

Different approaches for vitamin D determination in newborns by LC-MS/MS

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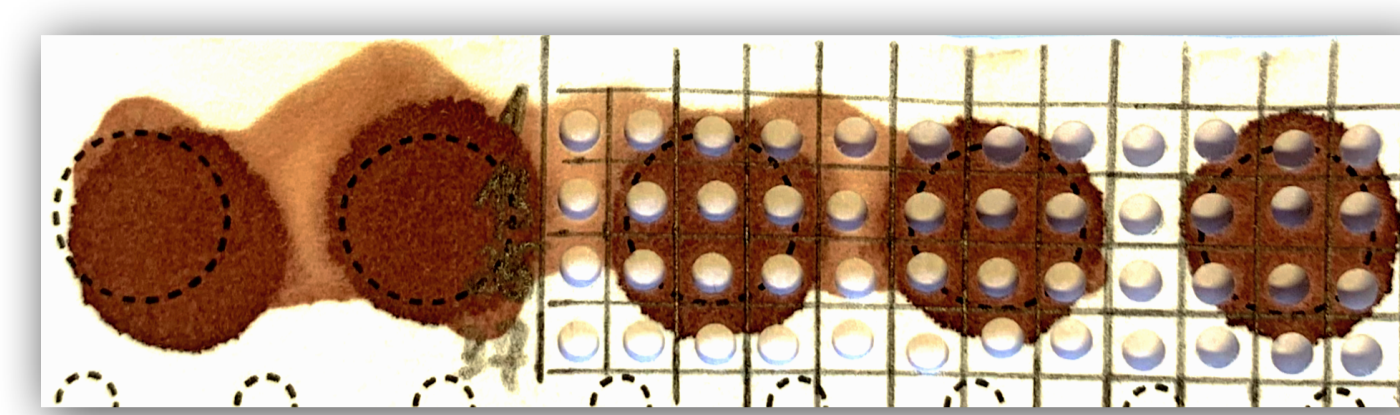
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Vitamin D plays a key role in metabolic processes in human body. Despite the growing social awareness, its large deficit is still being observed, which is particularly important in the case of pregnant women. Vitamin D concentration in the fetal period strictly depends on the maternal concentration. Deficiency of this vitamin in the newborn affects a number of diseases, including abnormal development of the bone or immune system. For this reason, preventing deficiency from the first days of life is extremely important.

The tested materials were dried blood spots collected from the newborn for routine screening and umbilical cord blood serum. Biological samples were analyzed using liquid chromatography coupled with tandem mass spectrometry. Quantitative analysis was carried out for four vitamin D metabolites, i.e. 25(OH)D₃, 3-epi-25(OH)D₃, 25(OH)D₂ and 24,25(OH)₂D₃. Application of the above technique was particularly important in the case of umbilical cord serum, because commonly available immunochemical methods give overstated results. Serum was prepared by liquid-liquid extraction, while DBS was extracted with an organic solvent in 96-well plate. For both materials, derivatisation using Cookson-type reagent (DAPTAD) was applied.

The purpose of the study was to investigate whether there is a relationship between the concentration of vitamin D metabolites in umbilical cord serum and DBS. Despite the correlation between these two materials, deviating values are observed. This may be due to the different effects of postpartum supplementation on the newborn, considering that the material in the form of DBS is collected within 3 days after delivery. The decisive advantage of both test materials is that they give the opportunity to assess the supply for vitamin D without additional harm to the infant.

