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**Title:** Rising Trend in Vitamin D status from 1993 to 2013: Dual Concerns for the Future

**Abbreviated title:** Rising trend in 25OHD

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**Abbreviations:** ARIMA, auto-regressive integrated moving average; DEQAS, vitamin D external quality assessment scheme; EAR, estimated average requirement; IOM, Institute of Medicine; LLD, lower level of detectability; RDA, recommended daily allowances; 25OHD, 25-hydroxyvitamin D.

## ABSTRACT

**Background:** The Institute of Medicine 2011 Report on Dietary Reference Intakes for Calcium and Vitamin D specified higher intakes for all age groups compared to the 1997 report, but also cautioned against spurious claims about an epidemic of vitamin D deficiency and against advocates of higher intake requirements. Over 40 years, we have noted marked improvement in vitamin D status but we are concerned about hypervitaminosis D.

**Objective:** We sought to evaluate the 25OHD trend over 20 years.

**Design:** We retrieved all results of serum 25-hydroxyvitamin D (25OHD) from 1993 to 2013 (n=69,012) that was trimmed to one sample per person (n=43,782). We conducted a time series analysis of the monthly averages for 25OHD using a simple sequence chart and a running median smoothing function. We modelled the data using univariate auto-regressive integrated moving average (ARIMA) and forecast 25OHD levels up to 2016.

**Results:** The time series sequence chart and smoother function demonstrated a steady upward trend with seasonality. The yearly average 25OHD increased from 36.1 nmol/L in 1993 to 57.3 nmol/L in 2013. The ARIMA model was a good fit for the 25OHD time series; it forecasted monthly average 25OHD up to the end of 2016 with a positive stationary R-squared of 0.377.

**Conclusions:** Vitamin D status improved over the past 40 years, but there is a dual problem: groups at risk of vitamin D deficiency, who need public health preventative measures; and, random members of the population who are taking unnecessarily high vitamin D intakes for unsubstantiated claims.

## INTRODUCTION

Vitamin D supply is changeable: being sourced from skin synthesis following solar exposure, which is curtailed seasonally in high latitude countries, and from oral intake of natural foodstuffs, fortified foodstuffs and supplements (1). Although sunlight exposure is the predominant natural source of vitamin D, the primacy of oral intake over sunlight exposure both in the prevention and correction of vitamin D deficiency has been known for some time (2). This is apposite given the concerns about sunlight exposure and skin cancer. For these reasons, the Institute of Medicine (IOM) 2011 Report specified dietary reference vitamin D intakes for those with minimal or no sunlight exposure (3). Individuals with intentional or inadvertent sunlight exposure have lesser dependence on oral sources. The recommended daily allowances (RDA) specified by IOM in 2011 are between 30% and three-fold higher compared to 1997 (4). Noting the trend for unsubstantiated claims regarding vitamin D, IOM cautioned against exceeding recommended intakes (3, 5).

The IOM gave guidance about the interpretation of the 25-hydroxyvitamin D (25OHD) result. First and foremost, they concluded that 25OHD is a biomarker of exposure and not a biomarker of effect: 25OHD is not a validated clinical outcome nor is it a surrogate of a clinical outcome. According to the IOM, 25OHD is a measure of risk: a concentration below 30 nmol/L (12 ng/ml) indicates increased risk of vitamin D deficiency; a concentration of 40 nmol/L (16 ng/ml) corresponds to the estimated average requirement (EAR) satisfying the needs of half the population; a concentration above 50 nmol/L (20 ng/ml) meets the requirements of 97.5% of the population; a concentration above 125 nmol/L (50 ng/ml) indicates risk of harm (5-7). Although some expert guidelines advocate higher thresholds and higher doses to achieve these thresholds (8), other recent systematic reviews support the IOM specifications with respect to skeletal and non-skeletal health (9-13).

We started measuring serum 25OHD in 1973 in clinical samples (14). We conducted a number of clinical studies up to the early 1980s, and noted an extremely high prevalence of hypovitaminosis D in the elderly that was easily corrected by low-dose daily vitamin D supplementation (14-17). Milk fortification with vitamin D started in Ireland in the mid-1980s,

76 although it was not mandatory. This fortification ameliorated greatly the decline in 25OHD over the
77 winter months (18). Over the past 20 years, supplements combining elemental calcium (500 mg) and
78 vitamin D (10 μg) have become readily available, initially on prescription-only basis but subsequently
79 on an over-the-counter basis. Most recently, manufacturers of high-dose vitamin D supplements up to
80 125 μg are seeking marketing licences.

81 While it is gratifying to witness a marked improvement in vitamin D status following
82 practices of fortification and supplementation, much attention is still needed in all countries to address
83 at-risk groups. The counterfactual to spurious claims about an epidemic of vitamin D deficiency is
84 increasing prevalence of hypervitaminosis D as a consequence of unnecessarily high intakes in excess
85 of IOM specifications (19). For this reason, we sought to evaluate the trend in vitamin D status in
86 Ireland over the past 20 years in order to forecast the future trend.

87 **METHODS**

88 **Samples and 25OHD Methodology**

89 Our hospital has kept a computerised record of all 25OHD results since May 1993. The
90 database includes the following additional variables: date of sample, forename, surname, date of birth,
91 hospital record number, and gender. We obtained permission from the Ethics Committee at St.
92 Vincent's University Hospital to extract the information. We opted to have no exclusion criteria. The
93 sole selection criterion was to ensure that only one sample per person was included in the database: if
94 a person had more than one sample, then an average was taken for that person. The total number of
95 results extracted was 69,012. Subsequent to trimming the database to one sample per person, the total
96 number was 43,782.

