VU medisch centrum

De ene vitamine D bepaling is de andere niet

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LWBA

Utrecht

Vitamin D

Calcium en phosphorus uptake by gut

Mineralisation of bone

Vitamin D deficiency

Rickets

Deficiency and supplementation

- 30-70% of the population is vitamin D deficient
  - Cut-off
  - Seasonal influences
  - Vitamin D assay used

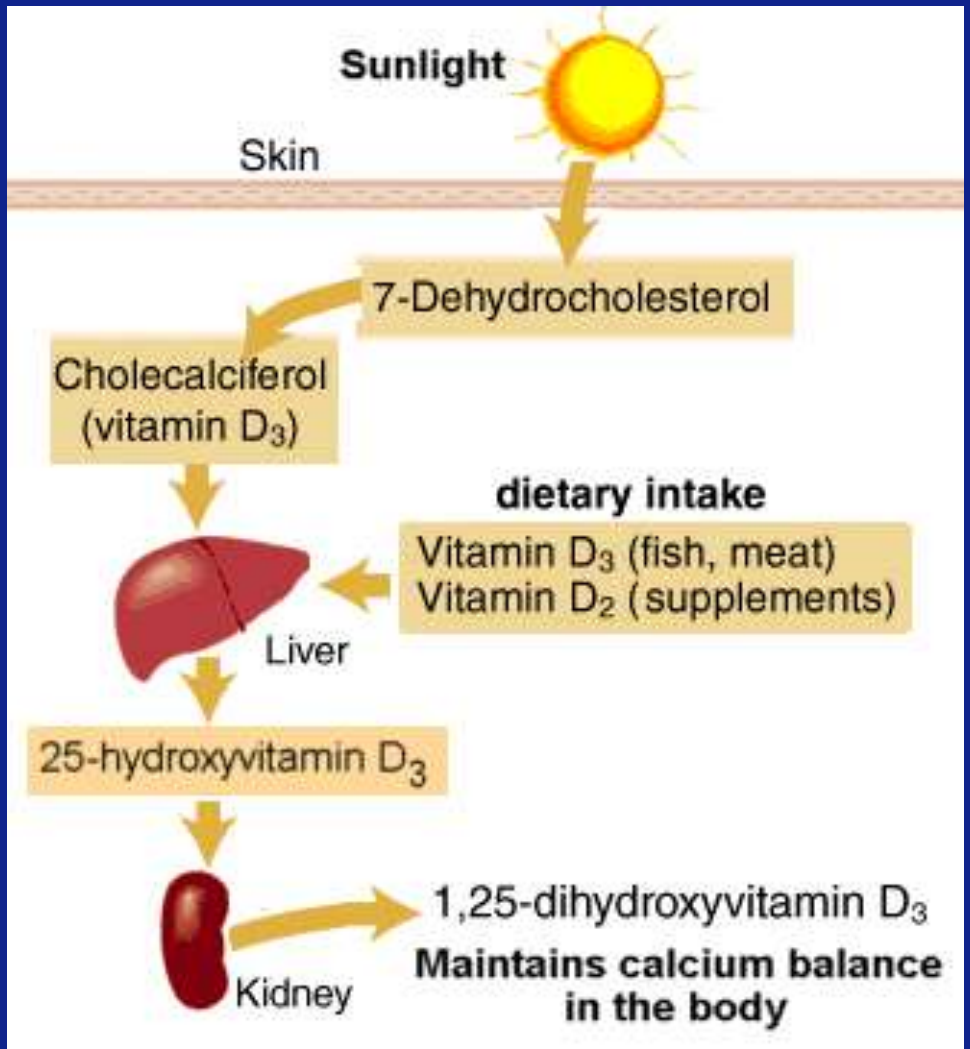
- Supplementation
  - Decreased risk of osteoporosis (and fractures)

Extraskeletal effects

- 3% of human genome is under control of vitamin D

- Muscle function
- Cancer
- Immune system
- Cardiovascular system
- Metabolic system
- Neuropsychiatric function

Vitamin D


Sunlight
Skin
7-Dehydrocholesterol
Cholecalciferol
(vitamin D_3)
dietary intake
Vitamin D_3 (fish, meat)
Vitamin D_2 (supplements)
Liver
25-hydroxyvitamin D_3
1,25-dihydroxyvitamin D_3
Kidney
Maintains calcium balance
in the body

Sources

- Sunlight (250 microgram production one day in sun)
- Fatty fish (10-25 microgram per portion)
- Supplements (recommended daily dose is 5 microgram)

Sun protection

Skin cancer versus vitamin D deficiency

Vitamin D assays

Clinical Chemistry 57:9
1227–1232 (2011)

Q&A

Clinical Applications for Vitamin D Assays: What Is Known and What Is Wished for

Moderator: Michael Kleerekoper[1*]
Experts: Rosemary L. Schleicher,[2] John Eisman,[3,4,5] Roger Bouillon,[6] Ravinder J. Singh,[7] and Michael F. Holick[8]

Vitamin D is a "hot topic," with the number of citations in PubMed exceeding 2400 in 2009, a 3-fold increase in 1 year. In the US, the number of requested 25-hydroxyvitamin D (25-OHD)[9] assays is increasing exponentially. Not all of the published material has validity, however. A panel of experts was invited to address a series of questions pertaining to laboratory methods and clinical applications of available assays

John Eisman: 25-OHD_2 and 25-OHD_3 should be measured in most clinical situations, although in many countries vitamin supplements and food fortification are moving from vitamin D_2 to D_3. I am un-

Vitamin D assays are laborious

Automation is desirable

Vitamin D binding protein (DBP)

- (25OH) Vitamin D transport
  - 85% bound to DBP
  - 15% bound to albumin
  - 0.03% free

Vitamin D assay

Extraction of vitamin D from DBP

Can automated immunoassays perform extraction well enough?

