Introduction

1,25-dihydroxyvitamin D, the hormonally active metabolite of vitamin D, tightly controls calcium blood levels. Measurement of 1,25-dihydroxyvitamin D is useful when disorders of 1α-hydroxylation or extrarenal 1α-hydroxylation, or vitamin D receptor defects are suspected.

Methods

Human reference values for serum total 1,25-dihydroxyvitamin D3 and D2 were determined from 96 heparinized plasma samples using a 2D ID-UPLC-MS/MS method consisting of an Acquity UPLC system (Waters) linked to a Xevo TQ-S tandem quadrupole mass spectrometer (Waters).

Sample preparation included sample purification using immunoaffinity columns and derivatization using PTAD. Intra- and inter-assay CVs for 1,25-dihydroxyvitamin D3 were 3.5% and 5.5%, respectively.

Our method was compared to a RIA, a LC-MS/MS method from the KU Leuven and the average DEQAS values for 1,25-dihydroxyvitamin D3.

Results

Fig. 2: Passing and Bablok regression analysis of our 2D ID-UPLC-MS/MS 1,25-dihydroxyvitamin D method compared to the IDS RIA (A), the LC-MS/MS method from the KU Leuven (B) and to the average LC-MS/MS DEQAS values (C).

Tab. 2: Reference values as determined by LC-MS/MS VUmc

<table>
<thead>
<tr>
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<th>LC-MS/MS VUmc (pmol/L)</th>
<th>LC-MS/MS VUmc (pmol/L)</th>
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</thead>
<tbody>
<tr>
<td>1,25-dihydroxyvitamin D3</td>
<td>59 – 159 pmol/L</td>
<td>59 – 159 pmol/L</td>
</tr>
<tr>
<td>1,25-dihydroxyvitamin D2</td>
<td>&lt; 7.2 pmol/L</td>
<td>&lt; 7.2 pmol/L</td>
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Fig. 3: Linear regression analysis of 1,25-dihydroxyvitamin D3 concentrations versus age in men and women.

Conclusion

We have determined reference values for 1,25-dihydroxyvitamin D3 in men and women.

A relationship between 1,25-dihydroxyvitamin D3 concentrations and age in women was apparent. However, closer examination suggested this relationship to be dependent on estrogen levels.

In view of the method comparisons, reference values have to be determined for every method individually.