97 Since 1974, we have measured serum 25OHD by four different techniques: Haddad and Chyu
98 competitive protein binding radioassay from 1974 to 1994 (20); Incstar/Diasorin radioimmunoassay
99 (Diasorin Inc. Stillwater, UK) from 1994 to 2008; Immunodiagnostic Systems (IDS)
100 radioimmunoassay (Immunodiagnostic Systems Limited, Boldon, Tyne & Wear, UK) from 2008 to
101 2011; and Elecsys Vitamin D Total (Roche Diagnostics GmBH, Mannheim, Germany) from 2011 to

current. Passing and Bablok method comparison and Bland-Altman test of method bias were performed on the comparative data. In addition, we performed a comparison between Elecsys Vitamin D Total and liquid chromatography-tandem mass spectrometry (LC-MS/MS). Details of these assays and method comparisons are given in the supplementary information. Since April 1991, we have participated in DEQAS (21) a period covering all four techniques and have been awarded proficiency certification throughout these years. In view of the difference in defining the lower level of detectability (LLD) with the assays over time, we decided to censor 25OHD levels at a functional sensitivity of 10 nmol/L (4 ng/ml), which was the highest LLD concentration determined for each of the four 25OHD methods used.

**Statistical Analysis**

Results are presented as mean and standard deviation (SD) or confidence intervals (CI), and number and frequency. Differences in means were tested using independent t test. The total group was divided into five ordered categories according to 25OHD levels in keeping with IOM specifications: <10 nmol/L (<4 ng/ml); 10-29.9 nmol/L (4-11.9 ng/ml); 30-50 nmol/L (12-20 ng/ml); >50-125 nmol/L (>20-50 ng/ml); >125 nmol/L (>50 ng/ml). The frequencies of 25OHD, according to these categories, were determined for the entire group, and for the first year and final year; regarding the latter two groups, the independence of row and column categories was tested using chi-square test.

Monthly averages for 25OHD were calculated from May 1993 to December 2013 (n=248), and yearly averages for 25OHD were calculated from 1993 to 2013 (n=21). In order to represent the moving average over the 20 years, a linear regression was fitted to the data with dependent variable being time and the independent variable being monthly-average 25OHD levels. The change in yearly-average 25OHD compared to baseline year 1993 was calculated; a linear regression was fitted with year being the dependent variable and change in yearly average being the independent variable.

We conducted a time series analysis of the monthly averages of 25OHD using a simple sequence chart. Then the same data was analyzed using a 4253H smoother, which is a running median smoothing function. Since we did not have independent predictors such as oral vitamin D intake or body mass index, we used the univariate auto-regressive integrated moving average (ARIMA) for time series modelling. ARIMA decides what the amount of lag for the series should be for both series values and errors. The model seeks to explain the following: trend, which is defined as the long term direction of the time series; seasonality, which is defined as repeated behavior that occurs at regular intervals; cycles, which are defined as up or down patterns that are not seasonal; and, natural variation. The performance of the ARIMA model was assessed in four ways: firstly, the plot of the model against the historic series was inspected; secondly, the errors of the model were examined on a sequence chart examining whether errors have a constant variance (homoscedastic) or a changing variance (heteroscedastic); thirdly, the distribution of the error terms both with and without outliers was tested for normality by Kolmogorov-Shapiro; fourthly, the model was rebuilt excluding the final 3 years followed by comparison of the forecasted values with the actual values. Finally, we used the ARIMA model to forecast 25OHD levels up to 2016. The stationary R-squared was chosen as the model fit statistic, because it compares the stationary part of the model to simple mean model, which is preferable to the usual R-squared when there is a trend or seasonal pattern. The stationary R-squared can be negative and has a range of negative infinity to +1; a negative value indicates that the forecasted model is worse than the baseline model, and a positive value means that the forecasted model is better than the baseline model. Statistical analysis was performed using IBM SPSS for Windows version 21.0 (Armonk, NY).

## RESULTS

### Descriptive statistics

The mean±SD for 25OHD was 54.6±31.4 nmol/L (22.1±12.8 ng/ml) with the range being <10 to 971 nmol/L (<4 to 395 ng/ml). The mean±SD for age was 49.8±25.6 (range: birth to 105) years; 66.7% were women and 32.3% were men. Women had higher 25OHD compared to men (56.2±31.2

vs 51.4±31.4 nmol/L, 22.8±12.7 vs 20.2±12.8 ng/ml, t=15.0, p<0.001). The yearly number of samples increased steadily from 741 in first full year (May 1993 to April 1994) up to 7,887 in 2013. Over the 20 years, sampling was evenly distributed throughout the 12 months of the year. The yearly mean±SD 25OHD increased from 36.1±24 nmol/L (14.4±9.6 ng/ml) in first year to 57.3±37.7 nmol/L (23.0±15.1 ng/ml) in 2013.

**Prevalence of 25OHD categories**

The frequencies, over the 20 years from 1993-2013, according to ordered 25OHD cut-points were as follows: 2.1% <10 nmol/L (4.0 ng/ml); 23.1% between 10-29.9 nmol/L (4.0-11.96 ng/ml); 26.7% between 30-50 nmol/L (12.0-20.0 ng/ml); 47.8% between >50-125 nmol/L (20-50 ng/ml); and 2.3% >125 nmol/L (50 ng/ml). In the first full year (May 1993 to April 1994) compared to the final year in 2013, the respective frequencies were as follows: 15.3% vs 2.7%; 32.3% vs 21.6%; 26.9% vs 23.7%; 24.9% vs 48.2%; and 0.7% vs 3.8% ($\chi^2$=414, p<0.001) (Figure 1).

