# Serum 25-hydroxy vitamin D levels in middle-aged women in relationship to adiposity and height trajectories over three decades

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## Abstract

BACKGROUND/OBJECTIVES:

The long-term chronology of the association between low serum concentrations of 25-hydroxy vitamin D (25(OH)D) and weight status is unclear. We examined whether lower 25(OH)D in middle-aged women drives upwards the weight, body mass index (BMI) and waist-hip ratio (WHR) over the next 32 years, and whether higher 25(OH)D might predict less decline in the mid- to late-life height trajectory.

SUBJECTS/METHODS:

The Population Study of Women in Gothenburg started in 1968-1969 (the baseline) in 38-60-year-old women residing in Gothenburg, Sweden. Anthropometric measures were taken at baseline and 4 re-examinations until 2000-2003. Levels of 25(OH)D were analyzed in serum stored since baseline in 1227 (84%) women. Repeated measures analyses were used to model associations between 25(OH)D (dichotomized, cut point 51.45 nmol/l) at baseline and anthropometric trajectories, adjusting for fixed and time-dependent covariates.

RESULTS:

At baseline, mean BMI was 25.2 kg/m(2) in women with low 25(OH)D and 23.8 kg/m(2) in the remaining women (P<0.001), but this difference did not increase over 32 years and longitudinal differences were explained by the baseline BMI. Similar results were observed for weight and WHR. In contrast, no association was seen for height at baseline or longitudinally.

CONCLUSIONS:

No relationship was observed between 25(OH)D height trajectory, but lower 25(OH)D was associated with higher BMI, weight and WHR differences that were maintained over three decades. This provides no evidence for the direction of causality, but for a life-long difference in adiposity-related measures according to the 25D level in middle-aged women.

**Keywords: 25D, Body Mass Index, Waist-Hip Ratio, Height, Weight, Longitudinal Studies**

**Background**

Serum concentrations of 25-hydroxy vitamin D (25(OH)D) (hereafter referred to as 25D for simplicity) level is well known to be associated with obesity but the direction of causality remains unclear, as concluded by at least four reviews in 2012-2013 (Earthman 2012, Soares 2012, Song 2012, Vanlint 2013). However, potential mechanisms towards both directions are manifold (Vanlint 2013, Earthman 2012, Foss 2009, Song 2012, Soares 2012, see also Drincic 2012, Wortsman 2000, Wamberg 2013).

Excluding 25D supplementation and weight loss studies, we are aware of six longitudinal observational studies on the association between serum 25D levels and measures of obesity in adult populations. In four studies, adiposity was considered as the exposure and serum 25D as the outcome. In all but one of these studies (Ding 2010, Jorde 2010, Jamal-Allial 2013, Gonzales-Moreno 2013), higher BMI (or other measures of adiposity) at baseline were related to smaller change in the serum 25D levels, or increased incidence of, and lower recovery from, 25D deficiency. In three studies, serum 25D was considered as the exposure and adiposity as the outcome. In two of these studies (Mai 2012, Gonzales-Moreno 2013), lower serum 25D status at baseline was associated with higher incidence of obesity during the 4-year (Gonzales-Moreno 2013) or 11-year follow-up (Mai 2012), whereas the third study (Young 2009) found a cross-sectional inverse association between serum 25D and various measures of adiposity at baseline but no associations longitudinally. In addition, the authors of a meta-analysis with a genetic approach (genetic markers for serum 25D and BMI, and a bi-directional Mendelian randomization analysis), concluded that higher BMI seems to lead to lower serum 25D level, whereas the opposite effect is likely to be small, if any (Vimaleswaram 2013).

Against this background, our aim was to evaluate the association between serum 25D level in middle-aged women and longitudinal development of adiposity in a study with a follow-up for as many as 35 years. We measured overall adiposity by body mass index (BMI) and central adiposity by waist-hip ratio (HWR). As height is known to decrease in older age (Sorkin et al. 1999 review), and changes in BMI might therefore result from changes in weight and/or height, we also evaluated if serum 25D level was associated with changes in weight and height during the follow-up. In addition, we studied if socioeconomic position (SEP) modifies the association between serum 25D and development of BMI and WHR.

**Methods**

The present study is part of the Population Study of Women in Gothenburg, Sweden. In 1968, women born in 1908, 1914, 1918, 1922 and 1930 were systematically sampled from the census register based on specific birth dates in order to yield a representative sample of 38-, 46-, 50-, 54- and 60-year-old women. Of those sampled, 1462 (participation rate 90%) women participated in a health examination in 1968-1969 (Bengtsson et al. 1973). This examination constituted the baseline for a prospective study with four re-examinations in 1974-1975, 1981-1982, 1992-1993 and 2000-2003. In 2000-2003, altogether 661 women participated in the 32-year follow-up (71% of those who were alive at that time). Details of the sampling, participation rates and data collection protocol have been described earlier (Bengtsson 1973, 1978, 1989 and 1997, Lissner 2003). The Ethics Committee of Gothenburg University approved the study. All subjects gave informed consent to participate, in accordance with the provisions of the Helsinki Declaration.

