tussen beide groepen na correctie voor de nierfunctie (p=0,03). Bovendien vertoonde de patiëntengroep een significante daling (p=0,02) in de gecorrigeerde natriumuitscheiding voor en na het volgen van het zoutbeperkt dieet gedurende 2 maanden. Hieruit kan geconcludeerd worden dat de patiënten zich netjes aan het zoutbeperkt dieet hebben gehouden.
Deze studie laat zien dat het volgen van een zoutbeperkt dieet gedurende 2 maanden, en een daarmee gepaard gaande verlaagde jodium inname, geen invloed heeft op de jodiumstatus.

Dankwoord

Dit onderzoek werd mogelijk gemaakt door de bereidwillige deelname van 3 huisartsenpraktijken uit de regio (Gezondheidscentrum Ubachsberg, Huisartsenpraktijk Voerendaal en Huisartsenpraktijk Hulsberg). Ook de onderzoeksverpleegkundigen Vera Post en Theo Thuis van de afdeling Cardiologie leverden een bijdrage aan het onderzoek.

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Ned Tijdschr Klin Chem Labgeneesk 2013; 38: 136-138

C3-epimer cross-reactivity of automated 25-hydroxyvitamin D immunoassays

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Presence of the 3-epi-25-hydroxyvitamin D_3 (3-epi-25 $(OH)D_3$; C_3-epimer) metabolite affects quantification of $25(OH)D_3$ in most routine liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods (1). The 3-epi-$25(OH)D_3$ metabolite is of unknown origin and its biological role, distribution and its clinical significance are hardly known (2). Historically, most clinical data on vitamin D research are based on 25(OH)D measurement using the DiaSorin RIA, an assay reported not to be affected by 3-epi-25(OH)D (1). For currently available automated immunoassays, the amount of cross-reactivity for 3-epi-$25(OH)D_3$ and its effects on 25(OH)D total measurement is yet unknown. Here, we examined 3-epi-$25(OH)D_3$ immunoassay cross-reactivity not only from exogenous addition of 3-epi-$25(OH)D_3$, but also by using human newborn samples with significant concentrations of endogenous C_3-epimer.

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Methods

We obtained neonatal heparin plasma and adult sera from leftover material. All samples were treated anonymously. A LC-MS/MS method separating $25(OH)D_3$ from 3-epi-$25(OH)D_3$ was used as a reference method (3). Immunoassays measurements were performed according to manufacturer's instructions and included DiaSorin LIAISON Vitamin D TOTAL assay (DiaSorin, Stillwater, MN, USA), IDS iSYS (IDS, Boldon, UK), Abbott ARCHITECT 25-OH Vitamin D (Abbott, Abbott Park, Deerfield, IL, USA), Siemens ADVIA Centaur Vitamin D total (lot. No 009, before re-standardisation) (Siemens, Deer Park, Deerfield, IL, USA), and Roche Elecsys Vitamin D Total (Roche Diagnostics, Mannheim, Germany). Use of serum and lithium heparin plasma produce similar results in all immuno- and CPB assays (info manu-

facturers and authors experience), with exception of the Liaison assay where a 22% bias between heparine plasma and serum is reported (Information from DiaSorin package insert, july 2012).
First we investigated the correlation between the immunoassay methods and LC-MS/MS using five adult serum samples covering the 25(OH)D concentration range between 25 and 125 nmol/L (P8-P12, table 1). Second, we used three pools of neonatal plasma at three concentration levels of 25(OH)D_3 (29, 54 and 84 nmol/L) with relatively low C_3-epi concentrations (3, 8, and 11 nmol/L, respectively)(P3-P5) and two neonatal plasma pools with relatively high percentages of endogenous 3-epi-25(OH)D_3 (P1 (38%) and P2 (21%)). Finally, 50 nmol/L of 3-epi-25(OH)D_3 was added to one neonatal plasma (P7) and two adult serum (P13 and P14) samples. Methods were compared using Pearson correlation coefficients (r), scatter (Passing and Bablok regression) and bias (Bland-Altman) plots were calculated by Analyse-it software (Microsoft Corporation).

