Tracking of serum 25-hydroxyvitamin D during 21 years

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Abstract

Objectives Our objective was to evaluate the degree of tracking for serum levels of 25-hydroxyvitamin D [25(OH)D] over time, by using data from three previously conducted surveys of the Tromsø study collected in the years 1994/1995 (Tromsø 4), 2007/2008 (Tromsø 6) and 2015/2016 (Tromsø 7).

Subjects and Methods Subjects with valid 25(OH)D measurements in all three surveys were included. 25(OH)D z-scores were used to adjust for seasonal variation. Z-scores and sextiles were used to illustrate tracking of 25(OH)D.

Results 1,702 subjects (572 males, 1,130 females) fulfilled the inclusion criteria. Median (5^{th}, 95^{th} percentiles) age for these subjects was 55 (33, 65) years in Tromsø 4, and mean (SD) 25(OH)D levels were 57 (18) nmol/L, 59 (19) nmol/L and 72 (21) nmol/L for Tromsø 4, Tromsø 6, and Tromsø 7, respectively. There was significant tracking of serum 25(OH)D over the 21 years period between the surveys of the Tromsø study. The correlation coefficient *r* between 25(OH)D z-scores from Tromsø 4 and Tromsø 6 was 0.40, and declined to 0.29 for the correlation between Tromsø 4 and Tromsø 7. 26 % of the subjects in the lowest 25(OH)D z-score sextile in Tromsø 4 were in the three highest sextiles of 25(OH)D in Tromsø 7. Similarly, 35 % of those in the highest sextile in Tromsø 4, were in the lowest three sextiles in Tromsø 7.

Conclusion The degree of tracking for serum 25(OH)D declines over time, and the use of a single serum 25(OH)D measurement as an indicator of the vitamin D status is questionable if used in long-lasting observational studies.

Introduction

The importance of vitamin D for skeletomuscular health is well established [1, 2]. The discovery of vitamin D receptors in almost all tissues of the body, also in those not related to calcium or bone metabolism [2], lead to discussions about its potential role in multiple heath related issues. Following this discovery there have been many studies published on the association between vitamin D status and an array of non-calcemic medical conditions, such as cardiovascular disease, cancer, obesity, depression and dementia [3]. Currently, serum levels of 25-hydroxyvitamin D [25(OH)D] are viewed as the standard method to evaluate a subject's vitamin D status and most often this vitamin D status assessment is based on a single measurement [4, 5].

In recent years there is new evidence that vitamin D levels are not only dependent on sun exposure and diet [1], but are also to a significant degree determined genetically [6, 7]. This, and vitamin D's possible association with numerous diseases, amplifies the value of being able to predict vitamin D levels in an individual over time. Preferably, this prediction would be based on a single or a few serum values, and enable an appraisal of a subject's vitamin D status over time. This constancy of 25(OH)D level in a specific individual over a longer period of time is referred to as tracking.

In a previous article from 2010, our research group confirmed tracking of serum 25(OH)D within individuals based on samples taken 13 years apart in 2,668 subjects. This was based on observations from the Tromsø study – an observational, longitudinal study, which is repeated on regular intervals in the form of surveys, examinations and collection of biological data from the population in the Tromsø municipality [8]. Since then, several papers have been published regarding tracking of 25(OH)D [9-21]. However, these studies have been considerably smaller, and/or have had much shorter observation time, or have focused on

population subgroups like pregnant women [11] or children/adolescents [17, 18, 20]. In general, they have confirmed our original observation of a high degree of tracking, as could be expected with a shorter observation time.

The seventh survey in the Tromsø study was performed in 2015/2016 and a considerable number of those included in our original tracking publication also participated in this survey. We therefore had the opportunity to evaluate the tracking of 25(OH)D over a 21 years time period.

Materials and method

The Tromsø survey is a longitudinal, observational population study conducted by the University of Tromsø – The Arctic University of Norway, and the Norwegian National Heath Screening Service. Initiated in mid-1970 as a means of mapping cardiovascular disease in north Norwegian males, it has since evolved to include a large portion of Tromsø municipality inhabitants of both sexes and with a wide range of age groups [22, 23]. The focus has also shifted; from the narrow emphasis on cardiovascular disease, to a multi-focus study with detailed data gathered on lifestyle, and a wide range of health issues [23]. The study has been repeated with regular intervals and recently finished its seventh survey in 2015/2016. In the present study we have used data from the fourth, sixth and seventh surveys:

- The fourth survey ("Tromsø 4") was performed in 1994/1995 and invited all citizens aged 25 years or older living in Tromsø municipality (n = 37,558) of whom 27,158 subjects participated in the first phase of the survey. Furthermore, all men aged 55 – 74 years, all women aged 50 – 74 years and 5 – 8 % random samples of the other age groups < 85 years were invited to a second visit with more extensive examinations.