Aim

- To test the accuracy of six available routine 25(OH)D assays by comparing these assays with an ID-LC-MS/MS method, using not only plasma from healthy individuals, but also plasma from patients with various DBP concentrations.

Methods: demographic details

Table 1. Demographic details of the subjects.

Group	N	Male/female	Age range (y)
Healthy individuals	51	23/28	20-64
Pregnant women	52	0/52	19-40
Haemodialysis patients	50	24/26	33-89
IC patients	50	28/22	18-89

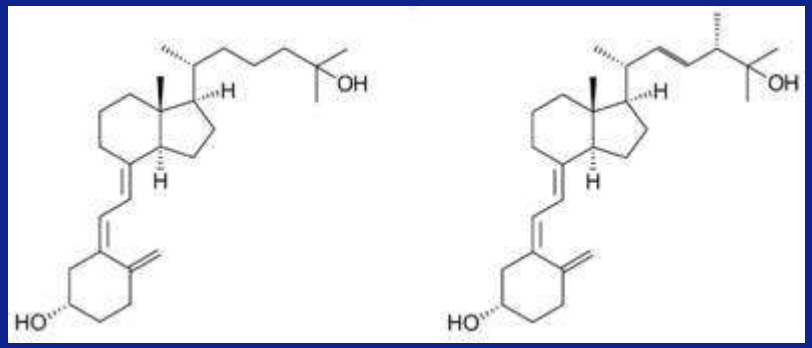
Methods: 25(OH)D assays

- Architect (Abbott)
- Centaur (Siemens)
- iSYS (IDS)
- Liaison (Diasorin)
- Modular (Roche)
- RIA preceded by extraction (Diasorin)

- ID-LC-MS/MS

Methods: ID-XLC-MS/MS

- 100 µl EDTA plasma
- Disruption D25 from DBP: proteolysis
- Deuterated internal standard (IS: 25(OH)D3-d6)
- Solid Phase Extraction (SPE)
- LC-MS/MS



25OHD$_3$ $C_{27}H_{44}O_2$ 25OHD$_2$ $C_{28}H_{44}O_2$

LC-MS/MS method

- Establishing accuracy by measuring standard and control with candidate reference method

Clinical Chemistry 57:3
441–448 (2011)

Automation and Analytical Techniques

Candidate Reference Measurement Procedures for Serum 25-Hydroxyvitamin D_3 and 25-Hydroxyvitamin D_2 by Using Isotope-Dilution Liquid Chromatography–Tandem Mass Spectrometry

Hedwig C.M. Stepman,[1] An Vanderroost, Katleen Van Uytfanghe,[1] and Linda M. Thienpont[1*]