During the baseline visit (1968-1969), a fasting blood sample (120 ml) was drawn from each study subject. A small quantity was used for immediate analysis and the remainder was stored for future analyses as follows: The sample was first allowed to cloth at room temperature and then centrifuged. After centrifugation, the serum was divided in 2.5 ml covered polystyrene cups enclosed together in small batches in firmly tied plastic bags which were then stored at -20°C. In 2013, the serum samples were retrieved from the storage freezer, and 25D levels were analyzed with the Cobas e601 instrument that uses the Roche Diagnostics 25D total assay and a competitive ECLIA protein-binding assay. The assay employs a 25D binding protein as a capture protein, which binds to both $25D_3$ and $25D_2$. Intra- and inter-assay coefficients of variation were below 5%. The analysis was performed at the Clinical Chemistry Laboratory, Sahlgrenska University Hospital, Mölndal. (Monica L et al 2015 **insert reference when available**). Among the 1462 participants of the 1968-1969 examination, with serum 25D levels were measured for 1227 women (84% of the participants)(Figure 1).

As described earlier (Bengtsson 1973, Lapidus et al. BMJ 1984), all anthropometric measures were taken with the woman standing and wearing only briefs. Body weight was measured to the nearest 0.1 kg with a balance scale. Body height without shoes was measured to the nearest 0.5 cm. Body mass index (BMI) was calculated as weight (kg)/height ($m^2$). Waist circumference (WC) was measured to the nearest 1 mm at the level midway between the lower rib margin and the iliac crest using a steel tape measure. Hip circumference was measured with the same steel tape measure to the nearest 1 mm at the widest point between hip and buttock. Waist-hip ratio (WHR) was calculated. In the present study, BMI was used as an indicator of overall adiposity, and WHR of central adiposity. In 1968-1969, weight and height were available for all the 1227 women with measured serum 25D, whereas WHR was available for 1211 women, respectively. In 2000-2003, the respective figures were 486 for weight and height, and 484 for WHR (Figure 1).

Country of origin was defined as Sweden or other, based on information about place of birth that was obtained from the Revenue Office Register. Information about education and SEP was obtained by questionnaire. For the purposes of the present study, educational level was dichotomized according to whether the woman had continued in school after elementary school or not. SEP was categorized into three categories (higher employee, employee, worker) based on the woman's or her husband's profession, whichever was higher. If the highest was domestic work only or pension, they were coded as missing. Information about smoking habits was obtained via a standardized interview (Bengtsson 1973), and smoking history was ascertained at each examination. In the present study, smoking habits were categorized at each examination according to whether the woman had never smoked, was an ex-smoker (stopped during the previous year or earlier), or a current smoker. Women who smoked ≥ 1 cigarette/pipe per day were defined as smokers. The women were also interviewed, at each examination, about physical activity during leisure time, which was dichotomized according to whether the woman reported having less than 4 hours, or at least 4 hours, leisure time physical activity per week. Age at the menopause was obtained from a questionnaire supplemented by an interview, and ascertained at each follow-up. In the present analysis, age at the menopause was dichotomized according to whether the woman had had menopause by the time of the examination in question. Finally, season of 25D measurement was defined as early summer (May-June), late summer (August-October) and winter (November-April). There were no 25D measurements made during July.

**Statistical methods**

Differences in mean values of 25D, height, weight, BMI and WHR according to different baseline background factors was tested with ANOVA. For the purpose of further analyses, 25D values were dichotomized into the lowest quartile (51.45 nmol/L) versus the upper three quartiles combined. Differences in mean values of the anthropometric measures at each study visit between the two categories of baseline serum 25D was tested with ANOVA (Student's t-test).

Cross-sectional associations between 25D level and BMI or WHR at baseline were modeled using linear regression. Adjustment for potential confounders (age, educational level, SEP, country of origin, smoking, leisure time physical activity, menopause and season of 25D measurement) was first done using stepwise regression. Then, each of the variables left out from the stepwise regression model was entered into the model one by one, in order to see how much they would change the parameter estimate for 25D.

General linear modeling (GLM) was used to analyze associations between baseline 25D level and development of BMI or WHR over time. The repeated measurement analyses were performed using the MIXED procedure in SAS, with UNSTRUCTURED covariance matrix. Two kinds of models were fitted: 1) including the baseline BMI/WHR s one of the visits in the repeated measurements model and; 2) including only the follow-up visits in the repeated measurements model, adjusting for the baseline BMI/WHR. In both models, we first included only 25D as an explanatory variable. Second, the baseline value of the anthropometric measurement in question was added to the model. After that, the models were adjusted also for other baseline covariates, adding them one by one. Finally, further adjusted models were built, where smoking, menopause and leisure time physical activity were treated as time-varying covariates in the repeated measurements models.