**Linear regression analysis**

The scattergrams of the monthly-average 25OHD and the difference in yearly-average 25OHD between 1993 and 2013 displayed an upward trend (Figure 2). The regression line for monthly-average 25OHD was as follows: 25OHD(nmol/L)=month*0.057 + 43(nmol/L), r=0.428, p<0.001. The increase in the average 25OHD level per 1 month was 0.057 nmol/L (0.023 ng/ml), implying an increase of 0.68 nmol/L (0.28 ng/ml) per year over the last 20 years. The regression line for the change in yearly-average 25OHD was as follows: Δ25OHD (nmol/L)=year*0.68(nmol/L), r=0.825, p<0.001 (Figure 2).

**Time series analysis**

Visual inspection of the sequence chart of the monthly average 25OHD time series demonstrated an upward trend, seasonality in the series, and a reduced variation in the data over time (Figure 3). The latter observation was consistent with an increase in sample size over time that narrows the confidence intervals. In order to visualize better the pattern of the data, the natural variation was

suppressed by smoothing the time series using a 4253H smoother; the seasonality of the data is more apparent as well as the upward trend (Figure 3). The ARIMA model was superimposed on the original data (Figure 4). The model does not attempt to fit the first 12 months of the data due to the fact that the model is seasonal and requires this initial information to begin the model. As can be seen in the sequence chart, it takes into account the seasonality of the data. It can be seen that the model fits the data. The residuals were plotted on a sequence chart (figure not shown). The mean value of the residuals was -0.39 (CI: -0.51 to 1.29) nmol/L but the distribution lacked normality (p=0.003). Following removal of the outliers, the mean value of the residuals was -0.03 (CI: -0.84 to 0.78) nmol/L and it passed the test of normality (p=0.200). The rebuilt model using data from 1993-2010 predicted accurately the monthly values through 2011, 2012 and 2013 (figure not shown).

In view of the above performance, the ARIMA model was used to forecast average monthly 25OHD through 2016 as shown in the sequence chart (Figure 4). As expected the forecasted values for 2014, 2015 and 2016 have the expected seasonality, trend and similar variance to the prior estimation period. The stationary R-squared was positive at 0.337 indicating that the forecast model was suitable. Table 1 contains the exact forecasts for 25OHD with upper and lower confidence limits for each month from 2014 to 2016.

## DISCUSSION

Vitamin D status has improved immensely in Ireland over the past 40 years following the advent of fortification of foodstuffs and the ready availability of low dose vitamin D supplements. Our earlier studies were conducted prior to the availability of fortified milk and vitamin D supplements. Milk fortification was initiated in the mid-1980s, and the range of vitamin D supplements have increased steadily starting in the early 1990s. In addition, inflated claims regarding the prevalence of vitamin D deficiency has led to undue public concern and has fuelled the practice of healthy individuals self-medicating with vitamin D supplements (3, 5). We have noticed immense improvement in vitamin D status over the past 40 years because our earlier studies showed that about 80% of infirm elderly had 25OHD levels below 30 nmol/L, but we still have a problem in 2013 with over 24% of the samples having 25OHD below 30 nmol/L (12 ng/ml). We now have a second concern

with 3.8% individuals having 25OHD >125 nmol/L (50 ng/ml). A recent population-based survey in Ireland from 2008, as compared with our laboratory-based survey, reported that 6.7% having 25OHD below 30 nmol/L (12 ng/ml) and 1.3% having 25OHD >125 nmol/L (50 ng/ml) (22).

The simplest approach to quantifying the magnitude of the increase in 25OHD is to compare yearly averages; over 20 years, the yearly average 25OHD level rose by 21.2 nmol/L (8.6 ng/ml) from 36.1 nmol/L to 57.3 nmol/L (14.4 ng/ml to 23.0 ng/ml). The next level of complexity is the linear regression model of yearly and monthly averages; this suggested an average yearly rise of about 0.7 nmol/L (0.28 ng/ml). The regression model is a poor fitting model for the historic data with respect to making specific monthly predictions; at best it represents the moving average values over time. Extrapolation of these regression results into the future could lead to non-sensible results when extending far beyond the range of the data. By comparison, the time series analysis is best for forecasting the upward trend in vitamin D status because it takes account of seasonality. Although seasonal variation of 25OHD is very well documented and easily explained as a consequence of seasonal terrestrial ultraviolet radiation, seasonality in time series analysis is a generic term for describing change over any recurring time period such as a day, a week, a month, a quarter of a year, or any other longer interval. Our ARIMA model performed well on the 1993-2013 dataset, indicating that the model should be accurate when forecasting future values. The model was extended to the end of 2016. There is no reason to believe that this trend will not be valid over the next three years.

Just as we previously highlighted two decades ago about the primacy of oral intake over sunlight exposure in the correction and prevention of hypovitaminosis D (2), the only explanation for the inexorable rise in 25OHD levels is the increase in oral intake. Increased travel to regions at lower latitudes, though not recorded, would have been a minor contributory factor. This increase must be consequent upon both fortification and supplementation. Fortification is a means to ensure that the vitamin D status of the population shifts upwards. Fortification is advantageous for all populations given the widespread concerns about hypovitaminosis D, regardless of latitude (23).

