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.
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 hydroxyl group in position C3 of the skeletal structure (Fig. 1).

Its active metabolite 3-epi-1,25-(OH)2-vitamin D3/D2 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-OHD3/D2 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-OHD3/D2 and 3-epi-25-OH-Vitamin D3/D2 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. 1: Chemical structures of the vitamins 25-OHD3, 25-OHD2, 3-epi-25-OHD3 and 3-epi-25-OHD2.

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 D3/D2 reagent kit. In addition to 25-OHD3 and 25-OHD2, the epimeric forms 3-epi-25-OH-Vitamin D3 and 3-epi-25-OH-vitamin D2 were found in significant quantities.
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-hydroxyergocalciferol 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-OH-vitamin D$_3$/D$_2$ and 25-OHD$_3$/D$_2$ analysis are traceable to the NIST-reference material 972.

**References**