Sensitivity analysis were made by leaving out from the model either educational level or SEP (they are closely correlated with each other), as well as by leaving out leisure time physical activity (because it could be a collider, affected by 25D and anthropometrics, and therefore cause a biased association between 25D and an anthropometric measure if conditioned on). Finally, we studied if SEP modifies the association between 25D and development of BMI or WHR. This was done by a) stratifying the regression analysis by SEP (worker vs. employee) and; b) by including an interaction term between SEP and 25D level into the regression model. For these analyses, SEP was dichotomized into higher (higher employee and employee) and lower (worker) SEP.

Drop-out analyses were performed such that participants and surviving non-participants at each follow-up study were compared as regards to their baseline characteristics. Concerning the first (1974-76) and second (1980-81) follow-up study, the difference between the participants and surviving non-participants was tested using a t-test for continuous variables (continuous 25D, weight, height, BMI, WHR) and a $X^2$-test for categorical variables (age, educational level, SEP, country of origin, smoking, leisure time physical activity, menopause, categorized 25D level and season of 25D measurement). At the third (1992-1994) and fourth (2000-2003) follow-up study, higher age was associated with higher proportion of non-participants; therefore, age was taken into account in the analysis as follows: Differences for continuous variables were age-adjusted based on the LS means; a GLM model was used. For categorical variables, differences between participants and surviving non-participants were calculated based on the observed frequencies. These differences were tested using age-adjusted logistic regression models, where the outcome was the probability of non-participating, and the characteristic of interest (e.g. season) was the independent variable.

Beta coefficients with their 95% confidence intervals (95%CI) are reported for all regression analyses, as well as $R^2$ values for the cross-sectional models. All statistical tests were 2-sided with $P<0.05$ considered as statistically significant. The statistical analyses were conducted with SAS 9.2 (SAS Institute Inc., Cary, NC, USA).

**Results**

At baseline, concentrations of 25D were statistically significantly lower in premenopausal women, as well as in women with a lower education level, SEP or level of leisure physical activity (Table 1). Women of Swedish origin had marginally higher 25D compared to other women. Season of examination was associated with 25D, with the highest mean concentration during late summer (August-October), and the lowest during the early summer (May-June, Table 1).

Increasing age was significantly associated with decreasing height and increasing weight, BMI and WHR at baseline (Table 1). Postmenopausal women and women with elementary education were shorter and heavier, and had higher BMI and WHR at baseline. Lower level of physical activity and lower SEP were also associated with higher weight, BMI and WHR (but not with height) at baseline. Smokers and ex-smokers were taller and had lower weight and BMI. Women of Swedish origin were somewhat taller than other women. In addition, WHR was significantly associated with season of 25D measurement, with the lowest WHR during the late summer and the highest WHR during the early summer. (Table 1)

The 25D level at baseline (≤ vs > 51.45 nmol/L) was not associated with height at baseline (163.4 cm vs 163.7 cm, p=0.48), nor at any of the follow-up visits (Figure 2, Table 2). In contrast, mean weight and BMI were higher at baseline in women with lower 25D (weight 67.5 kg vs 63.7 kg, $p<0.001$; BMI 25.2 kg/m$^2$ vs 23.8 kg/m$^2$, $p<0.001$), as well as at all subsequent study visits (statistically significant at all follow-up visits except for the third one). Mean WHR was also higher in women with lower 25D at baseline (0.75 vs 0.74, $p<0.001$) and at the two first follow-up visits (but not thereafter). (Figure 2, Table 2).

When adjusting for covariates, the baseline association for BMI was unchanged, with 1.45 (95%CI 0.97;1.92, $R^2$ 14.1%) kg/m$^2$ higher BMI for women with lower 25D (Table 2). The respective difference in BMI was 1.27 (95%CI 0.70;1.85) kg/m$^2$ for women with higher SEP, and 1.81 (95%CI 1.06;2.56) kg/m$^2$ for women with lower SEP. The interaction term between SEP and 25D was not significant (p=0.48). The baseline association for WHR was also unchanged when adjusting for covariates: WHR was 0.012 (95%CI 0.005;0.019, $R^2$ 11.8%) higher for women with lower 25D (Table 2). Stratified by SEP, the respective differences were 0.011 (95%CI

0.003;0.020) and 0.015 (95%CI 0.004;0.026)for women with higher and lower SEP. Again, the interaction term between SEP and 25D was not significant (p=0.68).

In the repeated measurement models, adjusted for covariates, there was no change in the estimate of 25D on BMI when all visits were included compared to the baseline association (β=1.47, 95%CI 0.99;1.94) (Table 2). When considering only the follow-up visits adjusted for baseline BMI, the association became negligible (β=0.01, 95%CI -0.27;0.24). The same was observed for high (β=0.03, 95%CI -0.29;0.35) and low (β=-0.06, 95%CI -0.48;0.35) SEP. Similarly to BMI, lower 25D was associated with higher WHR longitudinally when all visits were included (β=0.011, 95%CI 0.005;0.018), but not when baseline WHR was taken into account in the repeated measures model (β=-0.002, 95%CI -0.008;0.004). The estimate of 25D on WHR was negligible in women with higher (β=0.001, 95%CI -0.005;0.007) and lower (β=-0.003, 95%CI -0.013;0.006) SEP alike.