- Intra CV% <6% and inter CV% <8%; LOQ is 4 nmol/L

Methods: DBP

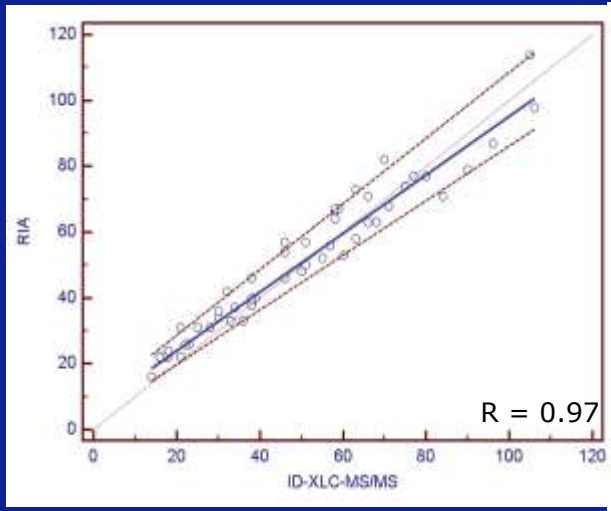
- ELISA (R&D systems)
- Intra CV <5%

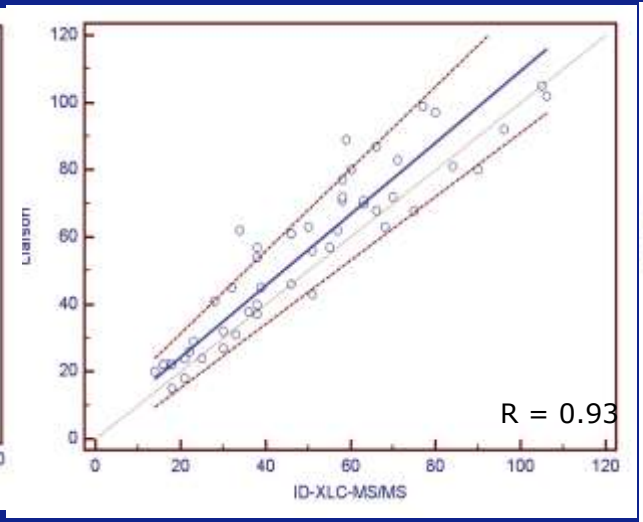
Accuracy

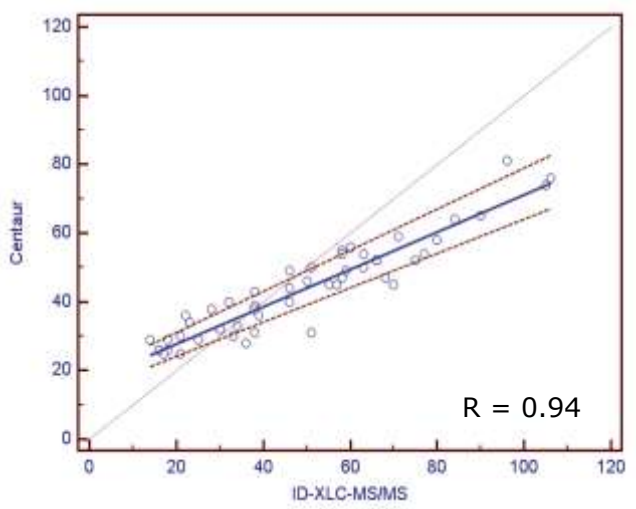
Results

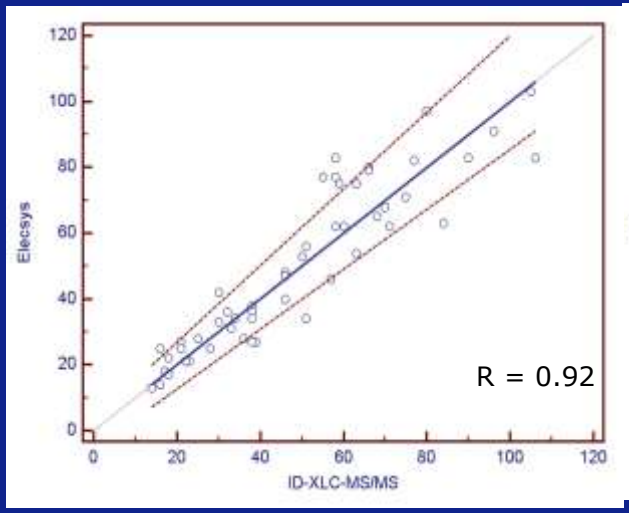
- ID-LC-MS/MS: 25(OH)D2 and 25(OH)D3
- Passing&Bablok
  - Immunoassay compared to ID-LC-MS/MS
    - Healthy individuals
    - Pregnant women
    - Dialysis patients
    - IC patients

