Serum 25-hydroxyvitamin D levels and the metabolic syndrome in older persons: a population-based study

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Abstract

Background
Recent evidence indicates that a lower plasma level of 25-hydroxyvitamin D (25OHD) is associated with a higher risk of the metabolic syndrome. It has not been studied in older people with a high prevalence of vitamin D insufficiency.

Objective
This study investigates the association between vitamin D status and the metabolic syndrome in community-dwelling older persons in the Netherlands.

Design and patients
The study is part of the Longitudinal Aging Study Amsterdam, an ongoing cohort study in a representative sample of Dutch older persons. A total of 1286 subjects (629 men and 657 women) between the ages of 65 and 88 years participated in the study.

Measurements
Metabolic syndrome (U.S. National Cholesterol Education Program definition) and its individual components were assessed as well as serum 25OHD levels.

Results
Among the participants, the prevalence of the metabolic syndrome was 37.0 %. The mean 25OHD level was 53.3 nmol/l. 47.8% had 25OHD levels below 50 nmol/l. There was a significantly increased risk for the metabolic syndrome in the subjects with serum 25OHD levels below 50 nmol/l, compared with that of subjects with levels over 50 nmol/l [odds ratio (OR)=1.54; 95% confidence interval (CI) 1.23-1.94]. After adjustment for founders, age, sex, season, years of education, alcohol use, total activity, smoking and PTH the OR was 1.29 (95% CI 1.00-1.68). The association between vitamin D deficiency and the metabolic syndrome was mainly determined by the components low HDL and (high) waist circumference.

Conclusions
Vitamin D deficiency is common in the older population in the Netherlands, and subjects with serum 25OHD below 50 nmol/l have a higher risk of the metabolic syndrome.
Introduction

Vitamin D is the main determinant of calcium absorption from the gut, which is important for bone mineralization. Currently, there has been growing interest in the additional metabolic roles of vitamin D, and evidence is accumulating that it may play a role in the development of the metabolic syndrome and diabetes mellitus type 2.

Vitamin D deficiency is associated with decreased insulin synthesis and secretion in animal models.(1,2) Cross-sectional studies have shown that low serum 25-hydroxyvitamin D (25OHD) is associated with glucose intolerance, insulin resistance, the metabolic syndrome and diabetes type 2, although data are not consistent. (3-10) Results from three prospective studies, the Women’s Health Study, the Nurses Health Study and the Australian Diabetes, Obesity and Lifestyle study, suggested a role for calcium and vitamin D in reducing the risk of type 2 diabetes and metabolic syndrome. (11-13) However, the results of small intervention studies, (8,14-17) as well as population-based studies have shown conflicting results, which may be due to different populations (e.g. younger populations, more obese subjects) that have been studied. (18-23) To our knowledge, this study is the first population-based study, in a representative sample of the older population with a high prevalence of vitamin D deficiency.

The purpose of our study was to investigate the association of the metabolic syndrome and its individual components with vitamin D deficiency in older men and women because they are both common in the older population. In addition, we investigated the threshold of serum 25OHD level with regard to the metabolic syndrome.

Subject and Methods

Study Sample

Data were collected within the Longitudinal Aging Study Amsterdam (LASA), an ongoing interdisciplinary cohort study on predictors and consequences of changes in autonomy and well-being in the aging population in the Netherlands (for more details see Deeg et al.)(24)

A random sample of men and women aged 55 years and over, stratified by age, sex, urbanization grade and expected 5-year mortality rate was drawn from the population registers of 11 municipalities in three regions of the Netherlands, being a representative sample of the Dutch population. In total, 3107 predominantly Caucasian (>99%) respondents were enrolled in the baseline examination 1992/1993.