There has been much debate on vitamin D requirements in health and disease, especially following the publication of the IOM report. The Clinical Practice Guidelines (CPG) of the Endocrine Society advocated higher vitamin D intakes (8). The IOM Committee countered with a critique of the CPG and disagreed on three principal points: (1) that 25OHD levels of 75 nmol/L (30 ng/ml) or higher compared with 50 nmol/L (20 ng/ml) provided increased health benefits; (2) that all persons are deficient if serum 25OHD levels are below 50 nmol/L (20 ng/ml); and (3) that the CPG incorrectly characterized several large at-risk subgroups, who are covered by the IOM specifications(24). A further weakness of the CPG is the method by which the vitamin D dose response was calculated that results in a two-fold or higher underestimate of the dose response and thereby an overestimate of intake requirements (25, 26). According to CPG, the vitamin D dose response is linear and is defined heuristically: 25OHD is expected to increase by 2.5 nmol/l (1 ng/ml) for each 100 IU/day of vitamin D ingested. IOM noted a curvilinear response between vitamin D intake and 25OHD as follows: 25OHD nmol/L=9.9*ln (total vitamin D intake (IU/day)). The curvilinear response has been confirmed by the Vitamin D Supplementation in Older Subjects study (ViDOS) (27, 28). By adhering to IOM advice on interpretation of 25OHD and on specification about intake requirements, it is possible to avoid the trend towards overreplacement. Infants and children seem to be the group at most risk of hypercalcemia due to overreplacement (29, 30). Whereas for the elderly, a prudent approach to vitamin D supplementation is likely to yield benefits for bone health (9, 31).

Three other studies have examined trends in 25OHD over time. In the Tromsø Study in the northern part of Norway, 2,668 subjects were studied in 1994 and again in 2008: 25OHD increased by a small but significant degree from 53.7±16.3 to 55.3±18.2 nmol/L (21.5±6.5 ng/ml to 22.1±7.3 ng/ml) ($p<0.01$) (32). Scandinavian countries in early studies had better baseline vitamin D status compared to countries at lower latitudes as a consequence of higher oral intake of vitamin D (2). The Tromsø Study again noted the importance of supplemental intake on vitamin D status. The yearly average 25OHD in our study was much lower in 1993 compared to Tromsø in 1994, but is slightly higher in 2013 than in Tromsø in 2008. The Third National Health and Nutrition Examination Survey (NHANES III) with data collected from 1988 through 1994 (n=18,883) was

compared with NHANES 2001-2004 (n=13,369). An initial study reported considerable decline in average 25OHD from 75 nmol/ L (28.0 ng/ml) during NHANESIII to 60 nmol/L (24.0 ng/ml) during NHANES 2001-2004 (33). This apparent decline was explained subsequently by assay drift; it was estimated that there was only a small decline of 1.0-1.6 nmol/L (0.4-0.64 ng/ml) between the two surveys (34, 35). Historically, vitamin D status has been better in the US than Ireland given the longstanding practice of milk fortification with vitamin D and being located at lower latitude(2). The Canadian Multicentre Osteoporosis Study (CaMOS), which is an ongoing prospective cohort study of 9,423 community-dwelling subjects, measured 25OHD in varying numbers of women and men at three time points: 1995-1997; 2000-2002; and 2005-2007. Over the three surveys, they noted an increase in both women from 59.5±20.7 nmol/L (23.8±8.3 ng/ml) to 64.4±23.2 nmol/L (25.8±9.4 ng/ml) to 70.7±24.7 nmol/L (28.3±9.9 ng/ml) and in men from 64.7±23.2 nmol/L (25.8±9.3 ng/ml) to 67.0± 23.7 nmol/L (26.8±9.5 ng/ml) to 69.9±25.0 nmol/L (28.0±10.0 ng/ml) (36). Vitamin D supplemental intake increased by a greater amount in women than in men, again demonstrating the relative importance of oral vitamin D intake over sunlight exposure on vitamin D status (36).

Our study has a number of limitations: there was no information about reason for test request, health status, about reason for sampling, about sunshine exposure, about ethnicity, about dietary intake of vitamin D, and about vitamin D supplementation. This was not a population-based sample. It is possible that the reason for testing changed over time: in the early years testing may have been requested in view of the concerns about vitamin D deficiency, and in later years testing may have been requested for casual reasons. This could have contributed to the 25OH trend. The strength of the study lies in the long duration of the study and the high standard of measurement, especially when initial assays were technically difficult to perform and highly variable (14). Over the past four decades, measuring 25OHD has become less arduous because of the availability of several commercial 25OHD assay manufacturers. This has led to several challenges including technical competency of laboratorians and assay performance. Assay performance parameters are maximised by participation in an accuracy-based and commutable proficiency scheme such as the vitamin D external quality assessment scheme (DEQAS), which uses the recently available Standard

Reference Materials by the American National Institute of Standards and Technology in order to objectively assess assay performance against assigned target values (21, 37, 38). The Vitamin D Standardization Program is advocating performance limits for both reference and routine laboratories (39).

Even though we were one of the early participants in DEQAS prior May 1993, the time span of this study used four assays with differences as outlined in the supplementary material. Using different assays, Barake et al have reported that change in 25OHD assays can lead to differences in interpretation of vitamin D status (40). Prior to conducting the trend analysis, we considered the need to quantify the variability between the different methodologies in order to vindicate the increase in 25OHD observed. Positive and negative biases existed between methods as outlined in the comparative data in the supplementary material. Taking into account these biases, the Haddad method, which was used for the year one baseline data point, should correlate well with IDS and Roche, which were used in the latter years of the study. We therefore deduce that the increase in 25OHD over the 20 years is accurate. Any attempt at standardising results between assays of the 20 years would likely have introduced further error.

Our clinical interpretation of vitamin D status, as judged by measurement of 25OHD, from the outset has been a probabilistic one (15, 41-43). The concept of 25OHD as a biomarker of nutrient supply and not as an outcome, which was the basis for the IOM report, is fundamental to the definition of inadequacy using a probabilistic method (44). This approach of describing the distribution of 25OHD and its subcomponents is being adopted for the comparison of diverse populations (45). The IOM report did not pursue an implementation strategy, but many experts have supported their position and societies such as the National Osteoporosis Society have drafted guidelines that incorporate the IOM positions (46).

