Special vitamin D diagnosis in infants: meaning of the C3-epimers

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The clinical picture of rickets in children has been known since the mid-17th century. Caused by vitamin D deficiency it leads to bone deformities and growth retardation due to a lack of mineralisation. Recent studies indicate that vitamin D and its active metabolites fulfil a number of additional important functions in the human organism. These include the promotion of epithelial cell differentiation in the skin, the influence on the activity of the immune system, the regulation of insulin secretion and the protection against cardiovascular diseases[1]. Beyond that, vitamin D deficiency is linked to many diseases such as breast and colon cancer, multiple sclerosis, dementia, rheumatoid arthritis, diabetes, Parkinson's and Alzheimer's disease, even though evidence for a direct connection is often lacking.

Intake and metabolism

Vitamin D is a fat-soluble vitamin, whose basic structure is derived from the cholesterol steroid skeleton. The main representative occurring in humans and animals is cholecalciferol (vitamin D_3) whereas in plants and fungi ergocalciferol (vitamin D_2) prevails. They only differ slightly in the chemical structure of the side chain (Fig. 1). Dietary intake plays a minor role for vitamin D supply. The organism is supplied with vitamin D to a greater degree by 7-dehydrocholesterol stored in the skin that is converted through sunlight into initially inactive vitamin D. Consequently, regular sun exposure is important for vitamin D intake and the serum concentration is therefore also subjected to seasonal fluctuations. Therefore, vitamin D supplementation for the western population is also often recommended during the winter months, whereby in a few countries only vitamin D_2 preparations are available for this purpose[2]. The metabolising of vitamin D_3/D_2 is carried out in the liver, where it is hydroxylated to 25-hydroxyvitamin D_3 and D_2 (25-OHD), respectively (Fig. 1). Both forms serve for transport and storage and have a half-life of a few weeks, which is why the serum level of 25-OHD_3/D_2 is the recognised parameter for determining vitamin D status. A further hydroxylation that can be carried out especially in the kidney but also locally and cell-specific, leads to the hormonally active 1, 25-dihydroxyvitamin D_3 and D_2, respectively.

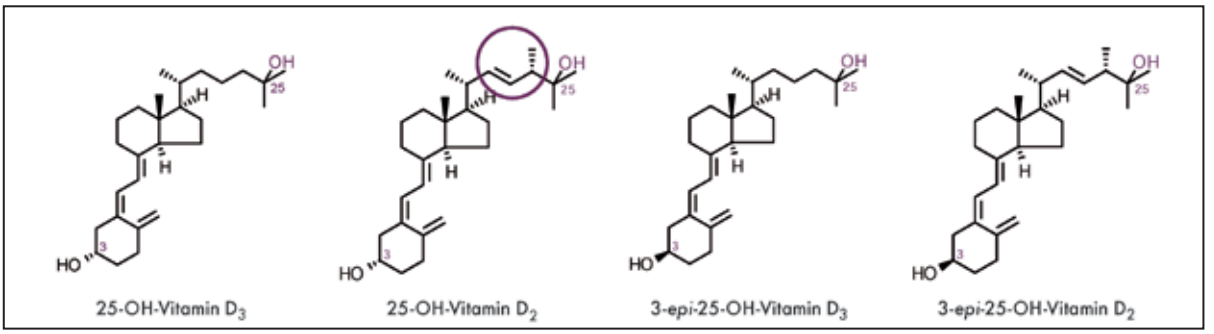


Fig. 1: Chemical structures of the vitamins 25-OHD$_3$, 25-OHD$_2$, 3-epi-25-OHD$_3$ and 3-epi-25-OHD$_2$.

The C3-epimer

In a study published in 2006, Singh and colleagues reported that in a significant proportion of infants and young children up to one year 25-OHD can exist as C3-epimer. The proportion of 3-epi-25-OH vitamin D can amount to up to 60 % out of total 25-OHD concentration. Tendencies: The younger the child, the higher the average proportion of epimeric forms, with strong variance observed. The C3-epimer of 25-OHD is only structurally different by the spatial orientation of the hydroxylgroup in position C3 of the skeletal structure (Fig. 1).

Its active metabolite 3-*epi*-1,25-(OH)^2-vitamin D$_3$/D$_2$ also draws attention to differences in biological activities. For example, the epimeric forms cause a comparable inhibition of parathyroid hormone secretion, however, calcium-induced effects in bone metabolism are significantly reduced. Due to this difference in effectiveness, it is of considerable importance to determine the concentration levels of 25-OHD$_3$/D$_2$ of the epimeric forms separately in order to identify the individual contribution of each of the different molecules to the total vitamin D status. At about one year, infants mostly have very low concentrations of 3-*epi*-25-OH-vitamin D [3–13] that are comparable to adults. It is believed that epimerisation is primarily a characteristic of a not yet matured vitamin D metabolism.

