A Four-Year Trend in Serum 25-Hydroxyvitamin D Levels in Western Connecticut

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ABSTRACT – Objective: This study describes the trends in blood vitamin D levels in a regional population from 2009–2012 through a cross-sectional study design.

Methods: Over a four-year period (2009–2012), serum levels of 25-hydroxyvitamin D [25(OH)D] have been measured using an automated enzyme immunoassay with a steadily increasing number of tests performed each year. A total of 54 700 tests were performed during this period, with a 90% increase in annual tests ordered.

Results: Mean and median serum levels of 25(OH) D showed statistically significant increases during this period. Those with 25(OH)D levels below 10 ng/mL represented 1.45% of the subjects in 2009 and 0.3% in 2012. The decrease in the proportion of subjects with 25(OH)D levels below 20 ng/mL and below 30 ng/mL was greatest out of all the proportioned subjects. Mean and median 25(OH)D levels increased with age in males and females.

Conclusion: These results likely reflect increased health awareness in Western Connecticut compared with national surveys showing a temporal decrease in 25(OH)D levels.

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Introduction

Rickets was described in the 17th century, but identification of vitamin D as a cure for rickets did not occur until the 1920s, and the elucidation of vitamin D's molecular structure and metabolism took decades longer.[1] Scientific publications about vitamin D have since increased, with a total of 17 340 from 2010 to 2014, compared with 9573 publications from 2005 to 2009.[2] Vitamin D sales have increased from $40 million in 2001, to $425 million in 2009, and the *Nutrition Business Journal* has labeled vitamin D the tenth most popular supplement sold from 1997 to 2011.[3] Coinciding with these changes, laboratory testing for serum levels of the vitamin D metabolite 25-hydroxyvitamin D [25(OH)D], the body's "storage" form of vitamin D, has also burgeoned in recent years,[4–6] and at considerable cost.[6–8] Generally healthy people are increasingly consuming inexpensive over-the-counter vitamin D supplements for presumed benefits to general health. This may be the result of reports in the scientific literature heavily emphasized in lay publications, which show disease associations with low serum levels of 25(OH)D.[9,10]

The lack of randomized, controlled trials prompted cautionary statements about vitamin D supplementation from the Institute of Medicine (IOM).[11] The Endocrine Society published a practice guideline for prevention, evaluation, and treatment of vitamin D deficiency in 2011.[12] While there have been different definitions of vitamin D insufficiency and deficiency, the Endocrine Society defines insufficient as a serum 25(OH)D level < 30 ng/mL and deficient as < 20 ng/mL. Still, the actual degree of insufficiency or deficiency relative to bone health and other issues continues to be a moving target.[12] The IOM committee suggests that the guideline may only apply to at-risk populations; thus disagreeing that serum 25(OH)D levels below 20 ng/mL are defined as deficiency.[13] The IOM report reveals that approximately 50% of the general population is assured adequate bone health with serum 25(OH)D concentrations of 16 ng/mL. Although the Endocrine Society defines deficient at < 20 ng/mL, the IOM claims that less than 3% of the general population is anticipated to need serum 25(OH)D levels above 20 ng/mL.[13] The cut-point value representing serum vitamin D deficiency continues to remain controversial. Evidence from future research focusing on the relationship between vitamin D intake, bone health, and dose-response across all age groups may strengthen the recommendations for the Dietary Reference Intake (DRI) intake values, as well as identify patients truly at risk for deficiency.[11]

We report our analysis of serum levels of 25(OH) D obtained from a population in the greater Danbury, Connecticut area over a four-year period (2009 to 2012). The major portion of the primary service area of our Western Connecticut Health Network region is located in northern Fairfield, Connecticut and Westchester, New York counties with a population of 275 000 and a secondary catchment population of 165 000 in more outlying counties of Connecticut and New York State (Figure 1).

Methods

All serum 25(OH)D measurements during the study period were performed using an enzyme immunoassay method (Immunodiagnostic Systems, Inc., Fountain Hills, AZ). This assay was automated on the Bio-Rad PhD microtiter plate processing system (Bio-Rad Laboratories, Hercules, CA). The test was performed per the assay manufacturer's instructions using internal quality control and external quality assessment (proficiency testing) practices that followed the commonly accepted laboratory standards of the College of American Pathologists (CAP).

