# Chapter 2.1

# BMI and body fat mass is inversely associated with vitamin D levels in older individuals

Sadaf Oliai Araghi, Suzanne C. van Dijk, Annelies C. Ham, Elske M. Brouwer-Brolsma, Anke W. Enneman, Evelien Sohl, Karin M.A. Swart, Nikita L. van der Zwaluw, Janneke P. van Wijngaarden, Rosalie A.Am. Dhonukshe-Rutten, Natasja M. van Schoor, M. Carola Zillikens, Paul Lips, Lisette C.P.G.M. de Groot, André G. Uitterlinden, Nathalie van der Velde

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## ABSTRACT

**Objective**

To assess the association between obesity (measured by Body Mass Index (BMI) and fat percentage) and serum 25(OH)D levels in older persons.

**Design**

Cross-sectional analysis of data from 'the B-PROOF study' (B-vitamins for the Prevention Of Osteoporotic Fractures).

**Participants**

2842 participants aged 65 years and older.

### Measurements

BMI and fat percentage, measured by Dual Energy X-ray, and serum 25(OH)D levels.

### Results

Mean age was 74 years (SD 6.5), with 50% women. Mean serum 25(OH)D levels were 55.8 nmol/L (SD 25). BMI and total body fat percentage were significant inversely associated with serum 25(OH)D levels after adjustment for confounders (ß-0.93; 95%CI [-1.15; -0.71], p<0.001 and ß-0.84; 95%CI [-1.04; -0.64], p<0.001). This association was most prominent in individuals with a BMI in the 'overweight' and 'obesity' range (ß -1.25 and -0.96 respectively) and fat percentage in the last two upper quartiles (ß-1.86 and -1.37 respectively).

### Conclusion

In this study, higher BMI and higher body fat percentage were significantly associated with lower serum 25(OH)D levels in older persons. This association was particularly present in individuals with overweight, and higher fat percentages, suggesting that these persons are at increased risk of vitamin D insufficiency.


Keywords: BMI, Fat percentage, vitamin D, elderly people

# INTRODUCTION

The percentage of individuals with overweight is growing in all age categories (1). This is an alarming issue (2), as overweight and obesity have been associated with a range of serious health consequences, including increased risk of metabolic syndrome, coronary heart disease, hypertension, type 2 diabetes, stroke and certain types of cancers (3,4). Furthermore, being overweight and obesity have been shown to alter the absorption, distribution, metabolism and/or excretion of micronutrients, which can cause several vitamin deficiencies (5-10). In particular in elderly, were vitamin deficiencies are more common (11,12). In this context, vitamin D deficiency has been associated with obesity (5-8). Because an accurate vitamin D level is important for calcium homeostasis5-8, and osteoporosis is a serious health problem in the older population (13), it is important to investigate the role of obesity in vitamin D deficiency.

A recent meta-analysis showed a significant inverse weak association between Body Mass Index (BMI) and serum 25-hydroxy vitamin D (25(OH)D) levels (14). However, this study did not analyze the relationship between body fat mass (fat percentage) and serum 25(OH)D levels. it needs to be emphasized that the way to measure overweight by BMI amongst the population of elderly is also under debate (15). Aging is associated with changes in body composition (16-18), this leads to loss in muscle mass and muscle strength (19). Therefore, BMI could underestimate the prevalence of obesity in this population, and fat percentage could be a better predictor for obesity than BMI in elderly individuals (20). So, dependent on the above mentioned arguments, body fat may be a better indicator of overweight than BMI. Consequently, we will investigated the association between BMI and fat percentage, and serum 25(OH)D levels, in a large population of older persons.

# MATERIALS AND METHODS

## Study participants

For the present cross-sectional analyses, baseline data of the 'B-PROOF study' (B-vitamins for the Prevention Of Osteoporotic Fractures) were used. B-PROOF is a multi-center, randomized, placebo controlled, double-blind, intervention study, investigating the effect of a 2-year daily oral vitamin B12 (500 µg) and folic acid (400 µg) supplementation on fracture incidence. The study was conducted in three research centers in the Netherlands: Vu University Medical Center (Amsterdam), Wageningen University (Wageningen), and Erasmus Medical Center (Rotterdam). This study included 2919 individuals, aged 65 years and older with an elevated homocysteine levels (12 - 50 µmol/l). Participants were excluded if they had a renal insufficiency (creatinine level > 150 µmol/l) or presence of a malignancy in the past 5 years. A detailed description of the trial has been reported elsewhere (21).