Among 10,542 eligible subjects 7,965 attended this second phase, and 7,156 (2,269 smokers and 4,887 non-smokers) had serum 25(OH)D measured.

- The sixth survey ("Tromsø 6") was performed in 2007/2008. All who had participated in the second phase of Tromsø 4, a 10 % random sample of those 20 – 39 years old, all subjects 40 – 42 or 60 – 87 years old, and a 40 % random sample of those 43-59 years old were invited. Among the 19,762 subjects invited, 12,984 subjects attended and 12,444 (2,389 smokers and 10,055 non-smokers) had serum 25(OH)D measured.
- The seventh survey ("Tromsø 7") was performed in 2015/2016 and all citizens aged 40 years or above living in Tromsø municipality (n = 32,591) were invited, among whom 21,084 participated and 20,720 (2,878 smokers and 17,842 non-smokers) had serum 25(OH)D measured.

The Tromsø surveys are binary in design, with a questionnaire part followed by physical examination and blood sampling. The details regarding the questionnaires and examinations can be found on http://tromsoundersokelsen.uit.no/tromso/ (May-November, 2019). The surveys contain information on age, sex, smoking habits, medication, use of cod liver oil and vitamin D supplements.

The wording in the questionnaires regarding cod liver oil and vitamin D supplements differed between the surveys. In Tromsø 4 the cod liver oil question was: "Have you used cod liver oil or fish oil capsules during the last 14 days ("yes"/"no")?". In Tromsø 6 and Tromsø 7 the corresponding question was "Do you use cod liver oil or cod liver oil capsules?". The answer options in Tromsø 6 were "yes, daily"/"sometimes"/"no", and in Tromsø 7 "no"/"sometimes"/"daily during the winter season"/"daily". In Tromsø 4 the vitamin D supplement question was "Have you used vitamin D supplements during the last 14 days?" and in Tromsø 7 "Do you use vitamin supplements with vitamin D?" with the same answer

options as for cod liver oil. Vitamin D supplements were not specifically asked for in Tromsø 6.

The Tromsø 6 and 7 surveys also included questions regarding sunny vacations last 8 weeks ("yes"/"no") and use of solarium or any form of light therapy during the last 7 days ("weekly"/"sometimes"/"never"). However, only 30 % of the subjects answered these questions in Tromsø 6 and sunny vacation/use of solarium therefore not included in the tracking analyses.

Height and weight were measured with light clothing, and body mass index (BMI) calculated as kg/m^2. Blood pressure was measured after a 2 min seated rest (in Tromsø 4 with Dinamap Vital Signs Monitor, Critikon Inc, Tampa, FL, USA; in Tromsø 6 and 7 with Dinamap ProCare 300 monitor, GE Healthcare, Oslo, Norway). The mean of the two last measurements was used in our analyses.

Blood samples were non-fasting. Serum cholesterol and serum calcium were analyzed as previously described [24, 25]. These methods have a total analytic coefficient of variation (CV) of < 2 % and 2.5 %, respectively.

The samples from Tromsø 4 were stored at -70 degrees C and together with the samples from Tromsø 6 analyzed for 25(OH)D in batch with ECLIA (Roche) using an automated clinical chemistry analyzer (Modular E170, Roche Diagnostics). This method, which overestimates serum 25(OH)D in smokers has been described in detail previously and has a CV of 7.3 % (26). The samples from Tromsø 7 were analysed consecutively with an in-house LC-MS/MS method, which has a CV of < 9 % [26, 27].

Statistical analyses

Only subjects with valid serum 25(OH)D measurements in all three surveys were included in the analyses. The effect of season was adjusted for by calculating month specific z-scores for serum 25(OH)D for each survey. Since the assay used in Tromsø 4 and Tromsø 6 overestimates serum 25(OH)D in smokers, the z-scores were calculated separately for smokers and non-smokers in all three surveys. Z-scores for smokers and non-smokers were then combined in the tracking analyses. We excluded subjects who changed smoking status between the surveys.

Normal distribution was evaluated with visual inspection of histograms and plots, and by assessing kurtosis and skewness. Distribution was normal for the dependent variables BMI, systolic blood pressure, serum calcium, serum cholesterol and serum 25(OH)D. The dataset was assessed using the Pitman-Morgan test for related samples, displaying homogeneity of variances.