Older age was associated with higher dropout at the two last (i.e. third and fourth) follow-up visits. Regardless of age, higher dropout was associated with lower 25D at all follow-up visits, as well as with higher BMI and weight (statistically significant at the third follow-up visit), and higher proportion of women with elementary education (at the third and fourth follow-up visit), women having been at menopause at baseline (at the second and third follow-up visit), and women who were current smokers (at the second and fourth follow-up visit). SEP, leisure time physical activity, country of origin and height were not different between participants and (surviving) non-participants at any follow-up visit.

**Discussion**

Lower 25D at baseline was associated with higher BMI and WHR at baseline and at all follow-up visits. When the baseline difference in BMI or WHR between the two 25D groups was taken into account, no growing or decreasing difference was observed in the longitudinal development of BMI. In other words, the inverse association between 25D and BMI or WHR observed at the baseline persisted unmodified across the 30-year follow-up. There was no indication of a statistical interaction between SEP and 25D on BMI or WHR, either at baseline or longitudinally. A similar association as for BMI was observed for weight, whereas no association was observed for height, either at baseline or at any of follow-up visits.

The main strengths of the present study include long follow-up with several repeated anthropometric measurements that allowed as to assess the longitudinal development of BMI/WHR over time; indicators of both general (BMI) and abdominal/central (WHR) adiposity; and high participation rate (90%) at baseline. Further, we were able to adjust the analyses for several potential confounders, including the season of 25D measurements.

Some potential weaknesses need to be considered when interpreting the results. The validity of the 25D values measured in serum samples stored for a period of 40 years has been studied previously (Monica L et al. 2015 **add reference when available**); it was found that levels were comparable with those expected in fresh blood in terms of mean, range and winter to summer variation. However, in the current data four study subjects had 25D levels above 200 nmol/L. Generally, such high values cannot be achieved through sun exposure exclusively even when such exposure is abundant (Binkley 2007). Vitamin D supplements were not available in 1968, and achieving such high values from food would have required for example a daily consumption of 100g cod liver oil or 250g cod liver (Garland et al. 2011, Heaney 2008, Huotari 2008). It is therefore possible that these values represent true high values exaggerated by the long storage time of the serum samples **(ref.)**. As such, all study subjects were included in the main analyses. Sensitivity analyses were performed were the four study subjects, or only the study subject with the highest value, were excluded. The results were the same as in the main analyses, and are therefore not shown **[not done yet]**.

Higher dropout was associated with lower 25D at all follow-up visits, and at some varying visits with age, lower education, menopause and current smoking.

Residual confounding by diet, diseases, medications, or because of broad categories of covariates?

Finally, as 25D was measured only at baseline, we were not able to analyze bi-directional associations between 25D and BMI/WHR. Therefore, we cannot exclude the possibility that our findings simply

represent repeated cross-sectional associations. A high Spearman correlation (0.85, p<0.001) between measurement taken in 1968 and 1974, i.e. 6 years apart was observed in a validation study of the current data (Leu M et al. 2015). Other previous studies with repeated measurement of 25D over long periods (up to 14 years) reported correlation coefficients between 0.39 and 0.55, depending on the method of adjusting for season (Saliba et al. 2012, Jorde R et al. 2010). **[Needs elaboration]**

As regards to the generalizability of our results, the data included only women.

Season of 25D assessment was independently associated with WHR (but not with BMI), i.e. also after adjusting for the 25D level. We are aware of four longitudinal observational studies where serum 25D was considered as the exposure and adiposity as the outcome (Mai 2012, Gilbert-Diamond 2010, Young 2009, Gonzales-Moreno 2013). Regarding the studies in adult populations, an inverse association (lower serum 25D – higher adiposity) between serum 25D status and later adiposity was found in one of the studies (Mai 2012) whereas another study (Young 2009) found a cross-sectional inverse association at baseline but no association longitudinally. In addition, one study had a bi-directional approach, that is, they looked at associations between 25D status and obesity to both directions within the same data (Gonzales-Moreno 2013). They did not find an association between obesity at baseline (or development of obesity during the follow-up period) and 25D status at follow-up, whereas low 25D status at baseline was associated with an increased risk of becoming obese during the 4-year follow-up. The fourth study (Gilbert-Diamont et al. 2010) consisted of children aged 5-12 years at the baseline, and is the only study where the development of adiposity was measured repeatedly (annually for a median of 30 months). Using multivariate mixed linear regression models to analyze the mean changes in the measures of adiposity, and controlling for baseline adiposity and other potential confounders, they found that "lower 25D serostatus was associated with greater increases in BMI and indices of central adiposity" (Gilbert-Diamont et al. 2010). In addition, the authors of a recent meta-analysis (with 42,000 study subjects from 21 adult cohort studies) using genetic markers for serum 25D and BMI, and a bi-directional Mendelian randomization analysis, concluded that higher BMI seems to lead to lower serum 25D level, whereas the opposite effect is likely to be small, if any (Vimaleswaram 2013). However, only linear associations were analyzed, while at least one study (Jorde et al. 2010) reported a non-linear cross-sectional association where 25D levels were lower both in those with low and high BMI values.

