the lower estimate, this metabolic effect would counterbalance a substantial component of the estimated 250- to 350-kcal/d increase in energy intake thought to underlie the obesity epidemic in the United States (assuming no other changes in energy balance components). Another area of agreement with Guyenet and Hall is the need for more research to resolve this controversy of fundamental importance to nutrition science and public health.

Author disclosures: DSL and CBE have conducted research studies examining the carbohydrate–insulin model funded by the National Institutes of Health and philanthropic organizations unaffiliated with the food industry; DSL received royalties for books on obesity and nutrition that recommend a low-glycemic load diet.
Abbreviations used: DLW, doubly labeled water; TEE, total energy expenditure; WRC, whole-room calorimetry.

David S Ludwig
Cara B Ebbeling

New Balance Foundation Obesity Prevention Center, Boston Children's Hospital and Harvard Medical School, Boston, MA, USA (DSL, e-mail: david.ludwig@childrens.harvard.edu).

## References

1. Ludwig DS, Ebbeling CB, Wong JMW, Wolfe RR, Wong WW. Methodological error in measurement of energy expenditure by the doubly labeled water method: much ado about nothing? Am J Clin Nutr 2019;110(5):1253–4.
2. Ludwig DS, Lakin PR, Wong WW, Ebbeling CB. Scientific discourse in the era of open science: a response to Hall et al. regarding the carbohydrate-insulin model. Int J Obes 2019;43(12):2355–60.
3. Ludwig DS, Greco KF, Ma C, Ebbeling CB. Testing the carbohydrate-insulin model of obesity in a 5-month feeding study: the perils of post-hoc participant exclusions. Eur J Clin Nutr 2020;74(7):1109–12.
4. Ebbeling CB, Bielak L, Lakin PR, Klein GL, Wong JMW, Luoto PK, Wong WW, Ludwig DS. Energy requirement Is higher during weight-loss maintenance in adults consuming a low- compared with high-carbohydrate diet. J Nutr 2020;150(8):2009–15.
5. Goldsmith R, Joanisse DR, Gallagher D, Pavlovich K, Shamoon E, Leibel RL, Rosenbaum M. Effects of experimental weight perturbation on skeletal muscle work efficiency, fuel utilization, and biochemistry in human subjects. Am J Physiol Regul Integr Comp Physiol 2010;298(1):R79–88.
6. Rosenbaum M, Ravussin E, Matthews DE, Matthews DE, Gilker C, Ferraro R, Heymsfield SB, Hirsch J, Leibel RL. A comparative study of different means of assessing long-term energy expenditure in humans. Am J Physiol 1996;270(3 Pt 2):R496–504.
7. Hall KD, Chen KY, Guo J, Lam YY, Leibel RL, Mayer LE, Reitman ML, Rosenbaum M, Smith SR, Walsh BT, et al. Energy expenditure and body composition changes after an isocaloric ketogenic diet in overweight and obese men. Am J Clin Nutr 2016;104(2):324–33.
8. Friedman MI, Appel S. Energy expenditure and body composition changes after an isocaloric ketogenic diet in overweight and obese men: a secondary analysis of energy expenditure and physical activity. PLoS One 2019;14(12):e0222971.
9. Leibel RL, Hirsch J, Appel BE, Checani GC. Energy intake required to maintain body weight is not affected by wide variation in diet composition Am J Clin Nutr 1992;55(2):350–5.
10. Rumpler WV, Seale JL, Miles CW, Bodwell CE. Energy-intake restriction and diet-composition effects on energy expenditure in men. Am J Clin Nutr 1991;53(2):430–6.
11. Ludwig DS, Ebbeling CB. The carbohydrate-insulin model of obesity: beyond "calories in, calories out." JAMA Intern Med 2018;178(8):1098–103.

doi: 10.1093/jn/nxab215.

# Contribution of Vitamin D–Binding Protein Polymorphism to Susceptibility and Outcome of COVID-19 Patients

We read with interest the paper of Bychinin et al. (1) in which they reported their investigation of the predictive value of serum 25-hydroxyvitamin D [25(OH)D] concentration for coronavirus disease 2019 (COVID-19) mortality in patients admitted to the intensive care unit (ICU). More in detail, serum 25(OH)D concentrations $\leq$9.9 ng/mL on admission could predict in-hospital mortality in COVID-19 patients. Here, we highlight the importance of the vitamin D–binding protein (DBP) polymorphism in the interpretation of the reported results.

