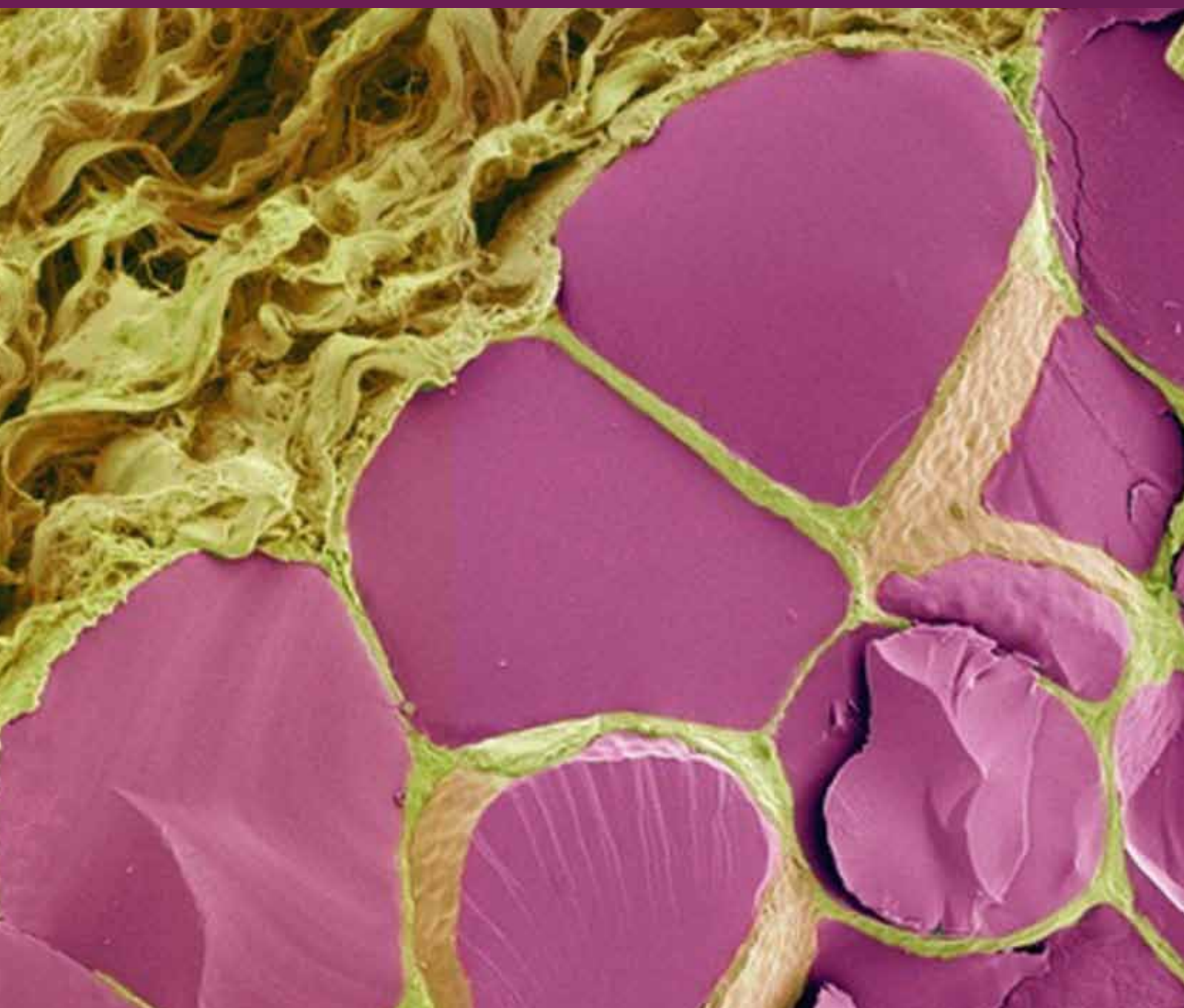


Vitamin D Binding Protein and Vitamin D Levels

Guest Editors: Xiangbing Wang, Arthur Santora, Sue A. Shapses, and Zhongjian Xie





Vitamin D Binding Protein and Vitamin D Levels

International Journal of Endocrinology

Vitamin D Binding Protein and Vitamin D Levels

Guest Editors: Xiangbing Wang, Arthur Santora, Sue A. Shapses,
and Zhongjian Xie



Copyright © 2014 Hindawi Publishing Corporation. All rights reserved.

This is a special issue published in "International Journal of Endocrinology." All articles are open access articles distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Editorial Board

Anil K. Agarwal, USA
John Ayuk, UK
Amelie Bonnefond, France
Donald W. Bowden, USA
Shern L. Chew, UK
Iacopo Chiodini, Italy
E. Christ, Switzerland
Sabrina Corbetta, Italy
Giuseppe D'Annunzio, Italy
Xavier Donadeu, UK
Maria L. Dufau, USA
Kristin Eckardt, Norway
Dariush Elahi, USA
Katherine Esposito, Italy
Riccarda Granata, Italy
Oreste Gualillo, Spain
Mahin Hashemipour, Iran

A. Höflich, Germany
M. Horowitz, Australia
Khalid Hussain, UK
Dario Iafusco, Italy
Giorgio Iervasi, Italy
Daniela Jezova, Slovakia
Janaka Karalliedde, UK
Andre P. Kengne, Australia
M. Kotula-Balak, Poland
Fernand Labrie, Canada
H. C. Lee, Republic of Korea
Mario Maggi, Italy
Ludwik K. Malendowicz, Poland
Matteo Monami, Italy
Robert D. Murray, UK
Constantinos Pantos, Greece
F. R. Perez-Lopez, Spain

Lorenzo Piemonti, Italy
Dario Pitocco, Italy
U. Ploeckinger, Germany
Andrew V. Schally, USA
Alexander Schreiber, USA
Muhammad Shahab, Pakistan
Kazuhiro Shiizaki, Japan
Kevin Sinchak, USA
Ajai K. Srivastav, India
Stuart Tobet, USA
Andreas Tomaschitz, Austria
Jack R. Wall, Australia
Matthew Watt, Australia
Aimin Xu, Hong Kong
Paul M. Yen, USA
N. Younis, United Kingdom

Contents

Vitamin D Binding Protein and Vitamin D Levels, Zhongjian Xie, Arthur C. Santora, Sue A. Shapses, and Xiangbing Wang
Volume 2014, Article ID 638263, 2 pages

Influence of Vitamin D Binding Protein on Accuracy of 25-Hydroxyvitamin D Measurement Using the ADVIA Centaur Vitamin D Total Assay, James Freeman, Kimberly Wilson, Ryan Spears, Victoria Shalhoub, and Paul Sibley
Volume 2014, Article ID 691679, 12 pages

Vitamin D Binding Protein and Bone Health, Ishir Bhan
Volume 2014, Article ID 561214, 5 pages

Vitamin D-Binding Protein Levels in Plasma and Gingival Crevicular Fluid of Patients with Generalized Aggressive Periodontitis, Xin Zhang, Huanxin Meng, Li Xu, Li Zhang, Dong Shi, Xianghui Feng, Ruifang Lu, and Zhibin Chen
Volume 2014, Article ID 783575, 6 pages

Vitamin D Binding Protein Impact on 25-Hydroxyvitamin D Levels under Different Physiologic and Pathologic Conditions, Pegah Yousefzadeh, Sue A. Shapses, and Xiangbing Wang
Volume 2014, Article ID 981581, 6 pages

Vitamin D Binding Protein Affects the Correlation of 25(OH)D and Frailty in the Older Men, Yi Wang, Yan-Jiao Wang, Jun-Kun Zhan, Zhi-Yong Tang, Wu Huang, Pan Tan, Shan Gao, Cai-Li Ma, Zai-Jin Jian, and You-Shuo Liu
Volume 2014, Article ID 543783, 6 pages

Association of High Vitamin D Status with Low Circulating Thyroid-Stimulating Hormone Independent of Thyroid Hormone Levels in Middle-Aged and Elderly Males, Qingqing Zhang, Zhixiao Wang, Min Sun, Mengdie Cao, Zhenxin Zhu, Qi Fu, Yuan Gao, Jia Mao, Yanyun Li, Yun Shi, Fan Yang, Shuai Zheng, Wei Tang, Yu Duan, Xiaoping Huang, Wei He, and Tao Yang
Volume 2014, Article ID 631819, 6 pages

Editorial

Vitamin D Binding Protein and Vitamin D Levels

Zhongjian Xie,¹ Arthur C. Santora,² Sue A. Shapses,³ and Xiangbing Wang⁴

¹ Institute of Metabolism and Endocrinology, The Second Xiangya Hospital, Central South University, Changsha, Hunan 410011, China

² Clinical Research, Diabetes and Endocrinology, Merck Research Laboratories, 126 East Lincoln Avenue, RY34B-148, Rahway, NJ 07065-0900, USA

³ Department of Nutritional Science, Rutgers University, New Brunswick, NJ 08901, USA

⁴ Division of Endocrinology, Metabolism and Nutrition, Robert Wood Johnson Medical School, Rutgers University, New Brunswick, NJ 08093, USA

Correspondence should be addressed to Xiangbing Wang; wangx9@rwjms.rutgers.edu

Received 21 May 2014; Accepted 21 May 2014; Published 25 June 2014

Copyright © 2014 Zhongjian Xie et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Over the past few decades, there has been a growing interest in understanding the multifunctional characteristics and clinical importance of vitamin D binding protein (DBP). Multiple studies have shed light on DBP, giving rise to hopes of identifying novel mechanisms as well as utilizing DBP as a potential therapeutic agent. The current issue is comprised of 6 manuscripts, two of which are review articles. The areas covered in this special issue mostly highlight the potential role of DBP in several conditions including periodontitis and frailty, implying that measurement of DBP may provide useful information in addition to total 25-hydroxy vitamin D concentration.

In this issue, the review by P. Yousefzadeh et al. titled “Vitamin D binding protein impact on 25-hydroxyvitamin D levels under different physiologic and pathologic conditions” calls for the implementation of DBP testing during the interpretation of 25-hydroxyvitamin D [25(OH)D] results under different clinical situations. I. Bhan reviews the recent findings regarding the association between the differences in vitamin D binding protein levels and bone density in his paper titled “Vitamin D binding protein and bone health.” The author has compiled studies concerned with DBP, including DBP polymorphisms in relation to bone health and its association with vitamin D bioavailability. These findings may impact not only strategies for designing novel therapeutic agents that influence DBP or its binding, but also our understanding of the mechanism of vitamin D in

the context of bone health. Evidence is emerging that the DBP polymorphisms are associated with race and ethnicity, resulting in differences in DBP levels and binding affinity that affect the transport and metabolism of vitamin D and its metabolites.

Using serum samples from various populations with varying DBP levels, J. Freeman et al., in their paper titled “Influence of vitamin D binding protein on accuracy of 25-hydroxyvitamin D measurement using the ADVIA Centaur vitamin D total assay,” assessed the agreement between the ADVIA Centaur vitamin D total assay for 25(OH)D testing and the liquid chromatography mass spectrometry (LS-MS/MS) method and concluded that the ADVIA Centaur vitamin D total assay demonstrated good performance compared to the LS-MS/MS method across the normal range of DBP concentrations. This is one example of methods development in the field to ensure accurate and simpler protocols for measuring circulating 25(OH)D in future study.

X. Zhang et al., in their paper titled “Vitamin D-binding protein levels in plasma and gingival crevicular fluid (GCF) of patients with generalized aggressive periodontitis,” examined the association of DBP with generalized aggressive periodontitis (GAgP). The authors found that GAgP patients had higher plasma DBP concentrations but lower GCF DBP concentrations than healthy controls, suggesting that decreased GCF-DBP level and increased plasma DBP level are associated with periodontitis. This study adds to

the growing evidence of the potential role of DBP in the pathogenesis of periodontitis.

Existing evidence shows an association of high circulating 25(OH)D levels with low TSH levels in younger individuals, but there is insufficient information on whether it is the same in the elderly and middle-aged. Q. Zhang et al., in their paper titled "*Association of high vitamin D status with low circulating thyroid-stimulating hormone independent of thyroid hormone levels in middle-aged and elderly males,*" report that such an association does exist in middle-aged and elderly males, independent of thyroid hormone levels. The authors also demonstrated the link between vitamin D insufficiency and serum thyroid antibody levels.

Y. Wang et al., in their paper entitled "*Vitamin D binding protein affects the correlation of 25(OH)D and frailty in the older men,*" assessed the frailty status in elderly men of Changsha city, China, and concluded that serum DBP levels should be measured when evaluating the 25(OH)D- frailty relationship.

We hope that these articles convey new insights to readers and researchers into the current renewed interest in vitamin D and DBP research.

*Zhongjian Xie
Arthur C. Santora
Sue A. Shapses
Xiangbing Wang*

Research Article

Influence of Vitamin D Binding Protein on Accuracy of 25-Hydroxyvitamin D Measurement Using the ADVIA Centaur Vitamin D Total Assay

James Freeman, Kimberly Wilson, Ryan Spears, Victoria Shalhoub, and Paul Sibley

Siemens Healthcare Diagnostics, 511 Benedict Avenue, Tarrytown, NY 10591, USA

Correspondence should be addressed to James Freeman; james.freeman@siemens.com

Received 13 December 2013; Revised 7 April 2014; Accepted 28 April 2014; Published 19 June 2014

Academic Editor: Arthur Santora

Copyright © 2014 James Freeman et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Vitamin D status in different populations relies on accurate measurement of total serum 25-hydroxyvitamin D [25(OH)D] concentrations [i.e., 25(OH)D₃ and 25(OH)D₂]. This study evaluated agreement between the ADVIA Centaur Vitamin D Total assay for 25(OH)D testing (traceable to the NIST-Ghent reference method procedure) and a liquid chromatography tandem mass spectrometry (LC-MS/MS) method for various populations with different levels of vitamin D binding protein (DBP). Total serum 25(OH)D concentrations were measured for 36 pregnant women, 40 hemodialysis patients, and 30 samples (DBP-spiked or not) from healthy subjects. ELISA measured DBP levels. The mean serum DBP concentrations were higher for pregnancy (415 µg/mL) and lower for hemodialysis subjects (198 µg/mL) than for healthy subjects and were highest for spiked serum (545 µg/mL). The average bias between the ADVIA Centaur assay and the LC-MS/MS method was -1.4% (healthy), -6.1% (pregnancy), and 4.4% (hemodialysis). The slightly greater bias for samples from some pregnancy and hemodialysis subjects with serum DBP levels outside of the normal healthy range fell within a clinically acceptable range—reflected by analysis of their low-range (≤ 136 µg/mL), medium-range (137–559 µg/mL), and high-range (≥ 560 µg/mL) DBP groups. Thus, the ADVIA Centaur Vitamin D Total assay demonstrates acceptable performance compared with an LC-MS/MS method for populations containing different amounts of DBP.

1. Introduction

Increasing awareness of the important role of vitamin D for bone and other diseases has led to increased 25-hydroxyvitamin D [25(OH)D] testing (D represents D₃ and D₂ forms). However, variability within and between methods and laboratories has often compromised correct diagnosis and the ability to compare results from different studies and national surveys [1–5]. Automated antibody-based, radioimmunoassays, high performance liquid chromatography (HPLC), and mass spectrometry methods for 25(OH)D testing are subject to variability issues that can arise from a variety of sources, such as differential detection of the D₃ and D₂ forms, interference by detection polyclonal antibodies, and nonspecific detection of other vitamin D metabolites such as the 3-epimer form of 25(OH)D [3-epi-25(OH)D] and 24,25(OH)₂D₃. In addition, incomplete release of 25(OH)D from the vitamin D binding protein

(DBP) has been identified as a potential source of variability for both manual and automated immunoassays [6].

Establishing an immunoassay for 25(OH)D is challenging because the majority of the highly hydrophobic 25(OH)D is tightly bound (dissociation constant, K_d , 5×10^{-8} M) to a vast excess of DBP from which it must be separated; almost no 25(OH)D is found “free” (non-protein bound) in the circulation, and less than 5% of the available DBP binding sites are occupied by vitamin D compounds [7]. In addition, DBP binds vitamin D₃ along with other metabolites and vitamin D₂, whose similar structures may be easier to release from DBP and difficult to differentiate; DBP has a higher affinity for vitamin D₃ than other metabolites and vitamin D₂ [8]; and generating specific antibodies against small antigenic molecules, such as 25(OH)D, is difficult, but it is mandatory because the Vitamin D Standardization Program (VDSP) states that 25(OH)D assays should measure

equimolar amounts of 25(OH)D₃ and 25(OH)D₂ (total vitamin D) [9]. Measuring total vitamin D is required because some supplements contain the D₂ form, and not measuring D₂ would lead to lower 25(OH)D values. In methods such as radioimmunoassay, HPLC, and mass spectrometry, an initial extraction step with organic solvents releases all bound 25(OH)D from DBP [10–13]. However, organic solvents are not compatible with most automated immunoassays, and alternative releasing agents, which are proprietary, are used instead. Recent studies performed in populations with different levels of DBP have questioned the effectiveness of these proprietary releasing agents to completely free 25(OH)D from DBP [6].

The goal of this study was to examine the ability of the ADVIA Centaur Vitamin D Total assay by comparison with an LC-MS/MS method to accurately measure 25(OH)D levels in serum samples from healthy adults (endogenous) and healthy adults with exogenous DBP (endogenous + spiked) and from pregnant women and chronic kidney disease (CKD) patients receiving dialysis, who have higher and lower than normal serum levels of DBP, respectively [7, 14, 15]. The ADVIA Centaur Vitamin D Total assay is traceable to the NIST-Ghent reference measurement procedure (RMP) for vitamin D testing. (This version of the ADVIA Centaur Vitamin D Total assay is not currently available commercially in all regions, including the USA.)

2. Materials and Methods

In order to determine the influence of DBP on a vitamin D immunoassay, a study examining DBP as an endogenous interference, similar to how hemoglobin, cholesterol, or total protein would be measured, following Clinical and Laboratory Standards Institute (CLSI) Document EP7-A2 [16] was performed at the Siemens R&D facility in Tarrytown, NY, USA. Human native DBP (>95% pure) was purchased from Athens Research & Technology, Inc.

2.1. LC-MS/MS. The LC-MS/MS method used in this study is traceable to the Esoterix ID-LC-MS/MS method, which is traceable to NIST. The LC-MS/MS method performed at Siemens used the Waters Acquity H-class ultrahigh performance liquid chromatography (UPLC) and triple quadrupole (TQD) tandem mass spectrometry (MS) with MassLynx and QuanLynx software (Waters Acquity TQD system, Waters Corporation, Manchester, UK). This method is able to separate, identify, and separately quantify the concentrations of 25(OH)D₂, 25(OH)D₃, and 3-epi-25(OH)D₃ in a serum sample. As reported by the manufacturer, the LC-MS/MS method demonstrated a dynamic assay range of 2.5–220 ng/mL (6.25–550 nmol/L) ($r^2 > 0.997$). Three levels of 25(OH)D₂ and 25(OH)D₃ concentrations tested over five consecutive days yielded intra-assay coefficients of variation (CVs) of $\leq 7.7\%$ and interassay precision CVs of $< 12\%$ for 25(OH)D₂ and 25(OH)D₃.

2.2. ADVIA Centaur Vitamin D Total Assay. The ADVIA Centaur Vitamin D Total assay used in this study is traceable

to the Ghent University ID-LC-MS/MS 25(OH)D RMP. (This version of the assay is not currently available commercially in all regions, including the USA.) The Ghent University is a reference laboratory for the Vitamin D Standardization Program (VDSP). The sample reference material (SRM) used for the Ghent University method is traceable to the NIST SRM 2972 [4, 9, 17]. Recently, Siemens received confirmation from the Centers for Disease Control and Prevention (CDC) that the ADVIA Centaur Vitamin D Total assay is now a certified procedure of the Vitamin D Standardization-Certification Program (VDSCP). Certification was achieved by demonstrating that the total vitamin D [25(OH)D] results for the 40 VDSCP samples (10 quarterly challenges) agreed with the results from the ID-LC-MS/MS RMP method. The ADVIA Centaur Vitamin D Total assay achieved a mean bias of 0.3% (acceptance criterion was $\pm 5.0\%$) and a mean imprecision of 5.5% (acceptance criterion was $< 10.0\%$). The ADVIA Centaur Vitamin D Total assay is a one-pass, 18-minute antibody competitive chemiluminescent immunoassay. Release of the 25(OH)D metabolites from the DBP is accomplished by denaturing and blocking agents. 25(OH)D in serum competes with a 25(OH)D analog (labeled with fluorescein) for an anti-25(OH)D monoclonal mouse antibody (labeled with acridinium ester). Detection occurs after the remaining anti-25(OH)D monoclonal antibody (labeled with acridinium ester) complexes with vitamin D analog (labeled with fluorescein) and anti-fluorescein monoclonal antibody covalently bound to paramagnetic particles. Results are inversely related to 25(OH)D serum concentrations. The standardized assay demonstrates equimolar cross-reactivity with 25(OH)D₂ (104.5%) and 25(OH)D₃ (100.7%), minimal cross-reactivity with 3-epimer-25(OH)D₃ (1.1%), and a broad dynamic assay range of 4.2–150 ng/mL (10.5–375 nmol/L) [18]. Precision was determined by assaying six samples twice a day in replicates of 4, over 20 days ($n = 160$ replicates per sample) according to the Clinical and Laboratory Standards Institute (CLSI) protocol EP5-A2 [19]; the run-to-run CVs were in the range of 4.2% and 11.9% [18]. All samples were run in singlicate on both the LC-MS/MS and a single ADVIA Centaur system.

2.3. Sample Population. Clinical serum samples from 18 healthy adults were purchased from a commercial vendor (ProMedDx, Norton, MA, USA). Serum samples from 36 pregnant women in their third trimester and 40 CKD hemodialysis patients were purchased from another commercial vendor (Research Sample Bank, Delray Beach, FL, USA).

2.4. Samples. Peripheral venous blood samples were collected, placed at 4°C, and centrifuged; serum aliquots were prepared and stored for less than four months at –20°C until analysis. Generally, no difference was found in the serum concentrations of DBP for men and women [11, 15].

2.5. Protocol. Serum samples were sent to Siemens Healthcare Diagnostics (Tarrytown, NY, USA) for DBP and 25(OH)D measurements. The serum samples from the 18 healthy adults were divided into five serum pools; each of

TABLE 1: Serum concentrations of vitamin D binding protein in healthy subjects, DBP-spiked samples from healthy subjects, pregnant women, and dialysis patients.

	Number of samples	Average \pm SD ($\mu\text{g}/\text{mL}$)	Range ($\mu\text{g}/\text{mL}$)	Median ($\mu\text{g}/\text{mL}$)	Interquartile (IQ) range ($\mu\text{g}/\text{mL}$)
Healthy not spiked (endogenous)	5	348 \pm 106	261–519	ND	ND
Healthy (endogenous and endogenous + spiked)	30	512 \pm 188 ^a	261–981	ND	ND
Healthy (endogenous + spiked)	25	545 \pm 185 ^a	261–981	ND	ND
Pregnancy	36	415 \pm 245 ^a	82–875	515	150–599
Dialysis	40	198 \pm 173	63–1116	142	100–262

^a $P < 0.0001$ compared to the dialysis group.

ND: not determined.

DBP: vitamin D binding protein.

the four pools contained 4 individual serum samples and one pool contained 2 individual serum samples. The 25(OH)D concentrations in these five serum pools were measured by using a LC-MS/MS method at Siemens Healthcare Diagnostics, (Tarrytown, NY, USA) according to a protocol that allowed resolution of 25(OH)D₂ and 25(OH)D₃ from 3-epi-25(OH)D₃. The LC-MS/MS values for the five individual pools (pools 1–5) resulted in mean 25(OH)D concentrations of 24, 32, 51, 41, and 75 ng/mL, respectively. The endogenous levels of DBP were measured in each of the five serum pools using the Quantikine ELISA Vitamin D Binding Protein BP kit, DVDBP0 (R&D Systems, Inc.). Subsequently, each of the five serum pools was divided into six aliquots, and DBP (ranging from 50 to 250 $\mu\text{g}/\text{mL}$ in 50 $\mu\text{g}/\text{mL}$ increments) was spiked into 5 of the 6 aliquots from each pool (Table 3). The DBP content in the resulting thirty samples was then reanalyzed to confirm higher DBP concentrations in spiked samples, and 25(OH)D measurements were performed according to routine procedures using the ADVIA Centaur Vitamin D Total assay traceable to the Ghent University ID-LC-MS/MS 25(OH)D RMP. (This version of the assay is not currently available commercially in all regions, including the USA.) Bias of 25(OH)D values to the original LC-MS/MS values was determined. In addition, the 36 clinical serum samples from third-trimester pregnancy patients and the 40 clinical serum samples from CKD patients were evaluated for endogenous DBP and 25(OH)D using the ADVIA Centaur Vitamin D Total assay; and bias of 25(OH)D values to the original LC-MS/MS values was determined. Only four samples from pregnancy subjects had detectable 25(OH)D₂ (3.2, 5.2, 8.0, and 10.7 $\mu\text{g}/\text{mL}$). Nineteen samples from dialysis patients had detectable 25(OH)D₂ (range 1.6 to 35 $\mu\text{g}/\text{mL}$), eight of which had levels above 10 $\mu\text{g}/\text{mL}$. The 3-epi-25(OH)D₃ was present at levels greater than 1.5 ng/mL in samples from 23 dialysis and 32 pregnancy subjects.

2.6. Statistics. Difference plots and bias values were obtained using Microsoft Excel (2010); Analyze-It add-in program in Excel was used to compare the different sets of data in order to obtain the 95% confidence interval (CI) and standard deviations (SD) for 95% limits of agreement. Correlation plots and correlation and Deming regression analyses were generated using GraphPad Prism, version 6.

3. Results

The mean serum concentrations of DBP in healthy subjects (endogenous and endogenous + spiked), pregnant women, and dialysis patients are presented in Table 1. For the five serum pools, the average endogenous serum DBP concentration (\pm SD) was 348 \pm 106 $\mu\text{g}/\text{mL}$ (range 260.7 to 519.0 $\mu\text{g}/\text{mL}$), which is consistent with the results of other studies [6, 20, 21]. For healthy serum samples spiked with DBP, the average DBP concentration was higher (545 \pm 185 $\mu\text{g}/\text{mL}$, range 261.2 to 980.6 $\mu\text{g}/\text{mL}$) than endogenous levels. For pregnancy samples, the average DBP concentration was also greater (415 \pm 245 $\mu\text{g}/\text{mL}$, range 82.2 to 874.5 $\mu\text{g}/\text{mL}$) than that for healthy serum samples. In contrast, for CKD patients receiving dialysis, the average DBP concentration was lower (198 \pm 173 $\mu\text{g}/\text{mL}$, range 63.4 to 1115.7 $\mu\text{g}/\text{mL}$; median 142.1 $\mu\text{g}/\text{mL}$) than levels in healthy serum and pregnancy samples.