In separate analyses of incidence of overweight and obesity, after excluding subjects that were overweight or obese at baseline, 25D was not identified as a risk factor for developing either of these conditions during the follow-up period (data not shown). This strengthens the finding of no further effect of 25D on the BMI development longitudinally once the baseline difference was taken into account.

Previous studies have suggested that 25D could affect fat mass and distribution, but have no or smaller effect on weight and BMI (Vanlint 2013, Arunabh 2003). BMI has also been critized for not being an optimal measure of adiposity, as it reflects both lean and fat mass. We were able to study BMI as well as WHR which reflects central adiposity (WHO report on WHR and WC); women with lower 25D had steadily higher values over time in both indicators and regardless of SEP. Further, BMI by definition reflects both weight and height. As height tends to decrease in older age (Sorkin 1999, Seidell & Visscher 2000), changes in BMI might results from changes in weight and/or height. In the present study, the cross-sectional associations between 25D and BMI at different study visits were reflected in similar associations between 25D and weight, while height was not associated with 25D, either at baseline or at any follow-up visit. This finding reinforces that what was observed for BMI is derived from differences in weight, not height.

Regarding height, we found only two previous, cross-sectional studies where height was considered in relation to 25D status. In the study of 18 to 76-year-old men and women from Greece (Pazaitou-Panayiotou et al. 2012), serum 25D was positively associated with height. However, the authors speculate that this relationship was a consequence of a positive relationship between body surface area (related to height and weight) and serum 25D, as height was not a statistically significant determinant of 25D in the multiple regression models (whereas body surface area was). In the study of 18 to 61-year-old men and women from Australia (Lucas et al. 2013), height (and hip circumference) were the best individual anthropometric determinants of serum 25D. In the multiple regression models, height was positively associated with serum 25D if hip circumference (with an inverse association with 25D) was also taken into account, but not alone. (Way more important determinants were sun exposure-related factors, genetic factors and skin phenotype.)

In conclusion, the inverse association between serum 25D level and BMI or WHR observed at the baseline persisted across the 30-year follow-up. These results provide no evidence for the direction of causality, but for a life-long difference in obesity-related measures according to the serum 25D level in middle-aged women.

Conflict of Interest:
None Disclosed.

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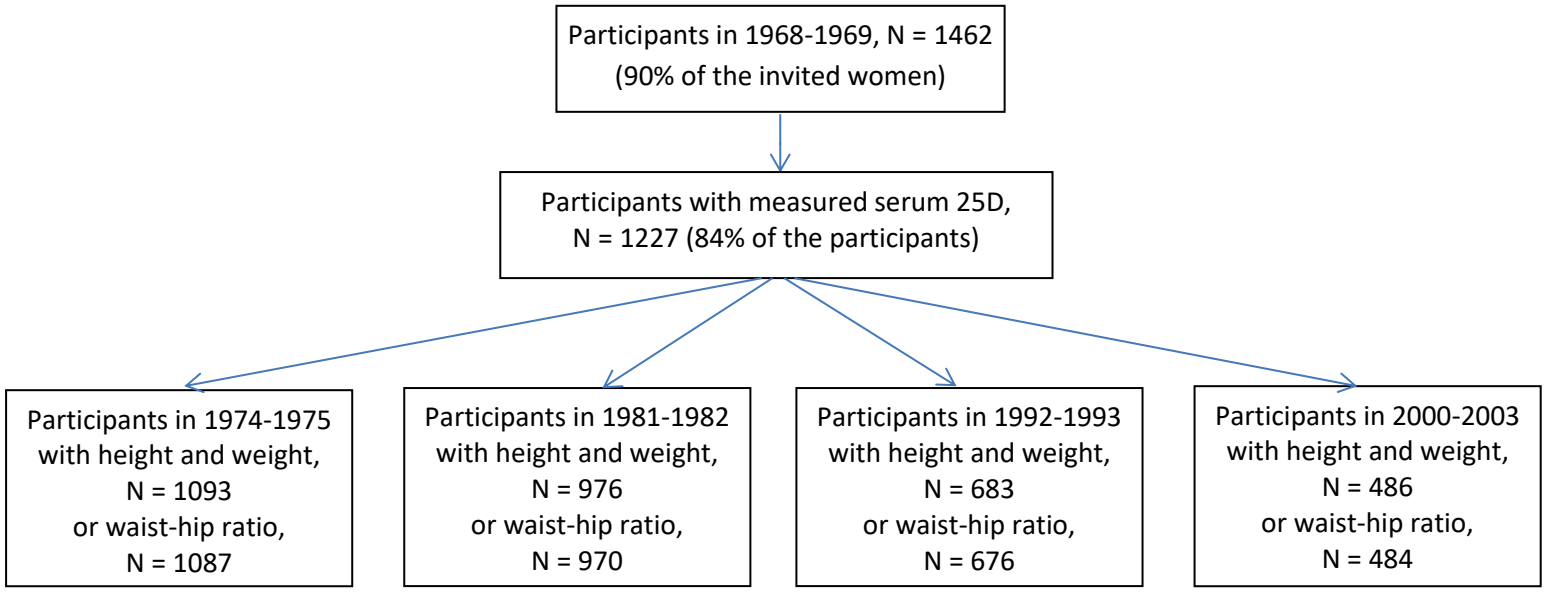