For statistical analysis, categorical variables were compared using the chi-square test and continuous variables using the student's t test, where a P value $< .05$ was defined as statistically significant. All analyses were conducted using JMP software, version 9.0 (SAS Institute, Cary, NC).

Figure 1. Western Connecticut Health Network Service Region

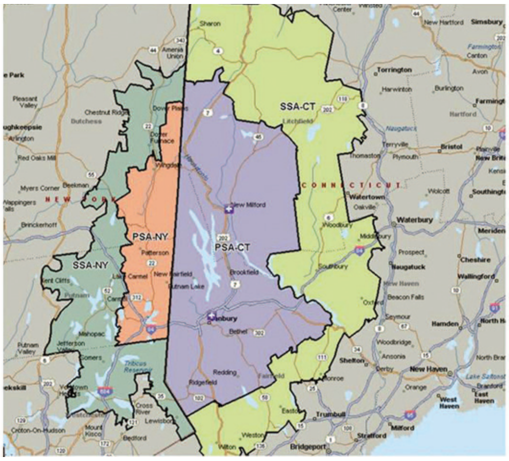


Table 1. 25-OH Vitamin D Test Volume, Mean and Median Levels, 2009–2012

Year	*No. Tests*	*Increase vs Previous Yr.*	*Median 25(OH)D (ng/mL)*	*Mean 25(OH)D (ng/mL)*	*SD (ng/mL)*	*25th Percentile 25(OH)D (ng/mL)*	*75th Percentile 25(OH)D (ng/mL)*
2009	9226	—	29.4	31.2	13.5	22.5	37.6
2010	13 322	44%	30.3	32.0	13.8	23.6	38.2
2011	14 655	10%	30.9	32.6	12.8	24.2	38.8
2012	17 497	19%	33.0*	35.0*	16.2	25.9*	41.4*
Total	54 700						

Abbreviation: 25(OH)D, 25-hydroxyvitamin D
*Statistically significant change from 2009 to 2012 ($P < .05$)

Table 2. Mean and Median 25-OH Vitamin D Levels by Gender

Gender	*No. Tests*	*% of Total*	*Mean 25(OH)D (ng/mL)*	*Median 25(OH)D (ng/mL)*	*SD*
Female	38 093	69.6%	33.5	31.7	14.84
Male	16 607	30.4%	31.8	30.2	13.19

Table 3. Distribution of 25-OH Vitamin D Levels, 2009–2012

Year	*# < 10 ng/mL (%)*	*# < 20 ng/mL (%)*	*# < 30 ng/mL (%)*	*# > 100 ng/mL (%)*
2009	134 (1.45)	1623 (17.6)	4767 (51.7)	13 (.14)
2010	90 (.68)	1878 (14.1)	6475 (48.6)	32 (.24)
2011	64 (.44)	1895 (12.9)	6773 (46.2)	14 (.10)
2012	52 (.30)	1728 (9.9)	6816 (39.0)	33 (.19)

Table 4. 25-OH Vitamin D Levels by Age

Age (yrs)	*No. Tests*	*% of Total*	*Mean 25(OH)D (ng/mL)*	*Median 25(OH)D (ng/mL)*	*SD*
1 – 9	1435	2.6%	33.0	29.4	20.77
10 – 19	2894	5.3%	28.0	26.2	10.44
20 – 49	14110	25.8%	29.9	28.4	11.69
50 – 69	23759	43.4%	34.1	32.3	14.81
≥ 70	12501	22.9%	35.5	34.0	15.29
Total	54699	100.0%			