The mean total serum 25(OH)D concentrations and range as measured by the LC-MS/MS method and the ADVIA Centaur Vitamin D Total assay are presented in Table 2. The mean 25(OH)D levels (\pm SD) were 44.6 \pm 19.8 and 44.8 \pm 20.1 ng/mL for healthy serum samples (endogenous), 44.6 \pm 18.0 and 43.5 \pm 16.7 ng/mL for healthy (endogenous + spiked) serum samples, and 44.6 \pm 18.0 and 43.7 \pm 17.0 ng/mL for both endogenous and endogenous + spiked healthy serum samples, and they were lower for pregnancy serum samples, 27.3 \pm 9.6 and 25.3 \pm 8.7 ng/mL, and dialysis serum samples, 28.1 \pm 14.8 and 29 \pm 15.3 ng/mL. Consistent with previous reports, no correlation was found between the DBP and 25(OH)D concentrations for serum from dialysis patients (Pearson's correlation coefficient $r = 0.1$) [11, 14, 15]. Pregnancy samples demonstrated a positive correlation ($r = 0.35$; $P = 0.013$) between serum concentrations of DBP and 25(OH)D for LC-MS/MS, but no correlation was found for ADVIA Centaur ($r = 0.15$; $P = 0.37$). There were too few non-spiked healthy samples for valid 25(OH)D and DBP correlation assessment.

The overall average bias of all samples from healthy individuals (endogenous and endogenous + spiked) for the ADVIA Centaur Vitamin Total assay to the LC-MS/MS method was -1.4% ; for all third-trimester pregnancy samples, the average bias was -6.1% ; and for all renal dialysis samples, the average bias was 4.4% . The results for bias, percent

TABLE 2: Serum concentrations of 25(OH)D in healthy subjects, DBP-spiked samples from healthy subjects, pregnant women, and dialysis patients.

	Number of samples	ADVIA Centaur Vitamin D Total assay Average \pm SD (ng/mL)	ADVIA Centaur Vitamin D Total assay Range (ng/mL)	LC-MS/MS Average \pm SD (ng/mL)	LC-MS/MS Range (ng/mL)
Healthy not spiked (endogenous)	5	44.8 \pm 20.1 ^a	24.0–75.0	44.6 \pm 19.8	24.3–75.3
Healthy (endogenous and endogenous + spiked)	30	43.7 \pm 16.7 ^{c,d}	22.9–75.3	44.6 \pm 18.0 ^e	24.0–75.0
Healthy (endogenous + spiked)	25	43.5 \pm 16.7 ^{b,d}	22.9–75.3	44.6 \pm 18.0 ^e	24.0–75.0
Pregnancy	36	25.3 \pm 8.7	3.7–40.8	27.3 \pm 9.6	4.0–44.9
Dialysis	40	29.0 \pm 15.3	6.5–72.6	28.1 \pm 14.8	6.0–67.0

To convert 25(OH)D concentrations to nanomoles per liter (nmol/L), multiply by 2.5.

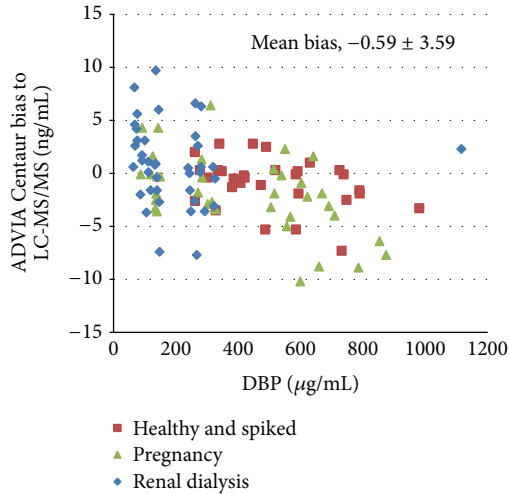
^a $P < 0.05$ compared to the pregnancy group; ^b $P < 0.01$ compared to the dialysis group; ^c $P < 0.001$ compared to the dialysis group; ^d $P < 0.0001$ compared to the pregnancy group; ^e $P < 0.001$ compared to the pregnancy and dialysis groups.

DBP: vitamin D binding protein.

TABLE 3: Serum concentrations of DBP in healthy subjects and DBP-spiked samples from healthy subjects.

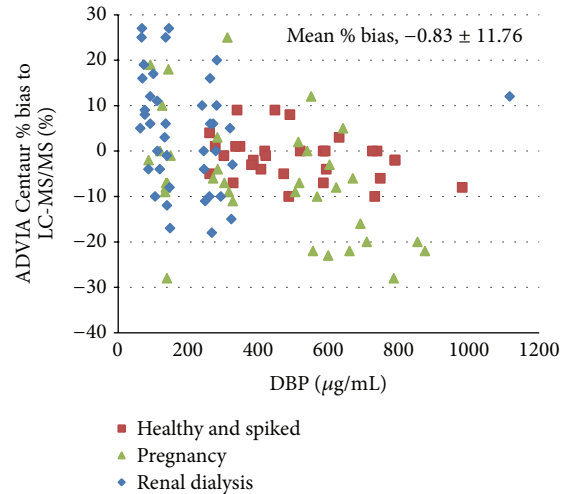
LC-MS/MS (ng/mL)	Concentration of spiked DBP in serum (μ g/mL)	DBP (mg/mL)	ADVIA Centaur (ng/mL)	ADVIA Centaur bias to LC-MS/MS
24		276.9	24.3	1%
24	50	347.2	24.2	1%
24	100	385.5	23.5	-2%
24	150	334.9	24.3	1%
24	200	407.1	23.1	-4%
24	250	472.1	22.9	-5%
32		301.6	31.6	-1%
32	50	339.1	34.8	9%
32	100	629.7	33	3%
32	150	446.8	34.8	9%
32	200	489.5	34.5	8%
32	250	584.4	31.9	0%
51		260.7	53	4%
51	50	261.2	48.4	-5%
51	100	327.8	47.5	-7%
51	150	417.7	50.8	0%
51	200	593.3	49.1	-4%
51	250	486.2	45.7	-10%
41		380.1	39.7	-3%
41	50	420.5	40.6	-1%
41	100	590.2	41.2	0%
41	150	747.3	38.5	-6%
41	200	738	40.9	0%
41	250	980.6	37.7	-8%
75		519	75.3	0%
75	50	584.8	69.7	-7%
75	100	724.9	75.3	0%
75	150	788.4	73.4	-2%
75	200	731.3	67.7	-10%
75	250	789.7	73.1	-2%

DBP: vitamin D binding protein. To convert 25(OH)D concentrations to nanomoles per liter (nmol/L), multiply by 2.5.



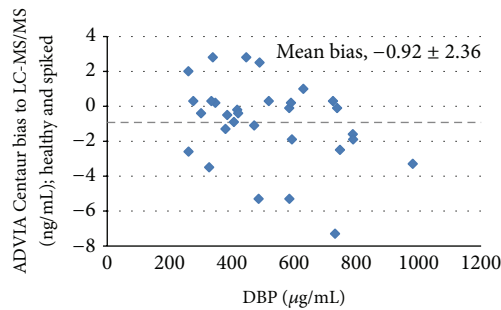
	<i>n</i>	Mean	95% CI	SE	SD
DBP spiked	106	360.93	314.38–407.49	23.480	241.742
All samples (ng/mL)	106	-0.591	-1.28–0.10	0.3487	3.5902

(a)



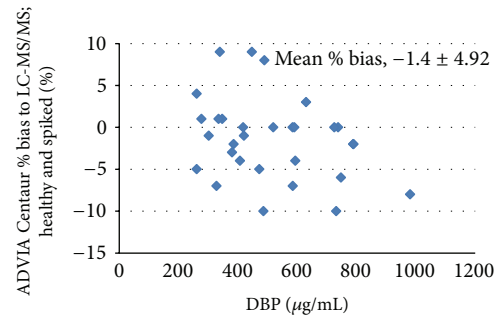
	<i>n</i>	Mean	95% CI	SE	SD
DBP spiked	106	360.93	314.38–407.49	23.480	241.742
All samples % bias	106	-0.830	-3.10–1.43	1.1423	11.7607

(b)



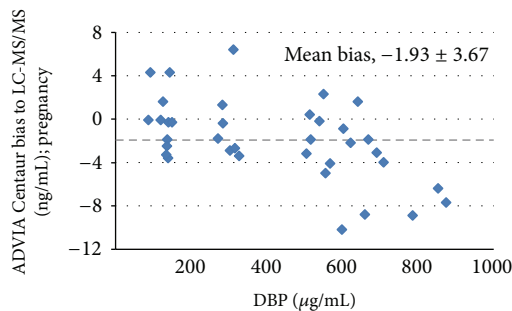
	<i>n</i>	Mean	95% CI	SE	SD
DBP spiked	30	511.88	441.62–582.14	34.354	188.163
Bias to LC-MS/MS (ng/mL)	30	-0.92	-1.80–0.03	0.431	2.363

(c)



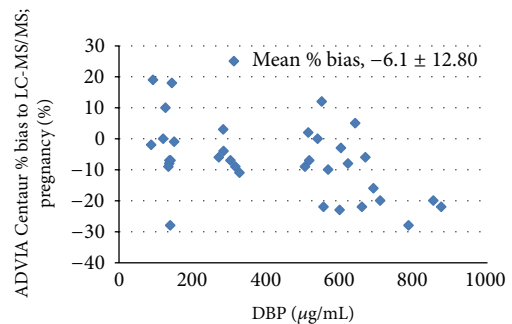
	<i>n</i>	Mean	95% CI	SE	SD
DBP spiked	30	511.88	441.62–582.14	34.354	188.163
Bias to LC-MS/MS (%)	30	-1.4	-3.20–0.47	0.90	4.92

(d)



	<i>n</i>	Mean	95% CI	SE	SD
Pregnancy	36	415.36	332.42–498.30	40.855	245.131
Bias to LC-MS/MS (ng/mL)	36	-1.933	-3.18–-0.69	0.6119	3.6712

(e)



	<i>n</i>	Mean	95% CI	SE	SD
Pregnancy	36	415.36	332.42–498.30	40.855	245.131
Bias to LC-MS/MS (%)	36	-6.1	-10.47–-1.81	2.13	12.80

(f)

FIGURE I: Continued.

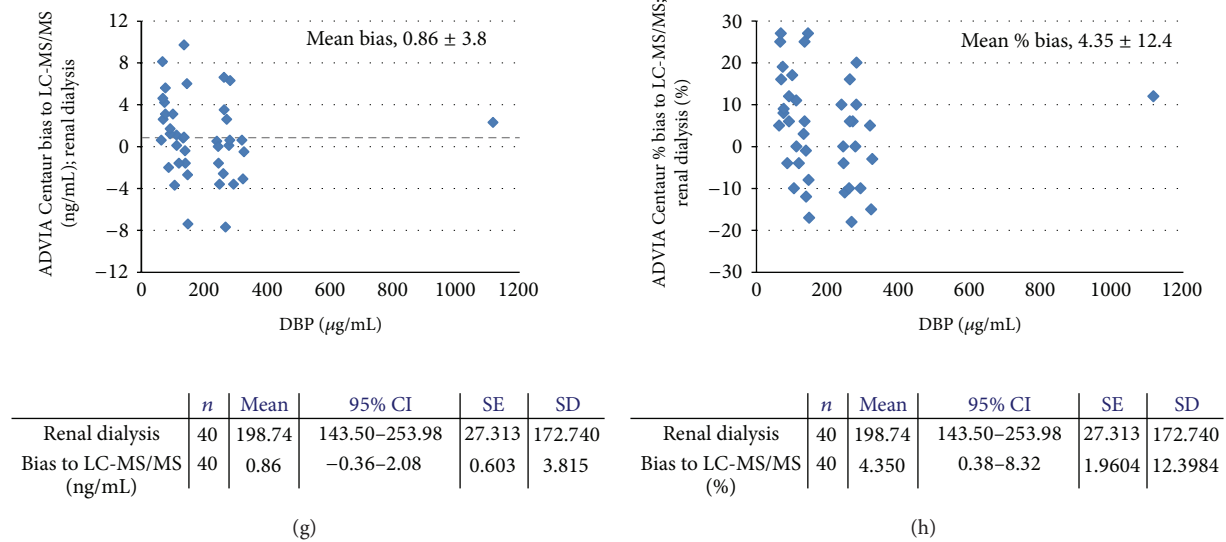


FIGURE 1: Bias and percent bias between the 25(OH)D results of the ADVIA Centaur Vitamin D Total assay and the LC-MS/MS method as a function of DBP concentration in healthy human serum pooled samples (endogenous and endogenous + spiked) (a, b, c, d), pregnancy (third trimester) samples (a, b, e, f), and renal dialysis samples (a, b, g, h). The bias ± 1.96 standard deviation (SD) represents the 95% limits of agreement. To convert 25(OH)D concentrations to nanomoles per liter (nmol/L), multiply by 2.5.

bias, 95% CI, and SD (95% limits of agreement = 1.96 SD) as a function of DBP concentration for each population—separate and combined—are presented in Figure 1. When all populations were combined, positive bias (versus LC-MS/MS) was observed at very low serum DBP concentrations and negative bias was observed at very high serum DBP concentrations (Figure 1).

With respect to dialysis samples with generally lower DBP concentrations, we do not know if uremic serum properties contributed to bias, and we question the validity of analyzing combined populations. Nevertheless, we examined how well the methods in subjects with serum DBP concentrations at extremes of the serum DBP concentration range (very low and very high)—for combined and separate populations (Figures 2, 3, and 4). Very low and very high serum DBP concentrations were defined as two SD below and above the mean for healthy subjects which is $348 \pm 106 \mu\text{g/mL}$; hence, the very low DBP group comprised samples having concentrations of $\leq 136 \mu\text{g/mL}$, and high DBP group comprised samples having concentrations of $\geq 560 \mu\text{g/mL}$. The middle range group had samples with DBP concentrations ranging from 137 to $559 \mu\text{g/mL}$. The following populations were analyzed: (1) healthy, spiked, pregnancy, and dialysis (Figure 2); (2) healthy (which had no low or high groups) [Figure 3(a)]; (3) healthy and spiked (which had no low group) [Figure 3(b)]; (4) spiked (which had no low group) [Figure 3(c)]; (5) pregnancy [Figure 4(a)]; (6) dialysis (which had no high group) [Figure 4(b)].

Analysis of 25(OH)D values for ADVIA Centaur and LC-MS/MS as a function of low, medium, and high serum DBP concentrations demonstrated that correlations between the two methods were acceptable at low and high serum DBP levels for all populations analyzed (combined and separate) (Figures 2, 3, and 4), with pregnancy samples demonstrating

the lowest correlation at very high serum DBP concentrations ($r = 0.87$, $P < 0.0002$). Healthy samples (endogenous and endogenous + spiked) showed very good correlations and agreement between methods. The mean bias obtained for combined populations and each population separately for their low, medium, and high range DBP groups represented acceptable assay performance (Table 4).

LC-MS/MS identified 25(OH)D₃, 25(OH)D₂, and 3-epi-25(OH)D₃ in samples. Only four samples from pregnant subjects had detectable 25(OH)D₂ (3.2, 5.2, 8.0, and $10.7 \mu\text{g/mL}$). Nineteen samples from dialysis patients had detectable 25(OH)D₂ (range 1.6 to 35 ng/mL), eight of which had levels above 10 ng/mL . The mean percent bias of the eight 25(OH)D₂ samples which had greater than 10 ng/mL was $9.0 \pm 0.12\%$ (mean \pm SD), whereas the mean percent bias of the remaining samples was $3.0 \pm 0.12\%$. Of the 40 dialysis patients, 17 had less than 1.5 ng/mL 3-epi-25(OH)D₃ and 23 (58%) had 3-epi-25(OH)D₃ concentrations ranging from 1.7 to 3.6 (mean \pm SD, $2.5 \pm 0.57 \text{ ng/mL}$). Of the 36 pregnancy subjects, four had less than 1.5 ng/mL 3-epi-25(OH)D₃ and 32 (89%) had 3-epi-25(OH)D₃ concentrations ranging from 1.6 to 6.3 ng/mL (mean \pm SD, 3.3 ± 1.3).

4. Discussion

This study addressed the influence of DBP on the accuracy of the ADVIA Centaur Vitamin D Total assay by comparison with an LC-MS/MS method. The ADVIA Centaur Vitamin D Total assay results in this study are traceable to ID-LC-MS/MS 25(OH)D reference method procedure and the standard reference materials established by NIST and the University of Ghent [4, 9, 17, 22].

In healthy individuals, endogenous serum DBP concentration ($347.6 \mu\text{g/mL}$) was found to be within the range

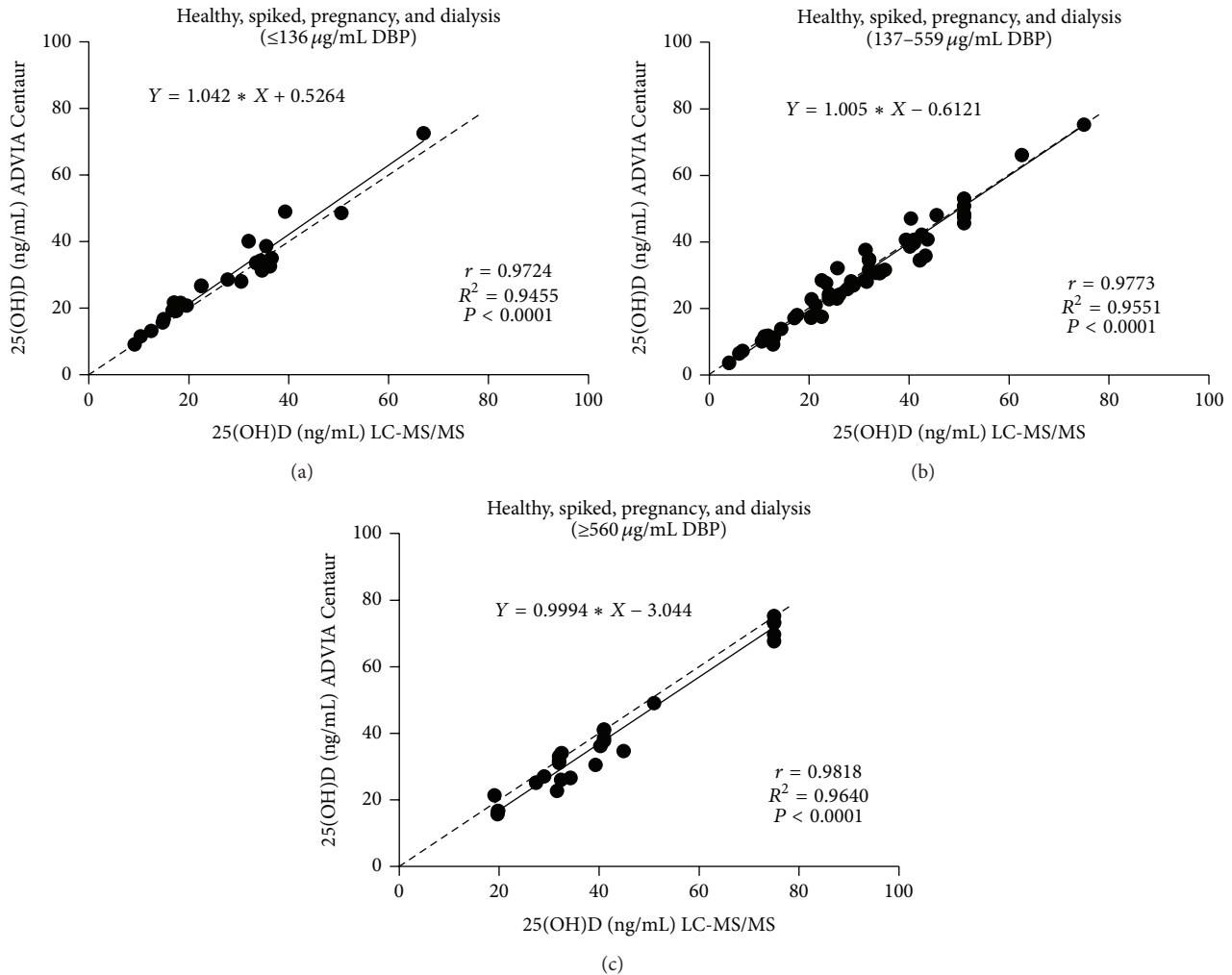


FIGURE 2: Correlation of 25(OH)D results obtained from the ADVIA Centaur Vitamin D Total assay and the LC-MS/MS method for combined normal human serum pooled samples (endogenous and endogenous + spiked), pregnancy (third trimester samples), and renal dialysis samples for (a) low, (b) medium, and (c) high DBP groups. Dotted line: line of identity. To convert 25(OH)D concentrations to nanomoles per liter (nmol/L), multiply by 2.5.

reported by others (300–600 $\mu\text{g/mL}$) [20] and increased after DBP spiking (545 $\mu\text{g/mL}$). Despite the increase in DBP concentrations after spiking, 25(OH)D measurements in individual samples were equivalent between the two methods. There were no healthy (endogenous or endogenous + spiked) samples in the low DBP range, as defined by two SD values below the mean of healthy samples (i.e., $\leq 136 \mu\text{g/mL}$) (Figure 3). The high DBP range, as defined by two SD values above the mean of healthy samples (i.e., $\geq 560 \mu\text{g/mL}$), comprised twelve spiked samples (Figure 3), and 25(OH)D agreement was good between the methods for 25(OH)D values ($r = 0.9927$, $P < 0.0001$; bias $-3.0 \pm 3.98\%$). Thus, there was not a significant bias observed for the ADVIA Centaur for healthy samples (endogenous + spiked). This demonstrates that DBP concentrations as high as 980 $\mu\text{g/mL}$ did not appear to interfere with the assay for this population. By comparison, endogenous serum DBP concentrations peaked at 519 $\mu\text{g/mL}$ in healthy subjects. Because the use of

DBP-spiked samples may be suspect, these results will be confirmed in future studies which evaluate a greater number of samples from healthy subjects containing endogenous serum DBP concentrations in the higher range (although it is unlikely that normal healthy subjects exist with DBP concentrations that can be achieved at the high spiking concentrations).

Depending on hormonal status or disease state serum matrix components may be different, and the levels of DBP may be higher or lower than those of healthy individuals [14, 15]. In women who are receiving estrogen therapy and those who are pregnant, higher serum estrogen levels correlate with increases in circulating DBP and total 1,25(OH)₂D. During pregnancy, increased 1,25(OH)₂D₃ occurs in response to the growing calcium demands of the fetus [14, 15]. Consistent with these reports, the mean DBP concentration was greater (415 $\mu\text{g/mL}$) for samples from pregnant women than for those from healthy subjects (347.6 $\mu\text{g/mL}$) and dialysis patients

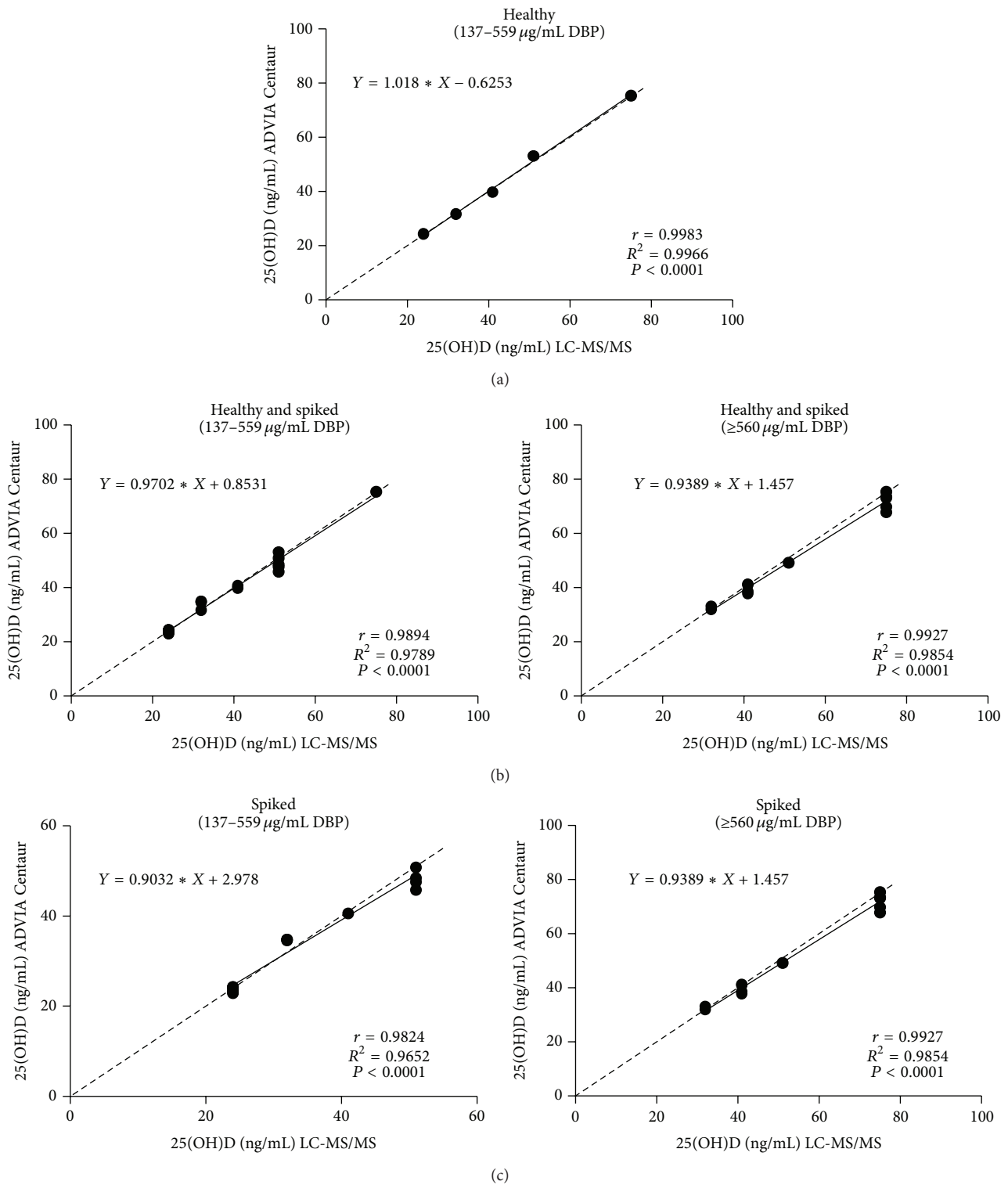


FIGURE 3: Correlation of 25(OH)D results obtained from the ADVIA Centaur Vitamin D Total assay and the LC-MS/MS method for normal human serum pooled samples: (a) endogenous, (b) endogenous and endogenous + spiked, and (c) endogenous + spiked. Dotted line: line of identity. To convert 25(OH)D concentrations to nanomoles per liter (nmol/L), multiply by 2.5.

