Journal of Steroid Biochemistry & Molecular Biology 173 (2017) 117–121

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry & Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

Review

Vitamin D measurement standardization: The way out of the chaos

N. Binkley[a], B. Dawson-Hughes[b], R. Durazo-Arvizu[c], M. Thamm[d], L. Tian[e], J.M. Merkel[f], J.C. Jones[g], G.D. Carter[g], C.T. Sempos[f,h,*]

[a] Osteoporosis Clinical Research Program and Institute on Aging, University of Wisconsin-Madison, Madison, WI, USA
[b] Bone Metabolism Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MI, 02111, USA
[c] Department of Public Health Sciences, Loyola University Stritch School of Medicine, Maywood, IL, 60153, USA
[d] Robert Koch Institute, 12101 Berlin, Germany
[e] Department of Health Research and Policy, Stanford University, Palo Alto, CA, USA
[f] Office of Dietary Supplements, National Institutes of Health, Bethesda, MD 20892, USA
[g] Vitamin D External Quality Assurance Scheme (DEQAS) Coordination Centre, Imperial College, London, UK
[h] Department of Population Health Sciences, University of Wisconsin-Madison, Madison, WI, 53726, USA

ARTICLE INFO

Article history:
Received 13 August 2016
Accepted 11 December 2016
Available online 12 December 2016

Keywords:
25-Hydroxyvitamin D
CAP
DEQAS
NIST
PT/EQA
Vdsp
Vitamin D

ABSTRACT

Substantial variability is associated with laboratory measurement of serum total 25-hydroxyvitamin D [25(OH)D]. The resulting chaos impedes development of consensus 25(OH)D values to define stages of vitamin D status. As resolving this situation requires standardized measurement of 25(OH)D, the Vitamin D Standardization Program (VDSP) developed methodology to standardize 25(OH)D measurement to the gold standard reference measurement procedures of NIST, Ghent University and CDC. Importantly, VDSP developed protocols for standardizing 25(OH)D values from prior research based on availability of stored serum samples. The effect of such retrospective standardization on prevalence of "low" vitamin D status in national studies reported here for The Third National Health and Nutrition Examination Survey (NHANES III, 1988–1994) and the German Health Interview and Examination Survey for Children and Adolescents (KIGGS, 2003–2006) was such that in NHANES III 25(OH)D values were lower than original values while higher in KIGGS. In NHANES III the percentage with values below 30, 50 and 75 nmol/L increased from 4% to 6%, 22% to 31% and 55% to 71%, respectively. Whereas in KIGGS after standardization the percentage below 30, 50, and 70 nmol/L decreased from 28% to 13%, 64% to 47% and 87% to 85% respectively. Moreover, in a hypothetical example, depending on whether the 25(OH)D assay was positively or negatively biased by 12%, the 25(OH)D concentration which maximally suppressed PTH could vary from 20 to 35 ng/mL. These examples underscore the challenges (perhaps impossibility) of developing vitamin D guidelines using unstandardized 25(OH)D data. Retrospective 25(OH)D standardization can be applied to old studies where stored serum samples exist. As a way forward, we suggest an international effort to identify key prior studies with stored samples for re-analysis and standardization initially to define the 25(OH)D level associated with vitamin D deficiency (rickets/osteomalacia). Subsequent work could focus on defining inadequacy. Finally, examples reported here highlight the importance of suspending publication of meta-analyses based on unstandardized 25(OH)D results.

Published by Elsevier Ltd.

Contents

1. Introduction 118
2. Standardizing 25 (OH)D measurements from the past 118

Abbreviations: 25(OH)D, serum total 25-hydroxyvitamn D; CDC, Centers for Disease Control and Prevention; EIA, enzyme immunoassay; IOM, Institute of Medicine; KiGGS, German Health Interview and Examination Survey for Children and Adolescents; LC–MS/MS, liquid chromatography–tandem mass spectrometry; NANS, Irish National Health/Nutrition Surveys; NHANES, National Health and Nutrition Examination Survey; NIST, National Institute of Standards and Technology; RMP, reference measurement procedure; UCC, University College Cork, Cork, Ireland; VDSP, Vitamin D Standardization Program.

* Corresponding author: NIH Office of Dietary Supplements, 6100 Executive Blvd Rm. 3B01, Bethesda, MD 20892-7517, USA.
E-mail address: semposch@mail.nih.gov (C.T. Sempos).

http://dx.doi.org/10.1016/j.jsbmb.2016.12.002
0960-0760/Published by Elsevier Ltd.