All participants gave written informed consent before the start of the study. The B-PROOF study has been registered in the Netherlands Trial Register (NTRNTR1333) and with ClinicalTrials.gov (NCT00696514). The WU Medical Ethics Committee approved the study protocol, and the Medical Ethics committees of Erasmus MC and VUmc gave approval for local feasibility (21).

## Clinical and anthropometrics measurements

Clinical and anthropometric measurements include height, weight and blood pressure. Height was measured in duplicate to the nearest 0.1 cm with the participant standing erect and without wearing shoes, using a stadiometer (21). Weight was measured to the nearest 0.5 kg using a calibrated weighing device (SECA 761) with the participant wearing light garments, empty pockets and without wearing shoes (21). BMI was calculated as weight in kilograms divided by square of height in meters and expressed as kg/m2. Participants were categorized in underweight (BMI < 20), normal weight (BMI 20 - 25.0), overweight (BMI 25.0 - 30) and obesity (BMI > 30) (22). Blood pressure measurements were performed two times on the left arm using an Omron M1 plus blood pressure device (Omron Healthcare Europe). The measurement with the lowest diastolic blood pressure was used for further analyses. Hypertension was defined as systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg.

Demographic characteristics and health status variables, which included age, sex, self-reported medical history (cardiovascular disease and diabetes), alcohol intake, smoking habits, and vitamin supplement use, were determined using a structured questionnaire. Alcohol intake was categorised into 'never', 'light', 'moderate' and '(very) excessive' drinkers, based on the number of days per week alcohol was consumed and the number of glasses per time, following the Dutch method of Garretsen et al. (23,24). Smoking habits were defined as never smoked, former smoker or current smoker and vitamin D supplement use was defined as users or non-users.

## Physical activity

At baseline, participants were asked to complete a questionnaire about their daily physical activity during the past two weeks, including walking, biking, light and heavy household work, gardening and sports using a validated questionnaire (LAPAQ) (25) and was calculated in kilocalories (Kcal) per day.

## Body composition

A subsample of participants underwent Dual Energy X-ray assessment (DXA) using the GE Lunar Prodigy device (GE Healthcare, USA, CV = 0.08%), (Erasmus MC) and the Hologic QDR 4500 Delphi device (Hologic Inc., USA, CV – 0.45%), (VuMC under standard protocols at baseline. The two devices were cross-calibrated by measuring a European spine phantom (ESP) five times on both devices and all results were adjusted accordingly. Total body composition was calculated by summing the amount of fat-free soft

tissue (i.e. lean mass minus bone mineral content) and fat mass. Fat percentage was also calculated from the DXA scan (21). For analyses, fat percentage was divided in quartiles.

## Biological sample collection and analysis

Venous blood samples were obtained in the morning, when the participants were in a fasted state, or had taken a restricted breakfast. Serum 25(OH)D was released from the protein through a denaturized internal standard (IS: 25(OH)D3-d6). Samples were extracted and analyzed by XLC-MS/MS (a Symbiosis online SPE system (Spark Holland, Emmen, the Netherlands) coupled to a Quattro Premier XE tandem mass spectrometer (Waters Corp., Milford, MA). The inter-assay coefficient of variation was 9% at the level of 10 ng/mL and 6% at the level of 25 ng/mL. All analyses were performed in the Endocrine Laboratory of the VU University Medical Center. The cut-off value for vitamin D deficiency was defined as a serum 25(OH)D levels < 50 nmol/L7,26 which was based on the current recommendations by the Institute of Medicine (27) and the recommendations for the older adults aged >70 years by the Dutch Health Council. Season of blood collection was dichotomized into summer (April – September) and winter (October – March) for the analyses.

## Statistical analyses

The total B-PROOF population was included to investigate the association between BMI and 25(OH)D levels (n=2842). To study the association between body fat percentage and serum 25(OH)D levels, a subsample of participants that underwent a DXA scans (n=1197) was used. Differences between subsamples were tested using the student t-test or Mann-Whitney U test, based on normally distributed or skewed data. Normal distribution for all variables was tested by visual inspection of histograms.

Second, linear regression analysis was used to determine associations between BMI, body fat mass and 25(OH)D levels (model 1, crude). Subsequently, age and gender were added as fixed confounders (model 2a). Thereafter, other potential confounders were added using the forward selection method (in model 2b). Potential confounders were smoking, alcohol intake, hypertension (yes or no), self-reported cardiovascular disease, total physical activity (in kcal/day), and season of blood collection. To address the potential mediating effect of vitamin D supplement use, this factor was added to the model. When the point estimate of interest changed >10%, vitamin D supplement use was regarded as a potential mediator and included in the final analysis. In addition, the interaction of age and total activity was tested in the crude

model, and a P value < 0.1 for the interaction was considered statistically significant. If the interaction term was statistically significant, stratified analyses were performed. Stratification was performed as follow: age was dichotomized as younger or older than 80 years; and for total activity we created quartiles; both only when the interaction term was significant.