Results

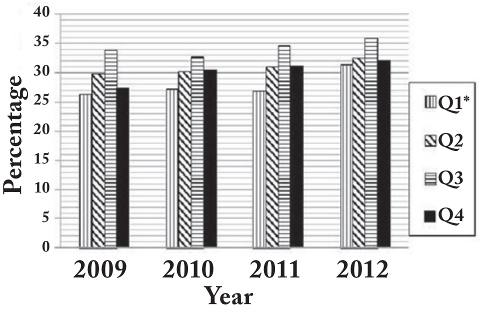
The annual number of 25(OH)D tests performed in our laboratory increased by 90% from 9226 tests in 2009 to 17497 tests in 2012 (Table 1). Mean and median serum levels of 25(OH)D in our regional population increased significantly during this period [median, 29.4 to 33.0 ng/mL; mean, 31.2 to 35.0 ng/mL; ($P < .0001$)]. 25(OH)D levels were tested more frequently in women than in men over the four years (69.6% vs 30.4%, respectively). 25(OH)D levels < 20 ng/mL were more common in men and levels < 10 ng/mL were more frequent in women (Table 2). The percentage of subjects with levels < 10 ng/mL, represented 1.45% of those tested in 2009, and declined significantly to 0.3% in 2012 ($P < .0001$) (Table 3). The proportion of subjects with 25(OH)D levels < 20 ng/mL, declined from 17.6% to 9.9% ($P < .001$). Subjects with 25(OH)D < 30 ng/mL levels declined from 51.7% to 39% over the four year period ($P < .0001$). The number of tests that showed levels > 100 ng/mL was small and there was no significant change during the four-year period. Both mean and median 25(OH)D levels increased with age (Table 4).

Analysis of the data by time of year showed a seasonal variation with the lowest 25(OH)D levels in the winter months. Over the period studied, the median 25(OH)D levels during the January–March and October–December quarters increased remarkably for these periods. The rise above 30 ng/mL during 2012 compared with 25 ng/mL in 2009 (Figure 2) was statistically significant for the January–March period, unlike data published in existing literature,[14,15] which frequently reports winter 25(OH)D levels < 25 ng/mL. All other seasons also showed increased vitamin D levels over the four years in our subjects, but less dramatically.

Discussion

Our analysis of 25(OH)D levels obtained from 54700 laboratory tests performed during 2009 through 2012 shows a significant increase in the median and mean serum 25(OH)D levels. The dramatic increase in vitamin D testing may be due to heightened health literacy and awareness among the public, increased consumer demand, and the substantial media representations of vitamin D supplementation.[16] The greatest improvement occurred among the subjects initially identified with the lowest 25(OH)D levels (< 10 ng/mL). A decrease in the proportion of subjects with levels < 30 ng/mL was seen in each of the four years ($P < .05$). The proportion of individuals with levels > 100 ng/mL was well below one percent each year of the study and will be the subject of ongoing analysis.

Figure 2. Seasonal Variations in Median 25-OH Vitamin D Levels.



Q1 = Jan-Mar Q2 = Apr-Jun Q3 = Jul-Sep Q4 = Oct-Dec

* Statistically significant change in 2012

Reasons for a two-fold greater number of 25(OH)D tests performed in women than in with men may be greater awareness of health issues and/or more health provider visits by women. A higher incidence of female primary care office visits and a higher morbidity burden were identified by Carretero, et al when compared with men through a retrospective study including 79 809 patients.[17] The results of seasonal variation show the uncommon phenomenon, in our location, of vitamin D levels > 30ng/mL in the fourth study year. Temporal changes in 25(OH)D levels in the US population were reported by Looker et al in 2008,[18] and further analyzed by Ginde et al[19] using data from the National Health and Nutrition Examinations Surveys (NHANES). The first national survey covered 1988–1994 (18 158 participants), and the follow-up survey was conducted from 2000–2004 (20 289 participants). The results revealed significantly lower mean 25(OH)D concentrations in 2000–2004 than in 1988–1994. The prevalence of 25(OH)D levels < 10 ng/mL increased significantly in all age, sex, and race/ethnicity groups. They reported a matching decline in levels < 30 ng/mL in all demographic characteristics. The general decline in 25(OH)D levels was attributed, in part, to higher BMI, more sun protection, and decreased milk consumption, especially in non-Hispanic whites. These same factors are also considered present in our subjects. These surveys may not be comparable to ours, which sampled a region with a more affluent population and with educational achievement which may be associated with greater health literacy. Vitamin D levels and intake have been related to several risk factors including low income,[20,21] which may be a difference from our study population. An accompanying editorial by Norman[22] acknowledges the importance of the detailed surveys while raising concerns about methodology of collection in different latitudes during different seasons and use of different analytic methods for measuring 25(OH)D in each survey. Nevertheless, the NHANES data provides an important source of vitamin D observations in their online supplementary data.

