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What can we learn from measuring 24,25-$(OH)_2D_3$?

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The vitamin D metabolite, 24,25-dihydroxyvitamin D_3 (24,25-$(OH)_2D_3$) is a catabolite of 25-OH-D_3 produced by the cytochrome P450, CYP24A1, present in all known vitamin D-target cells. 24,25-$(OH)_2D_3$ was first measured using a competitive protein binding assay in the late 1970s, but the assay fell out of favour because the method was laborious and the metabolite appeared to have little clinical value. With the advent of LC-MS/MS-based methods, the feasibility of rapid, specific and accurate assay of 24,25-$(OH)_2D_3$ has been realized. We have devised a LC-MS/MS assay that simultaneously measures several metabolites including 25-OH-D_3 and 24,25-$(OH)_2D_3$ and uses derivatization with DMEQ-TAD to increase the sensitivity 10-100 fold[1]. The novel assay was applied to 700 serum samples from normal women and gave a normal range for 24,25-$(OH)_2D_3$ of 3-13 nmol/L. Interestingly, the plot of serum 24,25-$(OH)_2D_3$ versus 25-OH-D_3 shows an X-axis intercept of 25 nmol/L, which we interpret as indicating the threshold for "true" vitamin D deficiency[1]. This parameter may be a more reliable indicator for vitamin D deficiency than serum PTH. Application of the new method to small 25-100 µL samples from mouse models permits the study of vitamin D metabolism in individual mice. One example of the usefulness of the assay of 24,25-$(OH)_2D_3$ was in the study of the VDR-knockout mouse where serum 1,25-$(OH)_2D_3$ is 100-fold x normal in untreated animals but is replaced by basal CYP24A1 expression and measurable serum 24,25-$(OH)_2D_3$ when the animals are fed a high Ca, high lactose diet[2]. This physiological change appears to be due to PTH suppression of CYP24A1 which when removed restores C-24-hydroxylation[2].

The most compelling argument for routine clinical chemistry use of serum 24,25-$(OH)_2D_3$ assay is in screening for the disease, idiopathic infantile hypercalcemia (IIH). IIH constitutes a broad group of diseases with a common outcome namely, hypercalcemia. It is now recognized that loss-of-function mutations of the vitamin D catabolic cytochrome P450, CYP24A1[3] represent one of the major causes of IIH. Patients often present in neonatal life with transient hypercalcemia, but adults with CYP24A1 defects and with hypercalciuria, renal stones & nephrocalcinosis are also being increasingly recognized[4]. Recently, there have been multiple reports that pregnant females with IIH can suffer hypercalcemic episodes during pregnancy, presumably due to increased synthesis of 1,25-$(OH)_2D_3$ by the placenta, that abate after pregnancy[5]. Estimates of the frequency of CYP24A1 gene mutations suggest 1:100 carriers and a 1:40,000 incidence of IIH. Genetic analysis of the *CYP24A1* locus represents the definitive diagnosis of this form of IIH, with over 20 mutations of the CYP24A1 gene recognized. However, the novel LC-MS/MS assay for serum 24,25-$(OH)_2D_3$ constitutes a rapid screening tool for detecting IIH patients with two defective CYP24A1 mutations. It consists of simultaneously measuring vitamin D metabolites: 25-OH-D_3 and 24,25-$(OH)_2D_3$ in serum by LC-MS/MS and then expressing the results as a 25-OH-D_3:24,25-$(OH)_2D_3$ ratio[1]. Heterozygotes and normal individuals have ratios between 5-25 while IIH patients have ratios >80. Refinements of this approach have further resolved IIH patients from individuals with a high ratio due to vitamin D deficiency and patients with chronic kidney disease on dialysis. Loss-of-function CYP24A1 mutations cause hyper-sensitivity to dietary vitamin D, so reduction of dietary vitamin D or exposure to UV light is recommended. Further insights into the causes of hypercalcemic episodes in IIH should come from studies of the CYP24A1-null mouse[6]. Reintroduction of a BAC clone representing a full wild-type human or mouse CYP24A1 gene into the CYP24A1 null mouse restores normocalcemia[7].

[1]Kaufmann et al JCEM, 2014; [2]Kaufmann et al Endocrinology, 2015; [3]Schlingmann et al NEngJMed, 2011; [4]Molin et al JCEM, 2015; [5]Shah et al JCEM, 2015; [6]St-Arnaud, JSBMB, 2010; [7]Carlson et al ASMR Seattle, October 2015.