Tracking was evaluated by Pearson's correlation coefficient *r*. Blood pressure and cholesterol analyses only included subjects not using blood pressure or lipid medication, respectively. A linear regression model was used to evaluate predictors of serum 25(OH)D and of change in serum 25(OH)D z-score (delta z-score: z-score in Tromsø 7 minus z-score in Tromsø 4) with covariates as appears in the tables. In addition, the 25(OH)D z-scores from Tromsø 4 and 7 were divided into sextiles and cross-tabled to illustrate degree of tracking.

$P < 0.05$ (two-tailed) is considered statically significant. Data are presented as mean (SD) for normally distributed values, and as median (5^{th}, 95^{th} percentiles) for non-normally distributed values. All statistical analyses are performed using IBM SPSS version 26 software.

Ethics

The Tromsø Study is approved by the Regional Committee for Medical Research Ethics (REK) and this investigation is covered by this approval. All included subjects signed a written informed consent.

Results

A total of 1,702 subjects (572 males, 1,130 females) with valid 25(OH)D measurements in the Tromsø 4, 6 and 7 surveys, and without change in smoking status were included in the present study. Their mean (SD) serum 25(OH)D levels were 57 (18) nmol/L, 59 (19) nmol/L and 72 (21) nmol/L for Tromsø 4, Tromsø 6, and Tromsø 7, respectively. As expected, the serum 25(OH)D levels were higher during the summer months, as shown for Tromsø 6 in Figure 1. Other characteristics from the separate surveys are displayed in Table 1.

In a linear regression model, sex, age, BMI, recent sunny vacation, intakes of cod liver oil and vitamin D supplements were significant predictors of serum 25(OH)D (Table 2).

The correlation coefficient *r* between serum 25(OH)D z-scores from Tromsø 4 and Tromsø 6 was 0.40, and declined to 0.29 for the correlation between Tromsø 4 and Tromsø 7 (Table 3). In comparison, correlations between Tromsø 4 – Tromsø 7 for BMI, systolic blood pressure (in subjects not using blood pressure medication), serum calcium and serum total cholesterol (in subjects not using lipid lowering medication) were slightly higher, at 0.78, 0.45, 0.31 and 0.53, respectively (Table 3). The degree of tracking for serum 25(OH)D was higher in males, non-smokers, age > 55 years in Tromsø 4, change in BMI < 1.1 kg /m^2, and with continuous use of cod liver oil or vitamin D supplements (Table 4).

The importance of BMI, use of cold liver oil, and vitamin D supplements for tracking of serum 25(OH)D was confirmed in a linear regression model where change in BMI and change

194 in intakes of vitamin D were significant predictors of change in serum 25(OH)D z-scores
195 (Table 5).

196 To explore reasons for the higher tracking for serum 25(OH)D for males and for subjects >
197 55 years, the serum 25(OH)D, BMI, intakes of cod liver oil and vitamin D supplement in
198 relation to sex and age are shown for the three surveys in Table 6. In the females there was an
199 increase in use of vitamin D supplementation from 8 % in Tromsø 4 to 29 % in Tromsø 7,
200 whereas in the males the corresponding increase was only from 3 to 14 %. This could possibly
201 explain the higher tracking in the males. However, there was in our data no obvious
202 explanation for the higher tracking of serum 25(OH)D in those with age > 55 years.

203 To further illustrate the degree of tracking, the change in distribution of sextiles of
204 25(OH)D z-scores from Tromsø 4 to Tromsø 7 is shown in Table 7. Among those in the
205 lowest sextile in Tromsø 4, 74 % were still in the three lowest sextiles of 25(OH)D in Tromsø
206 7, but accordingly, 26 % had shifted to the three highest percentiles. Similarly, among those in
207 the highest sextile in Tromsø 4, 65 % were still in the three highest sextiles in Tromsø 7, and
208 35 % were now in the three lowest.

209

210 Discussion

211

212 In the present study, based on data from three surveys in the Tromsø study, we have found
213 significant tracking of serum 25(OH)D over a 21-years period. Tracking was observed in both
214 sexes, and in all age groups, with a correlation coefficient *r* ranging from 0.25 to 0.40 in the
215 various subgroups between the first and last serum 25(OH)D measurement.

216 As expected, the degree of tracking declined over time. The correlation coefficient *r* was
217 for all subjects 0.40 between the fourth and the sixth surveys (13 years apart), and dropped to
218 0.29 between the forth and the seventh surveys (21 years apart). In comparison, one year

219 tracking data for participants receiving placebo in a vitamin D intervention study was as high
220 as 0.80 [8].