In conclusion, we have demonstrated a steady rise in vitamin D status since 1993 in Ireland, having already noted a substantive improvement from the early days of measuring 25OHD in the 1970s-1980s. This increasing trend has the potential to keep rising and to cause more harm than

benefit. Individuals, who are at risk of vitamin D deficiency, need public health strategies of fortification and supplementation with vitamin D in order to achieve IOM specified intakes. Whereas, the remainder of the population, who already had adequate vitamin D status, need to be cautioned against having intakes in excess of those advocated by IOM.

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## Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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## Legends to Figures

Figure 1

Histogram showing significant change in prevalence of 25OHD categories according to IOM specifications between the first year (May 1993 to April 1994) and the final year (2013) ($\chi^2$ =414, p<0.001).

Figure 2

Scattergram of monthly average 25OHD since 1993 (n=248) (upper panel) and scattergram of change in yearly average 25OHD compared to baseline of 1993 (n=21) (lower panel).

Figure 3

Sequence chart of monthly average 25OHD (upper panel) and sequence of same data after smoothing with 4253H smoothing function (lower panel).

Figure 4

Forecast of average monthly 25OHD levels for 2014-2016 based on the ARIMA model. Predicted 25OHD is depicted in red that is based on ARIMA modelling of monthly average 25OHD as depicted in light green.

Table 1

Predicted monthly average 25OHD with confidence limits over 3 years from 2014 to 2106

| Month | 25OHD nmol/L | | |
|---|---|---|---|
| | Average | LCL | UCL |
| Jan-14 | 50.7 | 38.0 | 63.5 |
| Feb-14 | 50.8 | 37.8 | 63.9 |
| Mar-14 | 50.1 | 37.0 | 63.1 |
| Apr-14 | 52.1 | 39.0 | 65.2 |
| May-14 | 55.2 | 42.2 | 68.3 |
| Jun-14 | 59.8 | 46.8 | 72.9 |
| Jul-14 | 63.9 | 50.9 | 77.0 |
| Aug-14 | 65.0 | 51.9 | 78.0 |
| Sep-14 | 66.4 | 53.3 | 79.4 |
| Oct-14 | 63.6 | 50.5 | 76.6 |
| Nov-14 | 56.4 | 43.4 | 69.5 |
| Dec-14 | 54.5 | 41.4 | 67.5 |
| Jan-15 | 51.1 | 38.0 | 64.1 |
| Feb-15 | 51.5 | 38.4 | 64.6 |
| Mar-15 | 50.8 | 37.7 | 63.8 |
| Apr-15 | 52.8 | 39.7 | 65.9 |
| May-15 | 55.9 | 42.9 | 69.0 |
| Jun-15 | 60.5 | 47.4 | 73.6 |
| Jul-15 | 64.6 | 51.5 | 77.7 |
| Aug-15 | 65.7 | 52.6 | 78.7 |
| Sep-15 | 67.0 | 54.0 | 80.1 |

| | | | |
|---|---|---|---|
| Oct-15 | 64.3 | 51.2 | 77.3 |
| Nov-15 | 57.1 | 44.1 | 70.2 |
| Dec-15 | 55.1 | 42.1 | 68.2 |
| Jan-16 | 51.8 | 38.7 | 64.8 |
| Feb-16 | 52.2 | 39.1 | 65.3 |
| Mar-16 | 51.5 | 38.4 | 64.5 |
| Apr-16 | 53.5 | 40.4 | 66.6 |
| May-16 | 56.6 | 43.5 | 69.7 |
| Jun-16 | 61.2 | 48.1 | 74.3 |
| Jul-16 | 65.3 | 52.2 | 78.4 |
| Aug-16 | 66.3 | 53.3 | 79.4 |
| Sep-16 | 67.7 | 54.7 | 80.8 |
| Oct-16 | 65.0 | 51.9 | 78.0 |
| Nov-16 | 57.8 | 44.7 | 70.9 |
| Dec-16 | 55.8 | 42.7 | 68.9 |