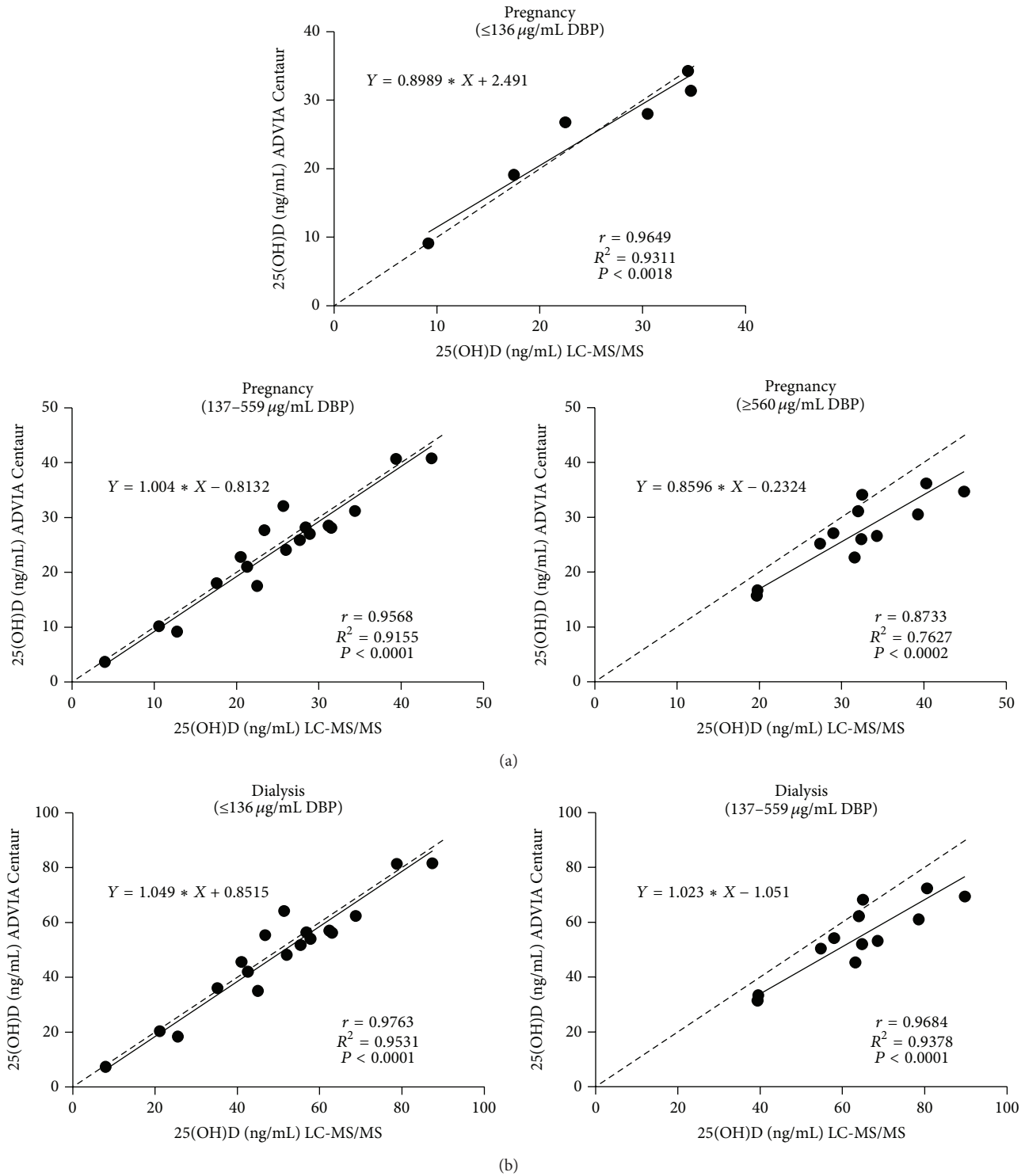


FIGURE 4: Correlation of 25(OH)D results obtained from the ADVIA Centaur Vitamin D Total assay and the LC-MS/MS method for (a) pregnancy (third trimester) samples and (b) renal dialysis samples. Dotted line: line of identity. To convert 25(OH)D concentrations to nanomoles per liter (nmol/L), multiply by 2.5.

(198 $\mu\text{g/mL}$). Despite the overall higher DBP concentrations in pregnancy serum, 25(OH)D results for those samples with low, medium, and high DBP concentrations demonstrated acceptable agreement between the ADVIA Centaur Vitamin

D Total assay and the LC-MS/MS method ($r = 0.96$, $P < 0.0018$, bias $2.0 \pm 10.9\%$; $r = 0.96$, $P < 0.0001$, bias $-3.0 \pm 12.6\%$; $r = 0.87$, $P < 0.0002$, bias $-14.0 \pm 9.9\%$, resp.). Although samples in the low and medium DBP range showed

TABLE 4: Mean bias (\pm SD) compared to LC-MS/MS for the low, medium, and high range DBP groups for combined populations and each population separately: healthy and DBP-spiked, DBP-spiked, pregnant women, and dialysis patients.

	DBP $\leq 136 \mu\text{g/mL}$	DBP 137–559 $\mu\text{g/mL}$	DBP $\geq 560 \mu\text{g/mL}$
Bias (ng/mL)			
Combined populations	1.67 \pm 3.33 (n = 24)	-0.45 \pm 3.07 (n = 57)	-3.0 \pm 3.98 (n = 25)
Healthy (endogenous and endogenous + spiked)		-2.78 \pm 2.12 (n = 18)	-1.88 \pm 2.47 (n = 12)
Healthy (endogenous + spiked)		-0.45 \pm 2.40 (n = 13)	-1.88 \pm 2.47 (n = 12)
Pregnancy	0.017 \pm 2.76 n = 6	-0.72 \pm 0.13 (n = 18)	-4.72 \pm 3.67 (n = 12)
Dialysis	2.23 \pm 3.38 (n = 18)	-0.38 \pm 3.91 (n = 21)	2.30 (n = 1)
% bias			
Combined populations	8.0 \pm 10.99% (n = 24)	-1.0 \pm 10.66% (n = 57)	-8.0 \pm 10.09% (n = 25)
Healthy (endogenous and endogenous + spiked)		0.0 \pm 5.30% (n = 18)	-3.0 \pm 3.98% (n = 12)
Healthy (endogenous + spiked)		0.0 \pm 6.10% (n = 13)	-3.0% \pm 3.98% (n = 12)
Pregnancy	2.0 \pm 10.9% (n = 6)	-3.0 \pm 12.6% (n = 18)	-14.0 \pm 9.9% (n = 12)
Dialysis	10.0 \pm 10.6% (n = 18)	0.0 \pm 12.4% (n = 21)	12.0% (n = 1)

DBP: vitamin D binding protein. To convert 25(OH)D concentrations to nanomoles per liter (nmol/L), multiply by 2.5.

less bias than those with very high DBP concentrations, the assay performance was acceptable for all groups. Four samples out of 36 contained measureable 25(OH)D₂; it is unlikely that 25(OH)D₂ influenced the assay bias because several samples lacking 25(OH)D₂ demonstrated similar levels of bias. In contrast to a previous study that found higher 25(OH)D levels in pregnant women compared to nonpregnant healthy women, this study found overall lower levels in pregnant women; this difference may relate to differences in vitamin D supplementation [6].

Nephrotic syndrome and CKD predialysis and dialysis patients demonstrate diminished serum levels of the bioactive 1,25(OH)₂D, likely due, in part, to impaired renal synthesis, nutritional deficit, and lower 25(OH)D substrate levels [23–26]. Although some studies report no change in serum DBP levels in renal failure patients compared with healthy individuals, other studies demonstrate lower serum levels and increased DBP urinary excretion; lower serum concentrations of DBP likely reflect increased urinary loss due to proteinuria, which is a common finding in CKD patients [15, 27, 28]. In this study, the overall mean 25(OH)D level was equivalent between the ADVIA Centaur Vitamin D Total assay and the LC-MS/MS method and for the low and medium range DBP groups ($r = 0.97$ overall; $r = 0.98$ low range DBP group; $r = 0.97$ middle range DBP group, $P < 0.0001$; bias was $4.35 \pm 12.4\%$ overall, $10.0 \pm 10.6\%$ for the low range DBP group and $0.0 \pm 12.4\%$ for the middle range DBP group, resp.), indicating acceptable

performance of the ADVIA Centaur Vitamin D Total assay in the presence of DBP and uremic serum. It is not known whether unique components of uremic serum contributed to the bias observed. Nineteen samples from dialysis patients had detectable 25(OH)D₂ (range 1.6 to 35 ng/mL), eight of which had levels above 10 ng/mL. The 25(OH)D₂ containing samples appeared to contribute to the positive bias in this patient population. This result is consistent with the performance of the ADVIA Centaur Vitamin D Total assay which demonstrates a slight difference in recovery for 25(OH)D₂ and 25(OH)D₃ (104.5% versus 100.7%) as stated in the Instructions for Use Manual [18]. Only one uremic sample was found in the higher range [1115.7 $\mu\text{g/mL}$ DBP; 19.1 ng/mL 25(OH)D₃ by LC-MS/MS and 21.4 ng/mL 25(OH)D by ADVIA Centaur; 12% bias of ADVIA Centaur to LC-MS/MS]. Whether an error in DBP measurement was the cause for the unusually high DBP concentration is not known. Although the serum 25(OH)D concentrations in dialysis patients were lower than those found in healthy individuals, the values were approximately normal (according to the Endocrine Society Guidelines). This is likely due to patient adherence to vitamin D supplementation which is indicated for end-stage renal disease patients on dialysis. It is worth noting that lower levels of serum 25(OH)D concentrations in predialysis patients correlate with a greater risk of mortality [29]. This underscores the need to accurately evaluate and monitor serum 25(OH)D levels in the CKD patient population.

A recent study implicated ineffective 25(OH)D-DBP extraction procedures as the cause of variability in an evaluation of five automated assays compared to an RMP LC-MS/MS method [6]. The study, which included samples from healthy individuals, pregnant women, dialysis patients, and intensive care patients, found that the bias was, at least in part, dependent on DBP concentration. The ADVIA Centaur Vitamin D Total assay in the present study differed from the assay in the previous study in that it has a different standardization; this version is standardized with internal standards traceable to the NIST-Ghent VDSP RMP. This may have had some impact on why the results of this study differ from those previously reported.

5. Conclusions

The small positive bias found in renal dialysis patients with DBP concentrations below those found in normal healthy subjects and small negative bias found in pregnant subjects with DBP levels above those found in normal healthy subjects were within the acceptable range for the assay. Thus, for populations with different levels of DBP, the 25(OH)D results obtained by the ADVIA Centaur Vitamin D Total immunoassay were equivalent to the sum of 25(OH)D₂ and 25(OH)D₃ using the LC-MS/MS method—especially for individuals with serum DBP concentrations within the range for the healthy population (137 to 559 µg/mL).

Disclosure

All authors are employees of Siemens Healthcare Diagnostics Inc.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgment

This study was supported by Siemens Healthcare Diagnostics Inc.

References

- [1] G. D. Carter, "Accuracy of 25-hydroxyvitamin D assays: confronting the issues," *Current Drug Targets*, vol. 12, no. 1, pp. 19–28, 2011.
- [2] G. D. Carter, J. L. Berry, E. Gunter et al., "Proficiency testing of 25-hydroxyvitamin D (25-OHD) assays," *Journal of Steroid Biochemistry and Molecular Biology*, vol. 121, no. 1–2, pp. 176–179, 2010.
- [3] B. W. Hollis, "Editorial: the determination of circulating 25-hydroxyvitamin D: no easy task," *Journal of Clinical Endocrinology and Metabolism*, vol. 89, no. 7, pp. 3149–3151, 2004.
- [4] C. T. Sempos, H. W. Vesper, K. W. Phinney, L. M. Thienpont, P. M. Coates, and Vitamin D Standardization Program (VDSP), "Vitamin D status as an international issue: national surveys and the problem of standardization," *Scandinavian Journal of Clinical and Laboratory Investigation*, vol. 72, no. 243, pp. 32–40, 2012.
- [5] A. M. Wallace, S. Gibson, A. de la Hunty, C. Lamberg-Allardt, and M. Ashwell, "Measurement of 25-hydroxyvitamin D in the clinical laboratory: current procedures, performance characteristics and limitations," *Steroids*, vol. 75, no. 7, pp. 477–488, 2010.
- [6] A. C. Heijboer, M. A. Blankenstein, I. P. Kema, and M. M. Buijs, "Accuracy of 6 routine 25-hydroxyvitamin D assays: influence of vitamin D binding protein concentration," *Clinical Chemistry*, vol. 58, no. 3, pp. 543–548, 2012.
- [7] N. E. Cooke and J. G. Haddad, "Vitamin D binding protein (Gc-globulin)," *Endocrine Reviews*, vol. 10, no. 3, pp. 294–307, 1989.
- [8] B. W. Hollis, "Comparison of equilibrium and disequilibrium assay conditions for ergocalciferol, cholecalciferol and their major metabolites," *Journal of Steroid Biochemistry*, vol. 21, no. 1, pp. 81–86, 1984.
- [9] L. M. Thienpont, H. C. M. Stepman, and H. W. Vesper, "Standardization of measurements of 25-Hydroxyvitamin D3 and D2," *Scandinavian Journal of Clinical and Laboratory Investigation*, vol. 72, no. 243, pp. 41–49, 2012.
- [10] US Renal Data System, *Annual Data Report: Atlas of End-Stage Renal Disease in the United States: International Comparisons*, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, Md, USA, 2007.
- [11] R. Bouillon, H. van Baelen, and P. de Moor, "The measurement of the vitamin D binding protein in human serum," *Journal of Clinical Endocrinology and Metabolism*, vol. 45, no. 2, pp. 225–231, 1977.
- [12] T. J. Gilbertson and R. P. Stryd, "High-performance liquid chromatographic assay for 25-hydroxyvitamin D3 in serum," *Clinical Chemistry*, vol. 23, no. 9, pp. 1700–1704, 1977.
- [13] V. Justova, L. Starka, H. Wilczek, and V. Pacovsky, "A simple radioassay for 25 hydroxycholecalciferol without chromatography," *Clinica Chimica Acta*, vol. 70, no. 1, pp. 97–102, 1976.
- [14] R. Bouillon, F. A. van Assche, H. van Baelen, W. Heyns, and P. de Moor, "Influence of the vitamin D-binding protein on the serum concentration of 1,25-dihydroxyvitamin D3. Significance of the free 1,25-dihydroxyvitamin D3 concentration," *Journal of Clinical Investigation*, vol. 67, no. 3, pp. 589–596, 1981.
- [15] J. G. Haddad Jr. and J. Walgate, "Radioimmunoassay of the binding protein for vitamin D and its metabolites in human serum. Concentrations in normal subjects and patients with disorders of mineral homeostasis," *Journal of Clinical Investigation*, vol. 58, no. 5, pp. 1217–1222, 1976.
- [16] Clinical and Laboratory Standards Institute (NCCLS), *Interference Testing in Clinical Chemistry*, NCCLS Document EP7-A2, Clinical and Laboratory Standards Institute, Wayne, Pa, USA, 2nd edition, 2005.
- [17] H. C. M. Stepman, A. Vanderroost, K. van Uytvanghe, and L. M. Thienpont, "Candidate reference measurement procedures for serum 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2 by using isotope-dilution liquid chromatography-tandem mass spectrometry," *Clinical Chemistry*, vol. 57, no. 3, pp. 441–448, 2011.
- [18] Siemens ADVIA Centaur XP Vitamin D Total (VitD) Assay, [Directional Insert 10699279_en rev a], Siemens Healthcare Diagnostics, Tarrytown, NY, USA, 2013.
- [19] Clinical and Laboratory Standards Institute (NCCLS), *Evaluation of Precision Performance of Quantitative Measurement Methods*, NCCLS Document EP5-A2, Clinical and Laboratory Standards Institute, Wayne, Pa, USA, 2nd edition, 2004.

- [20] M. Kawakami, C. B. Blum, R. Ramakrishnan, R. B. Dell, and D. S. Goodman, "Turnover of the plasma binding protein for vitamin D and its metabolites in normal human subjects," *Journal of Clinical Endocrinology and Metabolism*, vol. 53, no. 6, pp. 1110–1116, 1981.
- [21] M. Speeckaert, G. Huang, J. R. Delanghe, and Y. E. C. Taes, "Biological and clinical aspects of the vitamin D binding protein (Gc-globulin) and its polymorphism," *Clinica Chimica Acta*, vol. 372, no. 1-2, pp. 33–42, 2006.
- [22] W. G. Miller, G. L. Myers, M. L. Gantzer et al., "Roadmap for harmonization of clinical laboratory measurement procedures," *Clinical Chemistry*, vol. 57, no. 8, pp. 1108–1117, 2011.
- [23] W. Al-Badr and K. J. Martin, "Vitamin D and kidney disease," *Clinical Journal of the American Society of Nephrology*, vol. 3, no. 5, pp. 1555–1560, 2008.
- [24] E. A. González, A. Sachdeva, D. A. Oliver, and K. J. Martin, "Vitamin D insufficiency and deficiency in chronic kidney disease: a single center observational study," *The American Journal of Nephrology*, vol. 24, no. 5, pp. 503–510, 2004.
- [25] R. E. LaClair, R. N. Hellman, S. L. Karp et al., "Prevalence of calcidiol deficiency in CKD: a cross-sectional study across latitudes in the United States," *The American Journal of Kidney Diseases*, vol. 45, no. 6, pp. 1026–1033, 2005.
- [26] S. Williams, K. Malatesta, and K. Norris, "Vitamin D and chronic kidney disease," *Ethnicity & Disease*, vol. 19, no. 4, supplement 5, pp. S5-8–S5-11, 2009.
- [27] K. M. Thrailkill, C. Jo, G. E. Cockrell, C. S. Moreau, and J. L. Fowlkes, "Enhanced excretion of vitamin D binding protein in type 1 diabetes: a role in vitamin D deficiency?" *Journal of Clinical Endocrinology and Metabolism*, vol. 96, no. 1, pp. 142–149, 2011.
- [28] H. J. C. van Hoof, R. G. L. de Sévaux, H. van Baelen et al., "Relationship between free and total 1,25-dihydroxyvitamin D in conditions of modified binding," *European Journal of Endocrinology*, vol. 144, no. 4, pp. 391–396, 2001.
- [29] R. Mehrotra, D. A. Kermah, I. B. Salusky et al., "Chronic kidney disease, hypovitaminosis D, and mortality in the United States," *Kidney International*, vol. 76, no. 9, pp. 977–983, 2009.

Review Article

Vitamin D Binding Protein and Bone Health

Ishir Bhan

Massachusetts General Hospital, Harvard Medical School, 5 Suite 750, 50 Staniford Street, Boston, MA 02114, USA

Correspondence should be addressed to Ishir Bhan; ibhan@mgh.harvard.edu

Received 24 February 2014; Accepted 4 May 2014; Published 1 June 2014

Academic Editor: Xiangbing Wang

Copyright © 2014 Ishir Bhan. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Vitamin D binding protein (DBP) is the major carrier protein of 25-hydroxyvitamin D (25(OH) D) in the circulation, where it may serve roles in maintaining stable levels during times of decreased 25(OH) availability and in regulating delivery of 25(OH) D to target tissues. Several genetic polymorphisms of DBP have been described that lead to phenotypic changes in the protein that may affect affinity, activity, and concentration. These polymorphisms have been linked with alterations in bone density in several populations. One of the mechanisms by which DBP may alter bone health involves regulating vitamin D bioavailability. DBP-bound vitamin is thought to be relatively unavailable to target tissues, and thus alterations in DBP levels or affinity could lead to changes in vitamin D bioactivity. As a result, functional vitamin D status may differ greatly between individuals with similar total 25(OH) D levels. Additionally, DBP may have independent roles on macrophage and osteoclast activation. This review will summarize recent findings about DBP with respect to measures of bone density and health.

1. Introduction

Vitamin D binding protein (DBP) is a 58 kDa circulating alpha globulin produced primarily by the liver. While initially known as Gc-globulin (group-specific component of serum), it has been renamed for its ability to bind the vast majority (>85%) of circulating 25-hydroxyvitamin D (25(OH) D). DBP is a member of the same protein family as albumin and is produced at relatively stable levels throughout life, though high estrogen states like pregnancy can promote increased production [1]. While it is best known for its vitamin D binding properties, it may have roles in other biological processes as well. Additional actions attributed to DBP include binding of extracellular actin and transport of fatty acids. DBP also appears to protect the complement factor C5a from proteolytic degradation, effectively enhancing its action as a chemotactic protein [2]. A deglycosylated form of DBP, DBP-macrophage activating factor (DBP-MAF), is able to promote activation of macrophages and osteoclasts, and even native DBP may have effects on osteoclasts [3]. Given both its vitamin D binding characteristics and its potential direct actions on bone resorption, considerable interest has been generated in the scientific community surrounding the potential actions of DBP on bone metabolism and health.

Fueling this interest has been the discovery of significant interindividual differences in DBP levels. Early data points to associations between differences in DBP levels and bone density [4]. While some differences in DBP can be explained based on clinical characteristics, a moderate amount of variation in both levels and action appears to be driven by genetic polymorphisms.

2. Polymorphisms of DBP

Although over 120 variant forms of DBP have been recorded [5], three main phenotypic alleles have been described in the literature, initially identified based on their electrophoretic migration pattern. The slowest migrating is GC2, followed by GC1S (slow) and GC1F (fast) [6]. These phenotypic variants differ in both the associated concentration of DBP in the serum and their affinity for 25(OH) D and possibly other characteristics (see Table 1) [7]. In addition, there is substantial racial and geographic variation with these different forms. GC1F, which is associated with the lowest DBP concentration when present in homozygotes (though controversy remains about this topic), is more common in dark-skinned individuals, particularly those of African descent, while GC2 is more

TABLE I: Common phenotypic variants of DBP and associated characteristics.

Phenotype	rs7041 (D432E)	rs4588 (T436K)	DBP levels in homozygotes	25(OH) D affinity
Gc1F	t (D: asp)	c (T: thr)	Lowest	Highest
Gc1S	g (E: glu)	c (T: thr)	Highest	Intermediate
GC2	t (D: asp)	a (K: lys)	Intermediate	Lowest

The three most widely studied variants of DBP include Gc1F, Gc1S, and GC2, which are distinguished by their SNPs rs7041 and rs4588. The associated nucleotide and amino acid changes are presented, along with known data on DBP levels in homozygotes and affinity for 25(OH) D (derived from Powe et al. [9] and Arnaud and Constans [7]). Conflicting data regarding these relationships remain [10].

common in Caucasians [1]. The structural differences in these polymorphic forms are quite limited. Gc1S and GC2 differ from Gc1F only by single amino acid differences, tracked to single nucleotide polymorphisms. The rs7041 polymorphism leads to a substitution of g for t and, thus, a glutamate for an aspartate in GIS. The rs4588 polymorphism substitutes a for c and, thus, lysine for threonine in GC2 [8]. As each individual has two copies of the DBP gene, the combination of these various alleles may influence the levels and behavior of DBP on target tissues, including bone. Most studies of DBP's effects on bone in humans have focused on characterizing these allelic variants and attempting to correlate them with bone health.

3. DBP Polymorphisms and Bone Health

As women are at highest risk for the development of osteoporosis later in life, studies of DBP and bone disease have been concentrated in women. One of the first studies, originating over 20 years ago, looked at DBP in 258 nonblack elderly women, aged 65–90 [11]. The subjects were randomly selected as part of a prospective study of falls and fractures and underwent bone densitometry measurements at three sites (calcaneus, proximal radius, and distal radius) as well as height measurement. Phenotyping of DBP was performed based on electrophoretic analysis. Over two-thirds of the individuals in this study had either Gc1S/1S or Gc1S/2 diplotype, with less than three percent carrying the lowest-DBP allelic combination, Gc1F/1F. This lack of heterogeneity may have limited the ability of the authors to detect clinical differences between these alleles and, indeed, no statistically significant association with these allelic forms was found for bone mineral density (BMD), with respect to height or with respect to other bony measurements (os-calcis area and elbow breadth), even after multivariable adjustment for age and obesity.

Several more recent studies, however, are at odds with these early findings. While most studies have focused on the two major SNPs (rs7041 and rs4588) seen in Caucasians and people of African descent, Ezura et al. examined 13 DBP polymorphisms in a cohort of 384 postmenopausal Japanese women [12]. Five of these variants were significantly associated with BMD; one of these SNPs ($-39C > T$) was found in the promoter region, while the remainder were intronic. The combination of the exonic D432E (rs7041, which defines Gc1S) with another SNP (IVS1 + 827C > T) was most strongly associated with BMD, suggesting that

examining multiple SNPs simultaneously may be optimal for determining the effects of these polymorphisms on bone health, at least in some populations. Neither D432E nor T436K (rs4588) was significantly associated with DBP when examined in isolation in this population.

Studies in Western populations have yielded slightly different results. Lauridsen and colleagues used isoelectric focusing to determine Gc phenotype in 595 postmenopausal white Danish women as part of the Danish Osteoporosis Prevention Study, a 20-year partly randomized multicenter study of osteoporotic fracture prevention with hormone replacement therapy [13]. They identified large populations of Gc1/Gc1 and Gc1/Gc2 and a smaller population of Gc2/Gc2. They examined associations of these phenotypes with a historical evaluation of fracture number (prior to enrollment in the cohort), baseline bone mineral content (BMC), and baseline BMD at various sites (including forearm, lumbar spine, and hip). Subjects with Gc1/Gc1 were the most likely to have had a fracture, while Gc2/Gc2 subjects had the least, with Gc1/2 individuals falling into an intermediate group. The effects were particularly pronounced for low-energy fractures. Despite these historical clinical associations, there neither were differences in measured BMC or BMD between the subgroups nor were there any differences in circulating markers of bone turnover. The authors also examined the effects of plasma DBP concentration; while no effect was seen in the overall population, DBP concentrations were negatively correlated with BMD at all sites in women who had a history of fractures. These results suggest that there may be subpopulations in which DBP phenotype is particularly important. Notably, a separate analysis of this cohort demonstrated unusually high DBP levels in subjects with Gc1F/Gc1F, conflicting with more recent data [10]. This discrepancy may reflect changes in DBP assays over time or other biological differences in the relatively small number of individuals with this phenotype ($n = 17$).

While the mechanism underlying the observed associations was not clear, a subsequent analysis found that levels of both 25(OH) D and 1,25-dihydroxyvitamin D ($1,25(\text{OH})_2 \text{D}$) were lower in Gc2/Gc2 individuals compared with Gc1/Gc1 (with Gc1/Gc2 again falling between the two) [14]. Another study of 741 European white women similarly examined the relationship between the two common DBP SNPs and 25(OH) D concentrations [15]. This study similarly found that the SNP variant associated with GC2 (rs4588) was associated with lower plasma 25(OH) D and also found similar results for the polymorphism at rs7041 associated with Gc1F. However, DBP polymorphisms explained only 2% or less of the

variation in 25(OH) D levels, similar to the amount explained by vitamin D intake. A third study extended these findings to infants and toddlers. Carpenter et al. studied the relationship between DBP polymorphisms and 25(OH) D levels in over 750 urban US children, 6–36 months of age [8]. Children were genotyped based on the two most commonly studied SNPs (rs7041 and rs4588). Only the rs4588 SNP appeared to be associated with 25(OH) D levels. When assessed by diplotype, genetic variation in DBP did appear to be associated overall with variation in 25(OH) D level, though the effect was again mild. As with prior studies, the polymorphism associated with the GC2 phenotype was linked with lower 25(OH) D levels. The authors also concluded based on multivariate analysis that only some of the genotypic associations of DBP with 25(OH) D could be attributed to differences in plasma DBP concentrations. Additional factors may be differences in affinity for 25(OH) D between the phenotypes or in other aspects of DBP action.