The drying process is crucial for the **DBS homogeneity!**

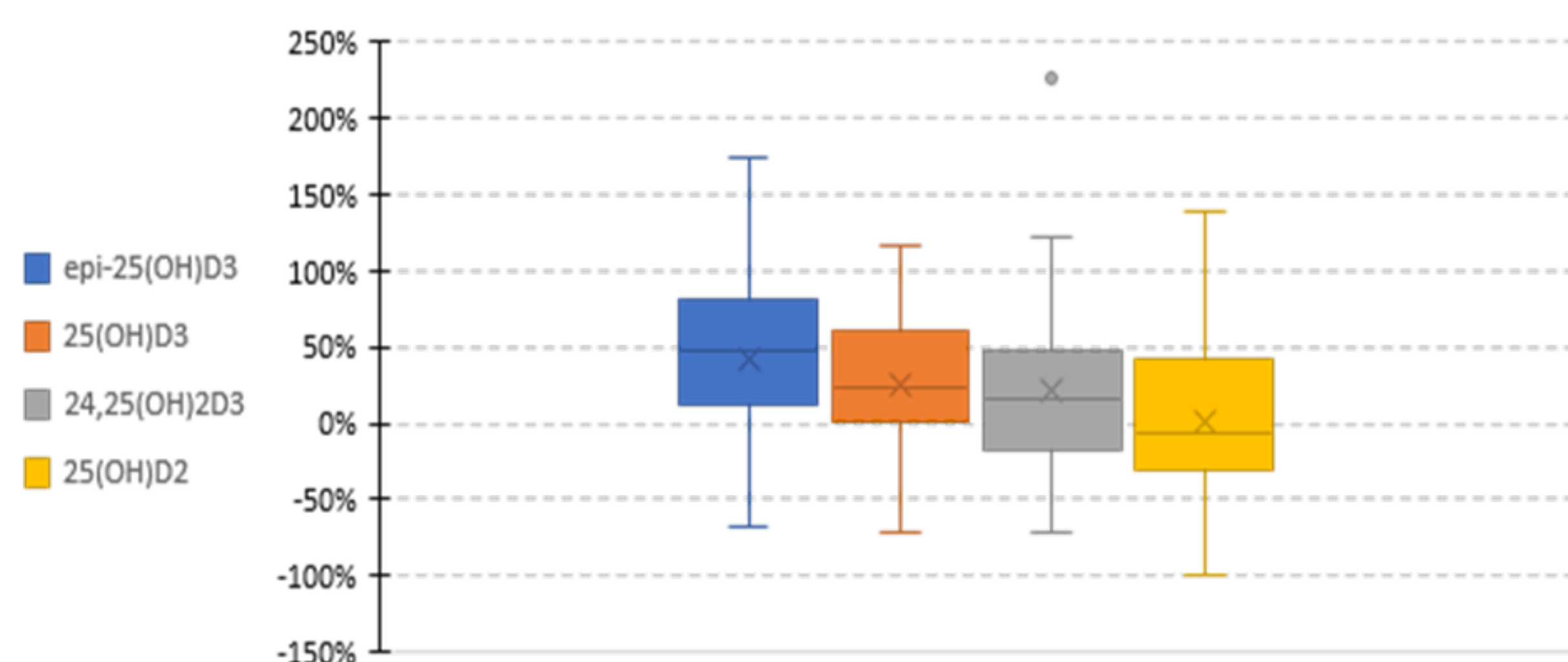


Concentration of 25(OH)D₃ [ng/ml]

