

Observational studies of vitamin D associations with asthma: Problems and pitfalls

In 2007, Litonjua and Weiss¹ hypothesized that vitamin D deficiency may have contributed to the rising prevalence of asthma and allergies. Since then, there have been hundreds of studies that have examined the relationship of vitamin D and asthma and allergies. A quick PubMed search of the terms “asthma” AND “vitamin D” revealed that from 1957 through 2007, there were 76 articles with these key words, while from 2008 through 2017, the number increased 10-fold to 764. Most of these studies have been observational; of these observational studies, many have examined the association of vitamin D and the development of asthma (mostly in birth cohort studies) and others have studied the association of vitamin D and asthma morbidity. Unfortunately, the results of these observational studies for both outcomes have been equivocal, with positive and null associations being reported.²

One of the reasons for the increase in the number of these studies has been the availability of a relatively low cost biomarker of vitamin D status 25 hydroxyvitamin D (25OHD). The attractiveness of 25OHD as a biomarker is that it is stable in storage, it reflects input from various exposures of vitamin D (eg, food sources, supplements, sun exposure), it can be measured from both plasma and serum, and it has a reasonably long half-life of about 4 weeks. The availability of this biomarker has allowed researchers to reach into their freezers, measure the biomarker, and perform analyses in their cohorts. However, the relative ease of measurement can sometimes blind the researcher into considering the appropriate study design and pitfalls of the use of the biomarker. First, while there are many methods to measure 25OHD, there was the realization that these methods were giving results that were too variable. DEQAS (Vitamin D External Quality Assessment Scheme) was launched to ensure the analytical reliability of 25OHD assays.³ Next, it is known that 25OHD levels vary by the season, particularly in high latitudes where there is great variability in the amount of UVB radiation that reaches the earth's surface (ie, northernmost and southernmost latitudes). Thus, there is no guarantee that 25OHD levels measured in the Spring will be close to levels measured in the Winter, unless the person is taking sufficient supplementation to abrogate this seasonal effect. Finally, because the half-life of 25OHD is about 4 weeks, one cannot assume that the levels, and any effects from these levels, stay the same from the time of measurement to more than a month in the future.

In this issue of *Pediatric Pulmonology*, Omand, and colleagues present an analysis from over 5000 children 0-6 years of age, recruited from seven primary care practices in Toronto, Canada, who were participants in the TARGet KIDS! research network. The children were

recruited from well child physician visits. The primary exposure was represented by 25OHD serum levels measured in the children on entry into the study (available in 2926 children), and secondary exposure was represented by a retrospective assessment of prenatal supplementation or a one-time assessment in the child of supplement use at baseline. Since the authors discuss the limitations of the assessment of the secondary exposure, we will not discuss this further. Outcome measures were several components of health services utilization (HSU) for asthma over a 2-year period from baseline, collected from administrative databases. The authors do not find a statistically significant effect of baseline vitamin D exposure on any of the asthma outcomes of hospital admissions, emergency department visits, or outpatient sick visits. The authors conclude that higher vitamin D levels may not decrease the burden of HSU for asthma in early childhood. However the effect estimates that the authors presented in their Table 2 show that a higher 25OHD level is associated with decreased hospital admissions (aOR = 0.76, 95%CI = 0.54-1.08) and emergency department visits (aRR = 0.92, 95%CI = 0.81-1.04). Although just missing statistical significance at $\alpha = 0.05$, the 95%CI suggests that the bulk of the evidence leans toward a protective association with higher 25OHD levels. These findings are consistent with the two largest pediatric asthma studies by Brehm et al^{4,5} that they cite.

Let us first examine whether the authors accounted for the potential pitfalls of the use of 25OHD as a biomarker of vitamin D status. First, the authors used a chemiluminescence assay to measure 25OHD. While not the current gold standard method,⁶ the laboratory that performed the assays participated in DEQAS, thus, we have confidence that the measures are reliable and reflect the true vitamin D status of the children. Next, while blood was drawn at various times of the year, the authors took into account the potential confounding by season by applying a method to account for the seasonal variability. Thus, the authors have insured against potential bias of seasonal variation in 25OHD levels. When it comes to the third pitfall, however, they fall short, since they associate baseline 25OHD levels with outcomes up to 2 years later. While they cite data that show that 25OHD levels are correlated when measured many years apart, these data were generated in middle aged to older adults, and thus, may not be applicable to young children. Data in 5-12 year-old children with asthma show that 25OHD measured 4 years apart, only had an intra-class correlation coefficient of about 0.33, even when taking account measurement in the same season.⁷ This makes sense, as early childhood is a period of rapid change in terms of diet, weight gain,

and sun exposure, all factors that can affect vitamin D status. Thus, it is not hard to see why an association was not found. A shorter time window for the outcomes would have made more biologic sense given what we know about the half-life of the biomarker and the ages of the subjects. For studies that aim to investigate outcomes over a longer time period, multiple measurements of vitamin D status would be ideal to uncover associations. For instance, a study of the association of vitamin D and asthma in children showed that the number of times that 25OHD levels were below 20 ng/mL over a 10-yr period was associated with the presence of asthma by 10 years of age.⁸ Thus, longitudinal observational studies should consider performing multiple measures of vitamin D status if we hope to move the field forward.