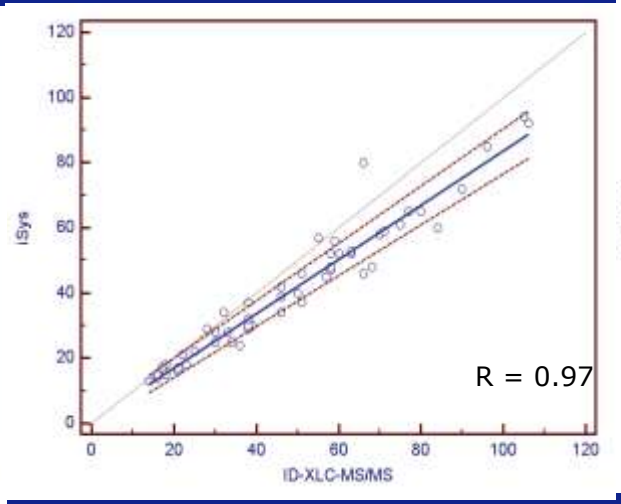
Healthy individuals

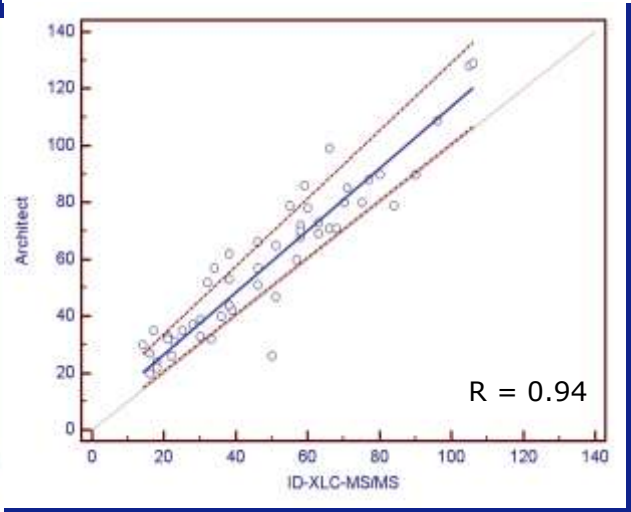




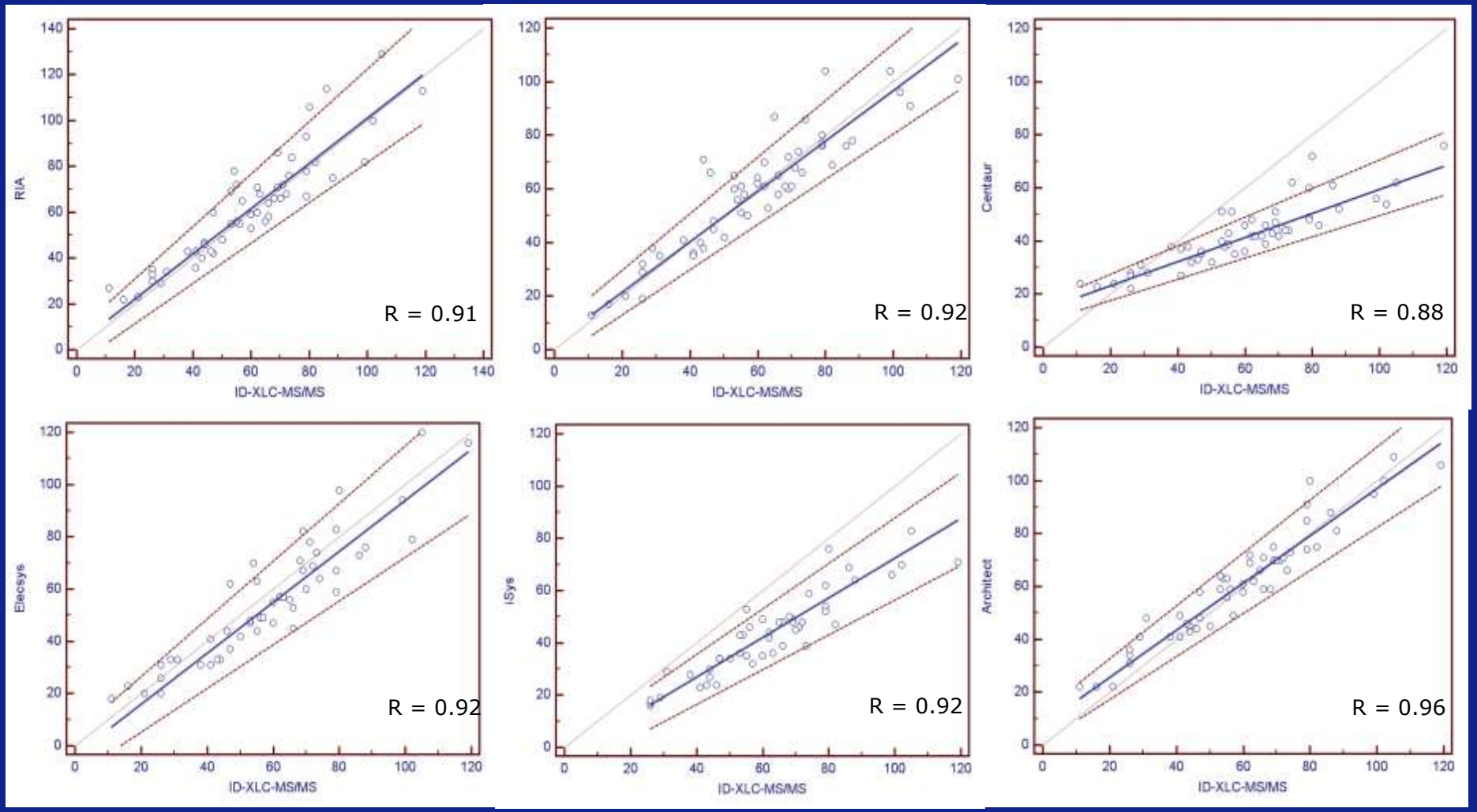




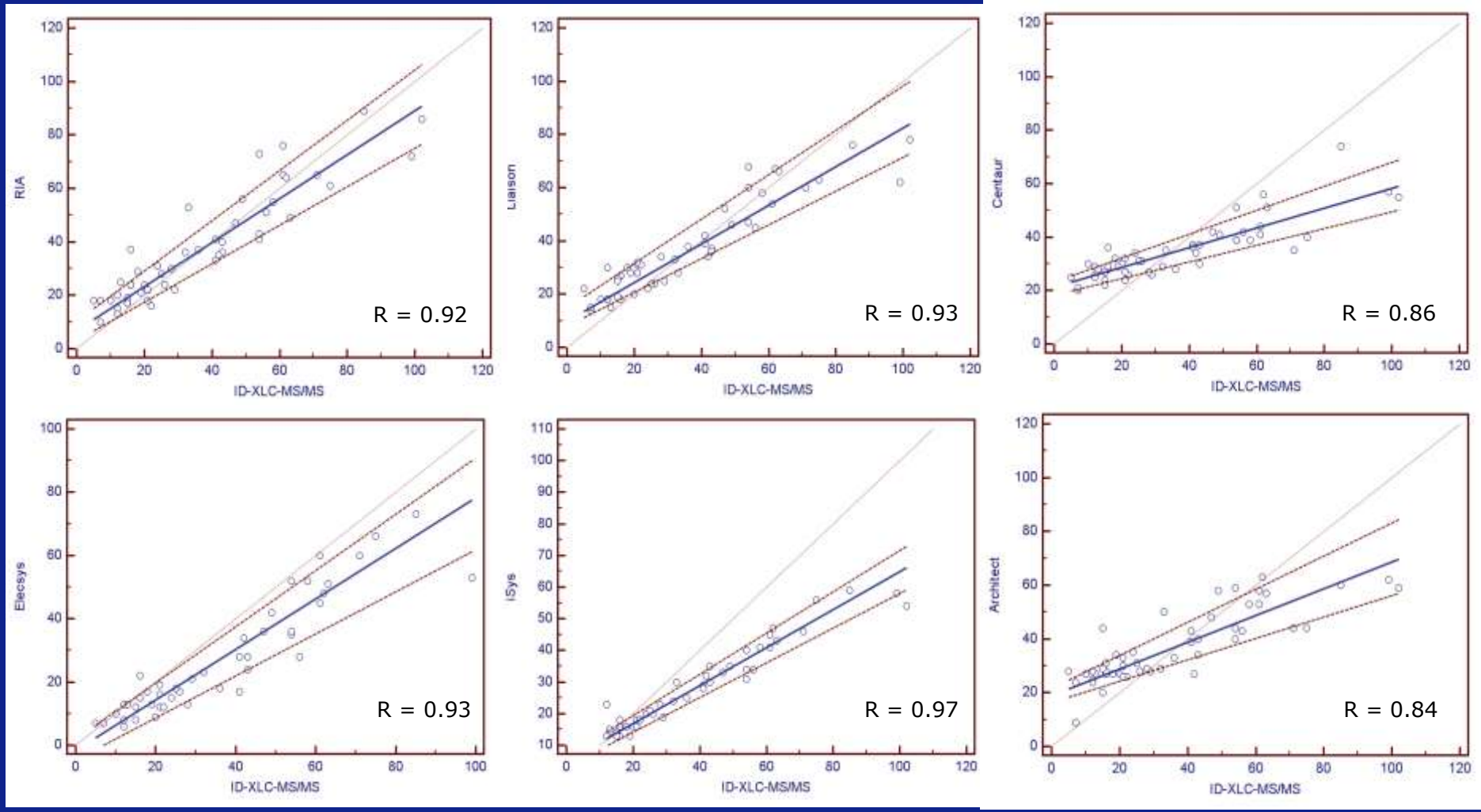




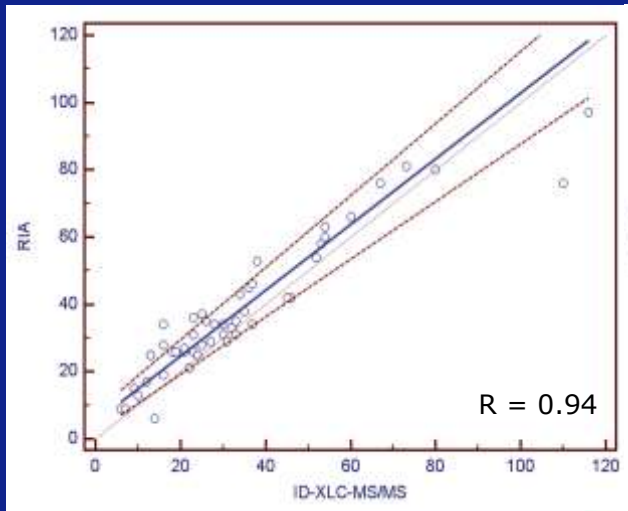
