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Metabolism

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Commentary

Vitamin D and insulin sensitivity: can gene association and pharmacogenetic studies of the vitamin D receptor provide clarity?

Increasing evidence suggests that the 1,25-dihydroxyvitamin D (1,25[OH]$_2$D)–vitamin D receptor (VDR) complex may play a role in a variety of human diseases beyond its established connection to skeletal health [1-6]. Among these is an association with insulin sensitivity and diabetes mellitus that has been supported by observational studies, but refuted by some interventional studies [1,7-14]. This discrepancy in outcomes is partially explained by limitations inherent to observational studies or by suboptimal study design in the few interventional trials that have been completed to date. Another potential explanation for these variable results is that the metabolism and physiology of vitamin D is complex, involving 25-hydroxyvitamin D (25[OH]D); 1,25(OH)$_2$D; VDR; parathyroid hormone; calcium; and other components [15]. Similarly, the physiology governing insulin sensitivity is equally complex and includes multiple known and unknown variables. The interplay between each of these variables, and the genetic polymorphisms that regulate their activity, may all contribute to phenotypic and disease outcome measurements in cross-sectional and interventional studies. Identifying causes for these heterogeneous results may guide the design of future definitive interventional studies.

Genetic susceptibility for a disease or phenotype may provide an attractive explanation for the variable outcome reports in the current literature. The case-control gene association study may provide an initial level of evidence to support a candidate gene's role in a disease. As with any observational study, gene association studies cannot draw conclusions related to causality and are subject to high type I error risks. In evaluating the role of vitamin D metabolites (circulating levels or supplementation) on insulin sensitivity, one quickly appreciates the complexity of the task at hand. There are many pathways to consider including factors that influence the level and activity of vitamin D metabolites, the interaction of 1,25(OH)$_2$D with the VDR, and its genomic or nongenomic target tissue effects. In addition, one must consider the many pathways that contribute to insulin sensitivity and diabetes. Finally, the gene of a "positive" association study may not contribute to disease, but merely represent a marker of a distal unidentified causal element or a reflection of poor analytical techniques.

One approach that can provide a degree of clarity is to select a gene whose expression resides in the known physiologic pathway involved in the disease state and test whether expression relates to variation of outcome. Most gene association studies focused on vitamin D have focused on the VDR; the VDR has been described in pancreatic β-cells and in relation with the insulin receptor [16]. Several polymorphic variants of the VDR have been identified including high-quality, well-studied mutations (*Fok*I, *Taq*I, *Bsm*I, *Apa*I) [17-21]. These potentially functional mutations are particularly important because they can provide an objective mechanistic readout of a gene product's influence in a particular condition. Observational studies have reported an inverse relationship between vitamin D_3 intake or circulating 25(OH)D concentrations and type 2 diabetes mellitus [2,9]; however, randomized controlled trials reveal a comparatively muted or absent effect, with some describing a modest effect on glycemic indices but only in insulin resistance states [12,13,22,23]. Because insulin resistance states like type 2 diabetes mellitus are largely heritable, it is logical to assume that genetic heterogeneity may explain a portion of conflicting reports within the literature. In this issue of *Metabolism*, Jain and colleagues [24] reported on allelic variation at the VDR gene and its association with insulin sensitivity. Instead of tagging the entire gene, they selected 5 well-described, potentially functional single nucleotide polymorphisms (SNPs) and genotyped 239 South Asian women from Auckland, New Zealand, who had participated in the Surya study. Study participants had a mean 25(OH)D level of 28 nmol/L, a mean body mass index of 25.7 kg/m^2, and a mean homeostasis model assessment of insulin resistance (HOMA-IR) of 2. Genotype status at 3 of 5 SNPs (*Bsm*I, ApaI, *Taq*I) was associated with HOMA-IR values (P values = .022-.035). Haplotypes derived from these 3 loci provided similar results.