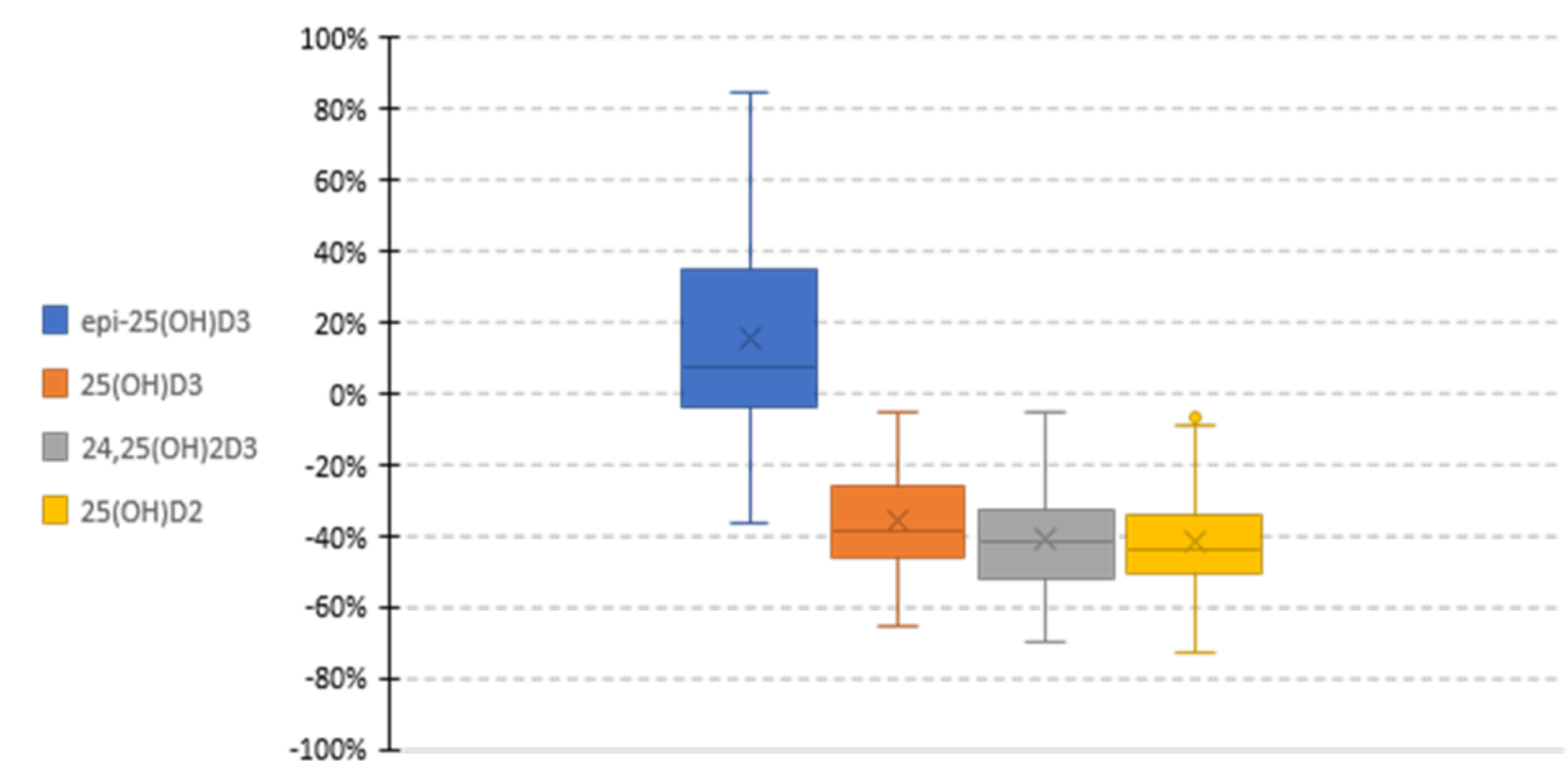
10,1	7,8	9,3	7,6	11,6	8,7	10,8	2,2	0,0	4,8	9,5	12,7
6,4	1,8	2,0	2,5	3,3	2,4	2,2	7,2	0,0	9,6	3,4	10,1
9,1	9,7	2,5	9,4	24,6	9,4	4,8	14,0	0,0	14,4	7,0	17,6
0,0	0,0	7,3	0,0	0,0	0,0	12,7	0,0	0,0	0,0	4,7	0,0