A recent study examined associations of DBP genotype with 25(OH) D levels, parathyroid hormone (PTH) levels, and bone density in a group of 231 Finnish children aged 7–19 [16]. Genotyping was done based only on rs4588, which identifies the GC2 phenotype as distinct from the GC1 forms, though only 6% of the study population was homozygous for GC2. GC2 homozygosity was again associated with the lowest 25(OH) D levels. DBP levels were not examined. The authors found that the effect of DBP genotype differed by gender. GC1 variants were associated with higher bone density only in boys, though another measure of bone health (strength and strain index) was significant in both genders. As expected, 25(OH) D levels were generally inversely associated with PTH levels, including within genotypes. Despite this, though GC2 homozygotes had the lowest 25(OH) D levels, they also had the lowest PTH levels. The authors hypothesize that this may reflect differential amounts of bound versus unbound 25(OH) D as a fraction of total 25(OH) D, thus affecting the PTH-vitamin D relationship. How this affects bone health itself is still unclear. Of note, rs4588 is located in exon 11, which is thought to affect the non-vitamin D binding activities of DBP, including potential effects on macrophage and osteoclast activation as part of DBP-MAF activity, so the effects on vitamin D biology may not be the only relevant factor to changes in bone density.

The gender differences in the association of DBP with BMD have also been seen in adult populations. Xu et al. studied 1873 individuals from 405 Caucasian European families, using the newer metric of compression strength index (CSI), which accounts for weight and the periosteal diameter of the femoral neck in the interpretation of femoral BMD [17]. Unlike most studies of DBP polymorphisms, the authors included 12 SNPs in the analysis, none of which were the common rs7041 and rs4588. Two were found to be significantly associated with CSI (rs222029 and rs222020; both are intronic), but gender-specific analysis revealed that these associations were driven entirely by men. The authors hypothesize that this may be due to a greater association between 25(OH) D and muscle strength in males, but the exact reason for the observed gender differences in this and other studies remains elusive.

Other studies have echoed the observations that DBP is associated with bone density in men. Rapado found that DBP concentration was positively associated with lumbar spine BMD in 140 elderly men (aged 55–90) [18]. Non-SNP variants of DBP may also be important in men. One polymorphism is a variable number of (TAAA)_n-Alu sequences in intron 8, which has been associated with an effect on plasma levels of DBP [4]. Al-Oanzi and colleagues studied this polymorphism in 170 male subjects that included 114 healthy males and 56 with idiopathic osteoporosis and low trauma fractures [19]. Specific alleles of the (TAAA)_n-Alu repeat expansion were associated with bone mineral density as well as DBP concentrations. However, studies of DBP and bone health in men have not universally been positive. A study of 211 men over 70 years of age found no association of either the conventional DBP phenotypes or (TAAA)_n repeat expansion genotypes in BMD or markers of bone turnover [20]. Replication of these genetic associations is key for validating early findings.

4. DBP and Vitamin D Bioavailability

The majority of both 25(OH) D and 1,25(OH)₂ D circulate in large part bound to DBP (85–90%). A lesser fraction is bound weakly to albumin, while less than 1% circulates in its free form [21]. Animal studies suggest that DBP serves to protect 25(OH) D from degradation, prolonging its half-life and protecting against vitamin D deficiency [22]. In addition to stabilizing vitamin D concentrations, however, DBP appears to slow the action of vitamin D in the intestine and reduce uptake by the liver. Additionally, other studies have demonstrated that DBP diminishes the action of vitamin D on target tissues such as monocytes and keratinocytes [23, 24]. These findings have supported the application of the free hormone hypothesis to vitamin D. This model postulates that only hormones that are not bound to the carrier proteins are free to enter cells and induce biological actions [25]. Following the identification of binding coefficients of 25(OH) D and 1,25(OH)₂ D with albumin and DBP [21, 26], relative fractions of these hormones in bound and unbound states could be calculated from their total concentrations and the concentrations of albumin and DBP. Vitamin D behaves similar to other hormones such as testosterone, where binding to a specific binding protein is much stronger than to albumin. Albumin-bound hormone can be considered similar to free hormone as it is available to act on target tissues, while binding-protein bound hormone cannot. Indeed, given this weak binding, fluctuations in albumin level (e.g., in many disease states) would not be expected to markedly affect vitamin D bioactivity. Our group adapted the previously validated formulae for bioavailable testosterone to vitamin D to define bioavailable vitamin D concentrations [27, 28]. In a study of 49 healthy young adults, we found that bioavailable 25(OH) D (not bound to DBP) was highly correlated with bone density, while total 25(OH) D was not [28].

A follow-up study in a cohort of incident hemodialysis patients similarly found that bioavailable 25(OH) D was positively correlated with serum calcium and negatively correlated with PTH levels, while 25(OH) D displayed no

such relationship [29]. In a recent publication, we examined the effects of race and DBP allelic variants in a large cohort of black and white Americans, focusing on the commonly studied SNPs rs7041 and rs4588 [9]. Blacks displayed lower 25(OH) D and DBP levels, but higher BMD. Genetic polymorphism explained the vast majority of the variation in DBP levels (79.4%). Though higher PTH levels were associated with lower 25(OH) D levels as expected, blacks had markedly lower 25(OH) D levels compared with whites within each quintile of PTH. This discrepancy appeared to be explained by assessment of bioavailable 25(OH) D, which was similar between the two races and displayed similar associations with PTH when examined by quintile. Some notable race-specific effects, however, were found. The T variant of rs7041 was associated with lower DBP in both races but lower 25(OH) D levels only in blacks, while the A variant at rs4588 was associated with higher DBP in both races but lower 25D only in whites. BMD was associated with total and bioavailable 25(OH) D levels only in whites. The DBP diplotype associated with the lowest DBP levels (Gc1F/1F) was common in blacks, but rare in whites. These marked racial differences are likely to be key for interpreting and assessing generalizability of studies involving both vitamin D and DBP.

Ongoing controversies remain with the interpretation of DBP levels. Assays are not standardized and can yield different results from one another, either due to differential binding to different DBP isoforms or due to assay nonspecificity [30–32]. While most studies of free or bioavailable vitamin D have relied on calculated values based on binding coefficients, more precise methods for assessing DBP binding are being developed. One recent study included a novel immunoassay that aims to directly measure free 25(OH) D and compared this with the conventional calculated approach in 155 individuals, including some cirrhotic and some pregnant subjects [33]. This study found that, as expected, DBP and albumin levels were the lowest in subjects with cirrhosis but that measured free 25(OH) D was the highest. Despite the higher DBP levels in pregnancy, however, measured free 25(OH) D was not different in pregnant women than in nonpregnant subjects. While calculated and measured free 25(OH) D levels were correlated, this correlation was relatively weak, explaining only 13% of the variability, and was particularly weak in African Americans. The authors also found that only measured and total 25(OH) D levels correlated with PTH. Additional assays to directly assess free and bioavailable vitamin D are under development, and all will require additional validation and comparison with clinical outcomes to determine the optimal assay.

5. Summary and Future Directions

A renewed interest in DBP has accompanied a general upswing in vitamin D research in recent years and, indeed, DBP may be key for understanding and interpreting vitamin D's action, particularly with respect to bone health. Numerous studies have found that DBP levels and phenotypic variants correlate with both total 25(OH) D levels and BMD and other measures of bone health. However, these

associations have not been consistent in the literature, and the magnitude of these effects is still of unclear clinical significance. Furthermore, many studies of DBP variants have been performed in specific, homogenous populations and how well these findings translate more broadly is not well known at this time. Additional large population studies are needed to better define the role of DBP and its variants on biological outcomes. The concept of bioavailable vitamin D, the fraction of vitamin D not bound to DBP, may explain some of DBP's effects on bone health and will be explored further as dedicated assays are developed. Additional actions, including DBP's actions on monocyte and osteoclast activation, may also be important in determining bone density. As yet unrealized is the potential for drugs that influence DBP or its binding that might be useful in novel approaches to the treatment or prevention of bone disease. This remains an exciting avenue for future investigation.

Disclosure

A provisional patent on behalf of the author has been filed on the measurement of bioavailable vitamin D and outcomes.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

References

- [1] M. Speeckaert, G. Huang, J. R. Delanghe, and Y. E. C. Taes, "Biological and clinical aspects of the vitamin D binding protein (Gc-globulin) and its polymorphism," *Clinica Chimica Acta*, vol. 372, no. 1-2, pp. 33–42, 2006.
- [2] C. A. Piquette, R. Robinson-Hill, and R. O. Webster, "Human monocyte chemotaxis to complement-derived chemotaxins is enhanced by Gc-globulin," *Journal of Leukocyte Biology*, vol. 55, no. 3, pp. 349–354, 1994.
- [3] O. A. Adebajo, B. S. Moonga, J. G. Haddad, C. L.-H. Huang, and M. Zaidi, "A possible new role for vitamin D-binding protein in osteoclast control: inhibition of extracellular Ca^{2+} sensing at low physiological concentrations," *Biochemical and Biophysical Research Communications*, vol. 249, no. 3, pp. 668–671, 1998.
- [4] S. S. Papiha, L. C. Allcroft, R. M. Kanan, R. M. Francis, and H. K. Datta, "Vitamin D binding protein gene in male osteoporosis: association of plasma DBP and bone mineral density with (TAAA)(n)-Alu polymorphism in DBP," *Calcified Tissue International*, vol. 65, no. 4, pp. 262–266, 1999.
- [5] H. Cleve and J. Constans, "The mutants of the vitamin-D-binding protein: more than 120 variants of the GC/DBP system," *Vox Sanguinis*, vol. 54, no. 4, pp. 215–225, 1988.
- [6] J. G. Haddad, "Plasma vitamin D-binding protein (Gc-globulin): multiple tasks," *Journal of Steroid Biochemistry and Molecular Biology*, vol. 53, no. 1-6, pp. 579–582, 1995.
- [7] J. Arnaud and J. Constans, "Affinity differences for vitamin D metabolites associated with the genetic isoforms of the human serum carrier protein (DBP)," *Human Genetics*, vol. 92, no. 2, pp. 183–188, 1993.

- [8] T. O. Carpenter, J. H. Zhang, E. Parra et al., "Vitamin D binding protein is a key determinant of 25-hydroxyvitamin D levels in infants and toddlers," *Journal of Bone and Mineral Research*, vol. 28, no. 1, pp. 213–221, 2013.
- [9] C. E. Powe, M. K. Evans, J. Wenger et al., "Vitamin D-binding protein and vitamin D status of black Americans and white Americans," *The New England Journal of Medicine*, vol. 369, no. 21, pp. 1991–2000, 2013.
- [10] A. L. Lauridsen, P. Vestergaard, and E. Nexø, "Mean serum concentration of vitamin D-binding protein (Gc globulin) is related to the Gc phenotype in women," *Clinical Chemistry*, vol. 47, no. 4, pp. 753–756, 2001.
- [11] J. E. Eichner, J. A. Cauley, R. E. Ferrell, S. R. Cummings, and L. H. Kuller, "Genetic variation in two bone-related proteins: is there an association with bone mineral density or skeletal size in postmenopausal women?" *Genetic Epidemiology*, vol. 9, no. 3, pp. 177–184, 1992.
- [12] Y. Ezura, T. Nakajima, M. Kajita et al., "Association of molecular variants, haplotypes, and linkage disequilibrium within the human vitamin D-binding protein (DBP) gene with postmenopausal bone mineral density," *Journal of Bone and Mineral Research*, vol. 18, no. 9, pp. 1642–1649, 2003.
- [13] A. L. Lauridsen, P. Vestergaard, A. P. Hermann, H. J. Møller, L. Mosekilde, and E. Nexø, "Female premenopausal fracture risk is associated with Gc phenotype," *Journal of Bone and Mineral Research*, vol. 19, no. 6, pp. 875–881, 2004.
- [14] A. L. Lauridsen, P. Vestergaard, A. P. Hermann et al., "Plasma concentrations of 25-hydroxy-vitamin D and 1,25-dihydroxy-vitamin D are related to the phenotype of Gc (vitamin D-binding protein): a cross-sectional study on 595—early postmenopausal women," *Calcified Tissue International*, vol. 77, no. 1, pp. 15–22, 2005.
- [15] M. Sinotte, C. Diorio, S. Bérubé, M. Pollak, and J. Brisson, "Genetic polymorphisms of the vitamin D binding protein and plasma concentrations of 25-hydroxyvitamin D in premenopausal women," *American Journal of Clinical Nutrition*, vol. 89, no. 2, pp. 634–640, 2009.
- [16] M. Pekkinen, E. Saarnio, H. T. Viljakainen et al., "Vitamin D binding protein genotype is associated with serum 25-hydroxyvitamin D and PTH concentrations, as well as bone health in children and adolescents in Finland," *PLOS ONE*, vol. 9, no. 1, Article ID e87292, 2014.
- [17] X.-H. Xu, D.-H. Xiong, X.-G. Liu et al., "Association analyses of vitamin D-binding protein gene with compression strength index variation in Caucasian nuclear families," *Osteoporosis International*, vol. 21, no. 1, pp. 99–107, 2010.
- [18] A. Rapado, F. Hawkins, L. Sobrinho et al., "Bone mineral density and androgen levels in elderly males," *Calcified Tissue International*, vol. 65, no. 6, pp. 417–421, 1999.
- [19] Z. H. Al-Oanzi, S. P. Tuck, S. S. Mastana et al., "Vitamin D-binding protein gene microsatellite polymorphism influences BMD and risk of fractures in men," *Osteoporosis International*, vol. 19, no. 7, pp. 951–960, 2008.
- [20] Y. E. C. Taes, S. Goemaere, G. Huang et al., "Vitamin D binding protein, bone status and body composition in community-dwelling elderly men," *Bone*, vol. 38, no. 5, pp. 701–707, 2006.
- [21] D. D. Bikle, P. K. Siiteri, and E. Ryzen, "Serum protein binding of 1,25-dihydroxyvitamin D: a reevaluation by direct measurement of free metabolite levels," *Journal of Clinical Endocrinology and Metabolism*, vol. 61, no. 5, pp. 969–975, 1985.
- [22] F. F. Safadi, P. Thornton, H. Magiera et al., "Osteopathy and resistance to vitamin D toxicity in mice null for vitamin D binding protein," *Journal of Clinical Investigation*, vol. 103, no. 2, pp. 239–251, 1999.
- [23] R. F. Chun, A. L. Lauridsen, L. Suon et al., "Vitamin D-binding protein directs monocyte responses to 25-hydroxy- and 1,25-dihydroxyvitamin D," *Journal of Clinical Endocrinology and Metabolism*, vol. 95, no. 7, pp. 3368–3376, 2010.
- [24] D. D. Bikle and E. Gee, "Free, and not total, 1,25-dihydroxyvitamin D regulates 25-hydroxyvitamin D metabolism by keratinocytes," *Endocrinology*, vol. 124, no. 2, pp. 649–654, 1989.
- [25] C. M. Mendel, "The free hormone hypothesis: a physiologically based mathematical model," *Endocrine Reviews*, vol. 10, no. 3, pp. 232–274, 1989.
- [26] D. D. Bikle, B. P. Halloran, and E. Gee, "Free 25-hydroxyvitamin D levels are normal in subjects with liver disease and reduced total 25-hydroxyvitamin D levels," *Journal of Clinical Investigation*, vol. 78, no. 3, pp. 748–752, 1986.
- [27] A. Vermeulen, L. Verdonck, and J. M. Kaufman, "A critical evaluation of simple methods for the estimation of free testosterone in serum," *Journal of Clinical Endocrinology and Metabolism*, vol. 84, no. 10, pp. 3666–3672, 1999.
- [28] C. E. Powe, C. Ricciardi, A. H. Berg et al., "Vitamin D-binding protein modifies the vitamin D-bone mineral density relationship," *Journal of Bone and Mineral Research*, vol. 26, no. 7, pp. 1609–1616, 2011.
- [29] I. Bhan, C. E. Powe, A. H. Berg et al., "Bioavailable vitamin D is more tightly linked to mineral metabolism than total vitamin D in incident hemodialysis patients," *Kidney International*, vol. 82, no. 1, pp. 84–89, 2012.
- [30] B. W. Hollis and D. D. Bikle, "Vitamin D-binding protein and vitamin D in blacks and whites," *The New England Journal of Medicine*, vol. 370, no. 9, pp. 879–880, 2014.
- [31] R. Bouillon, K. Jones, and I. Schoenmakers, "Vitamin D-binding protein and vitamin D in blacks and whites," *The New England Journal of Medicine*, vol. 370, no. 9, p. 879, 2014.
- [32] C. E. Powe, S. A. Karumanchi, and R. Thadhani, "Vitamin D-binding protein and vitamin D in blacks and whites," *The New England Journal of Medicine*, vol. 370, no. 9, pp. 880–881, 2014.
- [33] J. B. Schwartz, J. Lai, B. Lizaola et al., "A comparison of direct and calculated free 25(OH) vitamin D levels in clinical populations," *The Journal of Clinical Endocrinology and Metabolism*, vol. 99, no. 5, pp. 1631–1637, 2014.

Research Article

Vitamin D-Binding Protein Levels in Plasma and Gingival Crevicular Fluid of Patients with Generalized Aggressive Periodontitis

Xin Zhang,^{1,2} Huanxin Meng,¹ Li Xu,¹ Li Zhang,¹ Dong Shi,¹ Xianghui Feng,¹ Ruifang Lu,¹ and Zhibin Chen¹

¹ Department of Periodontology, Peking University School and Hospital of Stomatology, 22 Zhongguancun Nandajie, Haidian District, Beijing 100081, China

² Department of Stomatology, Peking Union Medical College Hospital, Beijing 100730, China

Correspondence should be addressed to Huanxin Meng; kqhxmeng@bjmu.edu.cn

Received 24 January 2014; Revised 20 March 2014; Accepted 2 April 2014; Published 12 May 2014

Academic Editor: Xiangbing Wang

Copyright © 2014 Xin Zhang et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Vitamin D-binding protein (DBP) is the main transport protein of vitamin D and plays an important role in the immune system and host defenses. The purpose of this study was to measure DBP levels in plasma and gingival crevicular fluid (GCF) of patients with generalized aggressive periodontitis (GAgP), in comparison to healthy controls, with the goal of elucidating the relationship between DBP and GAgP. Fifty-nine GAgP patients and 58 healthy controls were recruited for the study; clinical parameters of probing depths (PD), bleeding index, and attachment loss (AL) were recorded. DBP levels were measured by enzyme-linked immunosorbent assay. From the results, GAgP patients had higher plasma DBP concentrations ($P < 0.001$) but lower GCF DBP concentrations ($P < 0.001$) than healthy controls. In GAgP group, after controlling the potential confounders of age, gender, smoking status, and BMI index, GCF DBP concentrations correlated negatively with PD ($P < 0.001$) and AL ($P = 0.009$). Within the limits of the study, we concluded that decreased GCF DBP level and increased plasma DBP level are associated with periodontitis.

1. Introduction

Periodontitis is primarily a bacterial infection caused by a diverse group of microorganisms [1]. Generalized aggressive periodontitis (GAgP) is a subtype of periodontitis that mainly affects younger patients and is characterized by episodic and rapid loss of periodontal supporting tissues [2]. Though microorganisms are considered to be the etiologic agent that causes this inflammatory lesion, it is the chemical mediators of inflammation that play a pivotal role in the loss of periodontal connective tissue, as well as supporting alveolar bone [3].

Vitamin D has been shown to have immunomodulatory effects *in vitro* and in animal studies. Previous research from our group revealed a relationship between 25-hydroxyvitamin D (25[OH]D) and GAgP. Specifically, we reported that plasma 25(OH)D levels were higher in patients with

GAgP, as compared to healthy controls, and were positively correlated with an index of gingival bleeding [4]. It has also been shown that initial periodontal therapy reduces both local and systemic 25(OH)D levels [5]. Furthermore, polymorphisms in the gene encoding the vitamin D receptor have been reported in several studies to be associated with periodontitis [6–10]. Taken together, these studies suggest a potential role for the vitamin D pathway in maintaining periodontal health.

Vitamin D-binding protein (DBP), also known as group-specific component (Gc), is another important element in vitamin D pathways. It is the main transport protein of vitamin D in circulation and plays an important part in vitamin D bioavailability. Besides, it also has anti-inflammatory and immunomodulatory functions independent of vitamin D carriage, such as coactivating macrophages, enhancing the chemotactic activity of C5-derived peptides, and associating

with many immune cell surfaces including neutrophils. In addition it scavenges actins released from necrotic cells, binds fatty acids, and plays a part in bone modulation [11, 12]. The diverse roles of DBP, including its relationship with vitamin D levels, indicate its potential importance in a range of diseases. For example, DBP was found in the airways of patients with chronic obstructive pulmonary disease and acute respiratory distress syndrome and is believed to stimulate the monocyte inflammatory response in the lung by enhancing monocyte responses to C5-derived peptides [13]. In terms of cardiovascular disease, high expression of DBP protein has been found in fresh thrombotic plaques and in the serum of patients with ST elevation myocardial infarction [14]. Other disease states associated with altered DBP levels include tuberculosis, type 1 diabetes, sepsis, and cystic fibrosis [15–18].

With regard to periodontitis, there are few previous studies which have studied DBP levels in relation to this disease. A relatively small study (1987) reported elevated DBP concentrations in whole saliva of patients with periodontitis, as compared to dentulous or edentulous control subjects, and positively correlated DBP concentration with Gingival Index scores [19]. More recently, Wu et al. compared the proteomic profile of whole unstimulated saliva in GAgP patients with those of healthy controls and confirmed the rise of saliva DBP levels in periodontitis [20]. Our previous study firstly determined higher plasma DBP levels in patients with GAgP and found that the levels were associated with inflammatory cell types and inflammatory markers [21]. The present study was to assess DBP levels in GCF as well as plasma of patients with GAgP in comparison to healthy controls and to find out the link between DBP and GAgP.

2. Materials and Methods

2.1. Study Population. Fifty-nine GAgP patients were recruited from the clinic of the Department of Periodontology at Peking University Hospital of Stomatology from July 2001 to October 2007. Diagnostic criteria for GAgP were defined according to the classification proposed at the International Workshop for the Classification of Periodontal Diseases and Conditions in 1999. Specifically, (1) the onset of the periodontal disease occurred under 35 years of age; (2) at least eight teeth had a probing depth (PD) over 6 mm and at least three of them were not first molars or incisors; and (3) radiographic evidence of alveolar bone loss was present. Fifty-eight periodontal healthy controls were selected from staff and students at the School of Stomatology; none of them had any clinical evidence of periodontitis (PD \leq 3 mm; the percentage of sites with bleeding on probing (BOP) was $<$ 10%; no attachment loss (AL) and no bone loss visible on radiographs). Subjects with systemic disease, who had periodontal therapy within the previous year, who received antibiotics within the previous 3 months, were pregnant or were receiving vitamin D or calcium supplements (e.g., vitamin D, calcium carbonate, calcium lactate, or calcium gluconate) were excluded from the study. Each subject in the study completed a questionnaire at the beginning of the study, and their age, height, weight, body mass index (BMI),

and smoking status were recorded. Smokers were defined as subjects who were currently smoking (smoked routinely and never stopped or tried to stop smoking in recent three months) and nonsmokers were defined as subjects who had never smoked or had ceased smoking more than one year. The study was conducted with informed consent of all subjects and approved by the Ethics Committee of Peking University Health Science Center.

2.2. Clinical Examination. A full-mouth periodontal examination of each subject was conducted using a William's periodontal probe. PD and AL were recorded for each tooth at six sites (mesial, distal, and middle sites of facial and lingual sides), except wisdom teeth. AL was measured as the distance between the cemento-enamel junction and the bottom of the periodontal pocket. Bleeding index (BI) [22] was also recorded for each tooth. Sites with PD over 6 mm and AL over 5 mm were defined as sites of severe periodontitis. The mean PD, AL, and BI and the percentage of severe sites were calculated for each subject. All examinations were performed by two experienced practitioners. In addition to the dental exam, each patient had a set of full-mouth periapical radiographs taken.

2.3. Sample Collection and Processing. The blood samples were obtained from each subject by standard venipuncture using EDTA-containing collection tubes; all blood samples were collected between 8:00 and 10:00 am. Plasma was separated by centrifugation and immediately stored at -70°C until the time of the DBP assay. GCF was collected at two sites from 22 GAgP patients (seven males and 15 females) and 23 healthy controls (nine males and 14 females). The GCF collection sites included one around the right maxillary incisor and the other in the region of left mandibular molar. In GAgP patients, all of these sites were affected by periodontitis, with PD over 4 mm and AL over 1 mm. The method of GCF collection and volume determination were performed as previously described [5]. Briefly, the test site was air-dried and isolated with a roll of cotton, and supragingival plaque was removed without touching the marginal gingiva. A paper strip (Whatman, Maidstone, UK) was then inserted into the respective periodontal pocket until mild resistance was felt and was then left in place for 30 seconds. Strips with any visible contamination of blood were discarded. Before sampling, each strip was placed into a sterile 0.5 mL Eppendorf tube and weighed using an electronic scale (AE240S, Mettler, Zurich, Switzerland). After GCF collection, the strip was placed into the same tube and reweighed within 30 minutes of collection. The tube containing the strip was then stored at -70°C until the time of the DBP assay. The difference between the weight of the tube and strip before and after collection was used to calculate the volume of GCF collected.

GCF is considered to be a serum exudate, as such we established a standard curve for GCF volume with GCF weight using human serum. Specifically, 0.1, 0.3, 0.5, 1.0, 1.5, and 2.5 μL of human serum were dropped onto individual paper strips. The difference in the weight of each strip before and after addition of a defined drop of human serum was used

to calculate the weight of serum added. A linear regression model between weight and volume was then established ($r^2 = 0.993$; $P < 0.001$).

2.4. DBP Assay. At the time of the DBP assay, the 0.5 mL Eppendorf tubes containing GCF-saturated strips were thawed out at room temperature and a 100 μL of phosphate buffered solution (pH 7.4) was added to each tube. The tubes were gently shaken at 4°C for 20 minutes and then centrifuged at 13,000 rpm (rotor radius = 5.5 cm) for 10 minutes. The DBP levels in the GCF supernatant and plasma were measured using a commercially available enzyme-linked immunosorbent assay kit (BioSource Systems, Invitrogen, Grand Island, NY, USA). These assays were performed according to the manufacturer's protocol. The total GCF DBP level was calculated according to the measured DBP concentration. Calculation of DBP concentration in each GCF sample was performed by dividing the amount of DBP by the volume of GCF.