Pregnant women

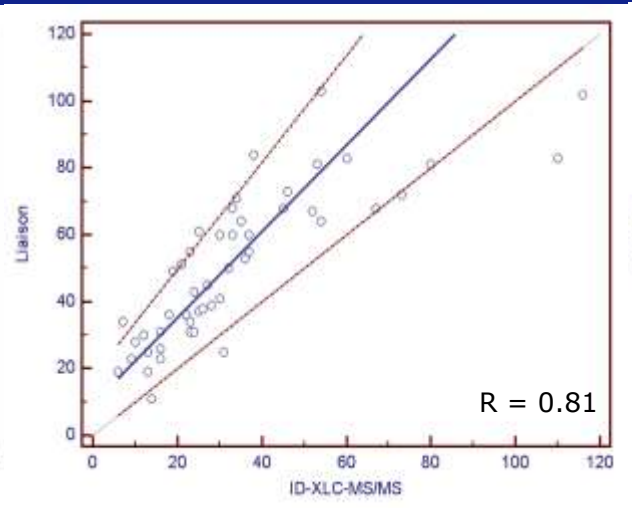


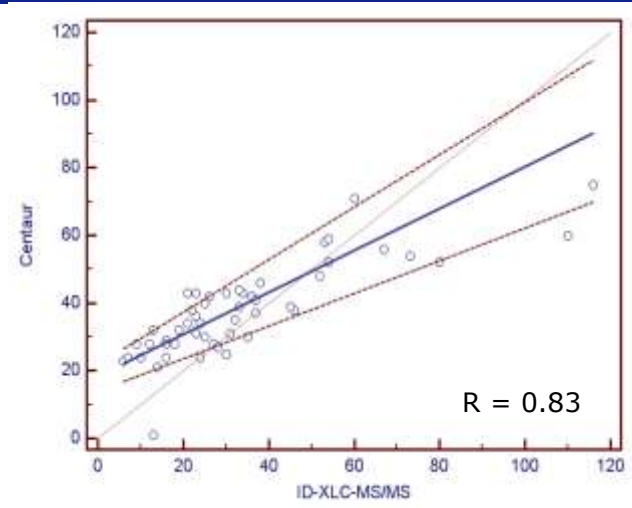
Hemodialysis patients

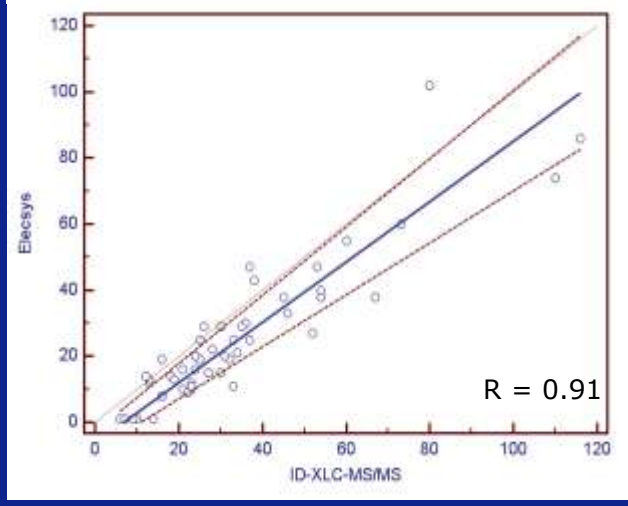


Intensive care patients