DBP, also called group-specific component (GC), is the oldest member of the albuminoid family. This $\alpha$-globulin is genetically very polymorphic, with 6 major phenotypes based on 3 frequent alleles [DBP1F (rs7041-T, rs4588-C), DBP1S (rs7041-G, rs4588-C), and DBP2 (rs7041-T, rs4588-A)], but in total, >120 different variants have been identified in human populations around the world (2). A near-perfect proxy for rs4588 is rs2282679: rs2282679-A is typically coinherited with rs4588-C, whereas rs2282679-C is typically coinherited with rs4588-A (3).

DBP is the major transport protein for vitamin D, binding >99% of the circulating vitamin D metabolites, along with albumin. In comparison with other transport proteins, the serum concentration of DBP is 20-fold higher than the serum concentration of the vitamin D metabolites, which results in a 5% occupation of the binding sites on DBP by vitamin D sterols, and in very low absolute and relative free concentrations of 25(OH)D [0.03% of total 25(OH)D] and 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] (0.4% of its total concentration) (2). A strong causal association ($P = 3.2 \times 10^{-19}$) between serum DBP and 25(OH)D concentrations has been reported (3).

The different DBP phenotypes have significant effects on total 25(OH)D, free 25(OH)D, and DBP concentrations. More specifically, the lowest total and free serum concentrations of 25(OH)D have been found in subjects with the DBP2 allele, who also tend to have the lowest serum DBP concentrations (4). Besides, the serum vitamin D concentration depends on the rs2282679 genotype, being highest in rs2282679-A/A subjects, intermediate in rs2282679-A/C carriers, and lowest in the rs2282679-C/C group (5). In a Mendelian randomization study (3), a strong association between rs2282679 and both serum 25(OH)D and DBP concentrations has been demonstrated. Individuals carrying the C-allele at rs2282679 had lower

serum DBP concentrations than those with the more common A-allele. So, the higher median serum 25(OH)D concentration in survivors than in nonsurvivors of COVID-19, as reported by Bychinin al. (1), could probably be partly explained by DBP and its polymorphism. Alterations in serum DBP concentrations and different DBP polymorphisms should be considered as potential confounders in the interpretation of serum total 25(OH)D concentrations. To support this statement, we have investigated in a previous report (6) the influence of the DBP phenotypes in patients with a severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection by comparing the frequency of the DBP1 allele (a mixture of DBP1F and DBP1S) with the prevalence and mortality data of COVID-19 in *55* countries. An association was observed between the DBP1 allele frequency and a lower prevalence and mortality due to a SARS-CoV-2 infection, which could be partly explained by the potential protective effects of vitamin D. In another study (7) that investigated the impact of patient genetic background related to vitamin D pathways on COVID-19 severity, the SNP rs2282679 could explain most of the positive correlation between the metabolism score (DBP rs2282679 + CYP24A1 rs17216707) and COVID-19 severity ($\rho = 0.13$, $P$ value $= 0.005$) (7). Besides the *DBP* gene, the *CYP2R1* gene (rs10741657), the *CYP24A1* gene (rs6013897), and the *DHCR7/NADSYN1* region (rs12785878) are also genetic determinants of the 25(OH)D concentration. However, no association has been found between the vitamin D total score (DHCR7 rs12785878 + CYP2R1 rs10741657 + DBP rs2282679 + CYP24A1 rs17216707 + AMDHD1 rs10745742 + SEC23A rs8018720) and the severity of COVID-19 (7).

As DBP is a multifunctional protein, the association of the reported DBP polymorphisms with disease severity might also be partly explained by DBP's actin scavenger capacity (8). During tissue injury, large quantities of actin can be released into extracellular fluids, resulting in the formation of actin filaments (F-actin) and leading to alterations in the coagulation and fibrinolytic systems, with occlusion and damage of the microcirculation (particularly in the lungs) as a consequence. High morbidity and mortality have been associated with coagulopathy in severe COVID-19 (9). DBP and plasma gelsolin act as part of the actin scavenging system and work in tandem (8). Gelsolin severs F-actin filaments into globular actin (G-actin) monomers, whereas DBP binds G-actin in a high-affinity (Kd of $10^{-9}$ M) 1:1 molar complex for transport and eventual clearance of actin from the circulation (8). Actin-induced depletion of plasma DBP correlates with a poor prognosis in cases of sepsis (10) and has a statistical correlation similar to the other outcome metrics such as the Acute Physiology and Chronic Health Evaluation II (APACHE II) score (sepsis), Kings College criteria (liver failure), and the trauma and injury severity score (TRISS) (multiple trauma) (10, 11). So, lower serum DBP concentrations might have a link with COVID-19 mortality in patients admitted to the ICU, not only by its causal association with vitamin D deficiency, but also by decreasing the actin-binding capacity.

The question of whether the reported relations between DBP (and its polymorphisms) and the outcome of COVID-19 are causal or consequential should be further investigated.

The authors reported no funding received.

Author disclosures: The authors report no conflicts of interest.