Further, we tested the associations between body fat percentage and serum 25(OH) D levels in different BMI categories (underweight, normal weight, overweight and obesity) and also per quartile of body fat percentage.

Statistical analysis was performed using the statistical software package of SPSS 21.0 (SPSS Inc., Chicago, Illinois, USA). P-values of < 0.05 were considered statistically significant for all the analyses other than the interaction analyses (<0.1).

# RESULTS

## Population characteristics

Population characteristics are presented in **Table 1**. Mean age was 74.0 years (6.5 SD) for the total population (n=2842) and 72.8 years (5.7 SD) for the DXA population (n=1197). Mean BMI for the total population was 27.2 (4.0 SD), and 27.0 (3.8 SD) for participants who underwent a DXA measurement. The participants who underwent a DXA scan were significantly younger, more active, largely included during summer; more likely to have hypertension and different alcohol consumption patterns (more moderate and excessive alcohol intake and less very excessive drinkers) when compared to the total B-PROOF population.

## BMI and serum 25(OH)D levels

Results of the linear regression analyses of BMI and serum 25(OH)D levels are showed in **Table 2**. BMI was inversely associated with serum 25(OH)D levels after adjustments for covariates, indicating that for each unit increase in BMI there was a decrease in 25(OH)D level of 0.93 nmol/L(β -0.93, $p < 0.001$). Age was a significant interaction-term ($p = 0.02$) in this association and total physical activity was not. Stratification for age showed that the association between BMI and serum 25(OH)D levels was most pronounced in participants younger than 80 years (β -0.97 $p<0.001$) compared to the participants older than 80 years (β -0.72 $p=0.006$), **Table 3**.

When considering the categories of BMI, we observed that in overweight and obese individuals, BMI was significantly associated with serum 25(OH)D levels (β -1.25, $p=0.004$ and β -0.96, $p=0.004$ respectively, as showed in **Table 4**).

## Fat percentage and serum 25(OH)D levels

The association between fat percentage and serum 25(OH)D levels is showed in Table 2. Fat percentage was inversely associated with serum 25(OH)D levels, after adjustments for covariates, (β -0.84, $p < 0.001$). No significant interaction effects were observed. We did observe a stronger association for the 3th and 4th quartile of body fat percentage (β -1.86, $p=0.01$ and β-1.37, $p<0.001$ respectively) and no association in the 1th and 2th quartiles (β-0.02, $p= 0.92$ and β-1.24, $p=0.16$ respectively, as showed in **Table 5**).

**Table 1. Population characteristics**

| | B-PROOF Participants (N = 2842) | DXA-test Participants (N = 1197) | Comparison B-PROOF participants and DXA scan participants p-value |
|---|---|---|---|
| Age (years)[a] | 74 (6.5) | 73 (5.7) | <0.001* |
| Gender | | | |
| Female (%) | 50 | 48 | 0.16 |
| Body Mass Index (kg/m$^2$) | 27.2 (4.0) | 27.0 (3.8) | 0.03 |
| Underweight (%) | 1 | 2 | |
| Normal weight (%) | 28 | 29 | |
| Overweight (%) | 51 | 50 | |
| Obesity (%) | 20 | 19 | |
| Fat | | | |
| Total Fat Mass (Kg) | NA | 25.6 (8.4) | NA |
| Total Fat Percentage (%) | | 32.5 (8.2) | |
| Smoking (%) | | | 0.56 |
| Current | 10 | 9 | |
| Former | 56 | 57 | |
| Never | 34 | 34 | |
| Alcohol intake (%) | | | 0.001* |
| Light | 67 | 64 | |
| Moderate | 29 | 32 | |
| Excessive | 3 | 4 | |
| Very excessive | 1 | 0 | |
| Self-reported medical history of | | | |
| Cardiac disease (% yes) | 25 | 25 | 0.97 |
| Diabetes (% yes) | 10 | 11 | 0.40 |
| Measured hypertension (% yes)* | 52 | 59 | 0.89 |
| 25(OH)D (nmol/L)[a] | 55.8 (25) | 55.1 (24) | 0.26 |
| Vitamin D <25 nmol/L (%) | 10 | 8 | 0.01* |
| Vitamin D <50 nmol/L (%) | 47 | 48 | 0.21 |
| Vitamin D supplement use (% yes) | 20 | 21 | 0.80 |
| Total activity (Kcal/day)$_a$ | 649 (477) | 714 (529) | <0.001* |
| Region (%) | | | <0.001* |
| Amsterdam | 26 | 34 | |
| Rotterdam | 44 | 66 | |
| Wageningen | 30 | 0 | |
| Season of blood collection(%) | | | <0.001* |
| Summer (April-September) | 51 | 43 | |
| Winter (October-March) | 49 | 57 | |