This study was conducted in a subgroup of the LASA population, including persons who participated in the medical interview of the second data collection (1995/1996), which was restricted to subjects who were aged ≥ 65 years(n=1509). Blood samples were obtained from 1352 subjects. Both vitamin D status and the metabolic syndrome were
assessed at the same interview. Metabolic syndrome and vitamin D status could be determined for 1286 respondents and these were included in the analysis. Informed consent was obtained from all respondents, and the study was approved by the Ethical Review Board of the VU University Medical Center.

Measurements

**Serum 25OHD**

Blood samples were collected in the morning in 1995/1996. Subjects were allowed to have tea and toast, but no dairy products. The blood samples were centrifuged and stored at -20 C. Serum 25OHD was determined using a competitive protein binding assay in 1997/1998 (Nichols Diagnostics, San Juan Capistrano, CA, USA). The coefficient of variation was 10%. All analyses were performed at the Endocrine Laboratory of the VU University Medical center. Values of serum 25OHD were dichotomized by 50 nmol/l, according to the currently used cut-off point for vitamin D insufficiency in the Netherlands.

**Metabolic syndrome**

Metabolic syndrome was defined as the presence of three or more of the following criteria: triglycerides ≥ 1.7 mmol/l, HDL cholesterol < 1.0 mmol/l for men and < 1.3 mmol/l for women; blood pressure ≥ 160/90 mmHg or antihypertensive medication; waist circumference >102 cm for men and > 88 cm for women; and fructosamine ≥ 0.247 mmol/l or antidiabetic medication in agreement with the definition established by the U.S. National Cholesterol Education Program (NCEP) Adult Treatment Panel III, with an increased cut-off for blood pressure, adjusted for an older population.(25) Furthermore, the cut-off of 0.247 mmol/l for fructosamine corresponds to the cut-off of 6.1 mmol/l for fasting plasma glucose in terms of sensitivity and specificity in discriminating subjects with glucose intolerance from subjects with normal glucose tolerance. Fructosamine was used instead of glucose because a fasting state was not required when blood samples were obtained and fructosamine is little affected by eating.(26)

**Assessment of components of the metabolic syndrome**

Blood pressure was measured using an Omron 706 automatic device while the subject was sitting. Waist circumference was determined as the average of two measurements calculated to the nearest 0.1 cm midway between the lower rib margin and the iliac crest after normal expiration. Medication use was assessed by recording the medications of the participant directly from the containers. Fructosamine was determined by a colorimetric test, and HDL cholesterol and triglycerides were determined by an enzymatic colorimetric test (Roche Diagnostics, Mannheim, Germany). The interassay coefficient of variation was < 2.8 % for fructosamine and triglycerides and < 6.4% for HDL cholesterol. All laboratory
analyses (HDL cholesterol, triglycerides and fructosamine) were performed in EDTA-plasma samples stored at – 80 ° C at the department of Clinical Chemistry of the VU University Medical Center in 2005. (27)

Potential confounders
Age, sex, season of blood collection, education, number of chronic diseases, smoking, alcohol use, total physical activity, physical performance and serum PTH were considered as potential confounders. Data on sex and age were derived from population registries at baseline. Because vitamin D status is partly dependent on sunlight exposure, we adjusted for season of blood collection. Education level was assessed by asking respondents for the highest educational level completed, which was converted into total number of years of education (range 5-18 years). Smoking status was categorized as never, former and current smoker. Alcohol consumption was categorized as none, light, moderate and excessive. Physical activity was assessed with the LASA Physical Activity Questionnaire. The following activities were included: walking outdoors, bicycling, light and heavy household activities, sports activities and a total activity score was calculated as time spent on physical activity in minutes per day. This variable was divided into tertiles for analysis, with the first tertile representing the lowest activity.(28) Diabetes and number of chronic diseases were assessed using algorithms in which information obtained from general practitioners, inspection of medicine bottles and self-report was combined. Self reported diabetes has been shown to be in good agreement with the general practitioner’s report (k=0.85). (29)

Statistical analyses
All analyses were performed using SPSS for Windows (version 15.0.1, SPSS, Inc., Chicago, IL, USA). Characteristics of the study sample were presented by metabolic syndrome status and were compared using independent t tests for normally distributed continuous variables and Pearson chi-square tests for dichotomous variables. Skewed distributed continuous variables were compared using Mann-Whitney U tests. The individual variables were checked for linearity. Spearman and Pearson correlation coefficients were calculated to examine multicollinearity ($r < 0.4$).