Abbreviations used: $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; APACHE II, Acute Physiology and Chronic Health Evaluation II; COVID-19, coronavirus disease 2019; DBP, vitamin D–binding protein; F-actin, filamentous actin; G-actin, globular actin; GC, group-specific component; ICU, intensive care unit; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; TRISS, trauma and injury severity score.

The authors' responsibilities were as follows–MMS, JRD: wrote the paper and both authors read and approved the final manuscript.

Marijn M Speeckaert
Joris R Delanghe

Department of Nephrology, Ghent University Hospital, Ghent, Belgium (MMS, e-mail: Marijn.Speeckaert@ugent.be); Research Foundation-Flanders (FWO), Brussels, Belgium; and Department of Diagnostic Sciences, Ghent University, Ghent, Belgium

## References

1. Bychinin MV, Klypa TV, Mandel IA, Andreichenko SA, Baklaushev VP, Yusubalieva GM, Kolyshkina NA, Troitsky AV. Low circulating vitamin D in intensive care unit-admitted COVID-19 patients as a predictor of negative outcomes. J Nutr 2021;151(8):2199–2205.
2. Bikle DD, Schwartz J. Vitamin D binding protein, total and free vitamin D levels in different physiological and pathophysiological conditions. Front Endocrinol 2019;10:317.
3. Leong A, Rehman W, Dastani Z, Greenwood C, Timpson N, Langsetmo L, Berger C, Fu L, Wong BYL, Malik S, et al. The causal effect of vitamin D binding protein (DBP) levels on calcemic and cardiometabolic diseases: a Mendelian randomization study. PLoS Med 2014;11(10):e1001751.
4. Schwartz JB, Gallagher JC, Jorde R, Berg V, Walsh J, Eastell R, Evans AL, Bowles S, Naylor KE, Jones KS, et al. Determination of free 25(OH)D concentrations and their relationships to total 25(OH)D in multiple clinical populations. The Journal of Clinical Endocrinology & Metabolism 2018;103(9):3278–88.
5. Wang TJ, Zhang F, Richards JB, Kestenbaum B, van Meurs JB, Berry D, Kiel DP, Streeten EA, Ohlsson C, Koller DL, et al. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. Lancet North Am Ed 2010;376(9736):180–8.
6. Speeckaert MM, De Buyzere ML, Delanghe JR. Vitamin D binding protein polymorphism and COVID-19. J Med Virol 2021;93(2):705–7.
7. Freitas AT, Calhau C, Antunes G, Araújo B, Bandeira M, Barreira S, Bazenga F, Braz S, Caldeira D, Santos SCR, et al. Vitamin D-related polymorphisms and vitamin D levels as risk biomarkers of COVID-19 infection severity. doi: 10.1101/2021.03.22.21254032 medRxiv
8. Delanghe JR, Speeckaert R, Speeckaert MM. Behind the scenes of vitamin D binding protein: more than vitamin D binding. Best Practice & Research Clinical Endocrinology & Metabolism 2015;29(5):773–86.
9. Rahi MS, Jindal V, Reyes S-P, Gunasekaran K, Gupta R, Jaiyesimi I. Hematologic disorders associated with COVID-19: a review. Ann Hematol 2021;100(2):309–20.
10. Dahl B, Schiødt FV, Ott P, Wians F, Lee WM, Balko J, O'Keefe GE. Plasma concentration of Gc-globulin is associated with organ dysfunction and sepsis after injury: Crit Care Med 2003;31(1):152–6.

11. Meier U, Gressner O, Lammert F, Gressner AM. Gc-globulin: roles in response to injury. Clin Chem 2006;52(7):1247–53.

doi: 10.1093/jn/nxab234.

# Reply to M Speeckaert and J Delanghe

Dear Editor:

We thank Marijn M Speeckaert and Joris R Delanghe for their clarifying comment on our article entitled "Low Circulating Vitamin D in Intensive Care Unit–Admitted COVID-19 Patients as a Predictor of Negative Outcomes." We agree with the authors that vitamin D binding protein (DBP) is important in determining serum 25-hydroxyvitamin D [25(OH)D] concentrations.

The authors supposed that lower serum DBP concentrations and its polymorphism might have a link with coronavirus disease 2019 (COVID-19) mortality in patients admitted to the intensive care unit (ICU), not only by its causal association with vitamin D deficiency, but also by decreasing the actin-binding capacity (1). In our opinion, this is a very important assumption, which allows us to look at this problem from a different angle.