[a]Presented as mean (SD) *significantly differences between total population and DXA-test participants

**Table 2. Linear regression results of obesity parameters (BMI and fat-percentage) and serum 25(OH)D levels**

| | Model 1 | | | Model 2[a] | | | Model 2[b] | | |
|---|---|---|---|---|---|---|---|---|---|
| Variable | ß | [95% CI] | p | ß | [95% CI] | p | ß | [95% CI] | P |
| Body Mass Index (kg/m$^2$) | -0.78 | [-1.01 ; -0.55]* | <0.001 | -0.84 | [-1.07 ; -0.62]* | <0.001 | -0.93 | [-1.15 ; -0.71]* | <0.001 |
| Total Body Fat Percentage (%) | -0.52 | [-0.68 ; -0.35]* | <0.001 | -0.84 | [-1.05 ; -0.64]* | <0.001 | -0.84 | [-1.04 ; -0.64]* | <0.001 |

Model 1: crude model. Model 2[a]: adjusted for age and sex. Model 2[b]: adjusted for total activities (sport and non-sport) in Kcal per day, smoking, alcohol and season of blood collection. *P-value <0.05.

**Table 3. Linear regression results of BMI and serum 25(OH)D levels, stratified for age**

| BMI | Model 1 | | | Model 2[a] | | | Model 2[b] | | |
|---|---|---|---|---|---|---|---|---|---|
| | ß | [95% CI] | p | ß | [95% CI] | p | ß | [95% CI] | p |
| <80 year N = 2302 | -0.91 | [-1.16; -0.66] | <0.001 | -0.91 | [-1.16; -0.65] | <0.001 | -0.97 | [-1.22; -0.73]* | <0.001 |
| ≥ 80 year N = 540 | -0.48 | [-0.99; 0.03] | 0.06 | -0.61 | [-1.12; -0.10] | 0.02 | -0.72 | [-1.22; -0.21]* | 0.006 |

Model 1: crude model Model 2[a]: adjusted for sex. Model 2[b]: adjusted for alcohol, total activities (sport and non-sport) in Kcal per day, smoking, alcohol and season of blood collection. *P-value <0.05.

**Table 4. Linear regression results of BMI and serum 25(OH)D levels, BMI in categories**

| BMI | Model 1 | | | Model 2[a] | | | Model 2[b] | | |
|---|---|---|---|---|---|---|---|---|---|
| | ß | [95% CI] | p | ß | [95% CI] | p | ß | [95% CI] | p |
| Underweight N = 45 | 1.20 | [-5.00; 7.35] | 0.70 | 1.27 | [-4.90; 7.44] | 0.68 | - | - | - |
| Normal weight N = 788 | -1.02 | [-2.50; 0.47] | 0.18 | -1.14 | [-2.60; 0.32] | 0.13 | -1.16 | [-2.59; 0.28] | 0.11 |
| Overweight N = 1444 | -0.99 | [-1.87; -0.10]* | 0.03 | -1.10 | [-1.97; -0.22]* | 0.01 | -1.25 | [-2.10; -0.40]* | 0.004 |
| Obesity N = 565 | -0.82 | [-1.43; -0.22]* | 0.001 | -0.89 | [-1.49; -0.28]* | 0.004 | -0.96 | [-1.54; -0.38]* | 0.001 |

Model 1: crude model. Model 2[a]: adjusted for age and sex. Model 2[b]: adjusted for alcohol, total activities (sport and non-sport) in Kcal per day, smoking, alcohol and season. *P-value <0.05.