Figure 1. Flow chart of the study participants of the Population Study of Women in Gothenburg, Sweden. [updated 11.12.2014]

Table 1. Distribution of the study subjects (N, %), serum 25D (25(OH)D) concentrations and baseline anthropometric measures (mean± sd), according to baseline characteristics of middle-aged women in 1968-1969, the Population Study of Women in Gothenburg, Sweden. (UPDATED 11.12.2014)

| Characteristic | N[1] | % | Serum 25(OH)D (nmol/l)[2] | Height (cm)[2] | Weight (kg)[2] | BMI (kg/m$^2$)[2] | Waist-hip ratio[2] |
|---|---|---|---|---|---|---|---|
| Overall mean ± sd | 1,227[3] | | 72.5 ± 30.5 | 163.6 ± 5.9 | 64.7 ± 10.8 | 24.1 ± 3.8 | 0.74 ± 0.05 |
| Age (y) | | | | | | | |
| 38 | 305 | 25 | 73.2 ± 31.9 | 164.6 ± 5.6 | 62.9 ± 9.8 | 23.2 ± 3.5 | 0.72 ± 0.05 |
| 46 | 342 | 28 | 70.0 ± 27.3 | 163.8 ± 6.1 | 63.4 ± 9.9 | 23.6 ± 3.4 | 0.73 ± 0.05 |
| 50 | 368 | 30 | 74.3 ± 33.3 | 163.6 ± 5.6 | 66.4 ± 11.3 | 24.8 ± 3.9 | 0.75 ± 0.05 |
| 54 | 133 | 11 | 75.1 ± 28.3 | 162.1 ± 6.5 | 66.2 ± 12.8 | 25.2 ± 4.3 | 0.76 ± 0.05 |
| 60 | 79 | 6 | 67.4 ± 26.9 | 162.0 ± 5.0 | 66.1 ± 11.1 | 25.1 ± 3.7 | 0.78 ± 0.06 |
| *p-value*[4] | | | 0.147 | <0.001 | <0.001 | <0.001 | <0.001 |
| Menopause | | | | | | | |
| Yes | 483 | 58 | 75.4 ± 31.6 | 163.1 ± 6.0 | 65.4 ± 12.0 | 24.5 ± 4.0 | 0.75 ± 0.06 |
| No | 677 | 42 | 71.4 ± 28.9 | 164.1 ± 5.7 | 64.0 ± 9.8 | 23.8 ± 3.4 | 0.73 ± 0.05 |
| *p-value*[4] | | | 0.025 | 0.004 | 0.026 | <0.001 | <0.001 |
| Education level | | | | | | | |
| Secondary school and higher | 375 | 31 | 76.8 ± 29.9 | 164.9 ± 5.8 | 63.2 ± 9.7 | 23.2 ± 3.9 | 0.73 ± 0.05 |
| Elementary school | 847 | 69 | 70.7 ± 30.5 | 163.1 ± 5.8 | 65.3 ± 11.2 | 24.5 ± 3.3 | 0.74 ± 0.05 |
| *p-value*[4] | | | 0.001 | <0.001 | 0.002 | <0.001 | <0.001 |
| Socioeconomic position | | | | | | | |
| Higher employee | 146 | 12 | 80.4 ± 27.6 | 164.5 ± 5.9 | 62.0 ± 7.7 | 22.9 ± 2.7 | 0.73 ± 0.04 |
| Employee | 543 | 46 | 74.4 ± 30.0 | 163.8 ± 5.8 | 63.4 ± 9.9 | 23.6 ± 3.4 | 0.74 ± 0.05 |
| Worker | 495 | 42 | 68.8 ± 30.9 | 163.4 ± 5.9 | 66.9 ± 11.7 | 25.0 ± 4.0 | 0.75 ± 0.06 |
| *p-value*[4] | | | <0.001 | 0.133 | <0.001 | <0.001 | <0.001 |
| Smoking | | | | | | | |
| Never | 647 | 53 | 72.6 ±28.6 | 163.2 ± 5.9 | 66.1 ± 11.2 | 24.8 ± 3.9 | 0.74 ± 0.05 |
| Ex-smoker | 90 | 7 | 76.3 ±30.8 | 165.1 ± 5.3 | 65.1 ± 9.6 | 23.9 ± 3.1 | 0.73 ± 0.05 |
| Current | 489 | 40 | 71.6 ± 32.8 | 164.0 ± 5.9 | 62.7 ± 10.2 | 23.3 ± 3.5 | 0.74 ± 0.05 |
| *p-value*[4] | | | 0.387 | 0.004 | <0.001 | <0.001 | 0.252 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Leisure time physical activity | | | | | | | |
| ≥ 4h/week | 1007 | 82 | 74.3 ± 30.6 | 163.6 ± 5.7 | 64.3 ± 10.2 | 24.0 ± 3.5 | 0.74 ± 0.05 |
| < 4h/week | 219 | 18 | 64.0 ± 28.5 | 163.9 ± 6.4 | 66.2 ± 13.2 | 24.6 ± 4.5 | 0.75 ± 0.06 |
| *p-value*[4] | | | <0.001 | 0.551 | 0.021 | 0.053 | <0.001 |
| Season of 25D measurement | | | | | | | |
| Early summer | 144 | 12 | 61.7 ± 25.1 | 162.8 ± 5.3 | 65.1 ± 10.9 | 24.5 ± 3.9 | 0.75 ± 0.05 |
| Late summer | 222 | 18 | 87.3 ± 34.3 | 163.5 ± 6.6 | 63.9 ± 9.8 | 23.9 ± 3.4 | 0.73 ± 0.05 |
| Winter | 861 | 70 | 70.5 ± 29.0 | 163.8 ± 5.7 | 64.8 ± 11.1 | 24.1 ± 3.8 | 0.74 ± 0.05 |
| *p-value*[4] | | | <0.001 | 0.153 | 0.511 | 0.375 | <0.001 |
| Country of origin | | | | | | | |
| Sweden | 1123 | 92 | 72.9 ± 30.2 | 163.7 ± 5.8 | 64.7 ± 10.7 | 24.1 ± 3.7 | 0.74 ± 0.05 |
| Other | 104 | 8 | 67.4 ± 33.5 | 162.6 ± 5.9 | 64.4 ± 11.7 | 24.4 ± 4.0 | 0.74 ± 0.06 |
| *p-value*[4] | | | 0.073 | 0.049 | 0.760 | 0.527 | 0.707 |