Results

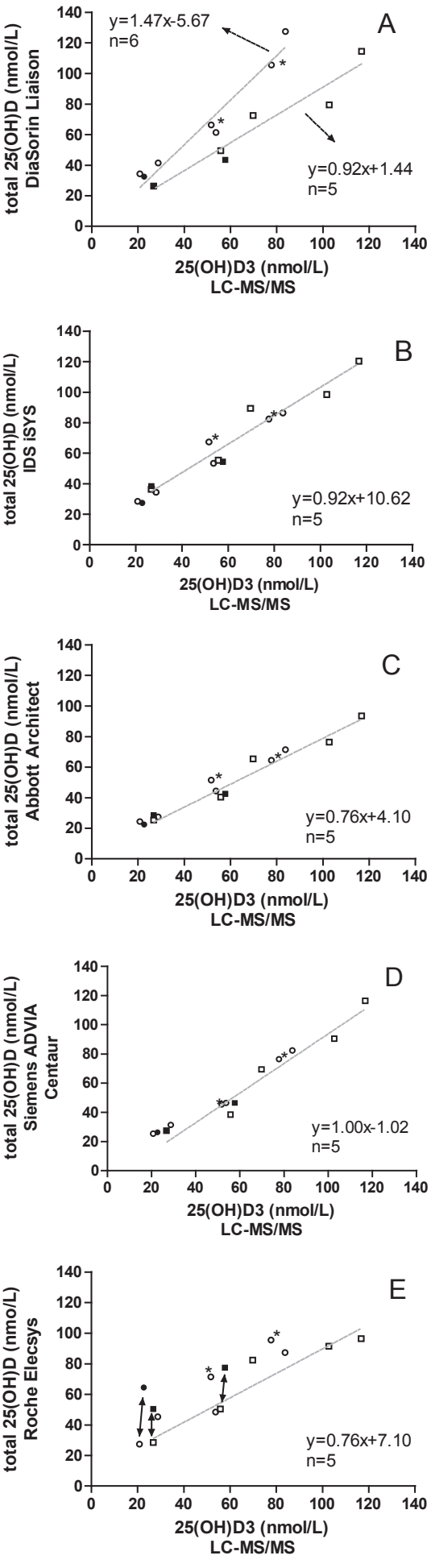
Figure 1 shows the relationship between the total 25(OH)D results for each immunoassay and the LC-MS/MS 25(OH)D_3 results in native and 3-epi-25(OH)D_3 spiked neonatal plasma and adult serum samples. The regression line shown in each plot represents the overall correlation for each immuno- or CBP assay with LC-MS/MS, based on five adult serum samples with increasing concentrations of 25(OH)D_3, not containing significant concentrations of 3-epi-25(OH)D_3 (<6 nmol/L; <5%) (P8-P12 in table 1).

No 3-epi-25(OH)D_3 cross-reactivity was observed in any of the immunoassays, neither from native or spiked samples. In three of the immunoassays (iSYS, Architect, and Centaur) the 25(OH)D results from neonatal samples with endogenous 3-epi-25(OH)D_3 up to 38% (P1-P6) and spiked neonatal and adult samples (P7, P13, P14) agreed with the 25(OH)D_3 LC-MS/MS results, since all data points are close to the respective regression line (figure 1B-D). In the Liaison assay, however, 25(OH)D results from neonatal plasma samples positively biased from those of adult serum samples in comparison to LC-MS/MS, as shown by a distinct regression line with a higher slope (figure 1A). Due to this positive bias, 25(OH)D results from neonatal plasma samples with the highest endogenous concentrations of 3-epi-25(OH)D_3 (P1 and P2), as well as the spiked neonatal sample (P7), were close to the neonatal plasma regression line, whereas 25(OH)D results from the adult samples spiked with 3-epi-25(OH)D_3 (P13,P14) were close to the adult serum regression line.
The Roche Elecsys CPB method showed a mean (range) 57% (44%-74%) cross-reactivity for exogenous addition of 3-epi-25(OH)D_3 in samples P7, P13 and P14 (table 1). The neonatal samples with the highest endogenous concentrations of 3-epi-25(OH)D_3 (P1 and P2) showed a minor, although not statistically significant, deviation from the regression line. A definitive conclusion on 3-epi-25(OH)D_3 recognition by the CPB method in native plasma requires further investigation.