Initially, we performed the analyses with commonly used cut-off points of serum 25OHD (<25 nmol/l, 25-49.9 nmol/l, 50-74.9 nmol/l, >75nmol/l). Due to power problems in the two extreme categories, we used the commonly used Dutch cut-off point for serum 25OHD level of 50 nmol/l and dichotomized the sample according to this cut-off point in all analyses. Dichotomous indicators were created for the individual components of the metabolic syndrome. Logistic regression analyses were performed to study the association between the metabolic syndrome and serum 25OHD levels, both unadjusted and adjusted for age, sex, season, education, smoking, alcohol, chronic diseases and physical ac-
tivity. The categorical variables smoking, alcohol and total physical activity were included in regression analysis as dummies. The analyses were repeated after exclusion of subjects with diabetes and lipid-lowering drugs. Additionally, we repeated the analysis after the adjustment for PTH. The associations between 25OHD and the individual components of metabolic syndrome were analyzed by including each component both separately and together in a logistic and linear regression model. All analyses were tested at the 0.05 level of significance, except for interaction term, for which 0.10 was tolerated. Effect modification by gender was evaluated and tested by adding the product term of serum 25OHD x gender to the univariate model.

In addition, we used restricted cubic spline functions to estimate an optimal cut-off point for 25OHD in relationship with the metabolic syndrome. Cubic splines are piecewise polynomial functions that are constrained to join smoothly at points called knots. These spline functions provide better insight in dose-response relationship as compared to analyses using categorized variables. Restricted cubic spline functions use all data points to estimate the risk at each level of exposure, as opposed to step functions using categorical variables, which assume a constant risk within categories. Cubic spline functions were tested in the logistic regression models at 3 - 5 knots using spline plots and likelihood ratio tests. Eventually, the best fitting spline regression model was identified. All spline regression analyses were performed using R version 2.10.0.(30)

Results

In table 1, the baseline characteristics are presented. In total, the prevalence of the metabolic syndrome among the 1286 participants aged 65-88 years was 37.0%. The prevalence of the individual components were 52 % for abdominal obesity, 62.8% for hypertension, 31.5% for high triglycerides, 36.4% for low HDL cholesterol and 25.1% for high fructoseamine. Subjects with the metabolic syndrome were more often women, had a significantly lower number of years of education, consumed less alcohol and suffered from more chronic diseases and diabetes. Furthermore, they had significantly lower levels of serum 25OHD levels (p=0.002). The mean serum 25OHD level was 53.5 ± 24.1 nmol/l, with 10.9% of the subjects below 25 nmol/l, 36.9% between 25 en 50 nmol/l, 34.4% between 50 and 75 nmol/l and 17.8% over 75 nmol/l. No interaction was found with sex (p=0.49).

The results of the logistic regression analyses are presented in Table 2. After adjustment for confounders, metabolic syndrome remained significantly associated with low serum 25OHD (OR =1.32, 95% CI 1.02-1.71). After additionally adjustment for PTH, the association remained unchanged (OR = 1.29, 95% CI 1.00-1.68). The direct association between PTH and the metabolic syndrome has also been explored. PTH was represented by dummy variables with the lowest quartile as reference group. None of the quartiles were associated with a higher risk of the metabolic syndrome. After exclusion of diabetic
Table 1. Baseline characteristics of the study sample by metabolic syndrome status

