25OHD$_3$ AND ITS EPIMER: DETERMINATION BY LC/MS-MS

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ABSTRACT

The C3-epimer of 25-OH-vitamin D$_3$ (25OHD$_3$) has been detected in significant concentrations in serum samples of infants under the age of one year (Fig.1).
Its downstream metabolite 3-epi-1,25-(OH)$_2$-vitamin D$_3$ (C-3 epimer) has been shown to have reduced calcemic effects in bone metabolism when compared to the physiologically active form 1,25-(OH)$_2$-vitamin D$_3$. Thus, monitoring the 25OHD status of this age group should also include the concentration of the epimeric form. Here we describe a new method suitable for daily routine analysis by LC-MS/MS that can distinguish between the forms 25OHD$_2$, 25OHD$_3$ and its C3-epimers (Fig.2).

INTRODUCTION

The D-vitamins are a group of secosteroids with the physiologically relevant forms vitamin D$_2$ and D$_3$. While the largest amount of vitamin D$_3$ is produced in humans' skin when induced by sunlight, vitamin D$_2$ occurs mainly in plants and plays only a minor role in human nutrition. Vitamin D is important for a lot of biological processes, such as mineralisation and growth of bones. Monitoring the vitamin D status is mainly performed by measuring the concentrations of the 25-OH metabolites in plasma or serum. Several methods are available for this purpose. Very recently, a British and an American Expert Panel – supported by Scandinavian colleagues – recommended LC-MS/MS as a method of choice (1, 2). Discrimination between 25OHD$_3$ and its C3-epimer of which is commonly detected in significant concentrations in serum samples of young infants under the age of one year, is possible only by using the LC-MS/MS technology. The C-3 epimer seems to be of clinical relevance, as it shows differences in biological activity when compared to 25OHD$_3$ (3, 4). Additionally, it contributes to an overestimation of the serum 25OHD$_3$ level which could lead to inappropriate treatment in newborns or very young children (5).

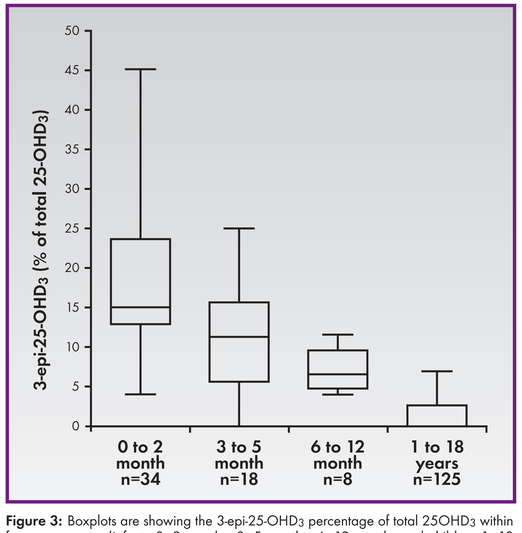
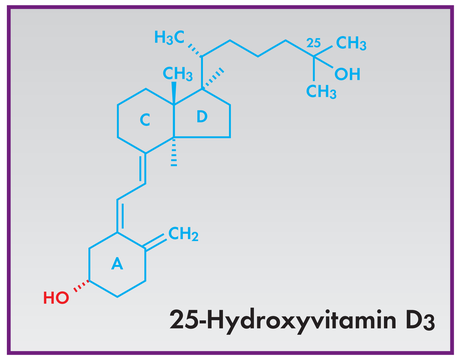


Figure 3: Boxplots are showing the 3-epi-25-OHD$_3$ percentage of total 25OHD$_3$ within four age groups (infants 0–2 months, 3–5 months, 6–12 months and children 1–18 years). The C3-epimer percentage seems to be age related with a trend to decreasing amounts with higher age.