Berger et al reported a 10-year observation among Canadians that related an increase in vitamin D intake (317 IU/day in women and 193 IU/day in men) to temporal changes in blood levels of 25(OH)D and parathyroid hormone (PTH).[5] The percentage of the 3896 participants with 25(OH)D levels < 50 nmol/L (< 20 ng/mL) fell from a mean of 29.7% to 19.8% at year 10; PTH levels also decreased, with a greater decrease seen amongst women. While area physicians and other providers usually recommend supplemental vitamin D, the public self-selects their nonprescription health supplements. Based on the Canadian study,[5] we estimate the median daily vitamin D intake in our subjects is approximately 1000 IU.

A trend of decreasing vitamin D intake from food sources was reported by Harnack et al for the Minnesota Heart Survey of risk factors for cardiovascular disease.[23] They conducted 24-hour recalls during six two-year study periods over 20 years and report significant decreases ($P < .001$) in daily intake of vitamin D. Our observational experience agrees that milk consumption is limited beginning in early teens; however the striking increases in serum 25(OH)D levels in our subjects necessitates increased Vitamin D intake.

Our study was not empowered to conduct interviews and does not have the detail and national overview of the NHANES, but there are important differences in our finding of overall increase in vitamin D concentrations across all age and sex groups within a smaller, more defined area in the Northeast US at latitude 41°. Most illuminating is the significant winter month vitamin D increase into the level of > 30 ng/mL compared to the NHANES winter level of 24 ng/mL during the follow-up survey in a subgroup likely similar to our population. Scientific publications expanded upon by popular media may be more influential in populations with higher health literacy who keep abreast of health issues.

The public's zeal for vitamin D supplementation represents a groundswell social phenomenon in response to lay publications about investigative endeavor that has linked low levels of vitamin D with a large number of chronic illnesses. With professional acquiescence and the ordering of a tsunami of 25(OH)D blood tests, plus the ready availability of a presumed safe and inexpensive treatment, our regional population has significantly increased its blood level of vitamin D in only four years. Median 25(OH)D levels of > 30 ng/mL in winter months at the latitude of the Danbury, CT region is especially striking. Baseline winter levels well below 30 ng/mL are reported in 14 of 15 cohorts in an international genome-wide study; only the Framingham, MA cohort was "at goal" with a mean vitamin D level of 78 nmol/mL (31.2 ng/L).[14] Framingham, MA, is a middle class, suburban region which resembles the population of this report.

Our data analysis did not account for repeat/follow-up 25(OH)D tests in the same subjects. We estimate repeat tests represented only 1% to 2% of the total data. Most tests were performed on ambulatory outpatients and very few tests were performed on hospitalized subjects. We did not have sufficient information to describe specific economic, educational, or racial influences, nor could we be more specific regarding the daily doses of vitamin D or calcium. There has been a change (mostly increase) in vitamin D content of multivitamin and calcium products in recent years. Whatever the dosing habits, average 25(OH)D blood levels in our region are significantly higher. If the trend in vitamin D consumption continues, future epidemiologic studies may show more clearly whether raising average 25(OH)D levels in unselected populations will be reflected in changes in incidence for the many clinical conditions which have been related to deficient or insufficient blood levels. Our study also shows that smaller, regional trends may differ greatly from national surveys that include diverse subpopulations.

Conclusion

This study found that the annual number of 25(OH)D tests, as well as the mean and median levels of serum 25(OH)D, significantly increased from 2009 to 2012 in our regional population. In addition, our study showed that vitamin D was tested more frequently in women than men over the four-year time period. This study also revealed the expected seasonal variation in 25(OH)D levels. The winter months (January through March) showed the lowest levels across all four years; however, the median 25(OH)D levels during this quarter increased significantly over the four-year time period.