**Table 5. Linear regression results of Fat% and serum 25(OH)D levels, fat% in quartiles**

| Fat% | Model 1 | | | Model 2[a] | | | Model 2[b] | | |
|---|---|---|---|---|---|---|---|---|---|
| | β | [95% CI] | p | β | [95% CI] | p | β | [95% CI] | p |
| Quartile 1 N = 298 | -0.06 | [-0.87; 0.75] | 0.88 | -0.04 | [-0.86; 0.78] | 0.92 | -0.02 | [-0.80; 0.77] | 0.97 |
| Quartile 2 N = 299 | -0.69 | [-2.45; 1.07] | 0.44 | -1.29 | [-3.03; 0.46] | 0.15 | -1.24 | [-2.95; 0.48] | 0.16 |
| Quartile 3 N = 299 | -1.12 | [-2.66; 0.42] | 0.15 | -1.96 | [-3.50; -0.42]* | 0.01 | -1.86 | [-3.29; -0.43]* | 0.01 |
| Quartile 4 N = 298 | -1.21 | [-1.87; -0.55]* | <0.001 | -1.33 | [-2.00; -0.66]* | <0.001 | -1.37 | [-2.02; -0.71]* | <0.001 |

Model 1: crude model. Model 2[a]: adjusted for age and sex. Model 2[b]: adjusted for alcohol, total activities (sport and non-sport) in Kcal per day, smoking, alcohol and season. *P-value <0.05.

# DISCUSSION

In our study we observed that BMI was significantly associated with serum 25(OH)D levels in older adults. After stratification for age, the association between BMI and serum 25(OH)D was only modest in the oldest group (>80 year), but stronger in the individuals younger than 80 years of age. This finding is consistent with the results of a recent meta-analysis, which showed that BMI inversely associated with 25(OH)D levels in a younger population14. Furthermore, we also observed that fat percentage was significantly associated with serum 25(OH)D levels and the association was more pronounced in the third and fourth quartiles of fat percentage. This finding supports the hypothesis that compared to BMI, body fat percentage is possibly a more accurate marker of obesity in our study group, to analyze the association between 'overweight' and serum 25(OH)D levels and could be used instead or next to BMI for measuring obesity in an older population.

A recent bi-directional genetic study, a design known to reduce the possibility of confounding, suggested that higher BMI leads to lower 25(OH)D levels in a younger population (mean age 53.4 years). Additionally, this study suggested an only modest relationship between lower 25(OH)D levels and BMI (28). The mechanism underlying the association between obesity and serum 25(OH)D levels is not yet completely understood. Several factors may be responsible for the observed association in this population of older adults, including limited sun exposure due to impaired mobility or clothing habits (29). In addition, it may be speculated that older obese persons have a lower vitamin D dietary intake, which may also lead to decrease in serum 25(OH)D levels (30,31). Moreover, laboratory findings indicated that adipose tissue is a storage site for 25(OH)D (32,33), and therefore it has been proposed that the

obesity-associated vitamin D deficiency may be due to the decreased bioavailability of vitamin D owing to its deposition in body fat compartments (34). Studies have also shown that weight loss and reduced body fat mass in obese persons is often accompanied with improvements in serum 25(OH)D levels (35).

The association between body fat and serum 25(OH)D levels may also be explained by metabolic pathways related to glucose intolerance. Particularly, it has been shown that a higher 25(OH)D levels may result in a higher insulin sensitivity, decrease in appetite and food intake, and thus a lower body fat percentage (36). Conversely, a higher body fat percentage may also result in lower serum 25(OH)D levels (36). As a result, vitamin D deficiency is more common in obese people (37), and this was also observed in our study. Based on the studies described above we can hypothesized that obese persons would require higher dosage of vitamin D supplementation to achieve accurate25(OH) vitamin D levels (38).

### Limitations and future research

The main strengths of our study are its large population, and the use of both BMI and fat percentage measured by DXA. Limitations include, the cross-sectional approach, which prevents us from drawing conclusions regarding causality and the direction of the association. Secondly, there were some differences between the sub-samples, which may be explained by the fact that the younger and fitter persons were the ones that were able to visit the hospital to undergo the DXA scan, and this may have biased our results.

## CONCLUSION

We observed an inverse association between BMI, body fat percentage, and serum 25(OH)D levels in elderly people. Thus, a higher BMI and a higher body fat percentage were associated with lower serum 25(OH)D levels. Although it is well known that elderly people overall are at risk for vitamin D deficiency, the results of the current study indicate that vitamin D deficiency is particularly important for obese older adults. This study suggests furthermore that, other anthropometric measurements, including fat mass percentage, may be more reliable measures of obesity than BMI, particularly if study outcomes are fat-mass related. Thus, it can be concluded that further research is needed to assess the direction and potential causality of this association in older persons and the effect of fat percentage on the dose-response effect of vitamin D supplementation in older persons.

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ETHICAL STANDARDS

We have worked according to the current laws in the Netherlands

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