Original Contribution

Tracking of Serum 25-Hydroxyvitamin D Levels During 14 Years in a Population-based Study and During 12 Months in an Intervention Study

Rolf Jorde*, Monica Sneve, Moira Hutchinson, Nina Emaus, Yngve Figenschau, and Guri Grimnes

* Correspondence to Prof. Rolf Jorde, Medical Clinic, University Hospital of North Norway, 9038 Tromsø, Norway (e-mail: rolf.jorde@unn.no).

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Low serum 25-hydroxyvitamin D (25(OH)D) levels are associated with risk factors for cardiovascular disease, and they also appear to predict later development of type 2 diabetes, cancer, and an increased mortality rate. These predictions are all based on a single 25(OH)D measurement, but so far there are no known reports on tracking of serum 25(OH)D levels. In the present Norwegian study, serum 25(OH)D levels were measured 1) in 2,668 subjects in the 1994 and 2008 Tromsø surveys and 2) every third month for 1 year in 94 subjects randomly assigned to placebo in a vitamin D intervention study. There was a marked seasonal variation in 25(OH)D, and, depending on the method of adjusting for season, the correlation coefficient between serum 25(OH)D measurements from 1994 and 2008 ranged from 0.42 to 0.52. In the 1-year intervention study, the correlation between baseline and 12-month values was 0.80. Apart from the effect of season, changes in weight, intake of vitamin D, and physical activity were related to change in serum 25(OH)D levels. Tracking of serum 25(OH)D appears similar to that for blood pressure and serum lipids, and it provides some support for the use of a single 25(OH)D measurement to predict future health outcomes.

follow-up studies; population; prospective studies; vitamin D

Abbreviations: SD, standard deviation; 25(OH)D, 25-hydroxyvitamin D.

The importance of vitamin D for skeletal health is well established (1, 2). Recently, receptors for 1,25-dihydroxyvitamin D, which is the active form of the vitamin (2), have been demonstrated in tissues not related to calcium metabolism, indicating a broader range of biologic effects (3). Accordingly, low serum levels of 25-hydroxyvitamin D (25(OH)D), which is the storage form of the vitamin and the one used to evaluate a subject’s vitamin D status (2), appear to be associated with risk factors such as blood pressure (4), blood glucose (5), and adiposity (6). Recently, several papers have also been published on the predictive value of serum 25(OH)D regarding later development of hypertension (7), cardiovascular disease (8), type 2 diabetes (9), cancer (10–13), and even mortality rate (14, 15). In most of these studies, only one 25(OH)D measurement in each individual was available at baseline, and repeated 25(OH)D measurements were not performed during the follow-up period. The predictive value of 25(OH)D has therefore been based on the assumption that an individual’s serum 25(OH)D level is relatively stable over time.

However, to our knowledge, no reports on tracking of serum 25(OH)D levels exist. Because serum 25(OH)D can safely be stored at −70°C for years (16, 17), we had the opportunity to address this issue by measuring 25(OH)D in 1994 and 2008 serum samples from 2,668 Norwegian individuals participating in The Tromsø Study. To evaluate changes occurring during a shorter period of time, serum 25(OH)D was measured every third month for 1 year in 94 subjects who received placebo while participating in a 1-year intervention study. All these serum 25(OH)D measurements were performed with the same assay and during the same time period to minimize effects of assay changes, which can be a problem when comparing serum 25(OH)D measurements made years apart (18).
MATERIALS AND METHODS

The Tromsø Study

The Tromsø Study, conducted by the University of Tromsø in cooperation with the National Health Screening Service, is a longitudinal, population-based, multipurpose study focusing on lifestyle-related diseases (19). In the fourth survey in 1994–1995 (hereafter referred to as 1994 for simplicity), 27,158 persons participated, providing an attendance rate of 77% among eligible inhabitants. All men aged 55–74 years, all women aged 50–74 years, and a 5%–10% sample of the remaining age groups between 25 and 84 years were preselected to participate in a second phase of the survey, and 7,965 persons (78% of those invited) attended (20). Sera from this second phase were stored for later analyses. In the sixth survey performed in 2008, 19,762 subjects were invited; 12,984 attended.