LCL = lower confidence limit; UCL = upper confidence limit

Figure 1

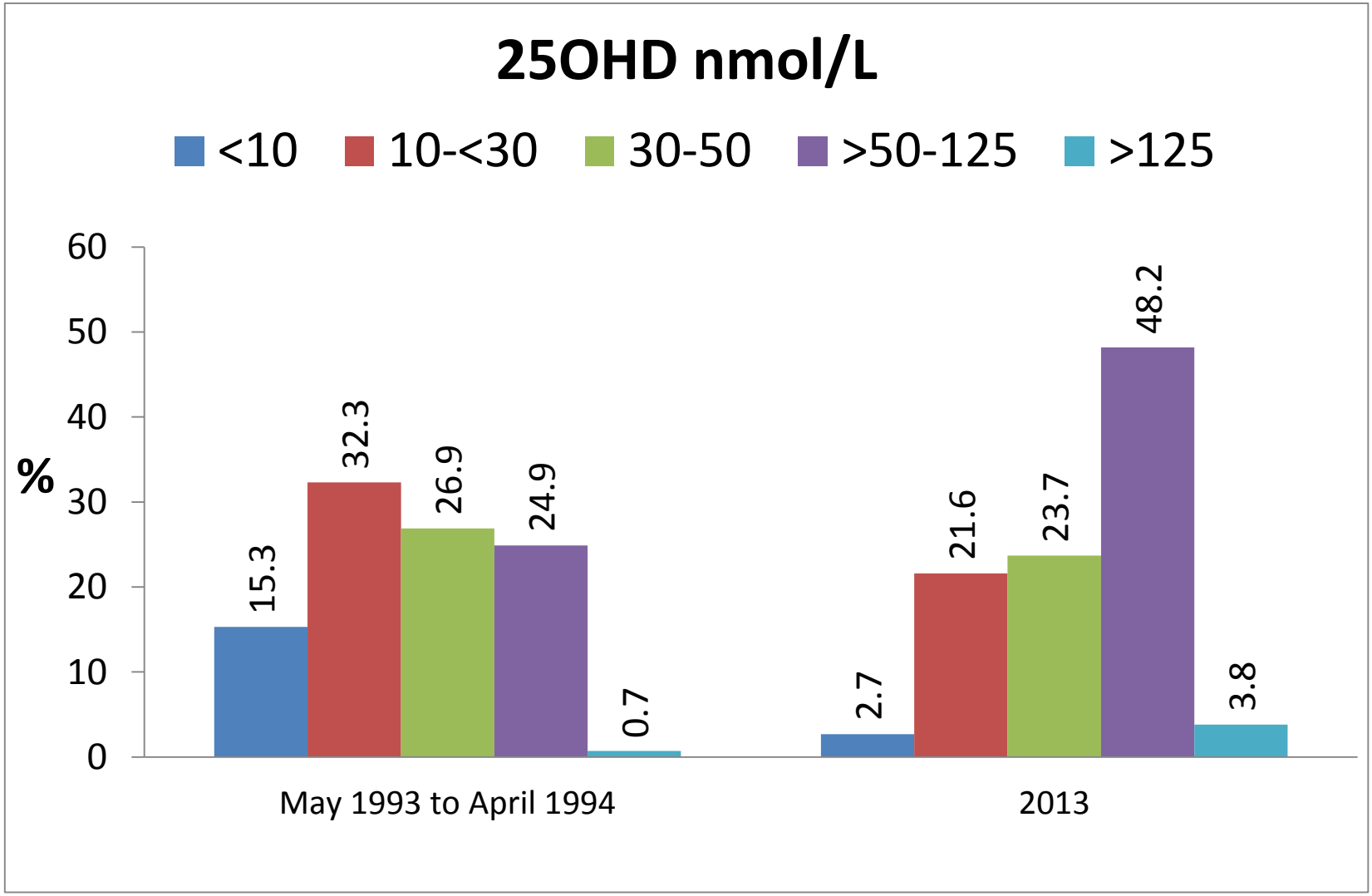

25OHD nmol/L
<10
10-<30
30-50
>50-125
>125
%
60
50
40
30
20
10
0
15.3
32.3
26.9
24.9
0.7
2.7
21.6
23.7
48.2
3.8
May 1993 to April 1994
2013