Aside from issues related to the use of 25OHD as a biomarker of vitamin D status, there are other issues in this study that relate to study design that need to be pointed out. While this was a study of asthma HSU, only a small minority of the children had these outcomes. That is not surprising since this is not a childhood asthma cohort, and majority of the children did not have asthma. Since a majority did not have asthma, they were not at risk for the outcomes of the study, and thus, a true effect among asthmatics may have been masked. A better design would have been to examine the association of vitamin D and asthma HSU among only the children with asthma. A second issue is that we feel that the authors missed on the opportunity to examine differential effects among the three age groups of children where, we surmise, the rates of the outcomes were likely different. Next, it does not appear from the description of the methods in the study that race was accounted for in the analyses, and this characteristic is related to both the exposure (vitamin D status) and the outcome (asthma HSU). Finally, while the authors conclude that they did not find an association, we disagree with this statement. As we alluded above, the results are consistent with the protective associations of higher 25OHD levels on these outcomes reported in two cohorts of asthmatic children. In interpreting results from studies, authors need to differentiate the effect that they are investigating from statistical significance. The flaws in "null hypothesis significance testing" have recently been addressed by the American Statistical Association statement,^{9,10} because of the improper application by the scientific community as a whole.

So what does this study add to the growing literature on vitamin D and asthma HSU? Despite its flaws, we think the results of this study are consistent with prior reports that higher 25OHD levels are associated with lower asthma hospitalizations and ED visits. In addition, we think that this study highlights the issues that need to be addressed when designing an observational study to investigate this association. Obviously the highest level of evidence will come from clinical trials, which are ongoing (ClinicalTrials.gov NCT02880930 and NCT02687815), but clinical trials cannot answer all the questions. For example, the question of whether preventing vitamin D deficiency over the first 10 years of life is beneficial, could not be feasibly answered by a clinical trial, but is amenable to a well-designed

observational longitudinal study.⁸ We need these studies to answer the questions that are not practical for clinical trials, but these need to be well-designed and well-executed studies that take into account the limitations of both the biomarker and the nature of observational studies.

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REFERENCES

1. Litonjua AA, Weiss ST. Is vitamin D deficiency to blame for the asthma epidemic? *J Allergy Clin Immunol*. 2007;120:1031–1035.
2. Litonjua AA. The role of vitamin D in the development, exacerbation, and severity of asthma and allergic diseases. In: Litonjua AA, ed. *Vitamin D and the Lung: Mechanisms and Disease Associations*. New York, NY: Humana Press; 2012:231–238.
3. Carter GD, Berry J, Durazo-Arvizu R, et al. Hydroxyvitamin D assays: an historical perspective from DEQAS. *J Steroid Biochem Mol Biol*. 2018;177:30–35.
4. Brehm JM, Celedon JC, Soto-Quiros ME, et al. Serum vitamin D levels and markers of severity of childhood asthma in Costa Rica. *Am J Respir Crit Care Med*. 2009;179:765–771.
5. Brehm JM, Schuermann B, Fuhlbrigge AL, et al. Serum vitamin D levels and severe asthma exacerbations in the Childhood Asthma Management Program study. *J Allergy Clin Immunol*. 2010;126:52–58. e55.
6. Black LJ, Anderson D, Clarke MW, Ponsonby AL, Lucas RM. Ausimmune Investigator G. Analytical bias in the measurement of serum 25-Hydroxyvitamin d concentrations impairs assessment of vitamin d status in clinical and research settings. *PLoS ONE*. 2015;10:e0135478.
7. Lasky-Su J, Lange N, Brehm JM, et al. Genome-wide association analysis of circulating vitamin D levels in children with asthma. *Hum Genet*. 2012;131:1495–1505.
8. Hollams EM, Teo SM, Kusel M, et al. Vitamin D over the first decade and susceptibility to childhood allergy and asthma. *J Allergy Clin Immunol*. 2017;139:472–481.
9. Nuzzo R. Scientific method: statistical errors. *Nature*. 2014;506:150–152.
10. Wasserstein RL, Lazar NA. The ASA's Statement on p-values: context, process, and purpose. *Am Stat*. 2016;70:129–131.