2.5. Statistical Analysis. Both clinical and biochemical parameters were reported as mean \pm standard deviation. Variables were tested for normality using the Shapiro-Wilk test. As they were not normally distributed, the data were analyzed using nonparametric tests. The levels of DBP were compared between GAgP group and control group using the Mann-Whitney *U*-test. Partial correlations between DBP levels and periodontal clinical parameters were analyzed with adjustment for age, gender, smoking status, and BMI index. Correlations between GCF DBP levels and clinical parameters were analyzed on site-level data, and clinical parameters of BI, PD, and AL of the sample site were used. While analysis about correlations between plasma DBP levels and clinical parameters was based on subject-level data, the calculated clinical parameters of mean BI, PD, and AL and percentage of severe sites for each subject were used. Statistical analyses were carried out using SPSS software, version 11.5 (SPSS Inc, Chicago, IL, USA), and *P* values < 0.05 were considered statistically significant.

3. Results

The basic clinical characteristics of the GAgP and healthy control groups are summarized in Table 1. The two groups were not different with respect to age, gender, smoking status, and BMI index. As expected, generally more severe clinical indices of periodontal disease were observed in the GAgP group. The GCF data was presented on site level. The mean PD of the 44 periodontitis sites selected for GCF sampling was 6.70 ± 1.89 mm, with a range from 4 mm to 10 mm. The mean AL of these sites was 6.39 ± 2.52 mm, with a range from 1 mm to 13 mm. As presented in Table 2 and Figure 1, the GAgP group had significantly lower GCF DBP concentrations (13.39 ± 10.20 $\mu\text{g}/\mu\text{L}$ GCF versus 166.01 ± 298.12 $\mu\text{g}/\mu\text{L}$ GCF; $P < 0.001$) and higher plasma DBP concentrations (231.75 ± 47.86 $\mu\text{g}/\text{mL}$ versus 111.12 ± 20.97 $\mu\text{g}/\text{mL}$; $P < 0.001$) compared to the healthy control group. In GAgP group, after controlling the potential confounders of age,

TABLE 1: Basic clinical characteristics of GAgP patients and healthy controls.

	GAgP group (<i>n</i> = 59)	Control group (<i>n</i> = 58)
Age (years)	26.7 \pm 4.9	25.4 \pm 3.6
Gender (<i>n</i> ; male/female)	20/39	25/33
BMI (kg/m ²)	21.58 \pm 3.43	21.22 \pm 2.04
Smoking (<i>n</i> ; smoker/nonsmoker)	5/54	1/57
Mean BI	3.69 \pm 0.33*	1.13 \pm 0.15
Mean PD (mm)	4.81 \pm 0.99*	1.63 \pm 0.35
Mean AL (mm)	4.75 \pm 1.84*	0
Severe site % (% of sites)	32.14 \pm 21.09*	0

Data are presented as mean \pm SD or number of subjects as indicated; * $P < 0.05$, compared to healthy controls.

TABLE 2: Comparisons of GCF DBP levels and plasma DBP levels between GAgP patients and healthy controls.

DBP levels	GAgP group Mean \pm SD	Control group Mean \pm SD	<i>P</i> value
GCF DBP ($\mu\text{g}/\mu\text{L}$ GCF)	13.39 \pm 10.20 <i>n</i> = 44	166.01 \pm 298.12 <i>n</i> = 46	< 0.001
Plasma DBP ($\mu\text{g}/\text{mL}$)	231.75 \pm 47.86 <i>n</i> = 59	111.12 \pm 20.97 <i>n</i> = 58	< 0.001

TABLE 3: Partial correlations between GCF DBP concentrations and clinical parameters after adjusting for age, gender, smoking status, and BMI index.

GCF DBP ($\mu\text{g}/\mu\text{L}$ GCF)	GAgP group (<i>n</i> = 44)		Control group (<i>n</i> = 46)	
	<i>r</i>	<i>P</i> value	<i>r</i>	<i>P</i> value
BI	-0.481	0.768	None	—
PD	-0.504	0.001	None	—
AL	-0.410	0.009	None	—

gender, BMI, and smoking status, GCF DBP concentration correlated negatively with PD ($r = -0.504$, $P = 0.001$) and AL ($r = -0.410$, $P = 0.009$) of the sample site (Table 3). No correlations were found between plasma DBP concentration and periodontal clinical parameters in neither GAgP group nor control group as shown in Table 4.

4. Discussion

DBP is a plasma α_2 -globulin with a molecular weight of 52–59 kDa and is recognized as a member of a multigene family that includes albumin, α -fetoprotein, and α -albumin/afamin. The DBP gene is expressed in a wide variety of tissues, but the vast majority of serum DBP is derived from gene expression and secretion by the liver. It is abundant in plasma and exerts multiple biological functions, which range from transporting vitamin D metabolites, scavenging actins, to recently most discussed roles in immune system and host defense [11].

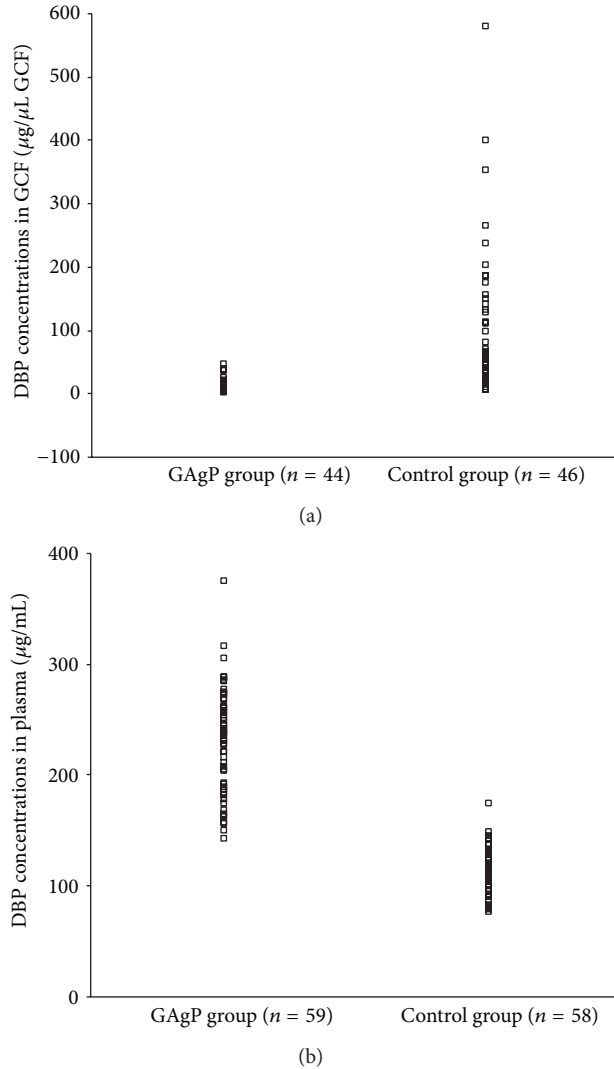


FIGURE 1: (a) DBP concentrations in GCF of GAgP group were significantly lower than in control group ($P < 0.001$); (b) DBP concentrations in plasma of GAgP group were significantly higher than in control group ($P < 0.001$).

TABLE 4: Partial correlations between plasma DBP concentrations and clinical parameters after adjusting for age, gender, smoking status, and BMI index.

plasma DBP ($\mu\text{g/mL}$)	GAgP group ($n = 59$)		Control group ($n = 58$)	
	r	P value	r	P value
Mean BI	0.1588	0.251	0.048	0.768
Mean PD	-0.1347	0.327	-0.190	0.216
Mean AL	-0.164	0.230	None	—
Severe sites %	-0.167	0.233	None	—

DBP was implied to be a normal constituent of parotid saliva and its levels in parotid saliva did not vary according to the presence or absence of periodontal disease and were irrespective of the presence or absence of the teeth [19].

While DBP concentrations in whole saliva of patients with periodontitis were increased compared to healthy controls and correlated positively with periodontal inflammation, the elevated DBP levels in whole saliva were reported less than $1 \mu\text{g/mL}$, which were far lower than those in GCF according to our reports and were proposed to come from GCF. It is reasonable considering the far differences between GCF and saliva DBP levels, and the fact that, in cases of periodontitis, both GCF volume and flow rate were increased significantly [23]. It was much easier for the high GCF DBP to flow into the mouth and finally contributed to the increased saliva DBP levels.

On healthy conditions, DBP presences in GCF were abundant and its levels were even higher than those in plasma. It is indicated that DBP might exert a role in periodontal health and that periodontal tissues might be another sources of GCF DBP other than serum. The significant decrease of GCF DBP levels in periodontitis patients might be a consequence of a lack of effective production or an increase of local consumption. For more information, it might be necessary to investigate the distributions or expressions of DBP in healthy and periodontitis tissues. Also, longitudinal study about how periodontal therapy influences local and plasma DBP levels is essential to confirm the relationship between DBP and periodontal inflammation. Nevertheless our data indicate that DBP is present in the GCF and available to participate in the inflammatory response to periodontal challenge.

DBP is the main transport protein of vitamin D in the circulation, and plasma 25(OH)D levels have been reported to be increased in patients with GAgP, as well as being associated with periodontal inflammation [4, 5]. In concordance with these previous studies, we observed that plasma DBP levels of patients with GAgP were higher than controls; however, no correlation between plasma DBP concentrations and the previously measured plasma 25(OH)D levels in the same 44 GAgP patients (data not shown) was found. We interpret this result to indicate that the increased DBP concentration might not be a direct response to a change in vitamin D status. Mechanisms independent of vitamin D levels might explain the rise in plasma DBP level seen in GAgP patients and also in the potential role of DBP in periodontitis. This finding is in agreement with a previous study in patients with type 1 diabetes, where altered DBP concentrations were not related to 25(OH)D levels [15].

In the current study, the plasma concentrations of DBP in healthy individual were assayed to be $77.51\text{--}175.10 \mu\text{g/mL}$, which were lower than those reported in the other race [11, 15]. It is worth noting that plasma 25(OH)D levels of Chinese people also seemed a little lower than in other people [4]. So one reason might be the differences caused by the human race. Besides the differences in the sensitivity of ELISA kits might make the data provided not always comparable.

DBP is implied to be an acute phase reactant produced by liver. Its hepatic synthesis could be upregulated by proinflammatory cytokines such as interleukin-6, and it is known that plasma interleukin-6 levels are increased in cases of GAgP, thus such a proinflammatory response may contribute to the increased DBP levels observed in patients with GAgP [24, 25].

In addition to the liver, several other tissues express DBP, including the kidney, adipose tissue, and gonads; however, it is not known whether DBP expression in these tissues can be regulated by the immune system [26]. In direct relation to cells of the immune system, it is known that DBP mRNA is expressed in activated monocytes [27]; it can be secreted by neutrophils and this level of secretion was enhanced after C5a stimulation [28]; it binds to the surface of neutrophils which is essential for DBP's chemotactic activity [29]; and neutrophils activated by LPS have an increased number of DBP-binding sites [30]. Taken together, these studies suggest that, in cases of periodontitis, increased numbers of neutrophils with an enhanced ability to secrete and bind DBP might also contribute to systemic increases in DBP levels.

From the results, GAgP patients have higher plasma DBP levels but lower GCF DBP levels when compared to healthy controls. But no significant correlations were found between these two presences in neither GAgP patients nor healthy controls, when taking the average of the two data as the GCF DBP levels for those 22 GAgP patients and 23 healthy controls (data not shown). According to the methods and materials, two sites of the individuals were chosen to collect GCF samples. These two sites were not always the most severe site of the patients with GAgP; on the other hand, the sampling sites were just two and may not represent the whole periodontal inflammation of the individual. Further research is needed to gain more detailed information about the potential roles of DBP in the pathogenesis of periodontitis.

5. Conclusions

In summary, DBP was measured in the GCF and plasma of patients with GAgP. In comparison to healthy controls, we found that patients with GAgP had higher plasma levels but lower GCF DBP concentrations. The greater the periodontal destruction is, the lesser the GCF DBP concentration is. Further research is needed to investigate the expressions of DBP in periodontium of periodontal health and disease and to study how periodontal therapy influences systemic and local DBP levels.

Conflict of Interests

The authors declare that there is no conflict of interests in this study.

Acknowledgments

This research was supported by the National Natural Science Foundations of China (30271411, 30471882, 30772420, 30973319, and 81271149); National Key Project of Scientific and Technical Supporting Programs of China (2002AA217091, 2007BAZ18B02); Clinical Research Fund, Ministry of Health P.R.C.

References

- [1] H. Loe, E. Theilade, and S. B. Jensen, "Experimental gingivitis in man," *Journal of Periodontology*, vol. 36, pp. 177–187, 1965.
- [2] "Parameter on aggressive periodontitis. American Academy of Periodontology," *Journal of Periodontology*, vol. 71, pp. 867–869, 2000.
- [3] R. J. Genco, "Host responses in periodontal diseases: current concepts," *Journal of Periodontology*, vol. 63, no. 4, pp. 338–355, 1992.
- [4] K. Liu, H. Meng, X. Tang et al., "Elevated plasma calcifediol is associated with aggressive periodontitis," *Journal of Periodontology*, vol. 80, no. 7, pp. 1114–1120, 2009.
- [5] K. Liu, H. Meng, R. Lu et al., "Initial periodontal therapy reduced systemic and local 25-hydroxy vitamin D3 and Interleukin-1 β in patients with aggressive periodontitis," *Journal of Periodontology*, vol. 81, no. 2, pp. 260–266, 2010.
- [6] B. J. W. Hennig, J. M. Parkhill, I. L. C. Chapple, P. A. Heasman, and J. J. Taylor, "Association of a vitamin D receptor gene polymorphism with localized early-onset periodontal diseases," *Journal of Periodontology*, vol. 70, no. 9, pp. 1032–1038, 1999.
- [7] S. Li, M. H. Yang, C. A. Zeng et al., "Association of vitamin D receptor gene polymorphisms in Chinese patients with generalized aggressive periodontitis," *Journal of Periodontal Research*, vol. 43, no. 3, pp. 360–363, 2008.
- [8] L. Nibali, M. Parkar, F. D'Aiuto et al., "Vitamin D receptor polymorphism (-1056 Taq-I) interacts with smoking for the presence and progression of periodontitis," *Journal of Clinical Periodontology*, vol. 35, no. 7, pp. 561–567, 2008.
- [9] K. S. Park, J. H. Nam, and J. Choi, "The short vitamin D receptor is associated with increased risk for generalized aggressive periodontitis," *Journal of Clinical Periodontology*, vol. 33, no. 8, pp. 524–528, 2006.
- [10] J. L. Sun, H. X. Meng, C. F. Cao et al., "Relationship between vitamin D receptor gene polymorphism and periodontitis," *Journal of Periodontal Research*, vol. 37, no. 4, pp. 263–267, 2002.
- [11] M. Speeckaert, G. Huang, J. R. Delanghe, and Y. E. C. Taes, "Biological and clinical aspects of the vitamin D binding protein (Gc-globulin) and its polymorphism," *Clinica Chimica Acta*, vol. 372, no. 1-2, pp. 33–42, 2006.
- [12] P. White and N. Cooke, "The multifunctional properties and characteristics of vitamin D-binding protein," *Trends in Endocrinology and Metabolism*, vol. 11, no. 8, pp. 320–327, 2000.
- [13] C. A. Piquette, R. Robinson-Hill, and R. O. Webster, "Human monocyte chemotaxis to complement-derived chemotaxins is enhanced by Gc-globulin," *Journal of Leukocyte Biology*, vol. 55, no. 3, pp. 349–354, 1994.
- [14] C. Gasparri, A. Curcio, D. Torella et al., "Proteomics reveals high levels of vitamin D binding protein in myocardial infarction," *Frontiers in Bioscience (Elite Edition)*, vol. 2, pp. 796–804, 2010.
- [15] D. Blanton, Z. Han, L. Bierschenk et al., "Reduced serum vitamin D-binding protein levels are associated with type 1 diabetes," *Diabetes*, vol. 60, no. 10, pp. 2566–2570, 2011.
- [16] M. Emmett, J. L. Miller, and A. J. Crowle, "Protein abnormalities in adult respiratory distress syndrome, tuberculosis, and cystic fibrosis sera," *Proceedings of the Society for Experimental Biology and Medicine*, vol. 184, no. 1, pp. 74–82, 1987.
- [17] L. Jeng, A. V. Yamshchikov, S. E. Judd et al., "Alterations in vitamin D status and anti-microbial peptide levels in patients in the intensive care unit with sepsis," *Journal of Translational Medicine*, vol. 7, article 28, 2009.

- [18] M. M. Speeckaert, C. Wehlou, S. Vandewalle, Y. E. Taes, E. Robberecht, and J. R. Delanghe, "Vitamin D binding protein, a new nutritional marker in cystic fibrosis patients," *Clinical Chemistry and Laboratory Medicine*, vol. 46, no. 3, pp. 365–370, 2008.
- [19] J. W. Krayner, D. L. Emerson, P. J. Goldschmidt-Clermont, A. E. Nel, P. A. Werner, and R. M. Galbraith, "Qualitative and quantitative studies of Gc (vitamin D-binding protein) in normal subjects and patients with periodontal disease," *Journal of Periodontal Research*, vol. 22, no. 4, pp. 259–263, 1987.
- [20] Y. Wu, R. Shu, L.-J. Luo, L.-H. Ge, and Y.-F. Xie, "Initial comparison of proteomic profiles of whole unstimulated saliva obtained from generalized aggressive periodontitis patients and healthy control subjects," *Journal of Periodontal Research*, vol. 44, no. 5, pp. 636–644, 2009.
- [21] X. Zhang, H. Meng, X. Sun et al., "Elevation of Vitamin D-binding protein in plasma of patients with generalized aggressive periodontitis," *Journal of Periodontal Research*, vol. 48, pp. 74–79, 2013.
- [22] J. E. Mazza, M. G. Newman, and T. N. Sims, "Clinical and antimicrobial effect of stannous fluoride on periodontitis," *Journal of Clinical Periodontology*, vol. 8, no. 3, pp. 203–212, 1981.
- [23] K. Tsuchida and K. Hara, "Clinical significance of gingival fluid measurement by 'Periotron,'" *Journal of Periodontology*, vol. 52, no. 11, pp. 697–700, 1981.
- [24] C. Guha, M. Osawa, P. A. Werner, R. M. Galbraith, and G. V. Paddock, "Regulation of human Gc (vitamin D-binding) protein levels: hormonal and cytokine control of gene expression in vitro," *Hepatology*, vol. 21, no. 6, pp. 1675–1681, 1995.
- [25] X. J. Sun, H. X. Meng, D. Shi et al., "Elevation of C-reactive protein and interleukin-6 in plasma of patients with aggressive periodontitis," *Journal of Periodontal Research*, vol. 44, no. 3, pp. 311–316, 2009.
- [26] J. F. McLeod and N. E. Cooke, "The vitamin D-binding protein, α -fetoprotein, albumin multigene family: detection of transcripts in multiple tissues," *Journal of Biological Chemistry*, vol. 264, no. 36, pp. 21760–21769, 1989.
- [27] A. Sabbatini, M. Petrini, L. Mattii, P. Arnaud, and R. M. Galbraith, "Vitamin D binding protein is produced by human monocytes," *FEBS Letters*, vol. 323, no. 1-2, pp. 89–92, 1993.
- [28] R. R. Kew, M. A. Sibug, J. P. Liuzzo, and R. O. Webster, "Localization and quantitation of the vitamin D binding protein (Gc-globulin) in human neutrophils," *Blood*, vol. 82, no. 1, pp. 274–283, 1993.
- [29] L. A. McVoy and R. R. Kew, "CD44 and annexin A2 mediate the C5a chemotactic cofactor function of the vitamin D binding protein," *Journal of Immunology*, vol. 175, no. 7, pp. 4754–4760, 2005.
- [30] S. J. DiMartino, G. Trujillo, L. A. McVoy, J. Zhang, and R. R. Kew, "Upregulation of vitamin D binding protein (Gc-globulin) binding sites during neutrophil activation from a latent reservoir in azurophil granules," *Molecular Immunology*, vol. 44, no. 9, pp. 2370–2377, 2007.

Review Article

Vitamin D Binding Protein Impact on 25-Hydroxyvitamin D Levels under Different Physiologic and Pathologic Conditions

Pegah Yousefzadeh,¹ Sue A. Shapses,² and Xiangbing Wang¹

¹ Division of Endocrinology, Metabolism & Nutrition, Department of Medicine, Rutgers University-Robert Wood Johnson Medical School, New Brunswick, NJ 08903, USA

² Department of Nutritional Sciences, Rutgers University, New Brunswick, NJ 08901, USA

Correspondence should be addressed to Xiangbing Wang; wangx9@rwjms.rutgers.edu

Received 18 January 2014; Revised 3 April 2014; Accepted 10 April 2014; Published 28 April 2014

Academic Editor: Zhongjian Xie

Copyright © 2014 Pegah Yousefzadeh et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

There is a high prevalence of vitamin D deficiency worldwide, but how to define vitamin D deficiency is controversial. Currently, the plasma concentration of total 25-hydroxyvitamin D [25(OH)D] is considered an indicator of vitamin D status. The free hormone hypothesis states that protein-bound hormones are inactive while unbound hormones are free to exert biological activity. The majority of circulating 25(OH)D and 1,25(OH)₂D is tightly bound to vitamin D binding protein (DBP), 10–15% is bound to albumin, and less than 1% of circulating vitamin D exists in an unbound form. While DBP is relatively stable in most healthy populations, a recent study showed that there are gene polymorphisms associated with race and ethnicity that could alter DBP levels and binding affinity. Furthermore, in some clinical situations, total vitamin D levels are altered and knowing whether DBP is also altered may have treatment implications. The aim of this review is to assess DBP concentration in different physiological and pathophysiological conditions. We suggest that DBP should be considered in the interpretation of 25(OH)D levels.

1. Introduction

Reports of the worldwide prevalence of low vitamin D status vary depending on the level of total 25-hydroxyvitamin D [25(OH)D] that is used to define vitamin D sufficiency. The plasma concentration of total 25(OH)D is considered an indicator of vitamin D status due to its long half-life of 15–35 days and lack of hormonal control of the hepatic 25-hydroxylase [1]. The free hormone hypothesis states that protein-bound hormones are relatively inactive while hormones not bound to binding proteins are available to exert biological activity [2]. The majority (85–90%) of circulating 25(OH)D and 1,25(OH)₂D is tightly bound to DBP, with a smaller amount (10–15%) bound to albumin. Less than 1% of circulating vitamin D exists in a free, unbound form [3, 4]. It is difficult to define vitamin D deficiency because circulating 25(OH)D levels vary in different physiologic and disease states. It is unclear whether a change in levels of DBP would have any effect on concentrations of vitamin D metabolites, as DBP circulates at a higher level than its

ligands. In a recent study, it was reported that despite the lower levels of total 25(OH)D and DBP in blacks compared to whites, both groups had similar concentrations of estimated bioavailable 25(OH)D and can be explained by a difference in polymorphisms in the DBP gene [5]. Another recent study used a novel immunoassay to directly measure free 25(OH)D, and they concluded that the current algorithms to calculate free 25(OH)D may not be accurate and further investigation is required [6]. A new assay that measures free serum 25(OH)D directly is now available by the Future Diagnostics (Wijchen, The Netherlands), but it is labor intensive, costly, and time-consuming. Alternatively, the bioavailable and DBP-bound 25(OH)D can be calculated using equations adapted from Vermeulen et al. [7] and is currently being used in clinical practice [8]. Recent studies have found that serum DBP and vitamin D levels are decreased in type 1 diabetes mellitus (T1DM) [9], chronic liver [10], and renal diseases [11], while pregnancy and oral contraceptive pills (OCP) increase DBP and vitamin D levels [12, 13]. On the other hand, in a large cohort of healthy adults with sufficient

vitamin D levels, the biological effects of vitamin D on PTH levels are mainly independent of DBP concentration [14]. This finding may provide useful information for studies investigating the relationship between 25(OH)D and DBP in different age groups, races, and disease states. Dynamic changes of DBP levels should be integrated into interpreting the serum levels of 25(OH)D in these conditions. Besides binding to vitamin D metabolites, DBP has other physiological functions, including interacting with target organ or cells. In addition, megalin, possibly in conjunction with cubilin, mediates uptake of DBP-bound 25(OH)D into the kidney for the intracrine conversion of 25(OH)D to 1,25(OH)₂D [4]. Megalin may also play a role in extrarenal tissues [4]. The aim of this review is to discuss the correlation between serum levels of DBP and 25(OH)D in various physiologic and pathologic conditions.

2. Physiological Factors Affecting DBP and 25(OH)D Levels

2.1. Race and DBP Genotype. There is a higher prevalence of vitamin D deficiency among African Americans, as their total 25(OH)D levels are lower than those in white individuals. This could be due to their lower cutaneous synthesis of vitamin D [15]. Levels of total 25(OH)D are in part genetically determined. Variations in the DBP originally referred to as GC1F, GC1S, and GC2 were first reported more than 50 years ago and may be associated with changes in binding affinity or serum concentration of DBP. The protein variants are now recognized as resulting from polymorphisms in the DBP binding protein gene GC. The phenotypic variations in the DBP amino acid sequence are distinguished by single nucleotide polymorphisms (SNPs) rs7041 and rs4588. Blacks and Asians are more likely to carry GC1F DBP, which has the highest affinity for 25(OH)D and is associated with low DBP levels. Whites are more likely to carry GC1S DBP. GC2, which has a lower affinity for 25(OH)D and is associated with higher DBP levels, is frequently found in whites and rarely found in blacks [4, 5]. The high prevalence of GC1F in blacks results in concentrations of bioavailable 25(OH)D similar to those in whites [5]. Engelman et al. [16] showed that homozygosity for the GC1F allele (DDTT) occurred in 53% of African Americans but only 6% of Caucasians and 13% of Hispanics. The rs4588 and rs7041 SNPs were associated with 25(OH)D levels in Hispanics and African Americans. Combinations of these alleles appear to alter DBP concentration, the affinity for 25(OH)D, and 25(OH) levels [17, 18]. In a cross-section study, Santos et al. found that the AA genotype of rs4588 and TT genotypic of rs7044 and CT-AT/AT-AT (GC1F-2/2-2) were associated with lower 25(OH)D levels in healthy girl [19]. The rs4588 and rs7041 SNPs were associated with 25(OH)D levels and rs4588 was associated with 1,25(OH)₂D levels in Hispanics and African Americans [16]. Cheung et al. [20] in a study of Southern Chinese women found that among four SNPs (rs2282679, rs10741657, rs12785878, and rs6013897), rs2282679 was associated with serum 25(OH)D levels and vitamin D insufficiency while rs12785878 was associated with vitamin D insufficiency only.