Table 1. Immunoassay cross-reactivity to 3-epi-25(OH)D_3

		LC-MS/MS					Immunoassays				
Sample Nr	Sample origin	3-epi 25(OH) D3 spiked	25(OH)D3 (nmol/L)	3-epi-25(OH)D3 (nmol/L)	25(OH)D3 + 3-epi-25(OH)D3 (nmol/L)	3-epi-25(OH)D3 (%)	DiaSorin LIAISON 25(OH)D (nmol/L)	IDS iSYS 25(OH)D (nmol/L)	Abbott Architect 25(OH)D (nmol/L)	Siemens ADVIA Centaur 25(OH)D (nmol/L)	Roche Elecsys 25(OH)D (nmol/L)
P1	neonatal		78	48	126	38	105	82	64	76	95
P2	neonatal		52	14	66	21	66	67	51	45	71
P3	neonatal		29	3	32	9,4	41	34	27	31	45
P4	neonatal		54	8	62	13	61	53	44	46	48
P5	neonatal		84	11	95	12	127	86	71	82	87
P6	neonatal		21	2	23	8,7	34	28	24	25	27
P7	neonatal	P6+50	23	61	84	73	32	27	22	26	64
P8	adult		27	1	28	3,6	26	36	25	27	28
P9	adult		56	2	58	3,4	49	55	40	38	50
P10	adult		70	2	72	2,8	72	89	65	69	82
P11	adult		103	5	108	4,6	79	98	76	90	91
P12	adult		117	6	123	4,9	114	120	93	116	96
P13	adult	P8+50	27	51	78	65	26	38	28	27	50
P14	adult	P9+50	58	47	105	45	43	54	42	46	77
3-epi-25(OH)D3 recovery (%)						90-118%	ND	ND	ND	ND	44-74%

ND = non detected (<5%)



Discussion

At present, the C_3-epimer of 25OHD$_3$ is regarded as a potential confounder in vitamin D measurement and thus in the assessment of vitamin D sufficiency and should not be included in the total 25(OH)D result. We have demonstrated that none of the current automated immunoassays showed 3-epi-25(OH)D$_3$ cross-reactivity, based on the findings that spiked samples as well as native newborn samples with up to 38% C_3-epimer did not alter the 25(OH)D$_3$ comparability between immunoassays and LC-MS/MS. The most likely explanation is that the 25(OH)D antibodies do not recognize the C_3-epimer.

It is believed that the 3-epi-25(OH)D$_3$ metabolite contributes to the total 25(OH)D results produced by the Roche CPB method, which uses a recombinant DBP rather than an antibody to detect 25(OH)D. These claims are mainly based on cross-reactivity studies from exogenously added 3-epi-25(OH)D$_3$. The Vitamin D total package insert describes a 93% cross-reactivity, and the DEQAS October 2011 results of a sample spiked with 50 nmol/L of 3-epi-25(OH)D$_3$ showed a mean 57% cross-reactivity to 3-epi-25(OH)D$_3$ by the CPB assay (4). We confirmed a 57% cross-reactivity to 3-epi-25(OH)D$_3$ from exogenous addition, but definitive proof on 3-epi-25(OH)D$_3$ recognition in native samples has yet to be delivered.

Acknowledgements

We thank Siemens and Abbott for the gift of kits free of charge for this study. G.J.P. van Dijk, M. Romeijn and L. Tax are acknowledged for performing the immunoassay analysis.

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Figure 1. Immunoassay cross-reactivity to 3-epi-25(OH)D$_3$. Shown is the relationship between the total 25(OH)D results of each immuno- and protein binding assay and the LC-MS/MS 25(OH)D$_3$ results in native (open) and 3-epi-25(OH)D$_3$ spiked (closed) neonatal plasma (circle) and adult serum (square) samples. Neonatal samples with the highest endogenous 3-epi-25(OH)D$_3$ concentrations (P1 and P2 in table 1) are marked by an asterisk. Paired samples (native and spiked) are indicated by arrows in the Roche Elecsys plot (E). Dotted line represents the Passing and Bablok regression equation from unadulterated adult serum (n=5) or, in case of the Liaison assay (A), also from neonatal plasma samples (n=6).