* target value: 9 ng/ml

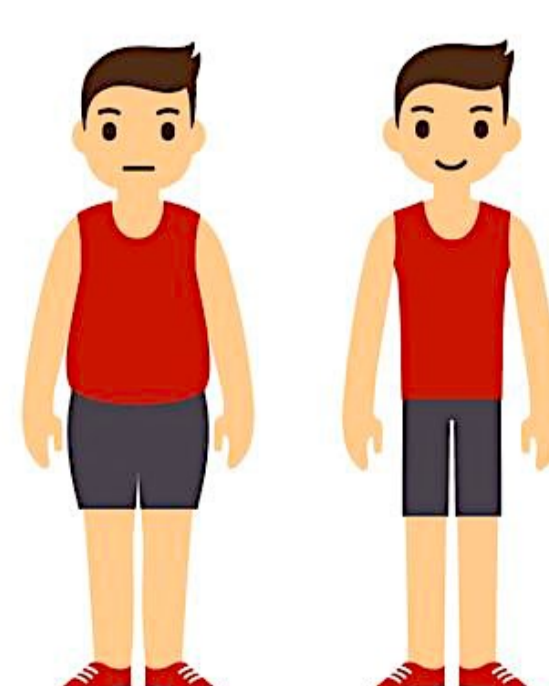
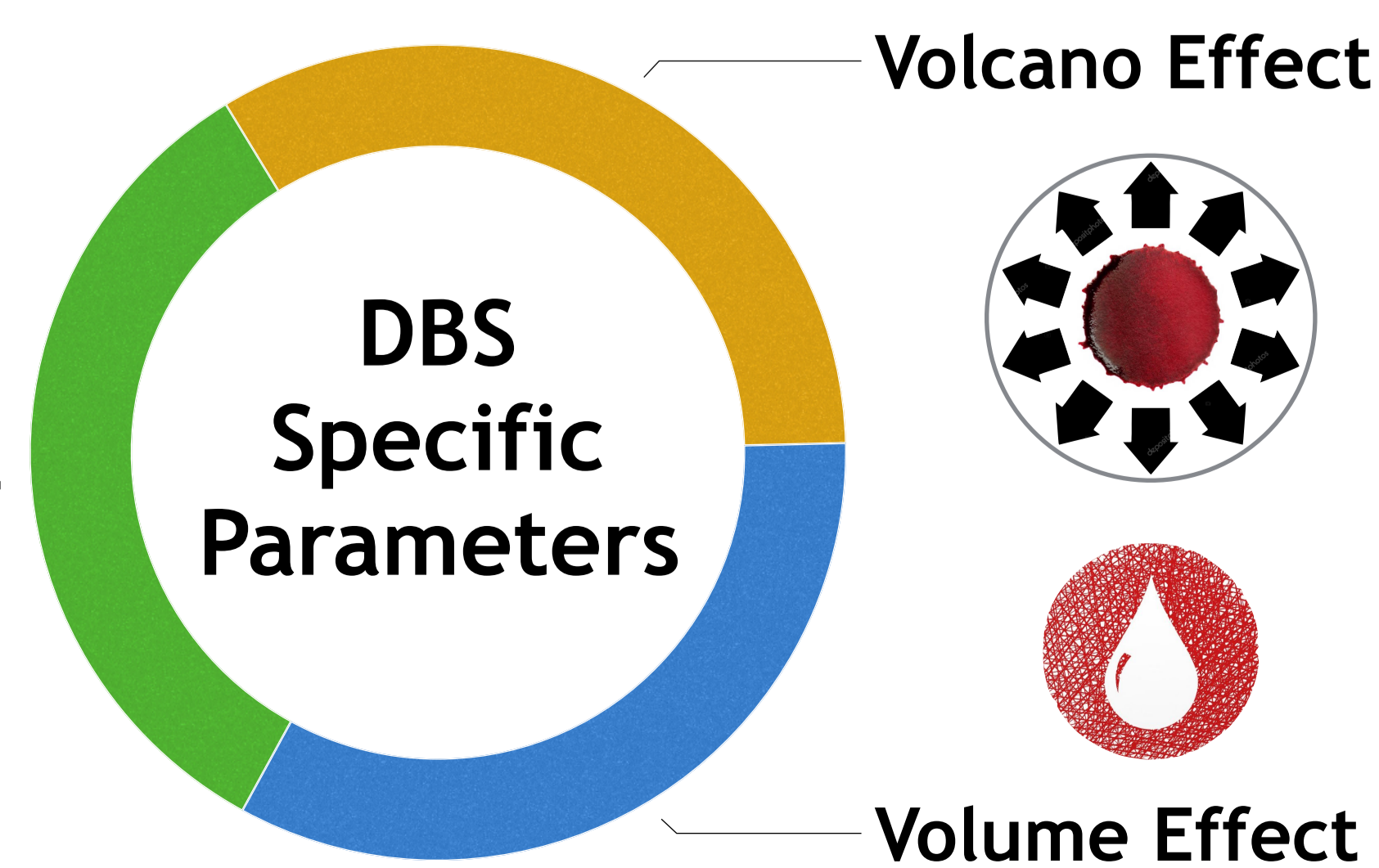