Structure



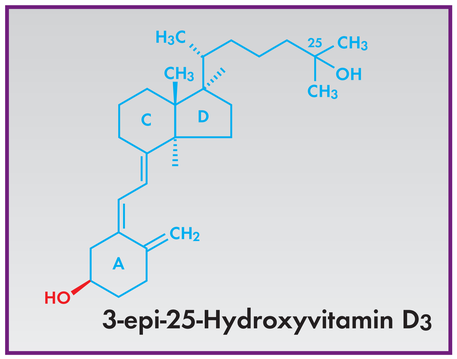
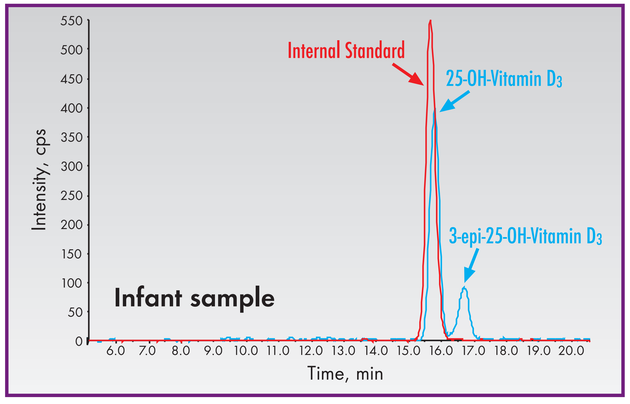


Figure 1: Chemical structures of vitamin 25OHD$_3$ and 3-epi-25OHD$_3$.



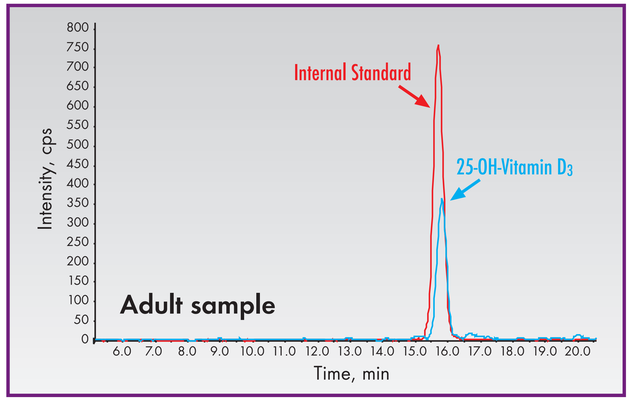


Figure 2: Typical chromatograms of an infant sample containing 42.3 µg/l 25OHD$_3$ and its epimer (15.3 µg/l) in contrast to an adult sample containing 26.2 µg/l 25OHD$_3$.

CONCLUSION:

- It is necessary to discriminate between the epimeric forms of 25OHD$_3$ when analysing infant serum samples as they show differences in biological activity in terms of bone metabolism.
- The Chromsystems ***MassChrom***® Kit fulfils all requirements for the vitamin D screening of all age groups.

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MATERIAL AND METHODS

Chromsystems' ***MassChrom***® Kit (Order number 62000) in combination with a high resolution HPLC column was used for all vitamin D measurements. 100 µl sample was mixed with 25 µl of Precipitation Reagent and 200 µl of Internal Standard solution. After incubating for 10 min at +4 °C the samples were centrifuged for 5 min at 15000 g. 50 µl of each supernatant was injected into the LC-MS/MS system (AB Sciex API 3200 triple/Shimadzu LC 20). Sample cleanup was performed online using a column switching technique. About 200 serum samples of very young infants (new born to 12 months), children and young adults (1 to 18 years) were screened for their 25OHD$_3$ and its C3-epimer amount. Additionally, serum samples of adult volunteers were analysed for the C3-epimer amount.

RESULTS AND DISCUSSION

Chromsystems provides a robust, precise and accurate high-throughput method (***MassChrom***® for 25OHD$_3$/D$_2$) for the daily routine analysis of 25OHD$_3$ and D$_2$ in plasma and serum. With the option of separating the C-3 epimer of 25OHD$_3$ in infant samples by simply replacing the standard HPLC column with a high resolution column it fulfils all requirements as demanded by expert panels in recently published reports including the NIST standardisation (1). Current measurements of around 200 infant and adolescent samples confirmed previously studies (5, 6): In infant samples the C3-epimer was found in high amounts. Moreover the C3-epimer percentage seems to be age related within this age group (Fig. 3). In contrast, only insignificant amounts could be detected in adolescents samples (1 to 18 years). In adult samples we generally did not find the epimeric form above the limit of quantification of 6.9 µg/l. As an exception a concentration of about 30 µg/l of C3-epimer was detected in a serum sample of a highly vitamin D supplemented 70-year-old person (391 µg/l).

Our data suggests that the C3-epimer plays a significant role in the vitamin D metabolite pattern of infants, whereas it does not in older children and adults. An international detailed study is ongoing in our laboratories to investigate the 25OHD$_3$ and its C3-epimer in infants and young adults.

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