[1] Totals may be less than 1,227 because of missing values. In childhood perception of weight, n(missing)=133; in menopause n(missing)=67; in SEP n(missing)=43; in other variables n(missing)≤5.
[2] Values are means ± SDs.
[3] Except for waist-hip ratio, N = 1211.
[4] ANOVA.

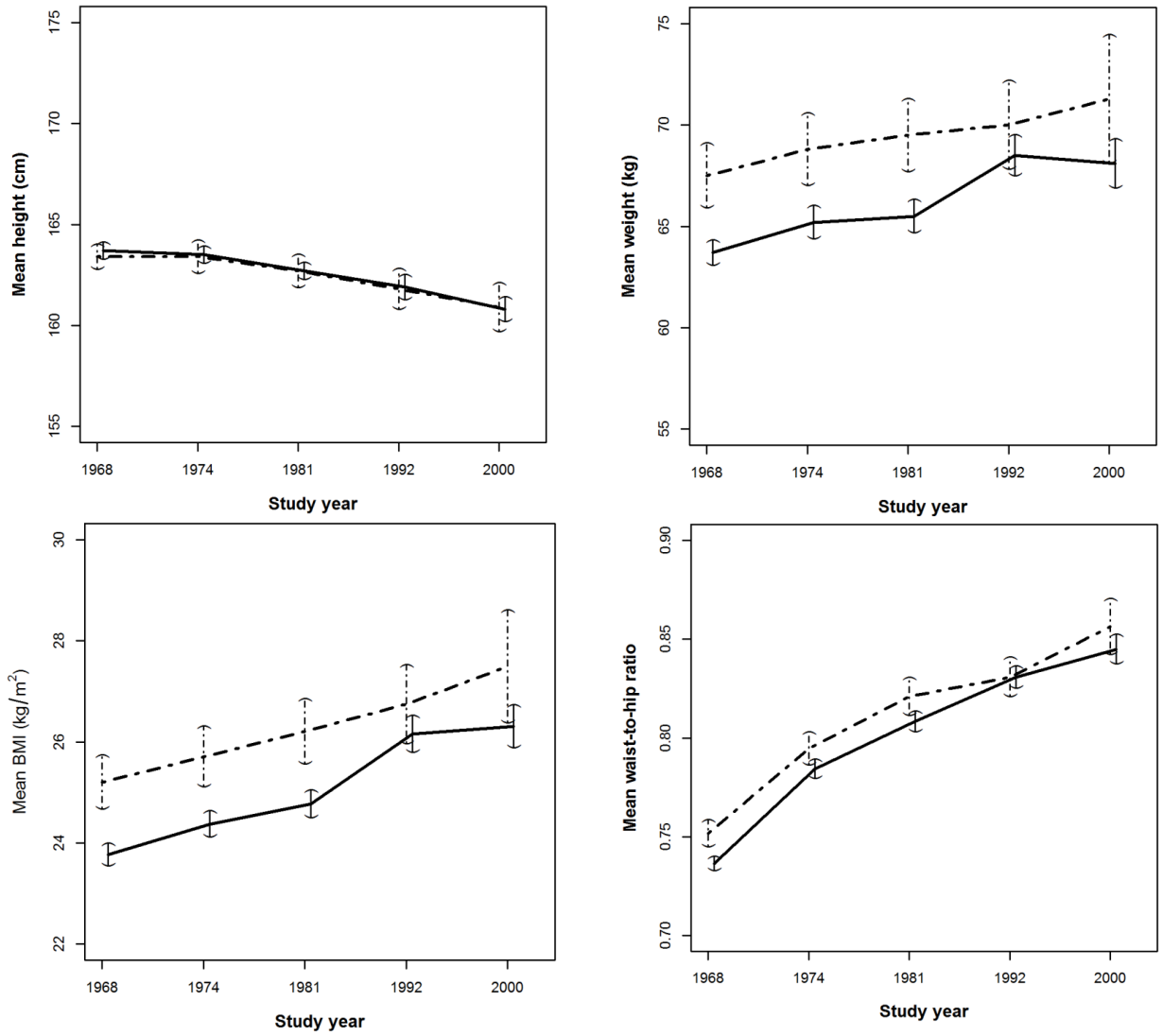


Figure 2. Mean height, weight, body mass index (BMI) and waist-hip ratio (WHR) by study visit and serum 25D levels (≤51.45 nmol/l and >51.45 nmol/l), the Population Study of Women in Gothenburg, Sweden.

Table 2. Results (beta coefficients, 95% CI, $R^2$?) from the regression models evaluating the association between low serum 25D levels (normal serum 25D level as the reference category) and baseline level or longitudinal development of height, weight, body mass index, and waist circumference.

| **Explanatory variable: Serum 25D** | | | **BMI** | | **Waist-hip ratio** |
|---|---|---|---|---|---|
| | | β | 95% CI | β | 95% CI |
| Baseline | | | | | |
| | Unadjusted | 1.43 | 0.95-1.91 | 0.015 | 0.009-0.022 |
| | Adjusted | 1.45[1] | 0.97-1.92 | 0.012[2] | 0.005-0.019 |
| Longitudinal | | | | | |
| | **All visits** | | | | |
| | Unadjusted | 1.44 | 0.95-1.93 | 0.013 | 0.006-0.020 |
| | Adjusted | 1.47[3] | 0.99-1.94 | 0.011[4] | 0.005-0.018 |
| | **Follow-up visits** | | | | |
| | Unadjusted | 1.37 | 0.82-1.91 | 0.009 | 0.001-0.017 |
| | Adjusted | 0.013[5] | -0.27-0.24 | -0.001 | -0.007-0.005 |
| | Adjusted | 0.01[7] | -0.25-0.27 | -0.002 | -0.008-0.004 |

Regression coefficients were derived from linear regression (baseline) or GLM (longitudinal).