<table>
<thead>
<tr>
<th></th>
<th>Metabolic syndrome</th>
<th>No metabolic syndrome</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>476</td>
<td>810</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>75.82±6.54</td>
<td>75.36±6.54</td>
<td>0.17</td>
</tr>
<tr>
<td>Men (%)</td>
<td>42.2%</td>
<td>52.8%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Season of 25OHD assessment%winter</td>
<td>59.5%</td>
<td>52.7%</td>
<td>0.02</td>
</tr>
<tr>
<td>Education (years) (5-18)</td>
<td>9[6-10]</td>
<td>9[6-11]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>85.5%</td>
<td>49.5%</td>
<td></td>
</tr>
<tr>
<td>High triglycerides (%)</td>
<td>67.0%</td>
<td>10.6%</td>
<td></td>
</tr>
<tr>
<td>High fructosamine (%)</td>
<td>43.0%</td>
<td>14.8%</td>
<td></td>
</tr>
<tr>
<td>Low HDL cholesterol (%)</td>
<td>76.4%</td>
<td>13.1%</td>
<td></td>
</tr>
<tr>
<td>Abdominal obesity (%)</td>
<td>81.5%</td>
<td>34.7%</td>
<td></td>
</tr>
<tr>
<td>Smoking status (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>38.4%</td>
<td>33.7%</td>
<td>0.10</td>
</tr>
<tr>
<td>Former</td>
<td>46.0%</td>
<td>46.7%</td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>15.5%</td>
<td>19.6%</td>
<td></td>
</tr>
<tr>
<td>Alcohol consumption (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>32%</td>
<td>19.8%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Light</td>
<td>46.9%</td>
<td>52.5%</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>15.8%</td>
<td>21.1%</td>
<td></td>
</tr>
<tr>
<td>Excessive</td>
<td>5.3%</td>
<td>6.7%</td>
<td></td>
</tr>
<tr>
<td>Physical activity (min/day)</td>
<td>135[77.5-203.7]</td>
<td>135[79.6-203.9]</td>
<td>0.36</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>17.1%</td>
<td>4.4%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Number of chronic diseases (0-5)</td>
<td>1 [1-2]</td>
<td>1[0-2]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>25OHD (nmol/l)</td>
<td>50.8±23.6</td>
<td>55.2±24.2</td>
<td>0.002</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>28.9±3.8</td>
<td>25.6±3.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PTH (pmol/l)</td>
<td>3.3[2.5-4.5]</td>
<td>3.1[2.4-4.1]</td>
<td>0.08</td>
</tr>
</tbody>
</table>

n=1286. Data are means ± SD, %, or median [interquartile range]. P values of independent t test for continuous variables and Mann-Whitney U tests for skewed distributed variables, differences in frequencies using Pearson Chi-square test.

Table 2. Association between serum 25OHD level and the metabolic syndrome

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
<th>Model 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>25OHD ≤50 vs &gt;50nmol/l</td>
<td>1.54 (1.23-1.94)</td>
<td>1.39 (1.08-1.79)</td>
<td>1.32 (1.02-1.71)</td>
<td>1.29 (1.00-1.68)</td>
</tr>
</tbody>
</table>

Presented as odd’s ratios and 95% confidence intervals.

Model 1: univariate.
Model 2: model 1 and adjusted for age, sex and season.
Model 3: model 2 and adjusted for smoking, alcohol, total activity, number of chronic diseases, education level.
Model 4: model 3, and adjusted for PTH.
patients and exclusion of subjects using lipid-lowering drugs, logistic regression analysis produced almost identical results (OR = 1.28, 95% CI 0.97-1.69).

Investigating the individual components of the metabolic syndrome in relation to 25OHD levels revealed that low HDL cholesterol was significantly associated with low serum 25OHD levels, even after adjustment for confounders (OR = 1.37, 95% CI 1.06-1.77). Waist circumference was significantly associated with serum 25OHD (OR = 1.55, 95% CI 1.24-1.94) and borderline after adjustment OR= 1.27, 95% CI 0.99-1.63). Hypertension, high triglycerides and high fructosamine were not significantly associated with vitamin D status. In conclusion, low HDL cholesterol and waist circumference were the most important components in relation to vitamin D status (Table 3).