2.2. Age and Gender. Age appears to have an impact on DBP concentration, as it does on other globulins such as sex hormone binding protein (SHBG) and insulin-like growth factor binding protein (IGFBP). Vitamin D insufficiency is common among older adults. Our recent study showed that DBP was negatively correlated with age in female subjects indicating that age might be an independent factor affecting DBP and 25(OH)D levels [21]. Carpenter et al. [22] suggested that in young children genetic variance of the common rs4588 affects circulating levels of the DBP, which in turn affects circulating 25(OH)D levels in infants and toddlers. The GC genotype may be related to the susceptibility to low 25(OH)D levels in female children and adolescents. The GC genotype also affects circulating 25(OH)D independent of its effect on circulating DBP. Genome-wide association studies have found an association between variants in the GC genotypes and serum levels of 25(OH)D and PHT in older adults [23]. Winters et al. [24] found that DBP was not an important determinant of circulating 25(OH)D in women, nor was it affected by race or adiposity. In a study in which DBP levels were assessed in 100 healthy, middle-aged, and older participants, it was found that women had higher mean DBP levels than men, but no associations were observed between DBP levels and age, body weight, BMI, fat mass, or fat percentage [25]. Blanton et al. [9] confirmed that DBP levels were lower in males than in females, which could be due to estrogen effects on DBP.

2.3. Pregnancy and Estrogen. The increase in DBP levels associated with pregnancy results from the stimulation of DBP synthesis by estrogen [12]. It has been shown that while DBP is higher in pregnancy, free 25(OH)D is not lower, which can be attributed to weaker binding to DBP [26]. The use of OCP may also affect 25(OH)D concentrations and DBP levels. The limited data available on the effects of OCP on 25(OH)D concentrations suggest no change or an increase in total 25(OH)D [13], whereas most studies consistently report an increase in levels of 1,25(OH)₂D and DBP [27].

DBP may be regulated by various hormone levels, as is the case with other globulins such as sex hormone binding globulin (SHBG) and thyroid binding globulin (TBG). Egawa et al. [28] reported that the DBP production might be regulated by insulin, estradiol, triamcinolone, dihydrotestosterone, or epidermal growth factor. The use of OCP is associated with 13%–25% higher concentrations of total 25(OH)D and 1,25(OH)₂D and DBP. The estrogen component of OCP may increase DBP synthesis or decrease its catabolism. The authors suggest that use of OCP should be considered in the interpretation of plasma concentrations of vitamin D metabolites [13]. Our recent preliminary findings showed that DBP and 25(OH)D were lower in postmenopausal women compared with premenopausal women [21]. Consistent with this finding, the initiation of estrogen therapy in postmenopausal women caused a significant 8% increase in DBP levels [29] also suggesting that estrogen is a factor affecting DBP and 25(OH)D levels.

2.4. Obesity. The association between reduced 25(OH)D concentrations and obesity is well established, although the mechanisms for the lower 25(OH)D concentrations are not fully described. In a recent report, it was shown that DBP was lower in obese adolescents compared to those with normal weight and there was a positive correlation between DBP and 25(OH)D levels. The authors also reported that there was an inverse relationship between insulin levels and DBP levels in obese adolescents even when corrected for adiposity. It was suggested that insulin suppresses the production of DBP [30]. Obese women had higher DBP concentrations and lower free 25(OH)D compared with normal-weight women and a lower free 25(OH)D level. The obese women were more likely to have 25(OH)D concentrations that could be considered suboptimal [31].

3. Pathologic Factors Affecting DBP and 25(OH)D Levels

3.1. Liver Disease. The liver is where 25-hydroxylation of vitamin D occurs and the majority of DBP is synthesized. The prevalence of vitamin D insufficiency is particularly high in patients with chronic liver disease. Vitamin D deficiency or low total vitamin D levels in chronic liver disease is likely to result from a number of mechanisms including lower levels of sun exposure and inadequate dietary intake of vitamin D. Serum 25(OH)D levels are inversely related to the severity of chronic liver disease [32]. Decreased production of DBP and albumin, which also carries vitamin D, might be critical in chronic liver disease [10, 33]. However, patients with cirrhosis and low albumin concentrations have higher free 25(OH)D levels [26]. Patients with end-stage liver diseases had a high prevalence of low total 25(OH) but maintained normal serum corrected calcium levels and did not develop secondary hyperparathyroidism [33]. Low total Vitamin D and low DBP levels in liver failure can be corrected with a liver transplant after which serum DBP and albumin increase substantially. Low DBP levels likely have a role in low total vitamin D levels in profound liver dysfunction [34].

3.2. Renal Disease. Low total vitamin D levels are common in patients with chronic kidney disease (CKD) at all stages. An alteration in megalin-dependent uptake of DBP in the kidney [4] in CKD would be expected to decrease the intracrine conversion of 25(OH)D to 1,25(OH)D leading to complications, such as renal osteodystrophy. Increased urinary excretion of DBP has been associated with tubular dysfunction, such as in patients with nephrotic syndrome [35] and renal Fanconi syndrome [36]. Increased urinary loss of DBP has been postulated to contribute to the low total vitamin D levels in patients with proteinuria. However, a recent study by Doorenbos et al. [37] found that antiproteinuric treatment reduced urinary loss of DBP, but did not affect 25(OH)D levels in CKD. This is consistent with the hypothesis that urinary DBP is a marker of renal interstitial inflammation and fibrosis [38]. When compared to white patients, black patients receiving hemodialysis had lower levels of total 25(OH)D, but similar levels of bioavailable 25(OH)D levels. Bioavailable

25(OH)D levels but not total 25(OH)D and 1,25(OH)₂D were significantly correlated with serum calcium [39]. It has also been shown that children with CKD exhibit altered concentration of DBP and bioavailable 25(OH), and there is an important impact of their underlying disease [11]. These findings suggest that bioavailable vitamin D levels are better correlated with measures of mineral metabolism than total vitamin D levels in patients receiving hemodialysis.

3.3. Diabetes. Blanton et al. [9] showed that serum DBP levels are decreased in patients with T1DM compared to healthy controls. They suggested that multiple components in the metabolic pathway of vitamin D may be altered in T1DM and, collectively, have the potential to influence disease pathogenesis. Another study showed that urinary loss of DBP in T1DM was higher compared to healthy individuals suggesting that urinary loss of DBP might contribute to the lower levels of serum 25(OH)D [40]. Tian et al. [41] found that DBP was higher in diabetic patients with nephropathy and microalbuminuria or macroalbuminuria compared to healthy controls or diabetic patients with normoalbuminuria. They suggested that urinary DBP is a potential biomarker for early detection of diabetic nephropathy.

3.4. Primary Hyperparathyroidism (PHPT). Low total 25(OH)D levels are commonly encountered in patients with PHPT. The causes are not totally understood, but proposed mechanisms include stimulation by PTH of the conversion of 25(OH)D to 1,25(OH)₂D by 1 α -hydroxylase and the increased catabolism of 25(OH)D by CYP24A1 induced by the high levels of 1,25(OH)₂D that prevail in PHPT [42]. We previously reported that both serum 25(OH)D and DBP levels were significantly lower in female patients with PHPT compared with control subjects. DBP levels were inversely correlated with intact PTH, suggesting that PTH regulates DBP production [43]. The findings suggest that a low DBP level contributes to the low 25(OH)D level observed in female PHPT patients.

3.5. Cancer. There are several reports that there is an association between vitamin D deficiency and risk of developing cancer. A recent meta-analysis suggested an inverse association of 25(OH)D with total cancer incidence and mortality [44]. However, Weinstein et al. [45] reported that 25(OH)D levels were positively correlated with pancreatic cancer risk and DBP was inversely associated with pancreatic cancer risk. Higher DBP concentrations may result in increased bound 25(OH)D and reduced free 25(OH)D. These authors suggest that simultaneous examination of DBP and 25(OH)D may be important in determining the association of vitamin D and cancer risk. In another study, the same authors found that serum DBP was not associated with prostate cancer risk overall; however, high serum DBP was associated with significantly decreased risk of prostate cancer in men with lower 25(OH)D concentrations and increased risk in men with higher 25(OH)D. These data suggest that the primary vitamin D carrier protein DBP modulates the impact of vitamin D status on prostate cancer [46]. Together, these data

suggest that the combination of both DBP and 25(OH)D is needed to understand cancer risk [45, 46]. Mondul et al. [47] reported that low serum 25(OH)D levels were associated with a higher risk of bladder cancer and provided data to support an etiologic role for vitamin D in bladder cancer. They suggested that free circulating vitamin D might be a more relevant factor when examining bladder and other cancers. Overall, the data for cancer is still emerging and the role of DBP requires further study.

3.6. HIV and Inflammation. Comparing over 200 HIV-infected youth on stable treatment with regimens containing tenofovir disoproxil fumarate (TDF) to those whose treatment lacked TDF, Havens et al. [48] found that the higher plasma tenofovir concentrations were associated with higher DBP and lower free 1, 25(OH)₂D. This might imply a functional vitamin D deficiency and can explain the TDF-associated increase in parathyroid hormone.

It is also common to see vitamin D insufficiency in hospitalized patients with an acute illness. DBP level is decreased in acute inflammatory conditions. One study showed that, following surgery, the mean CRP increased but both serums 25(OH)D and DBP decreased [49]. Patients with sepsis have a high mortality rate as well as a high prevalence of vitamin D deficiency. In addition, septic patients have decreased DBP levels, which further exacerbate the total low vitamin D levels but might attenuate the risk of vitamin D deficiency by maintaining normal free or bioavailable 25(OH)D [50]. Jeng et al. found that 25(OH)D and DBP levels were lower in patients in the intensive care unit with sepsis compared with healthy controls [51]. Serum 25(OH)D may be an unreliable biomarker of vitamin D status after acute inflammatory insult.

3.7. Procedures. Plasma exchange is a therapeutic procedure that is used to remove pathogenic circulating proteins from patients' plasma, and it often results in the depletion of physiologically important proteins including DBP. Plasma exchange induced an acute reversible decrease in plasma 1, 25(OH)₂D, DBP, and calcium and a sustained decrease in plasma 25(OH)D [52]. Another study showed that, in CKD stages 4-5 children on peritoneal dialysis (PD), urinary DBP losses were >300-fold higher than seen in age-matched healthy children and correlated with urinary albumin loss. They concluded that peritoneal DBP losses mirror both dialysate and urinary albumin losses and are associated with a longer dialysis vintage but do not contribute to vitamin D deficiency in children on PD [53]. These findings suggest that serum 25(OH)D may be an unreliable biomarker of vitamin D status after procedures such as plasma exchange and PD.

4. Conclusion

Different physiologic and pathologic conditions can affect DBP levels, which in turn affect circulating 25(OH)D levels. Therefore, alterations in DBP levels should be considered as potential confounders on the interpretation of plasma

total 25(OH)D concentrations. Further research is required to assess whether the free 25(OH)D index as compared to total 25(OH)D levels is a better marker of 25(OH)D tissue availability and if it has a higher correlation with indices of skeletal and nonskeletal outcomes.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The authors are grateful to Stephen H. Schneider, M.D. degree holder, Louis Amorosa, M.D. degree holder, and Xinjiang Cai, M.D. and Ph.D. degrees holder, and their insightful discussions. Vincent A. Rifici, Ph.D. degree holder, assisted in the preparation of this paper.

References

- [1] M. F. Holick, "Resurrection of vitamin D deficiency and rickets," *The Journal of Clinical Investigation*, vol. 116, no. 8, pp. 2062–2072, 2006.
- [2] C. M. Mendel, "The free hormone hypothesis: a physiologically based mathematical model," *Endocrine Reviews*, vol. 10, no. 3, pp. 232–274, 1989.
- [3] D. D. Bikle, P. K. Siiteri, and E. Ryzen, "Serum protein binding of 1,25-dihydroxyvitamin D: a reevaluation by direct measurement of free metabolite levels," *The Journal of Clinical Endocrinology & Metabolism*, vol. 61, no. 5, pp. 969–975, 1985.
- [4] R. F. Chun, B. E. Peercy, E. S. Orwoll, C. M. Nielson, J. S. Adams, and M. Hewison, "Vitamin D and DBP: the free hormone hypothesis revisited," *The Journal of Steroid Biochemistry and Molecular Biology*, 2013.
- [5] C. E. Powe, M. K. Evans, J. Wenger, A. B. Zonderman, A. H. Berg, M. Nalls et al., "Vitamin D-binding protein and vitamin D status of black Americans and white Americans," *The New England Journal of Medicine*, vol. 369, no. 21, pp. 1991–2000, 2013.
- [6] J. B. Schwartz, J. Lai, B. Lizaola et al., "A comparison of direct and calculated free 25(OH) Vitamin D levels in clinical populations," *The Journal of Clinical Endocrinology & Metabolism*, 2014.
- [7] A. Vermeulen, L. Verdonck, and J. M. Kaufman, "A critical evaluation of simple methods for the estimation of free testosterone in serum," *The Journal of Clinical Endocrinology & Metabolism*, vol. 84, no. 10, pp. 3666–3672, 1999.
- [8] D. D. Bikle, E. Gee, B. Halloran, and J. G. Haddad, "Free 1,25-dihydroxyvitamin D levels in serum from normal subjects, pregnant subjects, and subjects with liver disease," *The Journal of Clinical Investigation*, vol. 74, no. 6, pp. 1966–1971, 1984.
- [9] D. Blanton, Z. Han, L. Bierschenk et al., "Reduced serum vitamin D-binding protein levels are associated with type 1 diabetes," *Diabetes*, vol. 60, no. 10, pp. 2566–2570, 2011.
- [10] C. S. Stokes, D. A. Volmer, F. Grünhage, and F. Lammert, "Vitamin D in chronic liver disease," *Liver International*, vol. 33, no. 3, pp. 338–352, 2013.
- [11] M. R. Denburg, H. J. Kalkwarf, I. H. de Boer, M. Hewison, J. Shults, B. S. Zemel et al., "Vitamin D bioavailability and catabolism in pediatric chronic kidney disease," *Pediatric Nephrology*, vol. 28, no. 9, pp. 1843–1853, 2013.

- [12] U. K. Møller, S. Streym, L. Heickendorff, L. Mosekilde, and L. Rejnmark, "Effects of 25(OH)D concentrations on chances of pregnancy and pregnancy outcomes: a cohort study in healthy Danish women," *European Journal of Clinical Nutrition*, vol. 66, pp. 862–868, 2012.
- [13] U. K. Møller, S. V. Streym, L. T. Jensen et al., "Increased plasma concentrations of vitamin D metabolites and vitamin D binding protein in women using hormonal contraceptives: a cross-sectional study," *Nutrients*, vol. 5, no. 9, pp. 3470–3480, 2013.
- [14] Z. Dastani, C. Berger, L. Langsetmo, L. Fu, B. Y. Wong, S. Malik et al., "In healthy adults, biological activity of vitamin D, as assessed by serum PTH, is largely independent of DBP concentrations," *Journal of Bone and Mineral Research*, vol. 29, no. 2, pp. 494–499, 2014.
- [15] S. Nesby-O'Dell, K. S. Scanlon, M. E. Cogswell et al., "Hypovitaminosis D prevalence and determinants among African American and white women of reproductive age: third National Health and Nutrition Examination Survey, 1988–1994," *American Journal of Clinical Nutrition*, vol. 76, no. 1, pp. 187–192, 2002.
- [16] C. D. Engelman, T. E. Fingerlin, C. D. Langefeld et al., "Genetic and environmental determinants of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D levels in hispanic and African Americans," *The Journal of Clinical Endocrinology & Metabolism*, vol. 93, no. 9, pp. 3381–3388, 2008.
- [17] T. J. Wang, F. Zhang, J. B. Richards et al., "Common genetic determinants of vitamin D insufficiency: a genome-wide association study.," *The Lancet*, vol. 376, no. 9736, pp. 180–188, 2010.
- [18] G. P. Levin, C. Robinson-Cohen, I. H. De Boer, D. K. Houston, K. Lohman, Y. Liu et al., "Genetic variants and associations of 25-hydroxyvitamin D concentrations with major clinical outcomes," *The Journal of the American Medical Association*, vol. 308, no. 18, pp. 1898–1905, 2012.
- [19] B. R. Santos, L. P. Mascarenhas, M. C. Boguszewski, and P. M. Spritzer, "Variations in the vitamin D-binding protein (DBP) gene are related to lower 25-hydroxyvitamin D levels in healthy girls: a cross-sectional study," *Hormone Research in Paediatrics*, vol. 79, pp. 162–168, 2013.
- [20] C. L. Cheung, K. S. Lau, P. C. Sham, K. C. Tan, and A. W. Kung, "Genetic variant in vitamin D binding protein is associated with serum 25-hydroxyvitamin D and vitamin D insufficiency in southern Chinese," *Journal of Human Genetics*, vol. 58, pp. 749–751, 2013.
- [21] X. Wang and S. A. Shapses, "Vitamin D binding protein levels in normal pre-and post menopausal women," *Endocrine Reviews*, vol. 34, 2013.
- [22] T. O. Carpenter, J. H. Zhang, E. Parra, B. K. Ellis, C. Simpson, and W. M. Lee, "Vitamin D binding protein is a key determinant of 25-hydroxyvitamin D levels in infants and toddlers," *Journal of Bone and Mineral Research*, vol. 28, no. 1, pp. 213–221, 2013.
- [23] L. Perna, J. F. Felix, L. P. Breitling et al. et al., "Genetic variations in the vitamin D binding protein and season-specific levels of vitamin D among older adults," *Epidemiology*, vol. 24, no. 1, pp. 104–109, 2013.
- [24] S. J. Winters, R. Chennubhatla, C. Wang, and J. J. Miller, "Influence of obesity on vitamin D-binding protein and 25-hydroxy vitamin D levels in African American and white women," *Metabolism: Clinical and Experimental*, vol. 58, no. 4, pp. 438–442, 2009.
- [25] M. J. Bolland, A. B. Grey, R. W. Ames et al., "Age-, gender-, and weight-related effects on levels of 25-hydroxyvitamin D are not mediated by vitamin D binding protein," *Clinical Endocrinology*, vol. 67, no. 2, pp. 259–264, 2007.
- [26] J. B. Schwartz, J. Lai, B. Lizaola et al., "Variability in free 25(OH) vitamin D levels in clinical populations," *The Journal of Steroid Biochemistry and Molecular Biology*, 2013.
- [27] R. Bouillon, F. A. van Assche, H. van Baelen, W. Heyns, and P. de Moor, "Influence of the vitamin D-binding protein on the serum concentration of 1,25-dihydroxyvitamin D3. Significance of the free 1,25-dihydroxyvitamin D3 concentration," *The Journal of Clinical Investigation*, vol. 67, no. 3, pp. 589–596, 1981.
- [28] T. Egawa, H. Ito, H. Nakamura, H. Yamamoto, and S. Kishimoto, "Hormonal regulation of vitamin D-binding protein production by a human hepatoma cell line," *Biochemistry International*, vol. 28, no. 3, pp. 551–557, 1992.
- [29] L. Rejnmark, A. L. Lauridsen, C. Brot et al., "Vitamin D and its binding protein Gc: long-term variability in peri- and postmenopausal women with and without hormone replacement therapy," *Scandinavian Journal of Clinical and Laboratory Investigation*, vol. 66, no. 3, pp. 227–238, 2006.
- [30] A. P. Ashraf, C. Huisinigh, J. A. Alvarez, X. Wang, and B. A. Gower, "Insulin resistance indices are inversely associated with vitamin D binding protein concentrations," *The Journal of Clinical Endocrinology & Metabolism*, vol. 99, no. 1, pp. 178–183, 2014.
- [31] T. Karlsson, A. Osmanovic, N. Jansson, L. Hulthén, A. Holmång, and I. Larsson, "Increased vitamin D-binding protein and decreased free 25(OH)D in obese women of reproductive age," *European Journal of Nutrition*, vol. 53, no. 1, pp. 259–267, 2014.
- [32] M. Malham, S. P. Jørgensen, P. Ott et al., "Vitamin D deficiency in cirrhosis relates to liver dysfunction rather than aetiology," *World Journal of Gastroenterology*, vol. 17, no. 7, pp. 922–925, 2011.
- [33] R. L. Corey, M. D. Whitaker, M. D. Crowell, M. T. Keddis, B. Aqel, V. Balan et al., "Vitamin D deficiency, parathyroid hormone (PTH) levels, and bone disease among patients with end stage liver disease (ESLD) and normal serum creatinine awaiting liver transplantation (LT)," *Clinical Transplantation*, 2014.
- [34] P. P. Reese, R. D. Bloom, H. I. Feldman et al., "Changes in vitamin D binding protein and vitamin D concentrations associated with liver transplantation," *Liver International*, vol. 32, no. 2, pp. 287–296, 2012.
- [35] K. Colston, N. J. Williams, and H. J. W. Cleve, "Studies on vitamin D binding protein in the nephrotic syndrome," *Clinical Chemistry*, vol. 31, no. 5, pp. 718–721, 1985.
- [36] A. Vilasi, P. R. Cutillas, A. D. Maher et al., "Combined proteomic and metabolomic studies in three genetic forms of the renal Fanconi syndrome," *American Journal of Physiology—Renal Physiology*, vol. 293, no. 2, pp. F456–F467, 2007.
- [37] C. R. C. Doorenbos, M. M. de Cuba, L. Vogt et al., "Antiproteinuric treatment reduces urinary loss of vitamin D-binding protein but does not affect vitamin D status in patients with chronic kidney disease," *Journal of Steroid Biochemistry and Molecular Biology*, vol. 128, no. 1–2, pp. 56–61, 2012.
- [38] K. Mirkovic, C. R. Doorenbos, W. A. Dam, H. J. Lambers Heerspink, M. C. Slagman, F. L. Nauta et al., "Urinary vitamin D binding protein: a potential novel marker of renal interstitial inflammation and fibrosis," *PLoS ONE*, vol. 8, Article ID e55887, 2013.
- [39] I. Bhan, C. E. Powe, A. H. Berg et al., "Bioavailable vitamin D is more tightly linked to mineral metabolism than total vitamin D in incident hemodialysis patients," *Kidney International*, vol. 82, pp. 84–89, 2012.

- [40] K. M. Thrailkill, C.-H. Jo, G. E. Cockrell, C. S. Moreau, and J. L. Fowlkes, "Enhanced excretion of vitamin D binding protein in type 1 diabetes: a role in vitamin D deficiency?" *The Journal of Clinical Endocrinology & Metabolism*, vol. 96, no. 1, pp. 142–149, 2011.
- [41] X. Q. Tian, L. M. Zhao, J. P. Ge, Y. Zhang, and Y. C. Xu, "Elevated urinary level of vitamin D-binding protein as a novel biomarker for diabetic nephropathy," *Experimental and Therapeutic Medicine*, vol. 7, no. 2, pp. 411–416, 2014.
- [42] M. R. Clements, M. Davies, M. E. Hayes et al., "The role of 1,25-dihydroxyvitamin D in the mechanism of acquired vitamin D deficiency," *Clinical Endocrinology*, vol. 37, no. 1, pp. 17–27, 1992.
- [43] X. Wang, S. A. Shapses, S. Wei, D. Sukumar, and J. Ghosh, "Vitamin D binding protein levels in female patients with primary hyperparathyroidism," *Endocrine Practice*, vol. 19, no. 4, pp. 609–613, 2013.
- [44] L. Yin, J. M. Ordóñez-Mena, T. Chen, B. Schöttker, V. Arndt, and H. Brenner, "Circulating 25-hydroxyvitamin D serum concentration and total cancer incidence and mortality: a systematic review and meta-analysis," *Preventive Medicine*, vol. 57, no. 6, pp. 753–764, 2013.
- [45] S. J. Weinstein, R. Z. Stolzenberg-Solomon, W. Kopp, H. Rager, J. Virtamo, and D. Albanes, "Impact of circulating vitamin D binding protein levels on the association between 25-hydroxyvitamin d and pancreatic cancer risk: a nested case-control study," *Cancer Research*, vol. 72, no. 5, pp. 1190–1198, 2012.
- [46] S. J. Weinstein, A. M. Mondul, W. Kopp, H. Rager, J. Virtamo, and D. Albanes, "Circulating 25-hydroxyvitamin D, vitamin D-binding protein and risk of prostate cancer," *International Journal of Cancer*, vol. 132, no. 12, pp. 2940–2947, 2013.
- [47] A. M. Mondul, S. J. Weinstein, J. Virtamo, and D. Albanes, "Influence of vitamin D binding protein on the association between circulating vitamin D and risk of bladder cancer," *British Journal of Cancer*, vol. 107, no. 9, pp. 1589–1594, 2012.
- [48] P. L. Havens, J. J. Kiser, C. B. Stephensen, R. Hazra, P. M. Flynn, C. M. Wilson et al., "Association of higher plasma vitamin D binding protein and lower free calcitriol levels with tenofovir disoproxil fumarate use and plasma and intracellular tenofovir pharmacokinetics: cause of a functional vitamin d deficiency," *Antimicrobial Agents and Chemotherapy*, vol. 57, no. 11, pp. 5619–5628, 2013.
- [49] J. L. Waldron, H. L. Ashby, M. P. Cornes, J. Bechervaise, C. Razavi, O. L. Thomas et al., "Vitamin D: a negative acute phase reactant," *Journal of Clinical Pathology*, vol. 66, no. 7, pp. 620–622, 2013.
- [50] R. R. Watkins, A. V. Yamshchikov, T. L. Lemonovich, and R. A. Salata, "The role of vitamin D deficiency in sepsis and potential therapeutic implications," *Journal of Infection*, vol. 63, no. 5, pp. 321–326, 2011.
- [51] L. Jeng, A. V. Yamshchikov, S. E. Judd et al., "Alterations in vitamin D status and anti-microbial peptide levels in patients in the intensive care unit with sepsis," *Journal of Translational Medicine*, vol. 7, article 28, 2009.
- [52] T. F. Hiemstra, A. Casian, P. Boraks, D. R. Jayne, and I. Schoenmakers, "Plasma exchange induces vitamin D deficiency," *QJM*, vol. 107, no. 2, pp. 120–130, 2014.
- [53] A. Prytuła, D. Wells, T. McLean et al., "Urinary and dialysate losses of vitamin D-binding protein in children on chronic peritoneal dialysis," *Pediatric Nephrology*, vol. 27, no. 4, pp. 643–649, 2012.

Research Article

Vitamin D Binding Protein Affects the Correlation of 25(OH)D and Frailty in the Older Men

Yi Wang, Yan-Jiao Wang, Jun-Kun Zhan, Zhi-Yong Tang, Wu Huang, Pan Tan, Shan Gao, Cai-Li Ma, Zai-Jin Jian, and You-Shuo Liu

Geriatric Department of the Second Xiang-Ya Hospital, Institute of Aging and Geriatric, Central South University, Changsha, Hunan 410011, China

Correspondence should be addressed to You-Shuo Liu; liuyoushuo@yeah.net

Received 20 November 2013; Revised 19 February 2014; Accepted 20 March 2014; Published 13 April 2014

Academic Editor: Zhongjian Xie

Copyright © 2014 Yi Wang et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Vitamin D binding protein (DBP) may alter the biologic activity of 25-hydroxyvitamin D [25(OH)D]. The objective of our present study was to determine the joint effect of serum 25(OH)D and DBP on the risk of frailty. Five hundred sixteen male participants aged 70 years or older were recruited in Changsha city and its surrounding area in Hunan province of China. Frailty was defined as the presence of at least three of the five following criteria: weakness, low physical activity, slow walking speed, exhaustion, and weight loss. Multivariate linear regression analysis was performed to assess the relationship between 25(OH)D and DBP levels. Odds ratios (ORs) for frailty were evaluated across quartiles of 25(OH)D and DBP levels, adjusted age, education, and body mass index. The results showed that participants in the lowest quartile of 25(OH)D and the highest quartile of DBP levels, the lowest quartile of 25(OH)D and the lowest quartile of DBP levels, and those in the the lower quartile of 25(OH)D and lowest quartile of DBP levels had significantly higher OR of being frail compared with those in the highest quartile of 25(OH)D and lowest quartile of DBP, with OR of 3.18 (95% CI: 1.46–4.56, $P < 0.05$), 2.63 (95% CI: 1.31–3.68, $P < 0.01$), and 2.52 (95% CI: 1.22–3.52, $P < 0.05$), respectively. The results indicate that the joint effect of serum 25(OH)D and DBP levels is associated with the risk of frailty, and serum DBP levels affects 25(OH)D-frailty relationship in the older men.