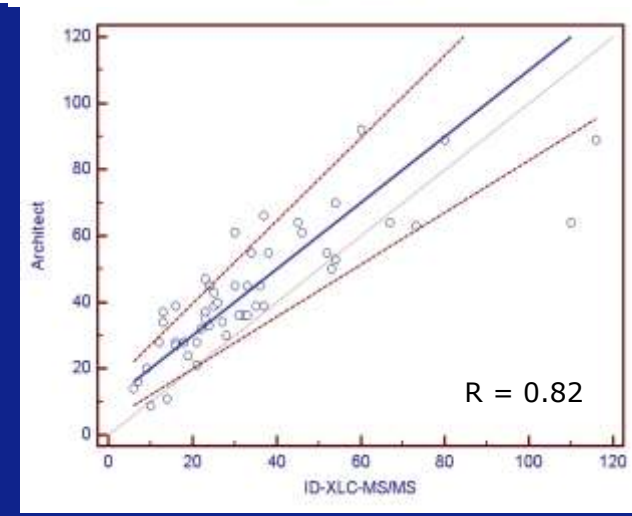






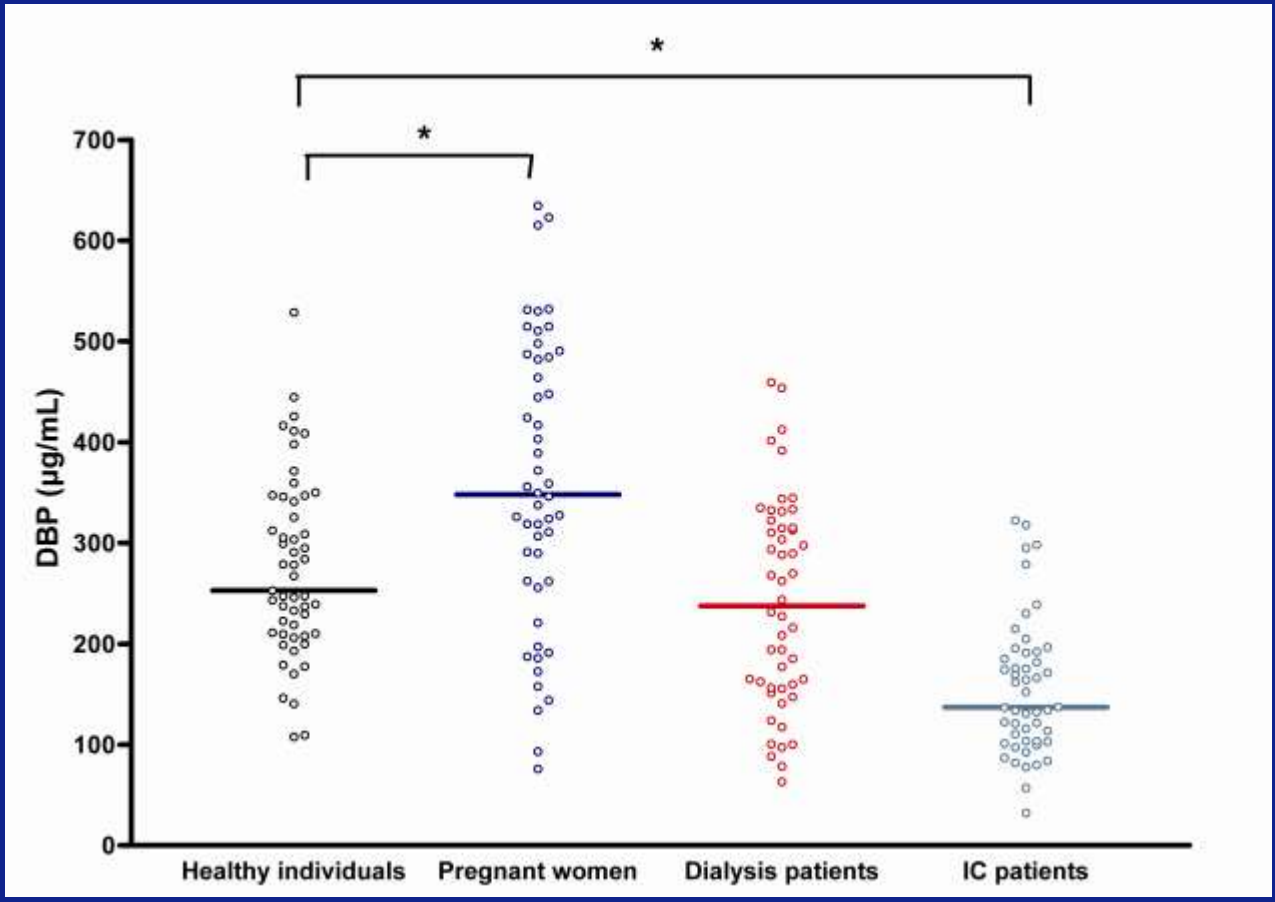


Intensive care patients not determined with iSYS

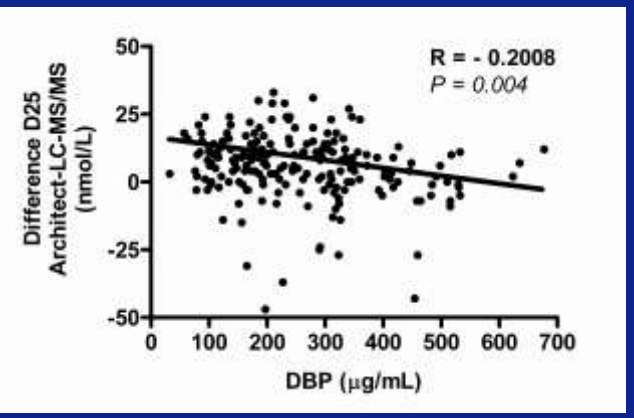


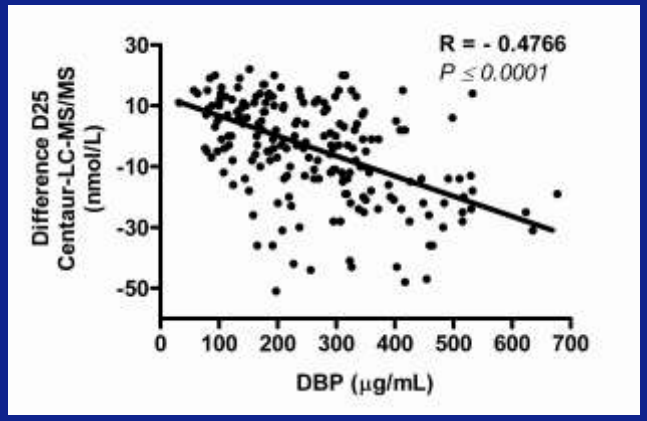
DBP concentrations