In the next step, we investigated the association using univariate and multivariable spline regression models. The model fit was best using 4 knots with a likelihood ratio of 16.24 for the adjusted model. Figure 1 shows the univariate association between serum 25OHD and the metabolic syndrome. The plot shows increasing spline coefficients for the metabolic syndrome in the serum 25OHD range of 25 nmol/l to 75 nmol/l. The spline coefficient was lowest at a 25OHD level of 75 nmol/l and highest at a 25OHD level of 25 nmol/l. Below 25 nmol/l and above 75 nmol/l, the plot is unreliable because of a wide confidence interval. In Fig. 2, the adjusted version is shown, which is very similar. As visible, the risk for metabolic syndrome increases about 2-fold with a 25OHD level decreasing from 75 nmol/l to 25 nmol/l.

Table 3. Association between serum 25OHD and the individual components of the metabolic syndrome

<table>
<thead>
<tr>
<th>25OHD≤50 nmol/l versus &gt; 50 nmol/l</th>
<th>Model 1</th>
<th>Model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td>1.33</td>
<td>1.06-1.68*</td>
</tr>
<tr>
<td>High triglycerides</td>
<td>0.98</td>
<td>0.78-1.24</td>
</tr>
<tr>
<td>Hyperglycaemia/fructosamine</td>
<td>1.39</td>
<td>1.08-1.79*</td>
</tr>
<tr>
<td>Low HDL</td>
<td>1.58</td>
<td>1.26-1.99*</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>1.55</td>
<td>1.24-1.94*</td>
</tr>
</tbody>
</table>

Data are presented as odd’s ratios with 95% confidence intervals.
Model 1: univariate.
Model 2: adjusted for age, sex, season, smoking, alcohol, total activity, number of chronic diseases, education level. The P value <0.05 was considered statistically significant and is depicted in the table as (*).
Serum 25-hydroxyvitamin D levels and the metabolic syndrome

Figure 1. Relationship between serum 25OHD and the metabolic syndrome in the spline regression model, univariate.

Figure 2. Relationship between serum 25OHD and the metabolic syndrome in the spline regression model, adjusted for sex, age, season, smoking, alcohol, total activity, number of chronic diseases and education level.
Discussion

In this population-based study of older individuals, metabolic syndrome was observed in more than one third of the subjects. A serum 25OHD level lower than 50 nmol/l was associated with an increased risk of the metabolic syndrome, independent of confounders and serum PTH. Low serum HDL levels and waist circumference were the main contributors of the association between the metabolic syndrome and serum 25OHD levels after adjustment for confounders.

The inverse association between serum 25OHD and the metabolic syndrome found in our study is consistent with the previously reported cross-sectional studies. (18,20,21,23,31-33) Two American studies (20,31) with large sample sizes included subjects aged >20 years. The British study (32) was conducted in participants aged > 45 years, the European study (21) included men between 40 and 79 years and the Chinese study (18) investigated Chinese aged 50-70 years. Serum 25OHD concentrations were most compatible with the British cohort, with mean 25OHD levels around 50 nmol/l. Our study investigated the oldest population, compared with other studies. In contrast, in another community-dwelling older population in the United States, an association between the metabolic syndrome and serum 25OHD was not observed; on the contrary, a relationship between serum PTH and the metabolic syndrome was shown in men. In that study, mean serum 25OHD levels were significant higher with a mean level >100 nmol/l, than in our study.(19) It is possible that a threshold exists for the association between serum 25OHD and the metabolic syndrome, where higher levels of serum 25OHD have no further implications. In a recent study performed in Norway in morbidly obese subjects, serum PTH was an independent predictor of metabolic syndrome while vitamin D status was not.(34) In our study we did not find a significant difference in PTH levels between persons with and without the metabolic syndrome. In that study no significant difference in vitamin D level between the metabolic syndrome groups was found.

