try methodology that they used to measure 25(OH)D. This assay does not distinguish between 25(OH)D and an epimer of 25(OH)D that is present in variable amounts in most adults, with levels of the epimer exceeding 7.5 nmol/L in 8% of the adults in one large study [10]. The epimer binds to the vitamin D receptor, but it lacks many of the activities of vitamin D [10], so it is likely that the epimer acts as a competitive inhibitor for some of the activities of vitamin D. Therefore, the differences in the relationship between the measured 25(OH)D level [i.e., 25(OH)D plus epimer] and the actual 25(OH)D level could have compromised the conclusions of their study. In contrast, most of the studies, in which a correlation between 25(OH)D level and the rate of SVR was identified, used a 25(OH)D assay that is unaffected by the epimer [4–7,10].

Considering the variation in vitamin D physiology between patients and the complexity of measuring vitamin D levels, a lack of a correlation between 25(OH)D level and the rate of SVR among a group of patients should not be interpreted as a lack of importance of vitamin D status in genotype 1 chronic hepatitis C treatment outcome. In the context of the evidence that vitamin D has a functional role as a determinant treatment response [8], it is important to consider the possibility that the level of 25(OH)D may be important in the response to treatment of an individual genotype 1 chronic hepatitis C patient, but that this may not be apparent when the relationship between 25(OH)D level and the rate of attaining an SVR is analyzed among a group of patients.

Conflict of interest

The author declared that he does not have anything to disclose regarding funding or conflict of interest with respect to this letter.

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Reply to: "Vitamin D status does not predict sustained virologic response or fibrosis stage in chronic hepatitis C genotype 1 infection"

To the Editor:

We thank Dr. Weintraub for his in-depth analysis of our paper and the insightful questions he has raised. Our study of 274 patients showing no independent association between baseline 25-hydroxyvitamin D [25(OH)D] status and sustained virologic response (SVR) in chronic hepatitis C genotype 1 (HCV-1) infection [1] was similar to 3 other published studies [2–4] involving 765 patients with HCV-1. Notably, published studies with the contrary finding of an association between baseline 25(OH)D level and SVR in HCV-1 have involved only 448 patients in total, with the largest including 171 patients.

Ethnic variation is one potential explanation for the differences observed between studies to date. However, in a further analysis of the sub-cohort of 234 Caucasian patients in our study, we found the results of multivariate analysis did not change. Moreover, the univariate association found between lower 25(OH)D level and SVR in the entire cohort lost statistical significance. This is not surprising as the Asian population (n = 34) in our study had a lower 25(OH)D level but a higher prevalence of rs12979860 interleukin-28B (*IL28B*) CC genotype. Indeed, our study found a trend association between *IL28B* CC genotype and lower 25(OH)D level.

Our study only comments on baseline 25(OH)D status and SVR and does not address whether the *in vitro* antiviral effect of vitamin D in HCV infection translates to improved treatment outcomes to PegIFN-based antiviral therapy in HCV-1. This question is best addressed by a randomized, placebo-controlled clinical trial. Unfortunately, this data is lacking and the few prospective studies on vitamin D supplementation are small and do not involve a placebo-control arm [5,6]. Any further studies address-

ing the issue of vitamin D supplementation on SVR in HCV-1 should, however, include evaluation of genetic polymorphisms in vitamin D synthesis and metabolism, given the potential influence of these factors on the results as detailed below.

The synthesis and metabolism of vitamin D is a complex and tightly regulated process, and there are many genetic and environmental determinants of vitamin D status. Genetic polymorphisms in key proteins such as vitamin D-binding protein [7] and 1α-hydroxylase [2,3] have been shown to influence SVR to PegIFN-based antiviral therapy in HCV-1 infection. Vitamin D-binding protein is highly polymorphic with extensive racial variation, and different isoforms have variable affinity for vitamin D metabolites. Furthermore, it has anti-inflammatory and immunomodulatory properties independent of its role as the main carrier protein for 25(OH)D and 1α,25-dihydroxyvitamin D [1α,25$(OH)_2$D] [8]. 1α,25$(OH)_2$D is the biologically active product of 1α-hydroxylase and ligand of the nuclear vitamin D receptor. It is intuitive that genetic polymorphisms in 1 α-hydroxylase influence downstream gene transcription of the >200 vitamin D target genes. Measurement of 25(OH)D level is a poor surrogate for measurement of 1α,25$(OH)_2$D level or genetic variation in vitamin D-binding protein and 1α-hydroxylase. We believe that further studies of genetic polymorphisms in these two genes are warranted, however, much like *IL28B* polymorphisms, the influence of any genetic variation on SVR in HCV-1 infection is likely to be attenuated in the direct-acting antiviral era [9].

With regards to the accuracy of the 25(OH)D assay used in our study, we agree that the interference of C-3 epimers can lead to overestimation of 25(OH)D level when liquid chromatography-tandem mass spectrometry (LC-MS/MS) methodology is used. However, samples were tested in a laboratory using LC-MS/MS methodology calibrated against the National Institute of Standards and Technology standard reference material for 25(OH)D assays. In this particular laboratory, adult C-3 epimer concentrations are <5 nmol/L, thus minimizing any 25(OH)D overestimation bias in our study. Furthermore, every other commercially available 25(OH)D assay has significant limitations in accuracy, which is why LC-MS/MS methodology currently remains the reference standard [10].

The results of our negative study appear to raise more questions than it answers and, much like the synthesis and metabolism of vitamin D, any relationship vitamin D status has with HCV-1 treatment outcome is likely to be complex.

Financial support

Dr. Matthew T. Kitson has received funding from the National Health and Medical Research Council of Australia.

Statistical analysis, provided by Infopeople Pty. Ltd., was supported by Roche Products Pty. Ltd. (Australia).

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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