221 When dividing the cohort into serum 25(OH)D z-score sextiles in the fourth and seventh
222 surveys, 26 % of those in the lowest sextile in Tromsø 4 were in the upper half of the cohort
223 in Tromsø 7. Conversely, 35 % of those in the highest sextile in Tromsø 4, were in the lower
224 half in Tromsø 7. Consequently, the use of a single serum 25(OH)D measurement as an
225 indicator of the vitamin D status, as has been frequently done in case-control studies [28, 29],
226 appears to be highly questionable, at least if the observation time is long. Likewise, in
227 prospective studies lasting for more than a few years, repeated measurements of serum
228 25(OH)D should be used for estimation of vitamin D status before occurrence of the outcome
229 in question. Furthermore, the high degree of tracking for serum 25(OH)D over shorter periods
230 of time, like a few years, is an argument against repeated serum 25(OH)D measurements in
231 clinical practice.

232 The serum 25(OH)D level is partly genetically determined, and several single nucleotide
233 polymorphisms (SNPs) in enzymes necessary for production, transport and degradation of the
234 active vitamin D metabolite 1,25-dihydroxyvitamin D have been described [6, 30]. These
235 genetic differences may account for a large part of the variation in serum 25(OH)D levels [7].
236 However, the main determinants of serum 25(OH)D levels are amendable factors related to
237 life-style, like time spent in the sun, intake of vitamin D rich food like fatty fish, and the use
238 of vitamin D supplement [1, 30]. Furthermore, it appears as body size, in particular adipose
239 tissue, is of importance by increasing volume of distribution [31]. It was therefore no surprise
240 that tracking was more pronounced in subjects who continuously used cod liver oil and /or
241 vitamin D supplements, and that changes in intake of these substances, as well as change in
242 BMI, were associated with greater change in delta serum 25(OH)D z-scores. In Norway,
243 which does not receive as much UV light during the summer as countries further south, the

importance of these factors are perhaps more important for 25(OH)D tracking than seasonal changes which are more pronounced in countries like England [32]. We also found a lower degree of tracking for serum 25(OH)D in the females, which could possibly be explained by an increase in their use of vitamin D supplements.

In addition to lifestyle factors, the degree of tracking is also influenced by the precision of the laboratory analyses, and for serum 25(OH)D the CV was higher than for serum calcium and total cholesterol. It should be noted that the tracking of serum 25(OH)D was lower than that found for serum calcium and serum total cholesterol, as well as for BMI and systolic blood pressure. For observational studies that rely on a single measurement these variables are therefore probably better suited than serum 25(OH)D.

In general, other studies have found a similar degree of tracking for serum 25(OH)D as we have [11, 17, 18, 20]. However, most of these studies have been of short duration, and to our knowledge, ours is the longest running by far. Some of these studies have included subjects in certain age groups or in particular periods of life. Thus, Thordisdottir et al. found in a group of 139 children a correlation coefficient between serum 25(OH)D at age 1 and 6 years of 0.34 [17]. Similarly, Zhu et al. found in a group of 821 children with serum 25(OH)D measured at ages 6, 14, 17 and 20 years, correlation coefficients ranging from 0.35 to 0.56 depending on time interval [20]. On the other hand, Poopedi et al. found no significant correlation for 25(OH)D between age 11 and 20 years in a group of 76 adolescents ($r = 0.15$), whereas between ages 15 and 20 the correlation was highly significant ($r = 0.65$) [18]. And finally, in 1,753 pregnant women, Moon et al. found a correlation coefficient of 0.53 between season corrected serum 25(OH)D measurements at 11 and 34 weeks of gestation [11].

The mean serum 25(OH)D levels were similar in Tromsø 4 and Tromsø 6 which were 13 years apart, but in Tromsø 7 eight years later the serum 25(OH)D was ~ 20 % higher. There was a change in 25(OH)D assay from Tromsø 6 to Tromsø 7, and in view of the time line, this

is the most likely explanation for the apparent increase in serum 25(OH)D. Additional factors could be the increase in use of daily vitamin D supplements from 7 to 24 % from Tromsø 4 to Tromsø 7, as well as nutritional changes that were not recorded in our study. Sunny vacation the last two months was a strong predictor of the serum 25(OH)D level in Tromsø 7, but was not asked for in the Tromsø 4 survey. Most likely such vacations were more frequent in 2015/2016 than in 1994/1995 and could thus have contributed to the apparent increase in serum 25(OH)D in this cohort.