3. Validity of VDSP algorithm . . . 119
4. Impact of assay standardization . . . 119
5. Moving forward: a proposal . . . 120
Disclaimer . . . 120
Conflict of interest . . . 120
References . . . 120

1. Introduction

The vitamin D field is in chaos. As an example, we have two very different sets of guidelines for defining clinically relevant states of vitamin D status – especially vitamin D deficiency and insufficiency; i.e. those of the Institute of Medicine and those released by the Endocrine Society (see Table 1) [1,2]. In both, serum total 25-hydroxyvitamin D [25(OH)D] concentration was used to interpret vitamin D status. As these guidelines are not concordant, a common question is: Which set of guidelines is correct? This is obviously a very important question for clinicians and patients, but it may not be the most important question to ask. A more fundamental question, given the multitude of clinical studies and meta-analyses that have been and continue to be published is: Why has the field not been able to reach consensus? In our opinion, the answer to this question is in large part due to the substantial variability both within and among the various laboratory assays for measuring 25(OH)D [3–5]. This lack of standardized 25(OH)D research data is a fundamental limitation in establishing consensus clinical and public health vitamin D guidelines [6].

To address this limitation and thus facilitate guideline development, the Vitamin D Standardization Program (VDSP) [7] since 2010 has coordinated an international effort to standardize the laboratory measurement of 25(OH)D in current and future measurement systems to the gold standard reference assays or reference measurement procedures (RMPs) developed by the National Institute for Standards and Technology (NIST), Ghent University and the Centers for Disease Control and Prevention (CDC) [8–11]. Thus, an approach is in place to produce standardized 25(OH)D results in current and future studies. However, given that some 60,000 papers on vitamin D have been published since 25(OH)D was first isolated and chemically identified in 1968 it is essential, where possible, to retrospectively standardize 25(OH)D measurements from key studies conducted in the past [12,13]. To meet that need the VDSP has developed a robust statistical sampling procedure for selecting a relatively small sample of properly stored serum samples ($n \approx 150$) from completed studies for re-analysis and use in calibrating all of the 25(OH)D values to gold standard reference measurement procedure values [7,14]. In this paper we demonstrate the potential impact of assay standardization on the distribution of 25(OH)D in national health/nutrition surveys, and in clinical and epidemiological studies.

2. Standardizing 25 (OH)D measurements from the past

Can old measurements of 25(OH)D from national health/nutrition surveys and clinical and epidemiological studies be calibrated (standardized) to the gold standard reference measurement procedures? Yes – if properly stored serum samples are available – the VDSP has developed and expanded two different protocols for calibrating those old values [14,15]. In general, the VDSP protocols consist of five basic steps: (1) Estimate the number of serum samples to be re-measured ($n \approx 150$ serum samples in most cases); (2) Select the specific stored samples to be re-measured from the sorted original 25(OH)D values of the complete data set using the VDSP's uniform sampling approach [14]; (3) Re-measure the 25(OH)D concentration in these samples using an assay that is certified to be traceable to the gold standard RMPs; (4) Develop a mathematical model to calibrate (standardize) the "Old"

Table 1
A Comparison of the Institute of Medicine (IOM) and Endocrine Society Guidelines [1,2[]] for interpreting the concentration of serum total 25-hydroxyvitamin D. Which is Correct?.

Vitamin D Status Interpretation	Institute of Medicine [1]	Endocrine Society [2]
	Serum Total 25-hydroxyvitamin D (ng/mL)[a]	
Deficient	<12 ng/mL	<20 ng/mL
Insufficient	12–20 ng/mL	20–29 ng/mL
Sufficient	20–30 ng/mL	30–100 ng/mL
No Added Benefit	30–50 g/mL	
Possible Harm	>50 ng/mL	>100 ng/mL

[a] Note: To convert ng/mL to nmol/L multiple by 2.5.

Table 2
Percent of Irish National Health/Nutrition Survey (NANS) with Serum 25-hydroxyvitamin D Concentrations Below Cut-points: A Comparison of Original Results (n = 1132) with VDSP Standardized Results Based on a Re-Measurement of a Statistical Subsample (n = 99), and with Standardized Results Based on a Re-Measurement of All Study Samples (n = 1132) using University College Cork's (UCC) CDC Certified Traceable LC–MS/MS Assay.

	Original IDS/EIA Assay Results	VDSP Statistical Standardization Protocols[a]	UCC's CDC Certified LC–MS/MS
Serum Total 25(OH)D Cutpoint	(n = 1132)	(n = 99)	(n = 1132)
<30 nmol/L	6.5%	11.4%	11.2%
<40 nmol/L	21.9%	25.3%	27.2%
<50 nmol/L	40.0%	43.7%	45.0%
>125 nmol/L	1%	0.3%	0.6%

[a] Mathematical model based on the results of 99 serum samples re-measured using UCC's CDC Certified Traceable Assay was used to Calibrate/Standardize all NANS 25(OH)D concentrations.