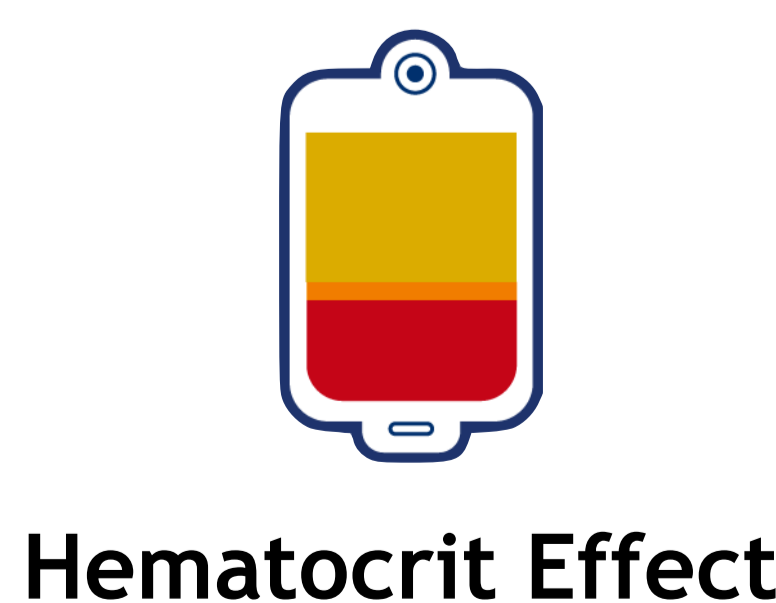
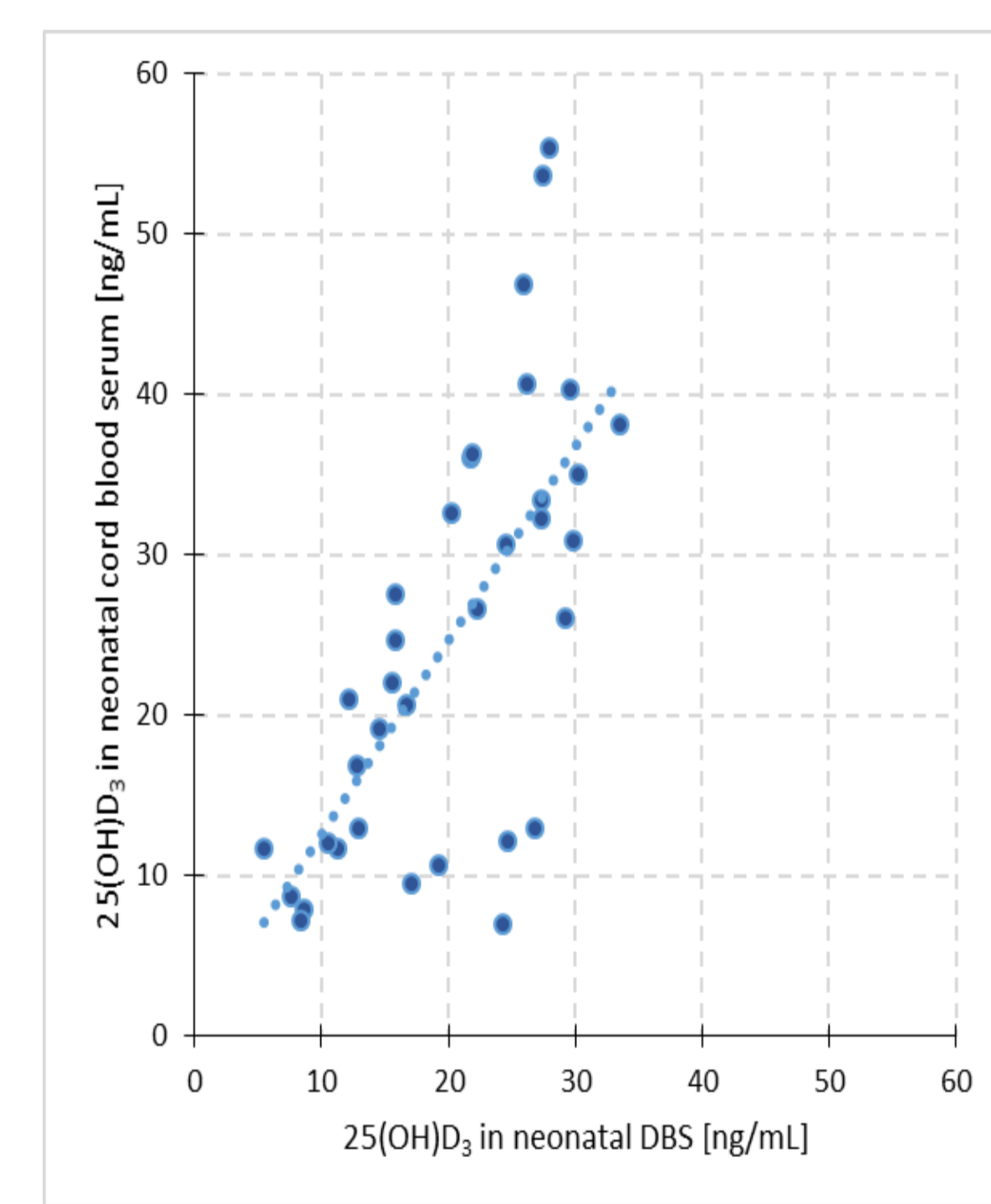