Literature showed inconsistent data on the relationship between the components of the metabolic syndrome and serum 25OHD. The British study found associations with all the components in the unadjusted model, while the association with HDL cholesterol disappeared after full adjustment, which is in contrast with our study.(32) In the American study, associations were found with adiposity, hypertriglyceridaemia and hyperglycaemia. (31) In the European study, an inverse association was found with waist circumference, systolic blood pressure, triglycerides and glucose levels.(21) In our study, fructosamine was used instead of glucose. This might explain why we did not find a significant association with the glucose/fructosamine component. In a recent study among Arab Americans, a positive association between serum HDL-cholesterol and serum 25OHD was observed in women, as was observed before in adolescents and morbidly obese people.(35-37) In the Women’s Health Initiative, however, vitamin D and calcium supplementation for 5
years did not change the lipid profile. Serum levels of vitamin D were not measured in this study. (38)

Recently, there has been much speculation about the optimal set point for serum 25OHD concentration related to its traditional roles as well as its metabolic roles.(39,40) In the present study, we found a linear relationship between vitamin D status and the metabolic syndrome in the range from 25 nmol/l to 75 nmol/l of serum25OHD. This suggests that 75 nmol/l might protect better against the metabolic syndrome rather than a level of 50 nmol/l, which is the currently used cut-off point in the Netherlands. However, this and other studies only show associations. A randomized clinical trial is needed to show whether vitamin D supplementation is able to reduce the risk of the metabolic syndrome, thereby proving that the relationship is causal.

Strengths of the present study include the large population-based sample of older individuals, including similar numbers of men and women, being a representative group of the Dutch population. The age-stratified enrolment facilitated exploration of age interactions. In this study, we adjusted for many potential covariates that might confound the observed association. We repeated our logistic regression analyses after excluding subjects with diabetes and subjects taking lipid-lowering drugs to investigate the influence on the association between 25OHD and the metabolic syndrome. Without excluding these subjects, a stronger relationship was observed, although the overall pattern did not change.

Nonetheless, there are several limitations. Because of the cross-sectional study design, we cannot conclude about a cause effect relationship. Unfortunately, we could not adjust for calcium intake. In the present study, we did not have glucose and HOMA-IR available and therefore we cannot conclude about possible insulin resistance. Third, our results are specific for a Caucasian population, so the results may not be valid in non-white older populations.

In conclusion, this study suggests that levels of serum25OHD below 50 nmol/l are associated with a higher risk of the metabolic syndrome in community-dwelling older persons, independently of serum PTH levels. In the present study, low serum HDL-cholesterol levels and high waist circumference were the more related individual components of the metabolic syndrome with serum 25OHD. In the range between 25 nmol/l serum 25OHD and 75 nmol/l we can conclude that a higher serum 25OHD level is associated with a lower risk of the metabolic syndrome. The results of this study do not allow any conclusions on the direction of a possible causal association. A low serum 25OHD level may be associated with obesity because obese persons simply avoid sunshine and have a more sedentary lifestyle.

More research is needed to fully understand these outcomes and to investigate the true threshold for optimal serum 25OHD levels with regard to the association with the metabolic syndrome. Sufficiently powered and well-designed randomized controlled clinical trials are needed to investigate the cause-and-effect relationships of serum 25OHD with glucose metabolism, lipid profiles and the metabolic syndrome.
Acknowledgments

The authors thank Jan Poppelaars for his assistance in providing the data. This study is based on data collected in the context of the Longitudinal Aging Study Amsterdam (LASA), which is largely funded by the Ministry of Health, Welfare and Sports of the Netherlands.

Disclosure

Paul Lips has consulted for Merck and Co regarding vitamin D in general and receives a research grant from ZON, a semi-government organization in the Netherlands. The other authors have nothing to declare.

Reference list

Serum 25-hydroxyvitamin D levels and the metabolic syndrome


Chapter 2