Figure 2

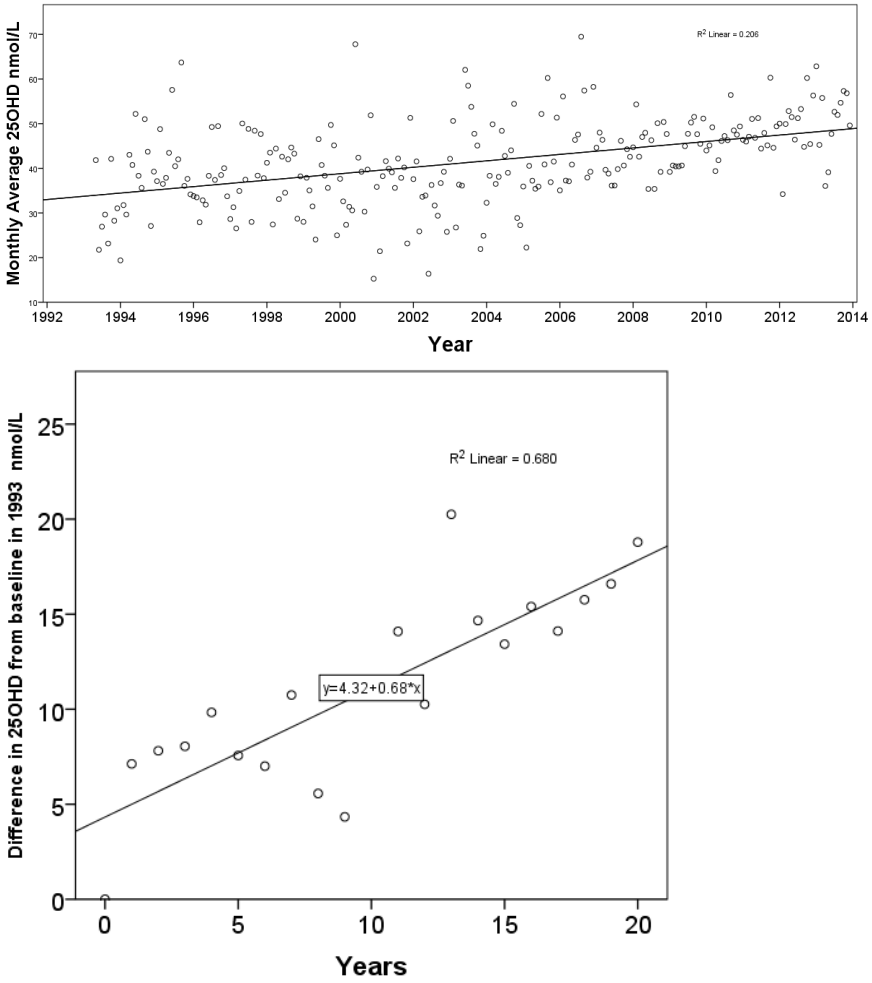
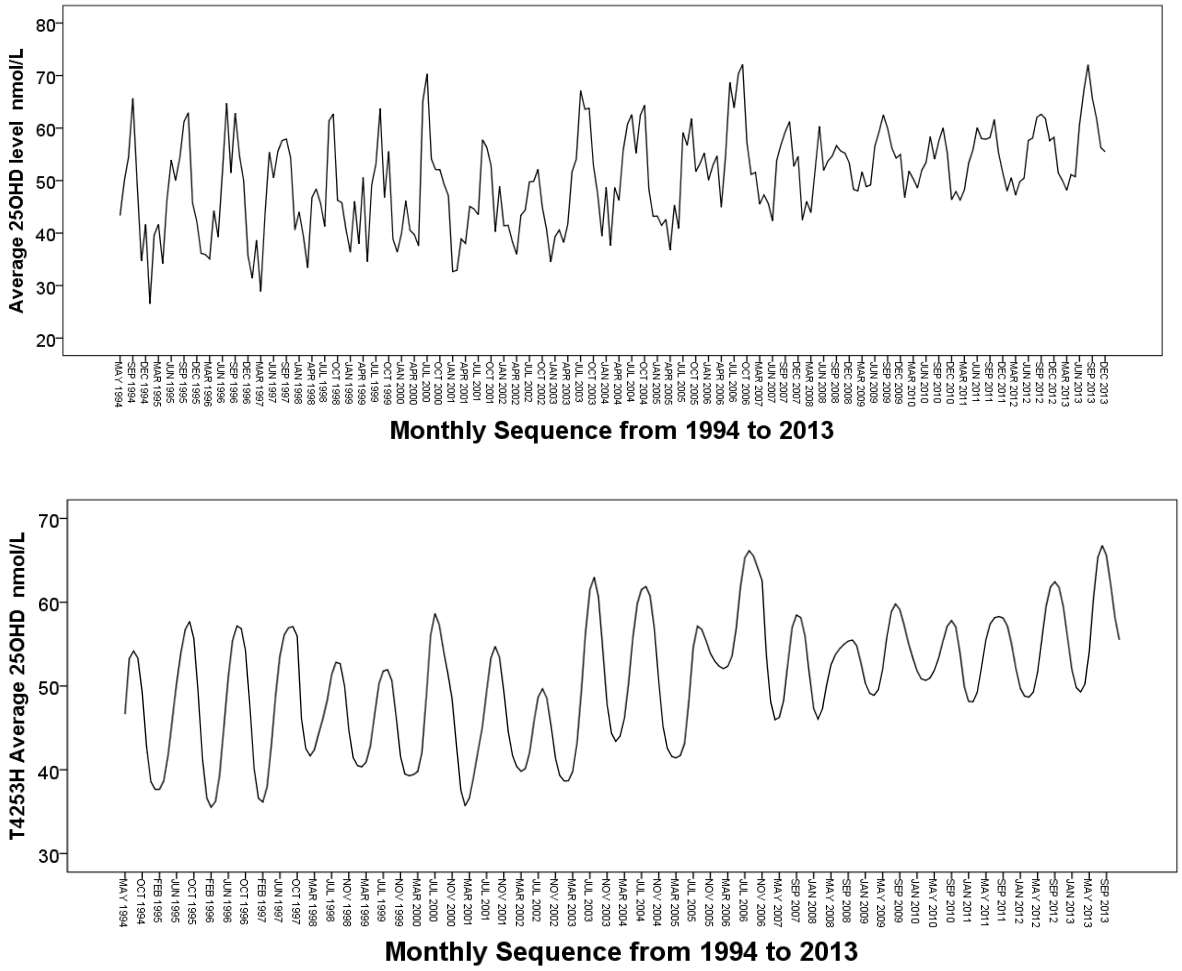
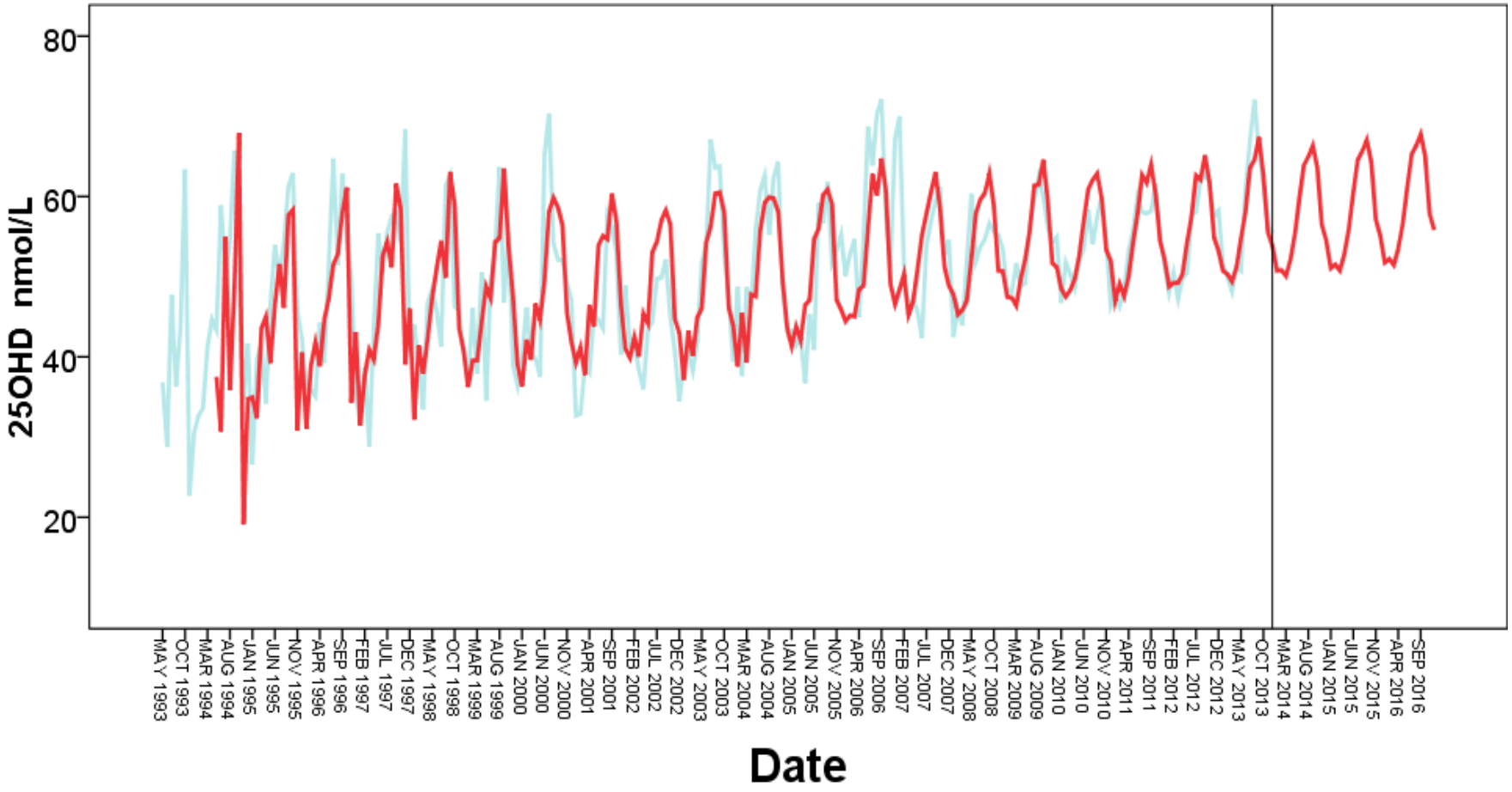