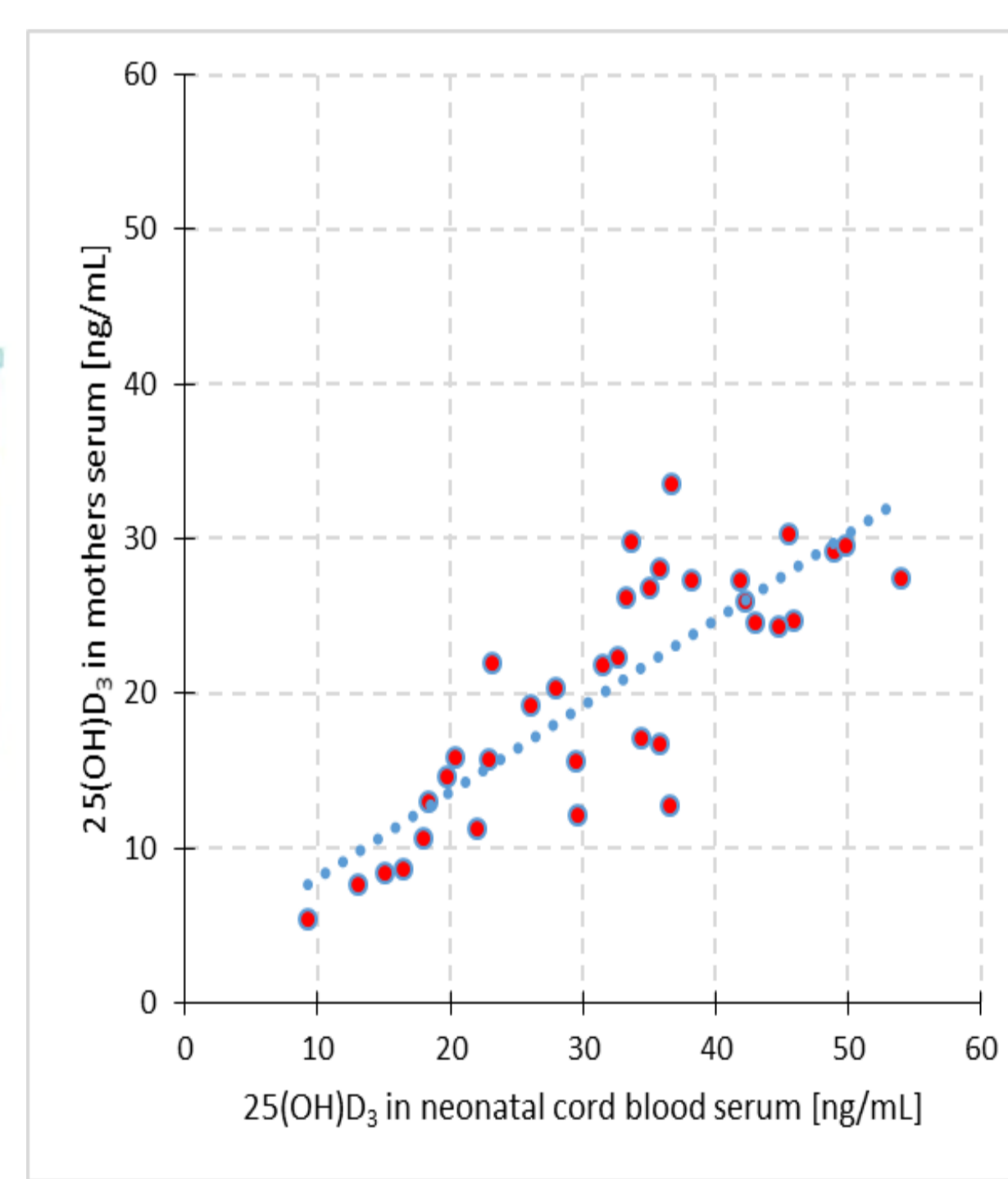
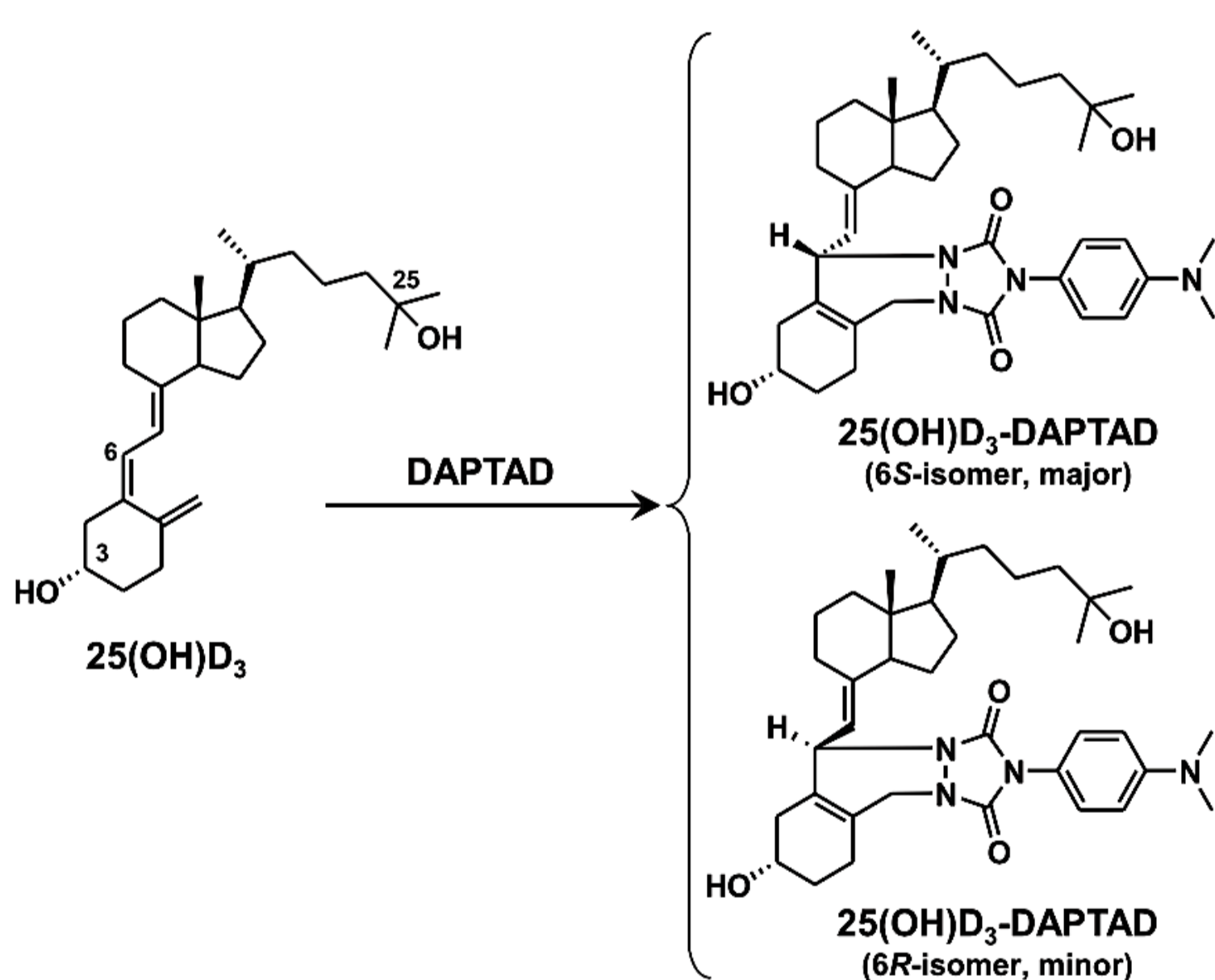
Differences between neonatal cord blood serum and neonatal DBS in vitamin D metabolites