The supply situation in children and adolescents

Thanks to systematic prophylaxis severe disease manifestations such as rickets and their side effects are rare. Nevertheless, a vitamin D deficiency is a problem which, even today, is paid far too little attention to in children. A recent extensive four-year representative U.S. study has shown that 61 % of 9- to 21-year-old Americans (about 50.8 million) have a vitamin D deficiency (37–72 nmol/l 25-OHD) and a further 9 % of this population (approximately 7.6 million) even suffer from an undersupply of vitamin D (< 37 nmol/l 25-OHD) [14].

Especially in children, the level of 25-OHD$_3$/D$_2$ and 3-*epi*-25-OH-Vitamin D$_3$/D$_2$ should be determined in serum samples to allow for a differentiated assessment of the supply situation in early childhood development. At this point, it is expressly highlighted that diagnostic tests

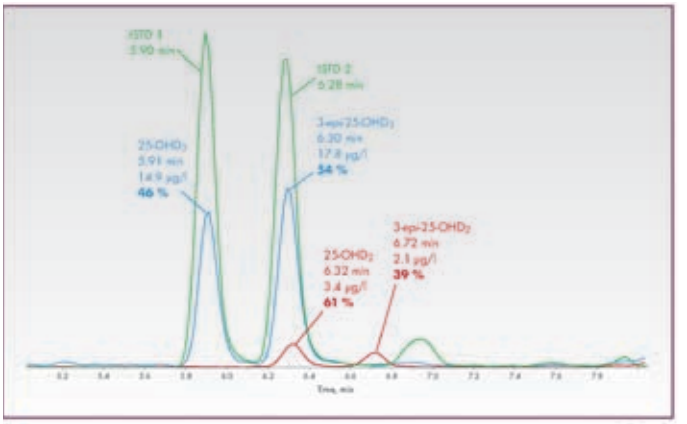
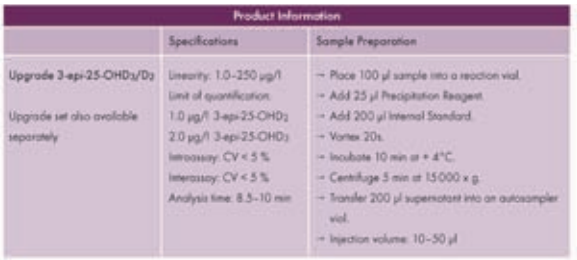


Fig. 2: An exemplary chromatogram of a serum sample from an infant that was analysed with the new epimer upgrade of the MassChrom® 25-OHvitamin D$_3$/D$_2$ reagent kit. In addition to 25-OHD$_3$ and 25-OHD$_2$, the epimeric forms 3-*epi*-25-OH-Vitamin D$_3$ and 3-epi-25-OH-vitamin D$_2$ were found in significant quantities.



Product Information		
	Specifications	Sample Preparation
Upgrade 3-epi-25-OHD3/D2 Upgrade set also available separately	Linearity: 1.0–250 µg/l Limit of quantification: 1.0 µg/l 3-epi-25-OHD2 2.0 µg/l 3-epi-25-OHD3 Intraassay: CV < 5 % Interassay: CV < 5 % Analysis time: 8.5–10 min	→ Place 100 µl sample into a reaction vial. → Add 25 µl Precipitation Reagent. → Add 200 µl Internal Standard. → Vortex 20s. → Incubate 10 min at + 4°C. → Centrifuge 5 min at 15000 x g. → Transfer 200 µl supernatant into an autosampler vial. → Injection volume: 10–50 µl

that do not consider or inadequately mirror the contribution of 3-*epi*-25-OH-Vitamin D_3/D_2 on total vitamin D status, at least for infants under one year, are unsuitable.

The method of choice

The new upgrade for the ***MassChrom***® analysis allows not only the main metabolites of vitamins D_3 and D_2, 25-hydroxycholecalciferol and 25-hydroxy-ergocalciferol to be determined, but also the rapid and simultaneous determination of 3-*epi*-25-OH-vitamin D_3/D_2 by LC/MS/MS in serum/plasma (Fig. 2). The manual sample preparation is limited to a simple and effective protein precipitation. Analogous to the 25-OHD_3/D_2-determination the analytes are systematically concentrated using a trap column and disturbing matrix components are separated. The trap column is associated with a particularly high resolution analytical column via a simple control valve, which enables chromatographic separation and reliable quantification of the analytes in less than 10 minutes (Fig. 2).