1 From the stepwise model. The covariates that were initially tested in the model are age (age 38 as ref) , vitD (Q1 (<=51.45 nmol/l) vs Q2-Q4; > 51.45 used as reference), smoking in 1968 (never-smokers as reference), season (months=8+9+10 as reference; other seasons: (11+12+1+2+3+4), (5+6)), menopause (no as ref), education (2 categories, 'elementary school' as ref), leisure PA ('less than 4h/w' as ref), SES ('Higher employee' as ref) and origin ('born in Sweden' as ref). Factors that stayed in the final model were Age at baseline (50, 54, 60 y), smoking (current), leisure PA, education, and SEP (employee and worker).

2 From the stepwise model. The covariates that were initially tested in the model are age (age 38 as ref) , vitD (Q1 (<=51.45 nmol/l) vs Q2-Q4; > 51.45 used as reference), smoking in 1968 (never smokers as reference), season (months=8+9+10 as reference; other seasons: (11+12+1+2+3+4), (5+6)), menopause ('no' as ref), education (2 categories, 'elementary school' as ref), leisure PA ( 'less than 4h/w' as ref), SES ('Higher employee' as ref) and origin ('born in Sweden' as ref). Factors that stayed in the final model were Age at baseline (46, 50, 54, and 60 y), smoking (current), leisurePA, season (Winter), SEP (employee and worker)

3 "Most complete model": age & education & season & SES + time-varying: leisure PA & smoking & menopause

4 age & education & season & SES + time-varying: smoking & menopause

5 BMI & age & education & season

6 WHR & age & education & season

7 BMI & age & education & season & SES + time-varying: leisure PA & smoking & menopause

8 WHR & age & education & season & SES + time-varying: smoking & menopause