1. Introduction

In the elderly, frailty is an extremely common clinical state and a serious health problem in which there is an increase in an individual's vulnerability for developing increased dependency and/or mortality when exposed to a stressor [1, 2]. Overall, it appears that low levels of vitamin D play an important role in frailty. Vitamin D insufficiency may exacerbate frailty by affecting mainly two aspects, bone formation and neuromuscular function [3, 4]. Vitamin D is expected to be a treatment for frailty in an aging society [5, 6].

Studies examining the relationship between total circulating 25-hydroxyvitamin D [25(OH)D] levels and frailty have yielded mixed results. Many epidemiologic investigations have suggested that lower levels of 25(OH)D have been linked to muscle strength and increased risk of frailty [7–15]. Additionally, vitamin D supplementation reduces falls and improves muscle function in people with low 25(OH)D

levels [16–18]. However, not all observational studies have confirmed the relationship between 25(OH)D and the risk of frailty. In several randomized trials, no effect of vitamin D supplementation on the risk of frailty was observed [19, 20]. It is possible that, in these studies by using total 25(OH)D levels as an only measure of vitamin D status, individuals may be misclassified as sufficient or insufficient in vitamin D.

The free hormone hypothesis postulates that only hormones liberated from binding proteins enter cells and produce biologic action [21]. 25(OH)D circulates bound to vitamin D binding protein (DBP) (85% to 90%) and albumin (10% to 15%), with less than 1% of circulating hormone in its free form [22]. DBP may alter the biologic activity of 25(OH)D. However, the link between the biologic activity of 25(OH)D and frailty is not clear yet. We hypothesized that the joint effect of serum 25(OH)D and DBP levels is tightly linked to the risk of frailty and serum DBP levels might affect the correlation of 25(OH)D and frailty.

TABLE 1: Elected baseline characteristics across quartiles of vitamin D binding protein (medians or proportions).

Characteristic	Quartile of serum vitamin D binding protein (nmol/L)				P value
	Q1 <4046	Q2 4046–<5269	Q3 5269–<6686	Q4 ≥6686	
Age (years)	76.5	75.1	76.8	77.3	Matched
Height (cm)	170	169	171	172	0.89
Weight (kg)	74.5	78.2	76.1	77.2	0.43
BMI (kg/m ²)	24.6	25.1	24.2	25.5	0.61
Education (% > elementary)	16.0	14.6	14.1	13.8	0.49
Physically active during leisure time (% yes)	18.5	16.9	22.1	23.3	0.21
Season of blood draw (% Nov.–Mar.)	100	100	100	100	Matched
Supplemental vitamin D use (% yes)	28.1	26.7	15.3	14.6	0.16
Supplemental calcium use (% yes)	29.7	30.0	23.3	20.1	0.23
Serum biomarkers					
25(OH)D (nmol/L)	46.1	42.5	41.2	45.6	0.27
DBP (nmol/L)	3642	4632	5861	7183	NA
Free 25(OH)D (pmol/L)	26.5	28.4	23.9	22.2	0.13
Albumin (g/L) (normal range: 40–55 g/L)	36.0	37.7	38.3	39.1	0.36
Serum calcium (mmol/L) (normal range: 2.03–2.54 mmol/L)	2.1	2.2	2.1	2.3	0.46
Parathyroid hormone (ng/L) (normal range: 20.3–46.8 ng/L)	26.6	30.1	27.9	29.1	0.29

BMI: body mass index; 25(OH)D: 25-hydroxyvitamin D; DBP: vitamin D binding protein.

2. Material and Methods

2.1. Participants. From November 2012 to March 2013, 1048 aged men who were ≥70 years old were recruited in Changsha city and its surrounding area in Hunan province of China. Individuals were originally excluded if they were unable to walk without the assistance of another person or their renal function and liver function were being abnormal. Five hundred sixteen subjects had sufficient blood samples for analysis, and their characteristics are presented in Table 1. The study protocol was approved by the Second Xiangya Hospital of Central South University Ethics Committees in accordance with the Declaration of Helsinki and Good Clinical Practices Guidelines.

2.2. Assessment Methods

2.2.1. Biochemical Analysis. Fasting morning blood was collected and serum was divided into aliquots and they were stored at –70°C until they were shipped on dry ice to a central laboratory, where they were stored at –70°C until analysis. Analysis was performed using the radioimmuno assay kit (DiaSorin, Stillwater, MN, USA) to measure 25(OH)D. Intra- and interassay coefficients of variation (CVs) for 25(OH)D were 6.3% and 9.1%, respectively. DBP was measured by commercial enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. The assay was conducted after diluting serum with intra- and interassay CV 6.1% and 10.2%, respectively, for DBP. The manufacturer reports that DBP has no significant cross-reactivity with human albumin, vitamin D₃, or α-fetoprotein. Intact PTH was measured

by electrochemiluminescence immunoassay on the Cobas E160 automated analyzer (Roche Diagnostics, Indianapolis, IN, USA). The intra- and interassay CVs for intact PTH measurement were 4.7% and 9.6%, respectively. Calcium and albumin levels were measured by dye-based photometric assays on an automated analyzer.

2.2.2. Calculation of Free 25(OH)D. Free levels of 25(OH)D were calculated using the following equation [23]:

$$\text{Free 25(OH)D} = \frac{\text{total 25(OH)D}}{1 + (6 \times 10^3 \times \text{albumin}) + (7 \times 10^8 \times \text{DBP})} \quad (1)$$

The reported correlation coefficient between calculated free 25(OH)D using this equation and measured free 25(OH)D by centrifugal ultrafiltration is 0.925 [23].

2.2.3. Other Measurements. Participants completed a questionnaire and were interviewed at the examinations and asked about health status, educational achievement, and smoking status. A selected medical history including a history of a physician diagnosis of stroke, cancer, dementia, hypertension, Parkinsonism, diabetes mellitus, coronary heart disease, and chronic obstructive lung disease was obtained. Participants were asked to bring all medications including nonprescription supplements to clinic for verification of use. Body weight and height measurements were used to calculate a standard body mass index (BMI).

2.2.4. Frailty Status. Participants were classified as frail, prefrail, and nonfrail according to a validated screening

TABLE 2: Selected demographic and study variables of the study sample across frailty categories.

Characteristics	Nonfrail (N = 160)	Prefrail (N = 182)	Frail (N = 174)	P*
Age (y), mean (SD)	72.7 (4.1)	74.6 (5.2)	81.9 (4.4)	<0.05
BMI (kg/m ²), mean (SD)	26.5 (3.6)	24.3 (5.2)	27.8 (6.1)	0.68
%BMI [†]				0.27
<20.0	4.6	10.3	18.1	
20.0–<25.0	40.2	25.8	26.0	
25.0–<28.0	33.9	29.1	25.6	
≥28.0	21.3	34.8	30.3	
Education (y), mean (SD)	7.8 (2.1)	5.8 (1.6)	4.1 (1.7)	<0.05
Smoking status (% current or previous smokers)	41.2	47.1	45.8	0.62
25(OH)D (nmol/L), mean (SD)	46.1 (11.2)	42.9 (8.3)	35.8 (10.1)	<0.05
DBP (nmol/L), mean (SD)	4576 (1676)	4876 (1319)	5323 (1213)	<0.05
Free 25(OH)D (pmol/L), mean (SD)	26.3 (7.2)	23.4 (5.2)	20.1 (4.3)	0.26
Albumin (g/L), mean (SD)	38.1 (2.1)	37.3 (2.3)	35.7 (2.6)	0.19
Serum calcium (mmol/L), mean (SD)	2.2 (0.2)	2.1 (0.3)	2.0 (0.3)	0.58
Parathyroid hormone (ng/L), mean (SD)	28.9 (7.8)	30.1 (11.6)	32.3 (9.1)	0.62

Notes: BMI: body mass index; 25(OH)D: 25-hydroxyvitamin D; DBP: vitamin D binding protein.

* P values were determined using Jonckheere-Terpstra trend test.

[†]BMI was considered a categorical variable as defined and was adjusted in all the regression models.

tool based on the presence or absence of five measurable characteristics by Fried and Walston [2]: weakness, low physical activity, slow walking speed, exhaustion, and weight loss. “Weakness” was defined as grip strength in the lowest quintile within groups defined by sex and BMI. Participants reported their level of daily leisure physical activity in the past year; “low physical activity” was defined as either complete inactivity or performing low-intensity activities less than 1 h/wk. “Slow walking speed” was defined as usual walking speed in the slowest quintile within groups defined by sex and height. Walking speed was measured on a 4-m course using photocell recordings at the course start and finish. The final measure averaged two walks. “Exhaustion” was indicated by a response of “occasionally” or “often/always” to the statement, “I felt that everything was an effort.” “Weight loss” was measured as self-reported unintentional weight loss more than 4.5 kg within the past year. Individuals with a critical mass of 3 or more of the five components were defined as frail, those with one or two components were defined as prefrail, and those with none were defined as nonfrail.

2.3. Statistical Analysis. Quartile cutpoints for DBP and 25(OH)D were determined based on the distribution among participation. Summary statistics were constructed for comparing baseline characteristics of the participation. Distributions of sociodemographic and health characteristics, total 25(OH)D, and free 25(OH)D and DBP levels were summarized according to frailty status. The Spearman rank correlation coefficient was used to describe the correlation between 25(OH)D and DBP levels. Linear regression analysis was used to study the relationship between total 25(OH)D (as independent variable) and DBP levels (as dependent variable), adjusting for age, education, BMI, and smoking status. Logistic regression models were used to assess the effects of 25(OH)D and DBP levels on the risk of being

frail versus nonfrail cross-sectionally at baseline. Because exploratory analyses suggested potential nonlinear associations of 25(OH)D and DBP levels with frailty, 25(OH)D and DBP levels were modeled as quartiles in association with frailty for ease of interpretation. Interaction terms were added to the main effects model to explore potential synergy between 25(OH)D and DBP levels in their associations with frailty.

3. Results

Characteristics of the subjects by quartiles of serum DBP are shown in Table 1. There was no significant difference in age, education, BMI, physical activity during leisure time, season of blood draw, vitamin D, and calcium supplementation among the four groups. 25(OH)D and DBP levels were correlated (Spearman correlation coefficient = 0.16, $P < 0.05$) adjusting for age, education, and BMI. Calculated free 25(OH)D levels were positively correlated with total 25(OH)D levels ($r = 0.24$, $P < 0.05$).

Table 2 reports baseline demographic and health-related characteristics, 25(OH)D, and DBP and free 25(OH)D levels of the study sample across frailty categories. There were significant differences in mean 25(OH)D and DBP levels across frailty categories ($P < 0.05$ for stepwise increase or decrease trend). Compared with nonfrail participants, frail participants were older ($P < 0.05$) and were less educated ($P < 0.05$).

To investigate potential joint association of 25(OH)D and DBP levels with frailty, odds ratios (ORs) of participants being frail versus nonfrail were assessed across quartile of 25(OH)D and DBP levels. As shown in Table 3, participants in the lowest quartile of 25(OH)D (Q1a) and the highest quartile of DBP (Q4b) levels, those in the lowest quartile of 25(OH)D (Q1a) and the lowest quartile of DBP (Q1b), and those in

TABLE 3: Odds ratios of being frail versus nonfrail of participants across quarties of 25(OH)D and DBP levels.

Quarties of 25(OH)D (nmol/L)	Quarties of vitamin D binding protein (nmol/L)			
	Q1b (<4046)	Q2b (4046–<5269)	Q3b (5269–<6686)	Q4b (≥6686)
Q1a (<31.5)	2.63 [†]	1.89	1.73	3.18*
Q2a (31.5.3–<41.8)	2.52*	1.95	2.17	2.02
Q3a (41.8–<56.6)	1.36	1.76	1.71	1.82
Q4a (≥56.6)	1.0 (reference)	1.22	1.29	1.37

Notes: 25(OH)D: 25-hydroxyvitamin D; DBP: vitamin D binding protein.

* $P < 0.05$.

[†] $P < 0.01$.

the lowest quartile of DBP (Q1b) and the lower quartile of 25(OH)D (Q2a) levels had significantly higher OR of being frail compared with those in the highest quartile of 25(OH)D (Q4a) and lowest quartile of DBP (Q1b) (reference group), with OR of 3.18 (95% CI: 1.46–4.56, $P < 0.05$), 2.63 (95% CI: 1.31–3.68, $P < 0.01$), and 2.52 (95% CI: 1.22–3.52, $P < 0.05$), respectively, adjusting for age, BMI, and education. These results showed that, in the setting of the lowest quartile of 25(OH)D levels, both the lowest and the highest DBP levels confer increased risk for frailty, suggesting a “U-” shaped joint association of 25(OH)D and DBP levels with frailty, and that, in the setting of the lowest quartile of DBP levels, both the lowest and lower 25(OH)D levels confer increased risk for frailty. The interaction terms between quartile of 25(OH)D and DBP, however, were not statistically significant.

4. Discussion

This study has observed multiplicative interaction in the associations of DBP and 25(OH)D levels with frailty. The results suggest an association of increased levels of DBP and decreased levels of 25(OH)D with frailty (Table 3). Therefore, a high level of DBP or low level of 25(OH)D may increase the risk of frailty, whereas a low level of DBP or high level of 25(OH)D may reduce the risk of frailty.

Vitamin D status is determined by vitamin D stores in vivo and its biologic activity. Circulating 25(OH)D levels generally are considered to better reflect overall vitamin D stores [24]. The free hormone hypothesis postulates that only hormones liberated from binding proteins enter cells and produce biologic action [21]. 25(OH)D circulates binds to vitamin D binding protein (DBP) (85% to 90%) and albumin (10% to 15%), with less than 1% of circulating hormone in its free form [22]. In the present study, we have found that calculated free 25(OH)D levels were positively correlated with total 25(OH)D levels. Consistent with the free hormone hypothesis, the results of our study suggest that circulating DBP is an inhibitor of the biologic action of vitamin D in frailty patients. Unlike binding to DBP, binding to albumin does not inhibit the action of 25(OH)D. DBP behaves similarly to other serum hormone carrier proteins and has broad clinical applications. Like thyroid hormone-binding globulin and sex hormone-binding globulin, DBP may act as

a serum carrier and reservoir, prolonging the circulating half-life of vitamin D while at the same time regulating its immediate bioavailability to target tissues [21]. Thus hormonal activity and sufficiency may be reflected by the amounts of bioavailable vitamin, not by total levels. So, a low DBP level may be beneficial due to the higher level of bioavailable 25(OH)D but may also be a bad thing considering vitamin D effects in tissues that express megalin. Indeed, as DBP is internalized in some cells through a megalin cubilin uptake, a higher DBP concentration may be a favourable point for these effects [25]. The affinity of DBP for 25(OH)D depends on the DBP genotype with important consequences on the calculation of free or bioavailable 25(OH)D [25]. A recent paper by Powe et al. [26] reports that community-dwelling black Americans had low levels of total 25(OH)D and DBP, resulting in similar concentrations of estimated bioavailable 25(OH)D as compared with whites. Racial differences in the prevalence of common genetic polymorphisms provide a likely explanation for this observation.

Currently, clinical testing for vitamin D deficiency is based on measurement of total serum concentrations of 25(OH)D [24]. Our data suggest that concentrations of total serum 25(OH)D may not be the best measure of assessing vitamin D insufficiency or sufficiency status in frailty patients. For example, aged men with high levels of DBP may appear to be 25(OH)D-sufficient but actually there may be higher risk of frailty due to deficient in bioavailable 25(OH)D. Joint examination of serum 25(OH)D and DBP concentrations could be better to reflect overall vitamin D stores and the biologic action of vitamin D.

Clinical trials of vitamin D supplementation in older frailty patients with low vitamin D status mostly report improvements in muscle performance and reductions in falls [16–18]. The underlying mechanisms are probably both indirect via calcium and phosphate and direct via activation of the vitamin D receptor (VDR) on muscle cells and bone by 1,25-dihydroxyvitamin D [$1,25(\text{OH})_2\text{D}_3$]. VDR activation at the genomic level regulates transcription of genes involved in calcium handling and muscle cell or osteoblast differentiation and proliferation [3, 4, 27, 28].

This study has three limitations. First, sample size of the subgroups in the analysis across quartiles of DBP and 25(OH)D levels is relatively small and provides limited statistical power. Cautious interpretation of these results is warranted, and further investigation of the joint effects

of DBP and 25(OH)D levels on frailty is needed. Second, genotyping of these subjects was not done. Third, other endocrine factors including IGF-1 and testosterone have been identified for their interactions with DBP and 25(OH)D as well as their associations with frailty [29]. Therefore, findings from this study should be interpreted in the context of the complexity of the nutrition and endocrine systems as well as multifactorial nature of the frailty syndrome. Despite these limitations, results from this study do support the free hormone hypothesis and provide a basis for further investigations into optimal vitamin D status in frailty individuals.

5. Conclusion

We conclude that the joint effect of serum 25(OH)D and DBP levels may be tightly linked to frailty, or serum DBP levels modify 25(OH)D-frailty relationship in the older men. Our findings support the hypothesis that the biologic activity of 25(OH)D may be altered by DBP in frailty patients and suggest that joint examination of serum 25(OH)D and DBP concentrations in future studies could shed additional light on the role of vitamin D and its pathway cofactors in the aetiology of frailty.

Conflict of Interests

The authors declare that there is no conflict of interests associated with this paper.

Acknowledgments

This work was supported by the Public Welfare Industry Fund of National Health and Family Planning Commission of the People's Republic of China (no. 201302008) and the National Natural Science Foundation of China (no. 81370931).

References

- [1] L. P. Fried, "Conference on the physiologic basis of frailty. April 28, 1992, Baltimore, Maryland, U.S.A.," *Aging*, vol. 4, no. 3, pp. 251–252, 1992.
- [2] L. P. Fried and J. Walston, "Frailty and failure to thrive," in *Principles of Geriatric Medicine and Gerontology*, W. R. Hazzard, J. P. Blass, and J. B. Halter, Eds., pp. 1487–1501, McGraw-Hill, New York, NY, USA, 5th edition, 2003.
- [3] F. R. Pérez-López, P. Chedraui, and A. M. Fernández-Alonso, "Vitamin D and aging: beyond calcium and bone metabolism," *Maturitas*, vol. 69, no. 1, pp. 27–36, 2011.
- [4] M. Montero-Odasso and G. Duque, "Vitamin D in the aging musculoskeletal system: an authentic strength preserving hormone," *Molecular Aspects of Medicine*, vol. 26, no. 3, pp. 203–219, 2005.
- [5] J. E. Morley, B. Vellas, G. A. van Kan, S. D. Anker et al., "Frailty consensus: a call to action," *Journal of the American Medical Directors Association*, vol. 14, no. 6, pp. 392–397, 2013.
- [6] R. Rizzoli, S. Boonen, M. L. Brandi, O. Bruyère et al., "Vitamin D supplementation in elderly or postmenopausal women: a 2013 update of the 2008 recommendations from the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO)," *Current Medical Research and Opinion*, vol. 29, no. 4, pp. 305–313, 2013.
- [7] K. E. Ensrud, S. K. Ewing, L. Fredman et al., "Study of Osteoporotic Fractures Research Group. Circulating 25-hydroxyvitamin D levels and frailty status in older women," *Journal of Clinical Endocrinology and Metabolism*, vol. 95, no. 12, pp. 5266–5273, 2010.
- [8] K. E. Ensrud, T. L. Blackwell, J. A. Cauley et al., "Circulating 25-hydroxyvitamin D levels and frailty in older men: the osteoporotic fractures in men study," *Journal of the American Geriatrics Society*, vol. 59, no. 1, pp. 101–106, 2011.
- [9] J. E. Morley, "Should all long-term care residents receive vitamin D?" *Journal of the American Medical Directors Association*, vol. 8, no. 2, pp. 69–70, 2007.
- [10] A. Tajar, D. M. Lee, S. R. Pye, M. D. O'Connell et al., "The association of frailty with serum 25-hydroxyvitamin D and parathyroid hormone levels in older European men," *Age Ageing*, vol. 42, no. 3, pp. 352–359, 2013.
- [11] V. Hirani, V. Naganathan, R. G. Cumming et al., "Associations between frailty and serum 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D concentrations in older Australian men: the Concord Health and Ageing in Men Project," *The Journals of Gerontology Series A: Biological Sciences*, vol. 68, no. 9, pp. 1112–1121, 2013.
- [12] E. Smit, C. J. Crespo, Y. Michael et al., "The effect of vitamin D and frailty on mortality among non-institutionalized US older adults," *European Journal of Clinical Nutrition*, vol. 66, no. 9, pp. 1024–1028, 2012.
- [13] E. R. Wilhelm-Leen, Y. N. Hall, I. H. Deboer, and G. M. Chertow, "Vitamin D deficiency and frailty in older Americans," *Journal of Internal Medicine*, vol. 268, no. 2, pp. 171–180, 2010.
- [14] C.-I. Chang, D. C. Chan, K.-N. Kuo, C. A. Hsiung, and C.-Y. Chen, "Vitamin D insufficiency and frailty syndrome in older adults living in a Northern Taiwan community," *Archives of Gerontology and Geriatrics*, vol. 50, no. supplement 1, pp. S17–S21, 2010.
- [15] M. Shardell, G. E. Hicks, R. R. Miller et al., "Association of low vitamin D levels with the frailty syndrome in men and women," *Journals of Gerontology A Biological Sciences and Medical Sciences*, vol. 64, no. 1, pp. 69–75, 2009.
- [16] M. H. Murad, K. B. Elamin, N. O. Abu Elnour et al., "The effect of vitamin D on falls: a systematic review and meta-analysis," *Journal of Clinical Endocrinology and Metabolism*, vol. 96, no. 10, pp. 2997–3006, 2011.
- [17] R. R. Kalyani, B. Stein, R. Valiyl, R. Manno, J. W. Maynard, and D. C. Crews, "Vitamin D treatment for the prevention of falls in older adults: systematic review and meta-analysis," *Journal of the American Geriatrics Society*, vol. 58, no. 7, pp. 1299–1310, 2010.
- [18] R. M. Daly, "Independent and combined effects of exercise and vitamin D on muscle morphology, function and falls in the elderly," *Nutrients*, vol. 2, no. 9, pp. 1005–1017, 2010.
- [19] N. K. Latham, C. S. Anderson, A. Lee, D. A. Bennett, A. Moseley, and I. D. Cameron, "Fitness Collaborative Group. A randomized, controlled trial of quadriceps resistance exercise and vitamin D in frail older people: the Frailty Interventions Trial in Elderly Subjects (FITNESS)," *Journal of the American Geriatrics Society*, vol. 51, no. 3, pp. 291–299, 2003.
- [20] D. C. Chan, H. H. Tsou, R. S. Yang et al., "A pilot randomized controlled trial to improve geriatric frailty," *BMC Geriatrics*, vol. 12, article 58, 2012.

- [21] C. M. Mendel, "The free hormone hypothesis: a physiologically based mathematical model," *Endocrine Reviews*, vol. 10, no. 3, pp. 232–274, 1989.
- [22] D. D. Bikle, P. K. Siiteri, and E. Ryzen, "Serum protein binding of 1,25-dihydroxyvitamin D: a reevaluation by direct measurement of free metabolite levels," *Journal of Clinical Endocrinology and Metabolism*, vol. 61, no. 5, pp. 969–975, 1985.
- [23] D. D. Bikle, E. Gee, and B. Halloran, "Assessment of the free fraction of 25-hydroxyvitamin D in serum and its regulation by albumin and the vitamin D-binding protein," *Journal of Clinical Endocrinology and Metabolism*, vol. 63, no. 4, pp. 954–959, 1986.
- [24] M. F. Holick, "Medical progress: vitamin D deficiency," *The New England Journal of Medicine*, vol. 357, no. 3, pp. 266–281, 2007.
- [25] R. F. Chun, B. E. Peercy, E. S. Orwoll et al., "Vitamin D and DBP: the free hormone hypothesis revisited," *The Journal of Steroid Biochemistry and Molecular Biology*, 2013.
- [26] C. E. Powe, M. K. Evans, J. Wenger et al., "Vitamin D-binding protein and vitamin D status of black Americans and white Americans," *The New England Journal of Medicine*, vol. 369, no. 21, pp. 1991–2000, 2013.
- [27] L. Ceglia, "Vitamin D and skeletal muscle tissue and function," *Molecular Aspects of Medicine*, vol. 29, no. 6, pp. 407–414, 2008.
- [28] L. Ceglia and S. S. Harris, "Vitamin D and its role in skeletal muscle," *Calcified Tissue International*, vol. 92, no. 2, pp. 151–162, 2013.
- [29] J. E. Morley and T. K. Malmstrom, "Frailty, sarcopenia, and hormones," *Endocrinology and Metabolism Clinics of North America*, vol. 42, no. 2, pp. 391–405, 2013.

Research Article

Association of High Vitamin D Status with Low Circulating Thyroid-Stimulating Hormone Independent of Thyroid Hormone Levels in Middle-Aged and Elderly Males

Qingqing Zhang,¹ Zhixiao Wang,¹ Min Sun,¹ Mengdie Cao,¹ Zhenxin Zhu,¹ Qi Fu,¹ Yuan Gao,² Jia Mao,¹ Yanyun Li,¹ Yun Shi,¹ Fan Yang,¹ Shuai Zheng,¹ Wei Tang,² Yu Duan,¹ Xiaoping Huang,¹ Wei He,¹ and Tao Yang¹

¹ Department of Endocrinology, The First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu 210029, China

² Department of Endocrinology and Metabolism, The Affiliated Jiangyin Hospital of Southeast University Medical College, Jiangyin, Jiangsu 214400, China

Correspondence should be addressed to Wei He; hewei1007@sina.cn and Tao Yang; yangt@njmu.edu.cn

Received 5 November 2013; Revised 25 December 2013; Accepted 8 January 2014; Published 16 February 2014

Academic Editor: Zhongjian Xie

Copyright © 2014 Qingqing Zhang et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Background. A recent study has reported that high circulating 25-hydroxyvitamin D [25(OH)D] is associated with low circulating thyroid-stimulating hormone (TSH) levels, but only in younger individuals. The goal of the present study was to explore the relationship between vitamin D status and circulating TSH levels with thyroid autoimmunity and thyroid hormone levels taken into consideration in a population-based health survey of middle-aged and elderly individuals. **Methods.** A total of 1,424 Chinese adults, aged 41–78 years, were enrolled in this cross-sectional study. Serum levels of 25(OH)D, TSH, thyroid hormones, and thyroid autoantibodies were measured. **Results.** The prevalence of vitamin D insufficiency was 94.29% in males and 97.22% in females, and the prevalence of vitamin D deficiency was 55.61% in males and 69.64% in females. Vitamin D status was not associated with positive thyroid autoantibodies after controlling for age, gender, body mass index, and smoking status. Higher 25(OH)D levels were associated with lower TSH levels after controlling for age, FT4 and FT3 levels, thyroid volume, the presence of thyroid nodule(s), and smoking status in males. **Conclusion.** High vitamin D status in middle-aged and elderly males was associated with low circulating TSH levels independent of thyroid hormone levels.