In both the 1994 and 2008 surveys, the participants completed questionnaires on lifestyle factors, including use of cod liver oil, use of vitamin D supplements, and smoking. A physical activity score was calculated by adding hours of moderate and hard physical activity per week, giving hard activity double weight. In 1994, the questions included activity also during working hours, whereas the questions in 2008 covered activity during leisure time only. Height and weight were measured with participants wearing light clothing and no shoes. Nonfasting blood samples were drawn. Blood pressure, pulse, serum total cholesterol, and triglycerides were measured, as previously described (20).

Intervention study

The primary endpoint of this study was weight reduction after supplementation with vitamin D (21). Males and females aged 21–70 years and with a body mass index of 28.0–47.0 kg/m² were included. Any previous supplements with calcium and vitamin D (including cod liver oil) were discontinued, and all subjects were given a daily 500-mg calcium supplement (Nycoplus Calcium; Nycomed, Oslo, Norway) throughout the 1-year intervention period. The participants were given oral information and written recommendations on healthy diet and physical activity. The subjects were randomly assigned to 40,000 IU of cholecalciferol per week, 20,000 IU of cholecalciferol per week, or placebo. The latter group was included in the present analysis. Blood samples to determine 25(OH)D were drawn at baseline and after 3, 6, 9, and 12 months. The trial was registered at ClinicalTrials.gov (NCT00243256).

Serum 25(OH)D measurements

Sera from 1994 were stored at −70°C and, after a median storage time of 13 years, were analyzed for 25(OH)D, whereas sera from 2008 were analyzed consecutively. The sera from 1994 and 2008 were analyzed in the same time period. Sera from the intervention study were analyzed in batch after a storage time of 2–30 months at −70°C. Serum 25(OH)D 3 was measured by immunometry (electrochemiluminescent immunometric assay) using an automated clinical chemistry analyzer (Modular E170; Roche Diagnostics GmbH, Mannheim, Germany). The total analytical coefficient of variation for the vitamin D assay was 7.3% as recorded by measuring a donor control (65.0 nmol/L) consecutively during the analytical period using a quality management program (QM; Tieto Enator, Helsinki, Finland). The cross-reactivity with 25(OH)D 2 was less than 10%, and the analytical sensitivity was 10 nmol/L. At present, the laboratory has no reference values for 25(OH)D but the manufacturer provides a population-based reference range of 27.7–107.0 nmol/L for adults as a guideline. This analysis was approved by the Norwegian Accreditation Authority. Because this method seems to overestimate serum 25(OH)D in smokers, we chose to exclude current smokers from the analyses.

Statistics

Normal distribution was evaluated with visual inspection of histograms and determination of skewness and kurtosis. All variables used as dependent variables were considered normally distributed.

Values from 1994 and 2008 were compared with the paired Student’s t test or the chi-square test. Correlations were evaluated with Pearson’s correlation coefficient r. To eliminate the effect of season and storage, z scores for the serum 25(OH)D values within each month were calculated for the 1994 and 2008 values. Delta values were calculated as the value in 2008 minus the value in 1994. Because the questions on physical activity differed in 1994 and 2008, z scores for 1994 and 2008 were used to calculate delta values for physical activity. Linear regression models to evaluate predictors for serum 25(OH)D or delta serum 25(OH)D z score were used with covariates.

Unless otherwise stated, all data in this paper are expressed as mean (standard deviation (SD)). All tests were 2-sided, and P < 0.05 was considered statistically significant. Statistical analyses were performed with SPSS version 15.0 software (SPSS Inc., Chicago, Illinois).

Ethics

Both studies were approved by the regional ethics committee. All participants gave written informed consent prior to the study.

RESULTS

The Tromsø Study

In 1994, 7,168 subjects had 25(OH)D measurements. A total of 2,337 were smokers and hence were excluded. Among the remaining 4,831 subjects, 2,712 had serum 25(OH)D measurements in 2008, 44 of whom had started smoking. Thus, 2,668 subjects remained for the present analyses. The baseline characteristics of these subjects in 1994 and 2008 are shown in Table 1.