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REFERENCES

1. Rajakumar K, Greenspan SL, Thomas SB, et al. SOLAR ultraviolet radiation and vitamin D: a historical perspective. *Am J Public Health*. 2007;97(10):1746–54.
2. PubMed [Internet]. Bethesda (MD): National Library of Medicine (US). [Table], "Vitamin D" Results by year to 2015; [cited 2015 Nov 9]; 38b. Available from: http://www.ncbi.nlm.nih.gov/pubmed by typing "vitamin d" in the search box and downloading the results by year CSV.
3. *Nutrition Business Journal*. Top 100 selling US supplements sales and growth: 1999-2008 - Chart 14. Penton Media, Inc. http://nutritionbusinessjournal.com/supplements/data-charts/top_100_selling_us_supplements_sales_growth/. Accessed February 25, 2015.
4. Shahangian S, Alspach TD, Astles JR, et al. Trends in laboratory test volumes for Medicare Part B reimbursements, 2000-2010. *Arch Pathol Lab Med*. 2014;138(20):189–203.
5. Berger C, Greene-Finestone LS, Langsetmo L, et al. Temporal trends and determinants of longitudinal change in 24-hydroxyvitamin D and parathyroid hormone. *J Bone Miner Res*. 2012;27(6):1381–9.
6. Sattar N, Welsh P, Panarelli M, et al. Increasing requests for vitamin D measurement: costly, confusing, and without credibility. *Lancet*. 2012;379(9811):95–6.
7. Pilon A, Lim S-K, Guechot J. Changes in serum vitamin D assay usage and the need for evidence-based recommendations. *Ann Biol Clin (Paris)*. 2012;70(4):451–5.
8. Bilinski K, Boyages S. The rise and rise of vitamin D testing. *BMJ*. 2012;345:e4743.
9. Holick MF. Vitamin D deficiency. *N Engl J Med*. 2007;357(3):266–81.
10. Rosen CJ, Adams JS, Bikle DD, et al. The nonskeletal effects of vitamin D: and Endocrine Society scientific statement. *Endocr Rev*. 2012;33(3):456–92.
11. IOM (Institute of Medicine) 2011 Dietary reference intakes for calcium and vitamin D. Washington, DC: The National Academies Press;260-262.
12. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2011;96(7):1911–30.
13. Rosen CJ, Abrams SA, Aloia JF, et al. IOM committee members respond to Endocrine Society vitamin D guideline. *J Clin Endocrinol Metab*. 201;97(4):1146–52.
14. Wang TJ, Zhang F, Richards JB, et al. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. *Lancet*. 2010;376(9736):180–8.
15. Shoben AB, Kestenbaum B, Levin G, et al. Seasonal variation in 25-hydroxyvitamin D concentrations in the cardiovascular health study. *Am J Epidemiol*. 2011;174(12):1363–72.
16. Caulfield T, Clark MI, McCormack JP, et al. Representations of the health value of vitamin D supplementation in newspapers: media content analysis. *BMJ Open*. 2014;4(12):e006395.
17. Carretero MT, Calderón-Larrañaga A, Poblador-Plou B, et al. Primary health care use from the perspective of gender and morbidity burden. *BMC Womens Health*. 2014;14:145.
18. Looker AC, Pfeiffer CM, Lacher DA, et al. Serum 25-hydroxyvitamin D status of the US population: 1988–1994 compared with 2000–2004. *Am J Clin Nutr*. 2008;88(6):1519–27.
19. Ginde AA, Liu MC, Camargo CA Jr. Demographic differences and trends in vitamin D insufficiency in the US population, 1988–2004. *Arch Intern Med*. 2009;169(6):626–32.
20. Navarro MdelC, Saavedra P, Jódar E, et al. Osteoporosis and metabolic syndrome according to socio-economic status, contribution of PTH, vitamin D and body weight: the Canarian Osteoporosis Poverty Study (COPS). *Clinical Endocrinol (Oxf)*. 2013;78(5):681–6.
21. Tolppanen AM, Fraser A, Fraser WD, et al. Risk factors for variation in 25-hydroxyvitamin D3 and D2 concentrations and vitamin D deficiency in children. *J Clin Endocrinol Metab*. 2012;97:1202–10.
22. Norman AW. A vitamin D nutritional cornucopia: new insights concerning the serum 25-hydroxyvitamin D status of the US population (Editorial). *Am J Clin Nutr*. 2008;88:1455–56.
23. Harnack LJ, Steffen L, Zhou X, et al. Trends in vitamin D intake from food sources among adults in the Minneapolis-St Paul, MN, metropolitan area, 1980-1982 through 2007-2009. *J Am Diet Assoc*. 2011;111:1329–34.