Monthly Average 25OHD nmol/L
Year
R² Linear = 0.206
Difference in 25OHD from baseline in 1993 nmol/L
Years
R² Linear = 0.680
y=4.32+0.68*x

Figure 3


Average 25OHD level nmol/L
80
70
60
50
40
30
20
MAY 1994
SEP 1994
DEC 1994
MAR 1995
JUN 1995
SEP 1995
DEC 1995
MAR 1996
JUN 1996
SEP 1996
DEC 1996
MAR 1997
JUN 1997
SEP 1997
DEC 1997
MAR 1998
JUL 1998
OCT 1998
JAN 1999
APR 1999
JUL 1999
OCT 1999
JAN 2000
APR 2000
JUL 2000
OCT 2000
JAN 2001
APR 2001
JUL 2001
OCT 2001
JAN 2002
APR 2002
JUL 2002
OCT 2002
JAN 2003
APR 2003
JUL 2003
OCT 2003
JAN 2004
APR 2004
JUL 2004
OCT 2004
JAN 2005
APR 2005
JUL 2005
OCT 2005
JAN 2006
APR 2006
JUL 2006
OCT 2006
MAR 2007
JUN 2007
SEP 2007
DEC 2007
MAR 2008
JUN 2008
SEP 2008
DEC 2008
MAR 2009
JUN 2009
SEP 2009
DEC 2009
MAR 2010
JUN 2010
SEP 2010
DEC 2010
MAR 2011
JUN 2011
SEP 2011
DEC 2011
MAR 2012
JUN 2012
SEP 2012
DEC 2012
MAR 2013
JUN 2013
SEP 2013
DEC 2013
Monthly Sequence from 1994 to 2013


T4253H Average 25OHD nmol/L
70
60
50
40
30
MAY 1994
OCT 1994
FEB 1995
JUN 1995
OCT 1995
FEB 1996
JUN 1996
OCT 1996
FEB 1997
JUN 1997
OCT 1997
MAR 1998
JUL 1998
NOV 1998
MAR 1999
JUL 1999
NOV 1999
MAR 2000
JUL 2000
NOV 2000
MAR 2001
JUL 2001
NOV 2001
MAR 2002
JUL 2002
NOV 2002
MAR 2003
JUL 2003
NOV 2003
MAR 2004
JUL 2004
NOV 2004
MAR 2005
JUL 2005
NOV 2005
MAR 2006
JUL 2006
NOV 2006
MAY 2007
SEP 2007
JAN 2008
MAY 2008
SEP 2008
JAN 2009
MAY 2009
SEP 2009
JAN 2010
MAY 2010
SEP 2010
JAN 2011
MAY 2011
SEP 2011
JAN 2012
MAY 2012
SEP 2012
JAN 2013
MAY 2013
SEP 2013
Monthly Sequence from 1994 to 2013

Figure 4


25OHD nmol/L
80
60
40
20
MAY 1993
OCT 1993
MAR 1994
AUG 1994
JAN 1995
JUN 1995
NOV 1995
APR 1996
SEP 1996
FEB 1997
JUL 1997
DEC 1997
MAY 1998
OCT 1998
MAR 1999
AUG 1999
JAN 2000
JUN 2000
NOV 2000
APR 2001
SEP 2001
FEB 2002
JUL 2002
DEC 2002
MAY 2003
OCT 2003
MAR 2004
AUG 2004
JAN 2005
JUN 2005
NOV 2005
APR 2006
SEP 2006
FEB 2007
JUL 2007
DEC 2007
MAY 2008
OCT 2008
MAR 2009
AUG 2009
JAN 2010
JUN 2010
NOV 2010
APR 2011
SEP 2011
FEB 2012
JUL 2012
DEC 2012
MAY 2013
OCT 2013
MAR 2014
AUG 2014
JAN 2015
JUN 2015
NOV 2015
APR 2016
SEP 2016
Date