DBP is a multifunctional glycoprotein, which regulates the total and circulating free vitamin D metabolite concentrations. DBP alleles differ in their affinity with the vitamin D metabolites and can have substantial impact on various clinical conditions. In the study of Batur and Hekim (2), which explored the influence of the DBP genotype, there was a difference in susceptibility for and mortality due to COVID-19 among the selected countries. The COVID-19 mortality might be explained by the presence of vitamin D deficiency due to a different vitamin D metabolism, orchestrated by the DBP polymorphisms of rs7041 and rs4588. The 2 most common alleles—Gc1s (rs7041 locus) and Gc2 (rs4588 locus)—differ in their affinity with the vitamin D metabolites and have been variably associated with several clinical conditions. Among these conditions, the G allele at the rs7041 locus was found to be related with increased susceptibility to hepatitis C viral infection. The individuals having an AA genotype within the rs4588 locus of the Gc2 polymorphic region showed a greater increase in 25(OH)D concentrations after vitamin D supplementation than those having the GG genotype (2). Several studies have reported that polymorphisms in the DBP gene were associated with vitamin D deficiency in different populations but not with severe deficiency (3–5). According to our data, severe vitamin D deficiency [serum 25(OH)D concentrations $\leq$9.9 ng/mL] upon admission to the ICU can predict in-hospital mortality in COVID-19 patients. The serum 25(OH)D concentration was greater in survivors (median: 13.3; IQR: 10.0–17.1 ng/mL) than in nonsurvivors (median: 9.6; IQR: 7.9–14.2 ng/mL) ($P = 0.044$).

Notably, the serum 25(OH)D concentrations in COVID-19 outpatients were significantly higher than those of patients admitted to the ICU (median: 22.5; IQR: 13.8–32.5 ng/mL and median: 12.0; IQR: 8.7–15.0 ng/mL, respectively; $P < 0.001$).

This indicates dysregulation of vitamin D metabolism in patients admitted to the ICU, whose vitamin D concentrations fall rapidly after admission to the ICU (5). We would like to focus on this and discuss it in more detail. A typical "portrait" of a patient admitted to the ICU with severe COVID-19 is as follows: this is an old patient (>59 y old) with overweight or obesity, concomitant diseases, and the presence of a hyperergic immune response, which is called a "cytokine storm" (6). Treatment for such patients includes glucocorticosteroids, anticoagulants, respiratory therapy, and different methods of cytokine elimination. Interestingly, this portrait corresponds to patients with vitamin D deficiency. The elderly patients tend to be severely vitamin D deficient (7). This occurs owing to the age-related downregulation of expression of vitamin D receptors, the decrease in 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] production in the kidneys, the decrease in vitamin D production in the skin, dietary insufficiency of vitamin D precursors, and the disruption of calcium metabolism (7). High prevalence of vitamin D deficiency in obese patients is a well-known finding that is most probably due to volumetric dilution into the greater volumes of fat, serum, liver, and muscle present in obese people (8). ICU-admitted COVID-19 patients are at risk of disruption of the vitamin D axis due to hepatic, parathyroid, and renal dysfunction impairing conversion of 25(OH)D to the active hormone (7, 9). Vitamin D deficiency may arise from vitamin D wastage secondary to loss of transport proteins. DBP concentrations were 30% lower in critically ill patients, especially among those with sepsis (5). Lower DBP concentrations lead to renal 25(OH)D wasting, because reabsorption of the vitamin D metabolites requires binding of the DBP–25(OH)D complex with megalin located in renal tubules (5). Besides critical illness itself, therapeutic interventions including surgery, fluids infusion, extracorporeal membrane oxygenation, and plasma exchange may significantly reduce vitamin D concentrations (9). Owing to the fact that the metabolism of 25(OH)D and 1,25$(OH)_2$D occurs in the liver with the help of cytochrome P-450 enzymes, the use of drugs with a similar metabolism can affect the concentrations of 25(OH)D and 1,25$(OH)_2$D (10).

In conclusion, there is substantial evidence that changes in vitamin D concentrations are common in ICU patients. It remains to be determined whether the concentration of vitamin D metabolites is reduced during critical illness or whether the concentration of vitamin D metabolites is the cause of comorbidity. A better understanding of the metabolism and regulation of vitamin D in critical illness could explain the therapeutic use of vitamin D to improve outcomes in critically ill COVID-19 patients.

The authors reported no funding received for this study.

Author disclosures: The authors report no conflicts of interest.

The authors' responsibilities were as follows – all authors: read and approved the final manuscript.

Mikhail V Bychinin
Tatiana V Klypa
Irina A Mandel

From the Intensive Care Unit, Federal Scientific and Clinical Center of Specialized Types of Medical Care and Medical Technologies, Federal Medical and Biological Agency of Russia, Moscow, Russia (MVB; TVK; IAM, e-mail: irina.a.mandel@gmail.com); and the Anesthesiology and Intensive Care Unit, IM Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russia (IAM).