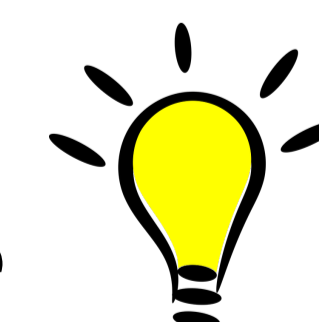
Differences between mothers serum and neonatal cord blood serum in vitamin D metabolites



2 x 3.1 µl of **blood**:
methanol extraction
OR
100 µl of **serum**:
LLE with hexane



You need to know what makes the difference



Least Significant Change:
serum: 3,2 ng/ml
 vs.
DBS: 6,4 ng/ml

	Mothers blood serum	Neonatal cord blood serum
epi-25(OH)D ₃ [ng/mL]	2.1 ± 1.1	2.4 ± 1.1
25(OH)D ₃ [ng/mL]	31.7 ± 11.5	20.1 ± 7.7
24,25(OH) ₂ D ₃ [ng/mL]	2.3 ± 0.9	1.3 ± 0.6
25(OH)D ₂ [ng/mL]	0.5 ± 0.3	0.3 ± 0.1
25(OH)D ₃ : epi-25(OH)D ₃	16.7 ± 4.7	9.1 ± 2.2
25(OH)D ₃ : 24,25(OH) ₂ D ₃	15.4 ± 4.9	16.4 ± 4.5