1. Introduction

Vitamin D is recognized to be an essential element for bone metabolism and skeletal health; however, its deficiency can cause rickets in children as well as an increased propensity for osteoporosis [1]. In addition, it may also affect extraskeletal health. Indeed, vitamin D deficiency has been identified as a risk factor for diabetes mellitus [2, 3], cancers [4], multiple sclerosis [5] and other autoimmune diseases [6, 7], atherosclerosis [8], and infectious diseases [9].

Few past studies have reported the impact of vitamin D deficiency on autoimmune thyroid disease and demonstrated inconclusive results [10, 11]. Besides affecting the thyroid gland through immune-mediated processes, vitamin D has

been shown to influence rat thyroid follicular cells by directly inhibiting thyrotropin-stimulated iodide uptake in a dose-dependent manner [12]. Recently, a population-based study has reported that high vitamin D status in younger individuals is associated with low circulating thyroid-stimulating hormone (TSH) [13]. However, it remains unknown as to why no relationship between vitamin D status and serum TSH levels in middle-aged and elderly individuals was found in this study. Therefore, in the present study, we examined the relationship between vitamin D status and circulating TSH levels in middle-aged and elderly individuals with thyroid autoimmunity, while taking thyroid function into consideration in addition to the relationship between vitamin D insufficiency and thyroid autoimmunity, the presence of

thyroid nodule(s) and thyroid volume in a cross-sectional study.

2. Materials and Methods

2.1. Study Subjects. From June to December 2011, we recruited a total of 9,982 Chinese living in Gulou, Nanjing. The study was approved by the ethics committee of the First Affiliated Hospital of Nanjing Medical University. All patients provided informed consent and completed a standardized questionnaire. Blood samples were collected and stored at -80°C .

The present study used a subsample collected from September to November 2011 to detect serum 25-hydroxyvitamin D [25(OH)D] levels, thyroid function, and thyroid autoantibodies. Subjects with a history of thyroid disease, without complete informations or taking medications that affected their thyroid function, such as oral contraceptives, oestrogen, glucocorticoids and iodine, were excluded.

Further exclusion criteria included overt hypothyroidism (TSH $> 5.29 \mu\text{IU/mL}$; free thyroxine (FT4) $< 8.5 \text{ pmol/L}$) and overt hyperthyroidism (TSH $< 0.35 \mu\text{IU/mL}$; FT4 $> 22.5 \text{ pmol/L}$). A total of 1,424 participants were included in our study.

2.2. Laboratory Measurements. Serum samples were collected from all 1,424 participants in the morning and all venous blood samples after an overnight fast. The serum samples were used to measure FT4, FT3, TSH, and 25(OH)D levels. Only 1,279 and 1,357 serum samples were used to measure thyroid peroxidase antibody (TPOAb) and thyroglobulin antibody (TgAb), respectively. Serum 25(OH)D levels were assessed using an enzyme immunoassay (IDS, UK). Serum TSH, TPOAb, and TgAb levels were measured using a chemiluminescent immunoassay (AutoBio Co., Ltd., Zhengzhou, China). Euthyroidism was defined as the absence of hypo- or hyperthyroidism. Serum TPOAb of $>40 \text{ IU/mL}$ and/or TgAb of $>110 \text{ IU/mL}$ were considered autoantibody positivity. High TPOAb and TgAb titres were defined as arbitrary values greater than 200 IU/mL and 550 IU/mL (four times greater than the normal values), respectively. We set vitamin D insufficiency and deficiency at levels below 75 nmol/L (30 ng/mL) and 50 nmol/L (20 ng/mL), respectively. The presence of thyroid nodule(s) and size of the thyroid gland were determined by thyroid ultrasonography. The thyroid volume was estimated by multiplication of its thickness, width, length, and a corrective factor (0.479) [14].

2.3. Statistical Analysis. Continuous variables are presented as means \pm standard deviation for continuous normally distributed variables and median (interquartile range) for nonnormally distributed variables. Categorical variables are presented as percentage. The Kolmogorov-Smirnov method was used to test for normality. Differences between two groups for continuous and categorical variables were separately assessed using the Student's *t*-test and χ^2 -test. The distribution of TSH levels deviated significantly from a Gaussian distribution ($P < 0.000$), while the logarithms of TSH levels

were found to follow an approximate Gaussian distribution ($P = 0.065$). Linear regression analysis was used to examine the relationship between vitamin D status and logTSH values. Furthermore, bivariate correlation analysis was performed to study the correlation between vitamin D status and logTSH values. All calculations were performed using SPSS 13.0 for Windows (Chicago, IL, USA). A probability (*P*) value of <0.05 was considered statistically significant for all tests.

3. Results

3.1. Clinical and Laboratory Characteristics. The clinical and laboratory characteristics of the included 1,424 subjects (863 females and 561 males; age, 41–78 years) are shown in Table 1. On an average, females had higher serum TSH levels and TgAb titres, but lower serum 25(OH)D levels and proportion of smokers than males (3.03 (range, 1.89–4.60) versus 2.06 (1.36–3.30) IU/mL, $P < 0.01$; 13.19 (7.90–35.66) versus 10.08 (6.87–19.22) IU/mL, $P < 0.01$; 42.90 (35.15–53.11) versus 47.50 (38.00–59.00) IU/mL, $P < 0.01$ and 1.85% versus 44.92%, $P < 0.01$, resp.). There were no significant differences in other laboratory characteristics between males and females. The overall median (interquartile range) serum 25(OH)D level was 44.68 (range, 36.2–55.3) nmol/L. The prevalence of vitamin D insufficiency was 94.29% in males and 97.22% in females, whereas the prevalence of vitamin D deficiency was 55.61% in males and 69.64% in females. In the present study, 138 (10.17%) subjects were positive for serum TgAb, of which 42 (30.43%) had high TgAb titres. A total of 152 (11.88%) subjects were positive for serum TPOAb, of which 55 (36.18%) had high TPOAb titres. The prevalence of thyroid nodule(s) was 36.03%.

3.2. Comparison of Clinical and Laboratory Characteristics between Vitamin D Insufficiency and Sufficiency Groups. Based on a cutoff value of 75 nmol/L (30 ng/mL), the subjects were grouped into vitamin D insufficiency and vitamin D sufficiency. Differences between the clinical and laboratory characteristics between the two groups are shown in Table 2. There were significantly fewer males in the vitamin D insufficiency group (38.67% versus 57.14%, $P = 0.006$) than in the vitamin D sufficiency group. The serum TSH level and TPOAb and TgAb titres in the vitamin D insufficiency group were significantly higher than those in the vitamin D sufficiency group. However, there were no significant differences in serum FT3 and FT4 levels, the presence of thyroid nodule(s), thyroid volume, and smoking status between the two groups. Multivariate logistic regression analysis revealed that vitamin D status was not associated with thyroid autoantibody positivity after controlling for age, gender, body mass index (BMI), and smoking status (Table 3). Based on a cutoff value of 50 nmol/L (20 ng/mL), the subjects were grouped into vitamin D deficiency and nonvitamin D deficiency. High TgAb titres, High TPOAb titres, and high TgAb and/or TPOAb titres were significantly more common in the vitamin D deficiency group compared with those in the nonvitamin D deficiency group (4.04% versus 1.43%, $P = 0.008$; 5.48% versus 2.18%, $P = 0.005$; 7.70% versus 3.60%, $P = 0.004$, resp.). And vitamin D deficiency was independently

TABLE 1: Clinical and laboratory characteristics of the participants.

Characteristics	Male (<i>n</i> = 561)	Female (<i>n</i> = 863)	Total (<i>n</i> = 1424)
Age (years)	59.22 ± 9.03	59.04 ± 8.20	59.11 ± 8.53
BMI (kg/m ²)	24.93 ± 3.38	24.66 ± 3.36	24.76 ± 3.37
Serum 25 (OH)D (nmol/L)	47.50 (38.00–59.00)	42.90 (35.15–53.11)*	44.68 (36.20–55.30)
Serum FT3 (pmol/L)	4.34 (3.99–4.69)	4.31 (3.98–4.72)	4.32 (3.98–4.69)
Serum FT4 (pmol/L)	15.87 (13.10–18.03)	15.81 (13.22–17.95)	15.83 (13.18–17.97)
Serum TSH (μIU/mL)	2.06 (1.36–3.30)	3.03 (1.89–4.60)*	2.65 (1.62–4.10)
Serum TPOAb (IU/mL)	3.09 (1.07–11.08)	4.18 (1.24–16.28)	3.66 (1.15–14.34)
Serum TgAb (IU/mL)	10.08 (6.87–19.22)	13.19 (7.90–35.66)*	12.06 (7.31–27.00)
Thyroid volume (mL)	8.44 (6.82–10.06)	7.24 (5.83–8.82)	7.74 (6.16–9.40)
Presence of thyroid nodule(s) (<i>n</i> (%))	174 (31.02%)	339 (39.28%)*	513 (36.03%)
Current smokers (<i>n</i> (%))	252 (44.92%)	16 (1.85%)*	268 (18.82%)

*Significantly different from males, $P < 0.01$.

TABLE 2: Comparison of clinical and laboratory characteristics based on vitamin D insufficiency.

	Vitamin D insufficiency (1368)	Nonvitamin D insufficiency (56)	<i>P</i> value
Age (years)	59.07 ± 8.55	60.07 ± 8.11	0.371
Male sex (<i>n</i> (%))	529 (38.67%)	32 (57.14%)	0.006
BMI (kg/m ²)	24.79 ± 3.40	24.21 ± 2.50	0.106
Serum FT3 (pmol/L)	4.39 (3.97–4.69)	4.48 (4.18–4.75)	0.094
Serum FT4 (pmol/L)	15.51 (13.14–17.93)	16.29 (14.01–18.79)	0.092
Serum TSH (μIU/mL)	3.42 (1.62–4.18)	2.47 (1.54–3.24)	0.000
Serum TgAb (IU/mL)	84.26 (7.26–27.77)	21.12 (8.38–22.40)	0.000
Serum TPOAb (IU/mL)	40.45 (1.17–14.33)	16.77 (0.99–14.99)	0.000
Thyroid volume (mL)	8.43 (6.12–9.35)	8.71 (6.53–10.62)	0.476
Presence of thyroid nodule(s) (<i>n</i> (%))	495 (36.18%)	18 (32.14%)	0.537
Current smokers (<i>n</i> (%))	814 (59.50%)	37 (66.07%)	0.326

associated with high thyroid autoantibody titres (see Supplementary Table 1 in the Supplementary Material available online at <http://dx.doi.org/10.1155/2014/631819>).

3.3. Relationship between Vitamin D Status and Serum TSH Levels according to Gender. With regard to the relationship between vitamin D status and serum TSH level, it was found that higher 25(OH)D levels were associated with lower TSH levels independent of age, FT3 and FT4 levels, TPOAb and TgAb titres, thyroid volume, the presence of thyroid nodule(s), and smoking status in males (Beta = -0.166 , $P = 0.004$; Table 4). In addition, the association was found between thyroid volume and serum TSH levels in males (Beta = -0.152 , $P = 0.009$) as well as the presence of thyroid nodule(s) and serum TSH levels in females (Beta = -0.116 , $P = 0.023$). However, there were no significant relationship between serum TSH, FT3 and FT4 levels, TgAb and TPOAb titres, and smoking status between genders.

3.4. Relationship between Vitamin D Status and Serum TSH Levels on the Basis of Negative Serum Thyroid Autoantibody Titres in Males. To determine the probable interaction between vitamin D status and thyroid autoantibody positivity on serum TSH levels, further analyses were performed based

on positive serum TPOAb, TgAb and TPOAb, and/or TgAb titres. The concentration of 25(OH)D was negatively correlated with serum TSH levels only in subjects with negative serum TPOAb, TgAb and TPOAb and/or TgAb titres ($P = 0.002$, 0.005 and 0.002 , resp.; Supplementary Table 2). Linear regression analysis showed that higher 25(OH)D levels were associated with lower TSH levels independent of age, serum thyroid hormone levels, thyroid volume, the presence of thyroid nodule(s), and smoking status in males with negative serum TPOAb, TgAb, and TPOAb and/or TgAb titres ($P = 0.004$, 0.004 , and 0.002 , resp.; Table 5), but not in females or subjects positive for serum thyroid autoantibodies (data not shown).

4. Discussion

In the present study, we explored the probable interaction between vitamin D status and thyroid autoantibodies on serum TSH levels in middle-aged and elderly Chinese population with euthyroidism. This report is the first, to the best of our knowledge, to show that a higher circulating 25(OH)D level was associated with lower TSH levels only in males with negative serum thyroid autoantibody titres independent of thyroid hormone levels.

TABLE 3: Determinants of positive serum thyroid antibodies.

	Serum TgAb		Serum TPOAb		Serum TgAb and/or TPOAb	
	Adjusted OR (95% CI)	<i>P</i> value	Adjusted OR (95% CI)	<i>P</i> value	Adjusted OR (95% CI)	<i>P</i> value
Age (years)	0.997 (0.974–1.022)	0.838	1.007 (0.983–1.032)	0.558	1.009 (0.987–1.030)	0.431
Male sex	2.738 (1.526–4.910)	0.001	2.200 (1.267–3.821)	0.005	2.524 (1.550–4.110)	0.000
BMI (kg/m ²)	1.035 (0.974–1.099)	0.266	1.040 (0.979–1.106)	0.204	1.039 (0.984–1.097)	0.166
Smoking status	0.786 (0.368–1.676)	0.532	1.308 (0.685–2.499)	0.415	1.052 (0.578–1.916)	0.868
Serum 25(OH)D (nmol/L)	0.992 (0.979–1.004)	0.188	0.999 (0.987–1.010)	0.818	0.997 (0.986–1.009)	0.660

TABLE 4: Standardized regression coefficients of variables in relation to serum TSH according to gender.

	Male (561)		Female (863)	
	Beta	<i>P</i> value	Beta	<i>P</i> value
Age (years)	−0.012	0.836	−0.023	0.647
Serum FT3 (pmol/L)	0.069	0.244	−0.043	0.399
Serum FT4 (pmol/L)	−0.040	0.501	−0.066	0.201
Presence of TgAb (%)	0.029	0.636	0.098	0.060
Presence of TPOAb (%)	−0.002	0.971	0.056	0.284
Thyroid volume (mL)	−0.152	0.009	−0.056	0.262
Presence of thyroid nodule(s) (%)	−0.025	0.669	−0.116	0.023
Current smokers (<i>n</i> (%))	−0.054	0.361	−0.001	0.985
Serum 25(OH)D (nmol/L)	−0.166	0.004	−0.001	0.983

TABLE 5: Standardized regression coefficients of variables in relation to serum TSH in males with negative serum antibodies.

	Serum TgAb		Serum TPOAb		Serum TgAb and/or TPOAb	
	Beta	<i>P</i> value	Beta	<i>P</i> value	Beta	<i>P</i> value
Age (years)	0.024	0.686	0.019	0.749	0.006	0.921
Serum FT3 (pmol/L)	0.030	0.593	0.092	0.122	0.074	0.228
Serum FT4 (pmol/L)	0.020	0.726	−0.037	0.535	−0.028	0.648
Thyroid volume (mL)	−0.165	0.003	−0.170	0.004	−0.154	0.011
Presence of thyroid nodule(s) (%)	−0.035	0.532	−0.020	0.736	−0.024	0.696
Current smokers (<i>n</i> (%))	−0.064	0.264	−0.060	0.313	−0.067	0.277
Serum 25(OH)D (nmol/L)	−0.157	0.004	−0.166	0.004	−0.184	0.002

The result that high vitamin D status was associated with low circulating TSH levels remained unknown. We hypothesized that vitamin D may influence the thyrotrophs by acting on vitamin D receptors, which are widely distributed through distinct portions of the brain system [15]. A previous study has showed that vitamin D modulated pituitary thyrotropin TSH secretion by binding to specific binding sites [16]. Smith et al. [17] also found that exogenous vitamin D administration significantly suppressed pituitary thyrotropin TSH secretion in the basal state. This study also found that serum TSH levels of middle-aged and elderly women were higher than those of same-age men, and this result was consistent with those of previous reports [18, 19]. This result may indicate that TSH secretion is regulated by sex hormones, genetic susceptibility, or environmental factors, which may also mediate the relationship between vitamin D

status and serum TSH levels. Another previous study [20] has showed that circulating oestrogen could induce acute serum TSH suppression in males by acting on the pituitary gland, and vitamin D was shown to be an important factor in oestrogen biosynthesis of both female and male gonads [21]. However, oestrogen administration has been reported to both increase [22] and decrease [23] or have no effect on thyroid activity in females [24]. Therefore, it may be safely inferred that oestrogens have a complex relationship with serum TSH secretion in females, which may explain the cause of this relationship only in men. We did not find the relationship between vitamin D status and serum TSH levels in subjects with positive thyroid antibody titres, which may be due to the limited number of participants, especially when divided into two groups according to gender. Further clinical investigation with a larger sample is required to elucidate the effects of

vitamin D on the thyrotrophs to provide further insight into this relationship.

Chailurkit et al. [13] reported that an increase in serum 25(OH)D concentration was independently associated with lower TSH, but only in younger (age, 15–44 years) individuals. However, we found that higher 25(OH)D levels were independently associated with lower TSH in males with euthyroidism aged over 40 years. The results of the present study and those of Chailurkit et al.'s study differed for some reasons. First, subjects with overt hyperthyroidism and hypothyroidism by measuring serum FT3, FT4 and TSH levels were excluded in our study, and the results of our study reflect the general population setting with euthyroidism. Second, Chailurkit et al. did not separately analyse the relationship between 25(OH)D and serum TSH levels in males and females. Third, thyroid volume, the presence of thyroid nodule(s), and smoking status were considered confounding factors in our statistical analyses. Lastly, ethnicity was previously reported to be related with TSH levels [18], which plausibly explained the difference between the present and Chailurkit's studies.

It is generally agreed that serum 25(OH)D levels of <75 nmol/L (30 ng/mL) should be considered as representative of vitamin D insufficiency, whereas serum 25(OH)D levels of <50 nmol/L (20 ng/mL) as an indicative of vitamin D deficiency [25]. Using these definitions, our data showed that the prevalence of vitamin D insufficiency was 94.3% in males and 97.3% in females, and the prevalence of vitamin D deficiency was 55.6% in males and 69.6% in females. However, there were no conclusive results on the impact of vitamin D deficiency on autoimmune thyroid disease [18, 26]. In addition, our study did not find a link between vitamin D status and thyroid autoantibody positivity after controlling for age, gender, BMI, and smoking status. However, vitamin D deficiency was independently associated with high-titre thyroid autoantibody positivity. We thought that this high-titre thyroid autoantibody positivity should be paid more attention.

There were a number of limitations to the present study. First, TSH receptor-stimulating antibodies were not measured. Second, our study focused on middle-aged and elderly individuals; therefore, the relationship between vitamin D status and serum TSH levels in younger individuals with negative serum thyroid autoantibodies remained unknown. Finally, because of the cross-sectional nature of the present study, the causative effect of vitamin D on serum TSH could not be readily determined.

5. Conclusion

There was a high prevalence of vitamin D insufficiency among healthy adults. This population-based study was the first, to the best of our knowledge, to report an association between vitamin D and serum TSH levels independent of thyroid hormone levels in middle-aged and elderly males with negative thyroid autoimmunity. Here, we demonstrated a link between vitamin D insufficiency and serum thyroid autoantibody levels; however, vitamin D status was not associated with positive

thyroid autoantibody titres after controlling for age, gender, and smoking status. Therefore, further longitudinal studies are required to clarify the relationship between vitamin D and serum TSH levels, particularly in subjects with negative-serum thyroid autoantibody titres.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Qingqing Zhang, Zhixiao Wang, and Min Sun contributed equally to this study.

Acknowledgments

The authors thank all the participants in this study. This work was supported by the grants from the Chinese Society of Endocrinology and the Project Funded by Jiangsu Provincial Special Program of Medical Science (BL2012026).

References

- [1] M. F. Holick, "Medical progress: vitamin D deficiency," *The New England Journal of Medicine*, vol. 357, no. 3, pp. 266–281, 2007.
- [2] A. G. Pittas, J. Lau, F. B. Hu, and B. Dawson-Hughes, "Review: the role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis," *Journal of Clinical Endocrinology and Metabolism*, vol. 92, no. 6, pp. 2017–2029, 2007.
- [3] J. Mitri, M. D. Muraru, and A. G. Pittas, "Vitamin D and type 2 diabetes: a systematic review," *European Journal of Clinical Nutrition*, vol. 65, no. 9, pp. 1005–1015, 2011.
- [4] C. F. Garland, E. D. Gorham, S. B. Mohr, and F. C. Garland, "Vitamin D for cancer prevention: global perspective," *Annals of Epidemiology*, vol. 19, no. 7, pp. 468–483, 2009.
- [5] K. L. Munger, S. M. Zhang, E. O'Reilly et al., "Vitamin D intake and incidence of multiple sclerosis," *Neurology*, vol. 62, no. 1, pp. 60–65, 2004.
- [6] M. Cutolo and K. Otsa, "Vitamin D, immunity and lupus," *Lupus*, vol. 17, no. 1, pp. 6–10, 2008.
- [7] M. F. Holick, "Vitamin D: extraskeletal health," *Endocrinology and Metabolism Clinics of North America*, vol. 39, no. 2, pp. 381–400, 2010.
- [8] T. J. Wang, M. J. Pencina, S. L. Booth et al., "Vitamin D deficiency and risk of cardiovascular disease," *Circulation*, vol. 117, no. 4, pp. 503–511, 2008.
- [9] J. J. Cannell, R. Vieth, J. C. Umhau et al., "Epidemic influenza and vitamin D," *Epidemiology and Infection*, vol. 134, no. 6, pp. 1129–1140, 2006.
- [10] G. Tamer, S. Arik, I. Tamer, and D. Coksert, "Relative vitamin D insufficiency in Hashimoto's thyroiditis," *Thyroid*, vol. 21, no. 8, pp. 891–896, 2011.
- [11] H. Orbach and Y. Shoenfeld, "Vaccination infection and autoimmunity: myth and reality VIAMR 2005-10-26-28, Beau-Rivage Palace Hotel, Lausanne, Switzerland," *Autoimmunity Reviews*, vol. 6, no. 5, pp. 261–266, 2007.
- [12] J. P. Berg, K. M. Liane, S. B. Bjorhovde, T. Bjoro, P. A. Torjesen, and E. Haug, "Vitamin D receptor binding and biological effects

- of cholecalciferol analogues in rat thyroid cells," *Journal of Steroid Biochemistry and Molecular Biology*, vol. 50, no. 3-4, pp. 145-150, 1994.
- [13] L. O. Chailurkit, W. Aekplakorn, and B. Ongphiphadhanakul, "High vitamin D status in younger individuals is associated with low circulating thyrotropin," *Thyroid*, vol. 23, no. 1, pp. 25-30, 2013.
- [14] W. Teng, Z. Shan, X. Teng et al., "Effect of iodine intake on thyroid diseases in China," *The New England Journal of Medicine*, vol. 354, no. 26, pp. 2783-2793, 2006.
- [15] K. Prüfer, T. D. Veenstra, G. F. Jirikowski, and R. Kumar, "Distribution of 1,25-dihydroxyvitamin D3 receptor immunoreactivity in the rat brain and spinal cord," *Journal of Chemical Neuroanatomy*, vol. 16, no. 2, pp. 135-145, 1999.
- [16] M. Sar, W. E. Stumpf, and H. F. DeLuca, "Thyrotropes in the pituitary are target cells for 1,25 dihydroxy vitamin D3," *Cell and Tissue Research*, vol. 209, no. 1, pp. 161-166, 1980.
- [17] M. A. Smith, C. McHenry, R. Oslapas, C. Hofmann, P. Hessel, and E. Paloyan, "Altered TSH levels associated with increased serum 1,25-dihydroxyvitamin D3: a possible link between thyroid and parathyroid disease," *Surgery*, vol. 106, no. 6, pp. 987-991, 1989.
- [18] J. G. Hollowell, N. W. Staehling, W. Dana Flanders et al., "Serum TSH, T4, and thyroid antibodies in the United States population (1988 to 1994): national Health and Nutrition Examination Survey (NHANES III)," *Journal of Clinical Endocrinology and Metabolism*, vol. 87, no. 2, pp. 489-499, 2002.
- [19] C. Li, H. Guan, X. Teng et al., "An epidemiological study of the serum thyrotropin reference range and factors that influence serum thyrotropin levels in iodine sufficient areas of China," *Endocrine Journal*, vol. 58, no. 11, pp. 995-1002, 2011.
- [20] H. A. Gross, M. D. Appleman Jr., and J. T. Nicoloff, "Effect of biologically active steroids on thyroid function in man," *Journal of Clinical Endocrinology and Metabolism*, vol. 33, no. 2, pp. 242-248, 1971.
- [21] K. Kinuta, H. Tanaka, T. Moriwake, K. Aya, S. Kato, and Y. Seino, "Vitamin D is an important factor in estrogen biosynthesis of both female and male gonads," *Endocrinology*, vol. 141, no. 4, pp. 1317-1324, 2000.
- [22] T. Yamada, Y. Takemura, I. Kobayashi, and K. Shichijo, "Re-evaluation of the effect of estrogen on thyroid activity in the rat and its mechanism," *Endocrinology*, vol. 79, no. 5, pp. 849-857, 1966.
- [23] E. M. Bogdanove and B. A. Eskin, "The influence of estrogen upon goiter induction in adult and immature rats," *Endocrinology*, vol. 59, no. 6, pp. 688-694, 1956.
- [24] D. Christianson, E. Roti, A. G. Vagenakis, and L. E. Braverman, "The sex-related difference in serum thyrotropin concentration is androgen mediated," *Endocrinology*, vol. 108, no. 2, pp. 529-535, 1981.
- [25] M.-C. Chapuy, P. Preziosi, M. Maamer et al., "Prevalence of vitamin D insufficiency in an adult normal population," *Osteoporosis International*, vol. 7, no. 5, pp. 439-443, 1997.
- [26] R. Goswami, R. K. Marwaha, N. Gupta et al., "Prevalence of vitamin D deficiency and its relationship with thyroid autoimmunity in Asian Indians: a community-based survey," *British Journal of Nutrition*, vol. 102, no. 3, pp. 382-386, 2009.