Our study has several weaknesses. In Tromsø 7 we used an LC-MS/MS assay for determination of serum 25(OH)D levels, whereas in Tromsø 4 and 6 an immunological assay was used. We could therefore not relate the degree of tracking to changes in nmol/L of serum 25(OH)D, but had to make a z-score transformation. The assay used in Tromsø 4 and Tromsø 6 overestimated serum 25(OH)D in smokers. We therefore calculated z-scores separately for smokers and non-smokers, before combining them into one group. Since we made month-specific z-scores to adjust for seasonal variation, some of the smoker-groups became small, which can have made these z-scores less accurate. We had limited information regarding intakes of vitamin D, as well as sun-exposure, which could have improved the analysis of factors affecting the degree of tracking. The questionnaires regarding cod liver oil and vitamin D supplements differed slightly between the surveys and made evaluation of changes in these intakes difficult. We had to exclude subjects with change in smoking habits, which might have resulted in an overestimation of the serum 25(OH)D tracking. Only subjects who lived in Tromsø at all three time points could be included, and in societies with a high degree of mobility the tracking of serum 25(OH)D is likely to be lower. On the other hand, our study also has strengths as it was performed on the general population, included a large number of subjects of both sexes and of different ages, and the follow-up period was 21 years.

In conclusion, we have found a significant degree of tracking for serum 25(OH)D over a period of 21 years. However, the degree of tracking declined over time, and using a single serum 25(OH)D measurement as an indicator of vitamin D status in long-term observational studies might be questionable.

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Conflicts of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Author Contributions

RJ and EK were responsible for designing the protocol. JK was responsible for doing the analyses and drafting the manuscript. JK, EK and RJ all participated in finalizing the manuscript.

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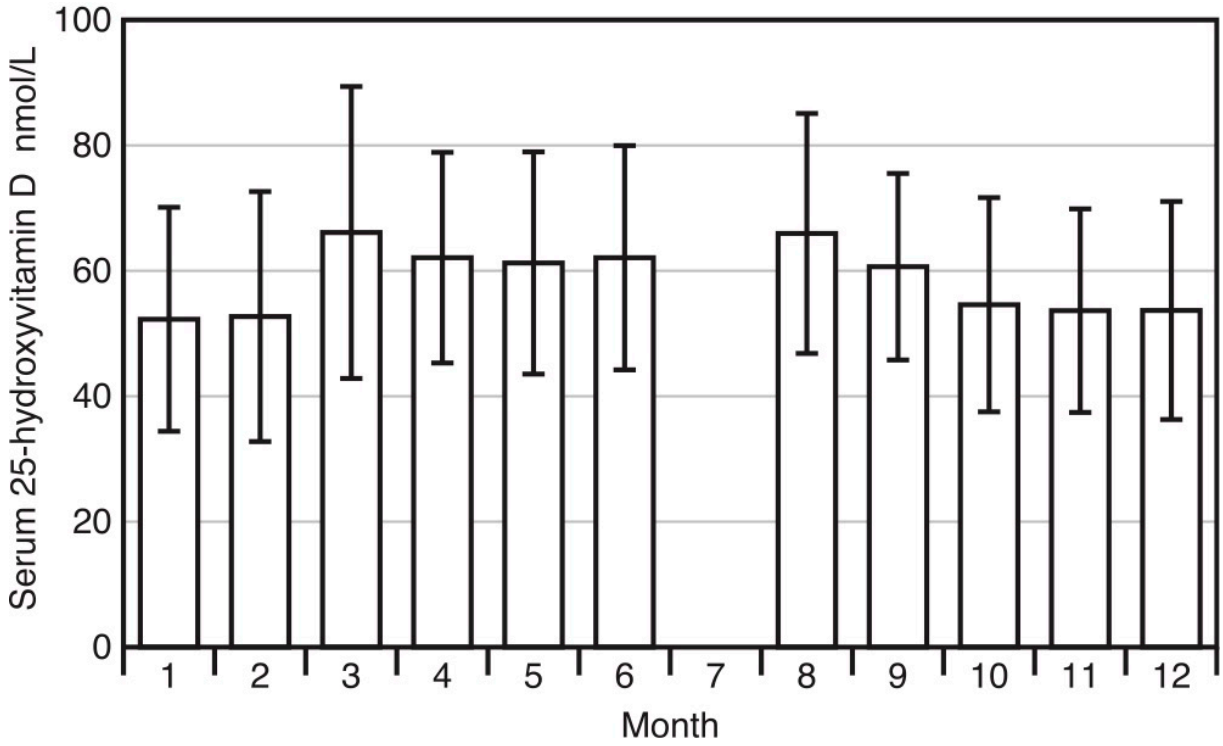
Legend to figure

Figure 1. Mean serum 25-hydroxyvitamin D in relation to month of blood sampling (1 = January, 2 = February etc.) in the 1702 subjects in the sixth survey of the Tromsø study. No blood samples were drawn in July. The error bars represent SD.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