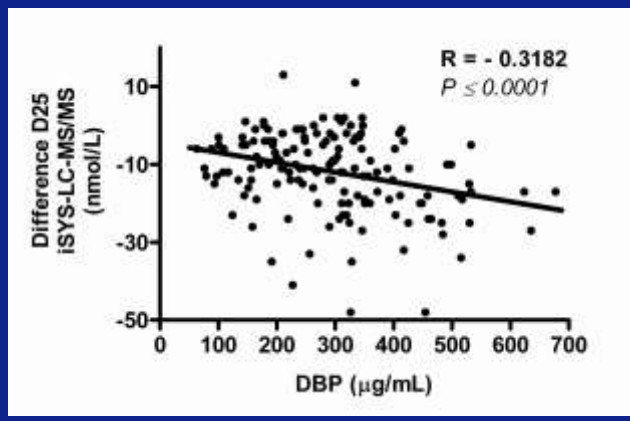
Results: DBP concentrations

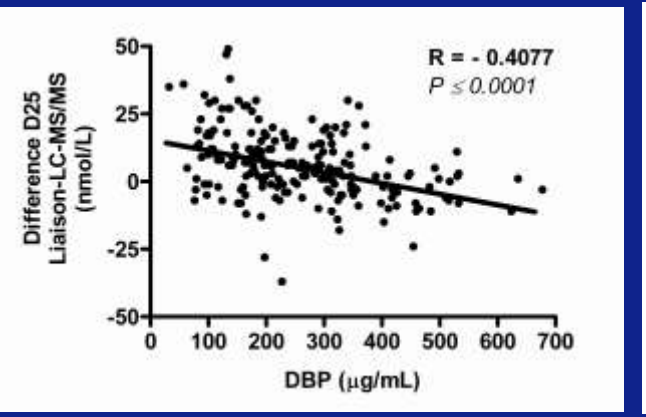


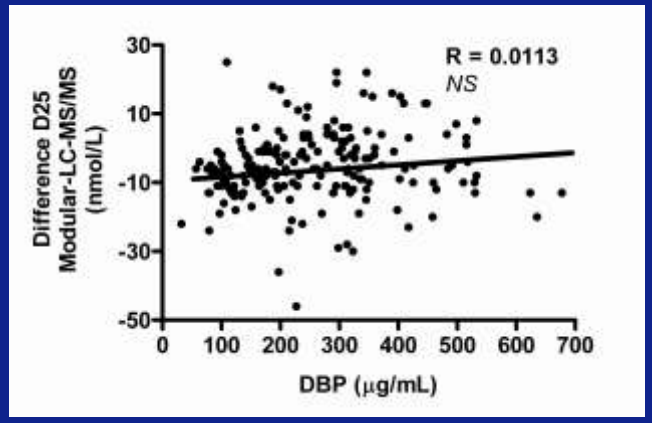
Relation between 25(OH)D deviation and DBP concentration

