm such a relationship [8,15]. Another finding of our study is the positive association between alcohol intake and $25(OH)D_3$ in women, which has also been reported by others [17,45]. Neither dietary nor supplemental vitamin D had an impact on $25(OH)D_3$ in our study, which is in contrast to some [15,17], but not all [8,11], previous studies. Given the low vitamin D intake from diet and supplements and the low percentage of supplement users in our study, these amounts may have been too low to affect $25(OH)D_3$ levels in non-vitamin D-deficient subjects.

Finally, we observed no age dependency of $25(OH)D_3$ levels although dermal vitamin D synthesis declines in advanced age [46]. It is possible that the age gradient was too small, but most likely, an age-related impairment of vitamin D synthesis is compensated for by adequate sun exposure in our subjects. This is in line with the observation that individuals aged ≥ 60 years are able to synthesise enough vitamin D during outdoor activities and thus show a 25(OH)D status similar to young adults [19]. However, we found that the association between the % TBF and $25(OH)D_3$ seems to strengthen after adjusting for age.

Our study has several strengths and weaknesses. For the strengths, this study examined both independently living elderly women and men without a vitamin D deficiency. A special feature in our approach was the consideration of age, iPTH and a variety of lifestyle factors as well as different cut-off levels of $25(OH)D_3$ that were used to evaluate the vitamin D status. In addition, confounding was minimised due to well-defined exclusion criteria. For the weaknesses, the cross-sectional design limited our ability to establish causal relationships. Due to the sample size, it is possible that some associations were classified as not statistically significant because of a type II error. Nevertheless, we found an independent and robust association between the % TBF and $25(OH)D_3$ levels in women. The subjects in this study were volunteers, had a higher educational level and were more aware of health issues than their peers in the general German population. In general, the study comparability is limited due to varying study designs among this and other studies, especially regarding the period of recruitment, assay methods and differences in the participants' ages and BMIs. The use of different 25(OH)D assays has been a matter of dispute [47]. It is possible that the ECLIA used in this study has systematically over- or underestimated the $25(OH)D_3$ levels of our subjects. However, the measurement results of the ECLIA are in a good overall agreement with those determined by tandem mass spectrometry [48]. Further limitations are the use of self-reported data, the indirect measurements of sun exposure and physical activity and missing information on the exact dosage and duration of the intake of vitamin D supplements. Considering the seasonal variation of $25(OH)D_3$ levels, our data may not clearly reflect the year-long vitamin D status of our participants. Finally, while dual-energy X-ray absorptiometry (DXA) may reflect the body composition better than BIA, our BIA equation has been validated against the DXA. In general, BIA works well in subjects with a relatively stable water and electrolyte balance when using an appropriate and validated BIA equation [49].

Conclusion

In conclusion, the present study provides evidence that, especially in elderly women, the % TBF can be an important negative determinant of the $25(OH)D_3$ status, which is for the most part independent of age, lifestyle factors and iPTH. Remarkably, we found this association during the summer in subjects who were primarily not obese and not vitamin D-deficient. Consequently, the requirements for vitamin D to achieve or exceed a defined cut-off level for $25(OH)D_3$ may depend on the % TBF, which may be highly variable even at a given BMI. Although our results indicate that, besides regular physical activity, adequate sun exposure and abstinence from smoking, a reduction in TBF may be one possible strategy to improve the vitamin D status, this may not easily be achievable or not even appropriate in the elderly. Therefore, and in view of the high prevalence of

overweight and obesity and potential associations of vitamin D with a variety of chronic age-related diseases, a clinical implication might be that especially overweight, post-menopausal women should be screened for vitamin D deficiency and, as the case may be, specifically advised to increase their vitamin D intake either by supplements or vitamin D-enriched foods.

Abbreviations
25(OH)D: 25-hydroxyvitamin D; 25(OH)D₃: 25-hydroxyvitamin D₃; iPTH: intact parathyroid hormone; GISELA: longitudinal study on nutrition and health status in senior citizens of Giessen, Germany; BMI: body mass index; WHR: waist-to-hip ratio; WC: waist circumference; HC: hip circumference; TBF: total body fat; FFM: fat-free mass; BIA: bioelectrical impedance analysis; ECLIA: electrochemiluminescence immunoassay; PAL: physical activity level; DXA: dual-energy X-ray absorptiometry; IoM: institute of Medicine.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
AJ performed the statistical analysis, interpreted the data and wrote the manuscript. HJR analysed the blood samples. MNB designed the study, conducted the research and proofread the manuscript. All authors approved the final manuscript.

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