There was a seasonal variation in serum 25(OH)D levels in both 1994 and 2008, with higher levels in the summer than during the winter. Thus, in 1994, the serum 25(OH)D levels in August and February were 60.6 nmol/L (SD, 18.5)
1994 and 2008, The Tromsø Study

As previously described in detail (21), 445 subjects were included and 149 were randomly assigned to placebo. Ninety-four of these subjects (aged 52.5 years (SD, 9.2), 34 males and 60 females) were nonsmokers and completed the 12-month study period. At baseline and after 12 months, their serum 25(OH)D levels were 52.4 nmol/L (SD, 15.6) and 50.0 nmol/L (SD, 14.0), respectively. The corresponding body mass index values were 35.2 kg/m² (SD, 4.1) and 35.3 kg/m² (SD, 4.6). There were highly significant

**Table 1. Characteristics of the 2,668 Norwegian Study Subjects in 1994 and 2008, The Tromsø Study**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1994</th>
<th>2008</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>56.8 (9.2)</td>
<td>56.1 (9.2)</td>
</tr>
<tr>
<td>Male gender</td>
<td>34.9</td>
<td>34.5</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.2 (3.7)</td>
<td>27.2 (4.3)*</td>
</tr>
<tr>
<td>Serum 25(OH)D, nmol/L</td>
<td>53.7 (16.3)</td>
<td>55.3 (18.2)*</td>
</tr>
<tr>
<td>Physical activity score, hours per week b</td>
<td>3.4 (2.6)</td>
<td>2.1 (2.1)</td>
</tr>
<tr>
<td>Use of cod liver oil or vitamin D supplements</td>
<td>38.3</td>
<td>28.9*</td>
</tr>
</tbody>
</table>

* P < 0.01.

Abbreviation: 25(OH)D, 25-hydroxyvitamin D.

Values are expressed as mean (standard deviation) or percent.

b In 1994, the questionnaire also included activity during working hours, whereas the questions in 2008 were on activity during leisure time only.

and 52.4 nmol/L (SD, 16.6), respectively, and the corresponding values in 2008 were 65.2 nmol/L (SD, 19.7) and 49.3 nmol/L (SD, 18.6). No relation was found between month of blood sampling in 1994 and 2008, and the crude correlation coefficient for the serum 25(OH)D values in 1994 and 2008 was 0.39 (P < 0.001). When z scores were used for serum 25(OH)D (calculated within each month of blood sampling), the correlation coefficient between 1994 and 2008 values increased to 0.42. In comparison, the correlation coefficients for systolic blood pressure, diastolic blood pressure, pulse, body mass index, serum total cholesterol, and triglycerides between 1994 and 2008 values were 0.47, 0.42, 0.46, 0.83, 0.37, and 0.49, respectively (in these analyses, correction for start of blood pressure medication and lipid-lowering drugs was not made).

The cohort was divided into z-score quintiles for 1994 and 2008 and also into groups based on actual serum 25(OH)D values in steps of 10 nmol/L. As shown in Tables 2 and 3, the subjects tended to stay within their z-score quintile or serum 25(OH)D group from 1994 to 2008. In particular, those with the highest or lowest levels were the ones most likely to remain within their group.

With no adjustment for month of sampling, 45.8% had a change in serum 25(OH)D of less than 10 nmol/L from 1994 to 2008, and 75.1% had a change of less than 20 nmol/L (Figure 1). When only those whose samples were drawn during the same time of year were examined (either the winter months November, December, January, or February in 1994 and 2008 or the summer months May, June, August, or September in 1994 and 2008) (n = 759), 50.6% had a change in serum 25(OH)D from 1994 to 2008 of less than 10 nmol/L, and 79.3% had a change of less than 20 nmol/L. For these 759 subjects, the correlation coefficient for the serum 25(OH)D values from 1994 and 2008 was 0.52.