For ionisation of the stable vitamin D molecules, the APCI (Atmospheric Pressure Chemical Ionisation) technique is used. The use of two isotope-labelled internal standards adapted to the epimeric forms compensates matrix effects and ensures the method's high accuracy and robustness.

Currently, only Chromsystems offers multilevel serum calibrators (3PLUS1®) and serum controls for this analysis. In addition, the calibrators and controls for the 3-*epi*-25-OHvitamin D_3/D_2 and 25-OHD_3/D_2 analysis are traceable to the NIST-reference material 972.

References

[1] Deutsche Gesellschaft für Kinder- und Jugendmedizin e.V. (2011) Stellungnahme: Vitamin D-Versorgung im Säuglings-, Kindes- und Jugendalter.

[2] Holick MF. (2007) Vitamin D deficiency. N Engl J Med 357(3): 266-81.

[3] Singh RJ, Taylor RL, Reddy GS, Grebe SK. (2006) C3-epimers can account for a significant proportion of total circulating 25-hydroxyvitamin D in infants, complicating accurate measurement and interpretation of vitamin D status. J Clin Endocrinol Metab 91(8): 3055-61.

[4] Strathmann FG, Sadilkova K, Laha TJ, LeSourd SE, Bornhorst JA, Hoofnagle AN, Jack R. (2012) 3-epi-25-hydroxyvitamin D concentrations are not correlated with age in a cohort of infants and adults. Clin Chim Acta 413(1–2): 203–6.

[5] Midasch O, Erlenfeld G, Yueksekdağ N, Fabian D, Burki D, Korall H, Wallner S, Lukačin R. 25-OHD3 and its C3-epimer: Determination by LC-MS/MS. MSACL 2011. 3rd Annual Conference & Exhibits, San Diego, CA (PosterSplash Track 3/29).

[6] Schleicher RL, Encisco SE, Chaudhary-Webb M, Paliakov E, McCroy LF, Pfeiffer CM. (2011) Isotope dilution ultra performance liquid chromatography-tandem mass spectrometry method for simultaneous measurement of 25-hydroxyvitamin D2, 25-hydroxyvitaminD3 and epi-25-hydroxyvitamin D3 in human serum. Clin Chim Acta 412(17–18): 1594–9.

[7] Shah I, James R, Barker J, Petroczi A, Naughton DP. (2011) Misleading measures in vitamin D analysis: A novel LC-MS/MS assay to account for epimers and isobars. Nutr J 10: 46.

[8] van den Ouweland JMW, Beijers AM, van Daal H. (2011) Fast separation of 25-hydroxyvitamin D3 from 3-epi-25-hydroxyvitamin D3 in human serum by liquid chromatography-tandem mass spectrometry: Variable prevalence of 3-epi-25-hydroxyvitamin D3 in infants, children, and adults. Clin Chem 57(11): 1618–9.

[9] Stepman HMC, Vanderroost A, Stöckl D, Thienpont LM. (2011) Full-scan mass spectral evidence for 3-epi-25-hydroxyvitamin D3 in serum of infants and adults. Clin Chem Lab Med 49(2): 253–6.

[10] Lensmeyer G, Poquette M, Wiebe D, Binkley N. (2012) The C-3 epimer of 25-hydroxyvitamin D3 is present in adult serum. J Clin Endocrinol Metab 97: 163–8.

[11] Baecher S, Leinenbach A, Wright JA, Pongratz S, Kobold U, Thiele R. (2012) Simultaneous quantification of four vitamin D metabolites in human serum using high performance liquid chromatography tandem mass spectrometry for vitamin D profiling. Clin Biochem 45(16–17): 1491–6.

[12] Wright MJP, Halsall DJ, Keevil BG. (2012) Removal of 3-epi-25-hydroxyvitamin D3 interference by liquid chromatography-tandem mass spectrometry is not required for the measurement of 25-hydroyvitamin D3 in patients older than 2 years. Clin Chem 58(12): 1719–20.

[13] Bailey D, Veljkovic K, Yazdanpanah, Adeli K. (2013) Review: Analytical measurement and clinical relevance of vitamin D3 C3-epimer. Clin Biochem 46(3): 190–6.

[14] DiMeglio LA. (2010) Pediatric endocrinology: Vitamin D and cardiovascular disease risk in children. Nat Rev Endocrinol 6(1): 12-3.

[15] Thomas L. Labor und Diagnose. 7. Aufl, Verlag TH-Books Frankfurt/Main (2008).

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