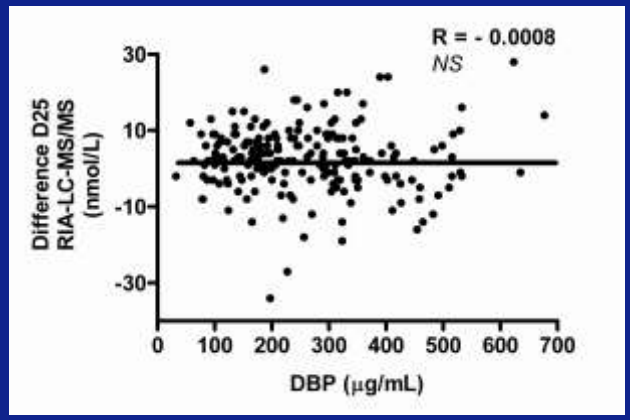












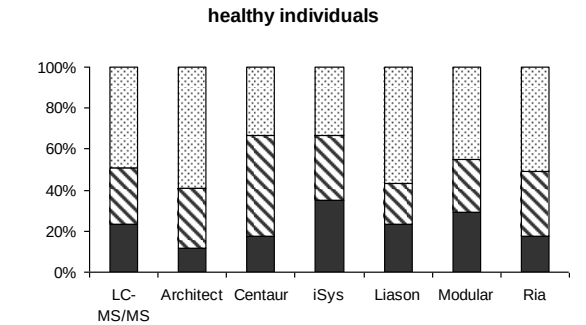
Discussion

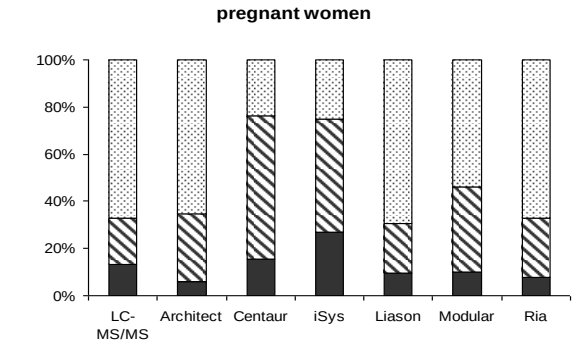
- Major differences between assays
  - Standardisation problems
  - Influence of DBP (4 of 5 automated assays)
  - Other unknown interference in immunoassays
- Not all assays are suitable for measuring 25(OH)D in all patient groups
- 25(OH)D2 is not causing differences
- Clinical significance
  - Advice on supplementation

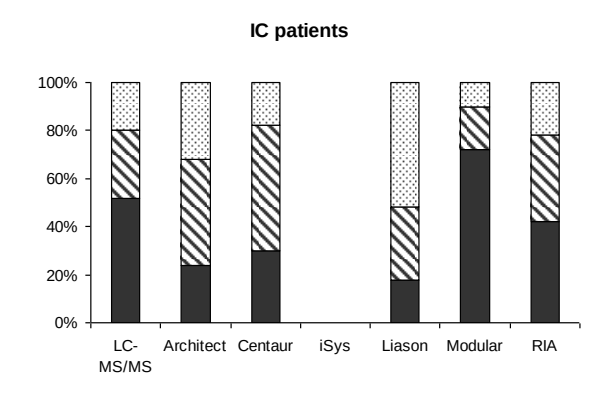
VU medisch centrum

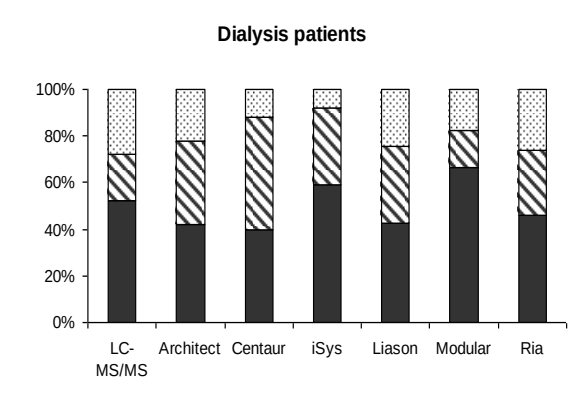
25(OH)D sufficient	>50 nmol/L
25(OH)D insufficient	30-50 nmol/L
25(OH)D deficient	<30 nmol/L

Sufficiency









Conclusion

- Some of the assays used to measure 25(OH)D are not well standardized and report significantly different results from measurements performed with ID-XLC-MS/MS.
- The deviations which are sometimes serious are different in various patient groups, and are dependent on the concentration of DBP and other still unknown interfering factors
- Laboratory specialists, clinicians, researchers, reviewers and authorities should carefully consider the method used when interpreting results of 25(OH)D measurements

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