In a multiple linear regression model, body mass index, physical activity score, and intake of cod liver oil and/or vitamin D supplements were significant predictors of serum 25(OH)D at baseline, whereas delta body mass index, delta physical activity (as z scores), and change in intake of cod liver oil and/or vitamin D supplements were predictors of delta z score for serum 25(OH)D (Table 4). In this model, age was not a significant predictor. However, if subjects older than age 65 years in 1994 were compared with those who were younger, the older subjects had a decrease in serum 25(OH)D of 0.3 nmol/L (SD, 18.0) from 1994 to 2008, whereas the younger subjects had an increase of 2.0 nmol/L (SD, 19.3) (P < 0.05, statistical evaluation performed on z scores). Subjects whose time of blood sampling changed from winter to summer (n = 361) had an increase in serum 25(OH)D from 1994 to 2008 of 6.9 nmol/L (SD, 16.7) versus a decrease of 9.8 nmol/L (SD, 19.1) for those whose sampling changed from summer to winter (n = 359) (P < 0.001). Subjects who discontinued cod liver oil or vitamin D supplementation (n = 581) had a decrease in serum 25(OH)D from 1994 to 2008 of 0.6 nmol/L (SD, 19.4), whereas those who started taking cod liver oil or vitamin D supplements (n = 331) had an increase of 3.7 nmol/L (SD, 17.3) (P < 0.001).

**Table 2. Numbers of Norwegian Study Subjects in Serum 25(OH)D z-Score Quintiles 1–5 in 1994 and 2008, The Tromsø Study**

<table>
<thead>
<tr>
<th>Quintile in 1994</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No. %</td>
</tr>
<tr>
<td>1</td>
<td>222</td>
<td>41.7</td>
<td>118</td>
<td>22.1</td>
<td>108</td>
<td>20.3</td>
</tr>
<tr>
<td>2</td>
<td>135</td>
<td>25.3</td>
<td>131</td>
<td>24.5</td>
<td>119</td>
<td>22.3</td>
</tr>
<tr>
<td>3</td>
<td>90</td>
<td>16.9</td>
<td>117</td>
<td>21.9</td>
<td>128</td>
<td>24.0</td>
</tr>
<tr>
<td>4</td>
<td>60</td>
<td>11.2</td>
<td>113</td>
<td>21.2</td>
<td>103</td>
<td>19.3</td>
</tr>
<tr>
<td>5</td>
<td>26</td>
<td>4.9</td>
<td>61</td>
<td>11.4</td>
<td>69</td>
<td>12.9</td>
</tr>
</tbody>
</table>

Abbreviation: 25(OH)D, 25-hydroxyvitamin D.
correlations ($P < 0.001$) between serum 25(OH)D at baseline and the values at 3, 6, 9, and 12 months (0.70, 0.55, 0.67, and 0.80, respectively). From baseline until the end of the study, 71.3% had a change in serum 25(OH)D of less than 10 nmol/L and 94.7% had a change in serum 25(OH)D of less than 20 nmol/L.

**DISCUSSION**

In the present study, we found, in a general population, the correlation between serum 25(OH)D levels from 1994 and 2008 to range from 0.39 to 0.52 depending on the method of calculation. In a 1-year intervention study, the correlation between serum 25(OH)D levels at baseline and after 12 months was 0.80 for subjects in the placebo group.

The higher correlation in the intervention study was to be expected for several reasons. First, that study lasted only 1 year, and, obviously, the closer the times of measurement, the better the correlation. The measurements 1 year apart also eliminated the effect of season, and, in this 1-year study, the effect of season was even more important than a short time between the measurements. Thus, the correlation between baseline and 6 months in the intervention study was only 0.55, or close to that seen in the general population when only those subjects with samples drawn during the same season in 1994 and 2008 were included.

In epidemiologic studies, tracking is used to describe the stability of a characteristic over time (22). If the variable is a risk factor with a high tracking coefficient, a single measurement can be used to identify subjects at risk and to start prophylactic treatment. Generally, the tracking correlation is highest for characteristics with the lowest measurement error, such as body mass index, and is lowest for lifestyle factors that are difficult to measure, such as nutrient intake (23) or physical activity (24). For serum 25(OH)D, the correlation between values from 1994 and 2008 was similar to that seen for cardiovascular risk factors such as blood pressure and lipids (25, 26) but was much lower than for body mass index, as observed in our study. If a low serum 25(OH)D level is a risk factor for future diseases, as has been suggested (27), it would strengthen the need for treatment if those with the lowest levels remained at low levels over time. That was the case in our study; 75% of those with serum 25(OH)D of less than 30 nmol/L had serum 25(OH)D of less than 50 nmol/L 14 years later. Similarly, 87% of subjects with serum 25(OH)D levels higher than 79 nmol/L had serum 25(OH)D levels higher than 50 nmol/L 14 years later. Accordingly, measured levels of serum 25(OH)D are likely to persist in individuals.