The authors then explored whether vitamin D_3 supplementation altered insulin sensitivity over a 6-month period in a subset of 81 participants and whether genotype status modified this relationship. In this intervention, women had insulin sensitivity measured via HOMA2-IR and HOMA2%S before and after receiving 6 months of vitamin D_3 4000 IU daily (n = 42) or placebo (n = 39). Genotype variation at *Fok*I, and to a lesser

extent *Taq*I, predicted whether vitamin D_3 supplementation improved insulin sensitivity when compared with placebo. The authors concluded that the described polymorphic variants in the VDR were associated with insulin sensitivity and the change in insulin sensitivity with vitamin D_3 supplementation. They suggested that haplotype analysis may be an important pharmacogenetic predictor of who may benefit from the insulin-sensitizing effects of vitamin D_3 supplementation.

There are limitations to the conclusions drawn from these results, beginning with those related to very modest sample sizes and the observational nature of association studies. As the authors correctly point out, the apparent high-significance values reported in the observed vitamin D_3 treatment study are likely the consequence of very low minor allele frequencies. The authors found an association between HOMA-IR and the *Bsm*I, *Apa*I, and *Taq*I SNPs; but improvements in insulin sensitivity with vitamin D_3 therapy were associated mainly with the *Fok*I SNP. This observation could be considered as either internally inconsistent or suggestive of a differential mechanism of action associated with each polymorphism, as the authors suggest. The study population was composed of women of South Asian descent; to increase confidence in the authors' findings, the results would have to be extrapolated to male sex and possibly other ethnicities. Lastly, the authors used approximate measures of insulin sensitivity in homeostasis model assessment 2 for insulin resistance (HOMA2-IR) and homeostasis model assessment 2 for insulin sensitivity (HOMA2%S); however, in prior studies by Muscogiuri et al [25] and Grimnes et al [26] that used hyperglycemic clamp techniques, neither 25(OH)D concentrations nor vitamin D_3 supplementation was associated with insulin sensitivity.

Still, there are several aspects of this study that in combination are noteworthy. For example, the haplotype results did not weaken the individual SNP findings. One might expect that a combination of affected alleles would strengthen an association, but that would typically be true only if each individual variant was contributing uniquely to an effect estimate. It is more likely that each SNP tested in this case was in linkage disequilibrium with an element that was the true causal variant. The concordance of vitamin D_3 supplementation with improved insulin sensitivity coupled with the finding of a gene interaction, albeit of marginal statistical significance, is additional evidence for a nonspurious association. Hence, although the effect and sample sizes individually appear underpowered for the testing conducted, their collective consistency provides more confidence.

True to any gene association study, confirmation is critical. Ideally, a confirmation population would be of sufficient size so as to adequately test whether haplotype status modifies the insulin sensitivity response to vitamin D_3 supplementation. Ultimately, one would hope to conduct a prospective randomized controlled trial of vitamin D_3 or VDR-agonist supplementation according to VDR genotype status with changes in insulin sensitivity as the primary outcome measure. As the authors point out, this would likely have the largest impact in individuals who are 25(OH)D deficient at baseline.

Thought-provoking and hypothesis-generating studies such as that published in this issue of *Metabolism* by Jain et al [24] continue to suggest a link between vitamin D and insulin sensitivity in humans; however, like many prior studies, these findings remain inconclusive. Future studies should focus on confirming findings in larger cohorts followed by properly designed randomized controlled trials. Alternatively, pharmacogenomic studies using VDR-agonist therapy to evaluate the change in insulin sensitivity by VDR genotype may serve as intermediate studies (proof of concept) to further support (or refute) the need for and design of larger vitamin D_3 interventional studies. To some, the burden of proof may seem unnecessary when considering the relative "benign" intervention of vitamin D_3 supplementation. However, understanding the basis of these relationships will not only identify susceptible individuals but could also facilitate discovery of novel pathways in the pathogenesis and treatment of type 2 diabetes mellitus.

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