Although the study was not designed to evaluate predictors of change in serum 25(OH)D levels over time, observing that changes in serum 25(OH)D corresponded to changes in the known predictors (2) adds external validity to the study. Thus, serum 25(OH)D level is mainly the result of sunlight exposure and intake of vitamin D (2), and one would therefore expect season to affect the serum levels and that the correlation between measurements to be highest if sera were drawn at similar times of the year. In our study, both of these relations were found. We included a physical

<table>
<thead>
<tr>
<th>Serum Level in 2008, nmol/L</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>&lt;30</td>
<td>39</td>
</tr>
<tr>
<td>30–39</td>
<td>47</td>
</tr>
<tr>
<td>40–49</td>
<td>30</td>
</tr>
<tr>
<td>50–59</td>
<td>27</td>
</tr>
<tr>
<td>60–69</td>
<td>9</td>
</tr>
<tr>
<td>70–79</td>
<td>4</td>
</tr>
<tr>
<td>&gt;79</td>
<td>1</td>
</tr>
</tbody>
</table>

Abbreviation: 25(OH)D, 25-hydroxyvitamin D.
activity score, which is a crude measure of outdoor activity, and, as expected, a reduction in physical activity was associated with a lowering of serum 25(OH)D level. Regarding vitamin D intake, we did not have a detailed food frequency questionnaire. However, questions about use of cod liver oil and vitamin D supplements were included, and the difference in change in serum 25(OH)D from 1994 to 2008 between those who started and stopped these vitamin D intakes was 4.3 nmol/L. In comparison, the difference in change in serum 25(OH)D between subjects with measurements in summer 1994 and winter 2008 compared with those with measurements in winter 1994 and summer 2008 was 16.7 nmol/L, which again emphasizes the impact of season.

It is known that production of vitamin D in the skin is reduced with age (2). Accordingly, a decrease in serum 25(OH)D levels over time should be found in the older compared with the younger subjects. We observed a difference in change from 1994 to 2008 of 2.3 nmol/L between those older than 65 years of age in 1994 compared with those younger. Finally, body mass index is a negative predictor of serum 25(OH)D levels, probably because of storage of 25(OH)D in adipose tissue (6). In line with that theory, an increase in body mass index from 1994 to 2008 was associated with a decrease in serum 25(OH)D levels in our study.

Apart from change in sun exposure and intake (and absorption) of vitamin D, the variation coefficient of the vitamin D assay, or change of assay, will obviously also influence the relation between measurements made at separate times. In a recent analysis of serum 25(OH)D levels from the National Health and Nutrition Examination Surveys in 2000–2004 and 1988–1994, there was, depending on the age of subjects, a significant 5–20 nmol/L reduction in serum 25(OH)D levels. However, most of this apparent reduction was due to assay differences (18). Therefore, it is a strength of our study that we used the same vitamin D assay for both the 1994 and 2008 samples and that they were analyzed during the same time period. In addition, serum 25(OH)D is known to be stable for years when stored at −70°C (16, 17), and the similar mean serum 25(OH)D levels in samples from 1994 and 2008 demonstrates that the effect of storage was of minor importance.

In conclusion, this study shows that tracking of serum 25(OH)D is similar to that for blood pressure and serum lipids and, in particular, that most subjects with low serum 25(OH)D levels are unlikely to have a substantial improvement in their vitamin D levels over time. The study therefore provides some support for the use of a single serum 25(OH)D measurement in epidemiologic studies in which low serum 25(OH)D levels are related to future diseases and mortality.

**ACKNOWLEDGMENTS**

Author affiliations: Institute of Clinical Medicine, University of Tromsø, Tromsø, Norway (Rolf Jorde, Moira Hutchinson); Medical Clinic, Department of Endocrinology, University Hospital of North Norway, Tromsø, Norway (Rolf Jorde, Guri Grimnes); Department of Ophthalmology and Neurosurgery, Division of Ophthalmology, University Hospital of North Norway, Tromsø, Norway (Monica Sveve); Institute of Community Medicine, University of Tromsø, Tromsø, Norway (Nina Emaus); Diagnostic Clinic, Department of Medical Biochemistry, University Hospital
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