

# Determination of human reference values for serum total 1,25-dihydroxyvitamin D<sub>3</sub> and D<sub>2</sub>

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## 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 1 $\alpha$ -hydroxylation or extrarenal 1 $\alpha$ -hydroxylation, or vitamin D receptor defects are suspected.

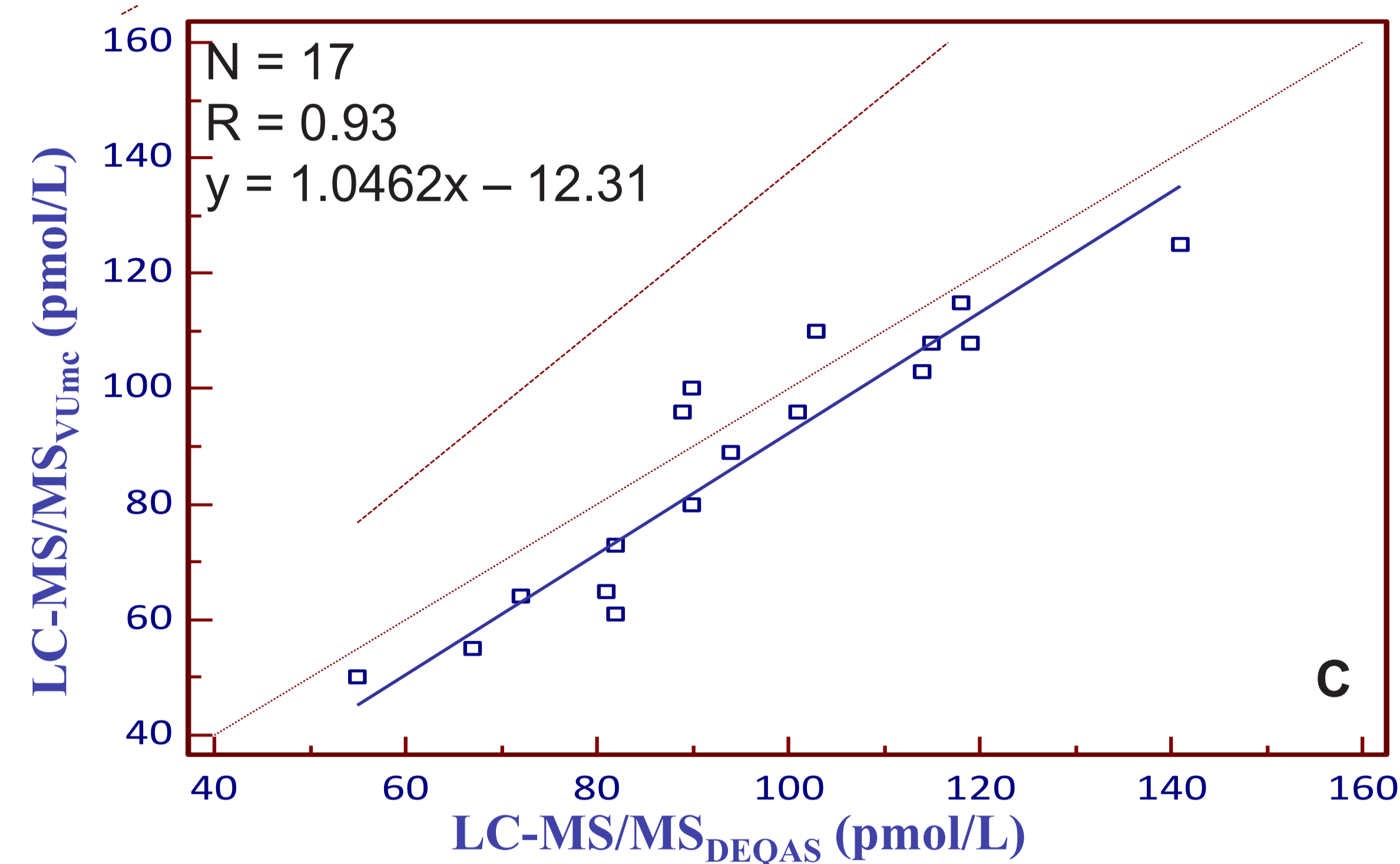
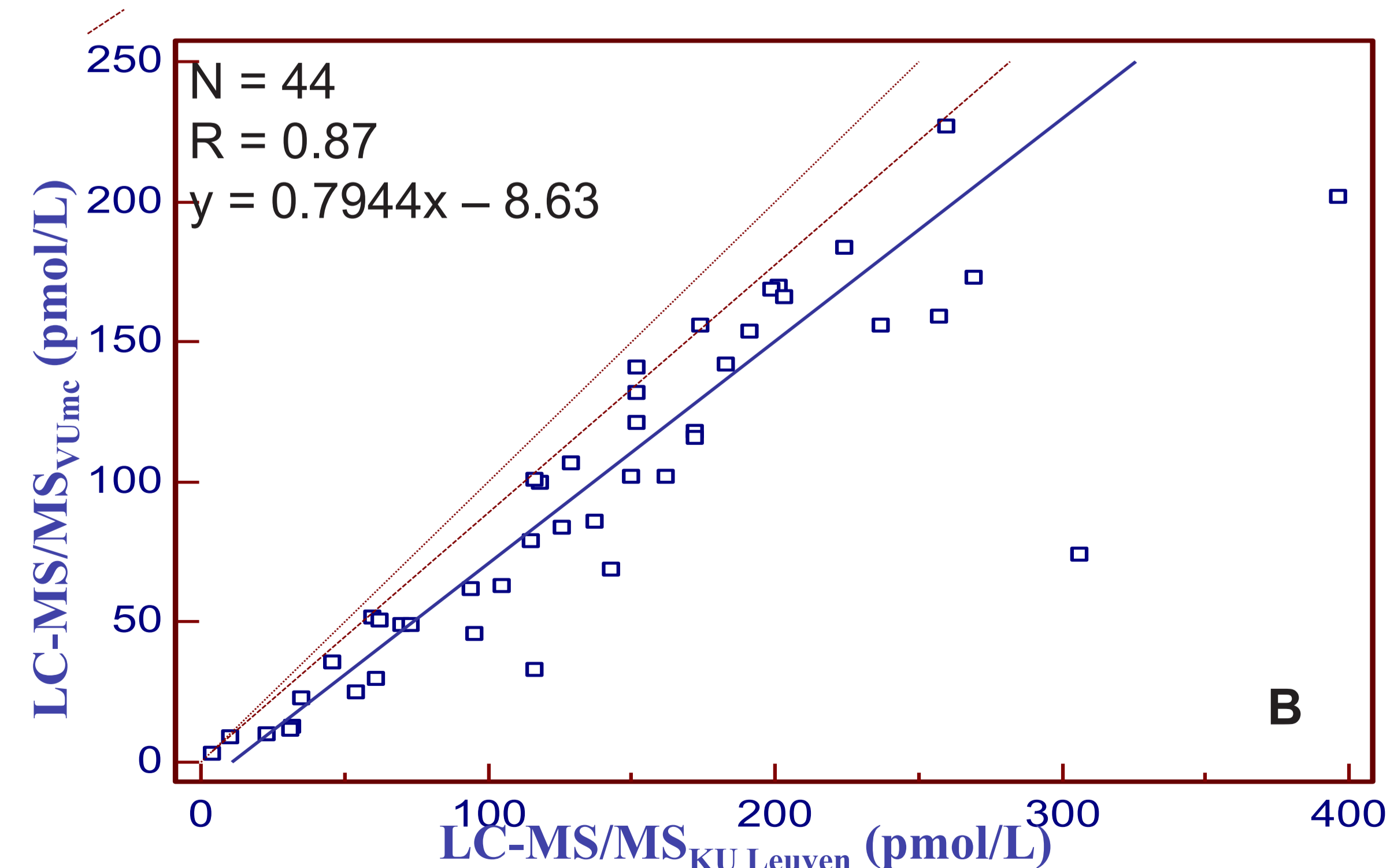
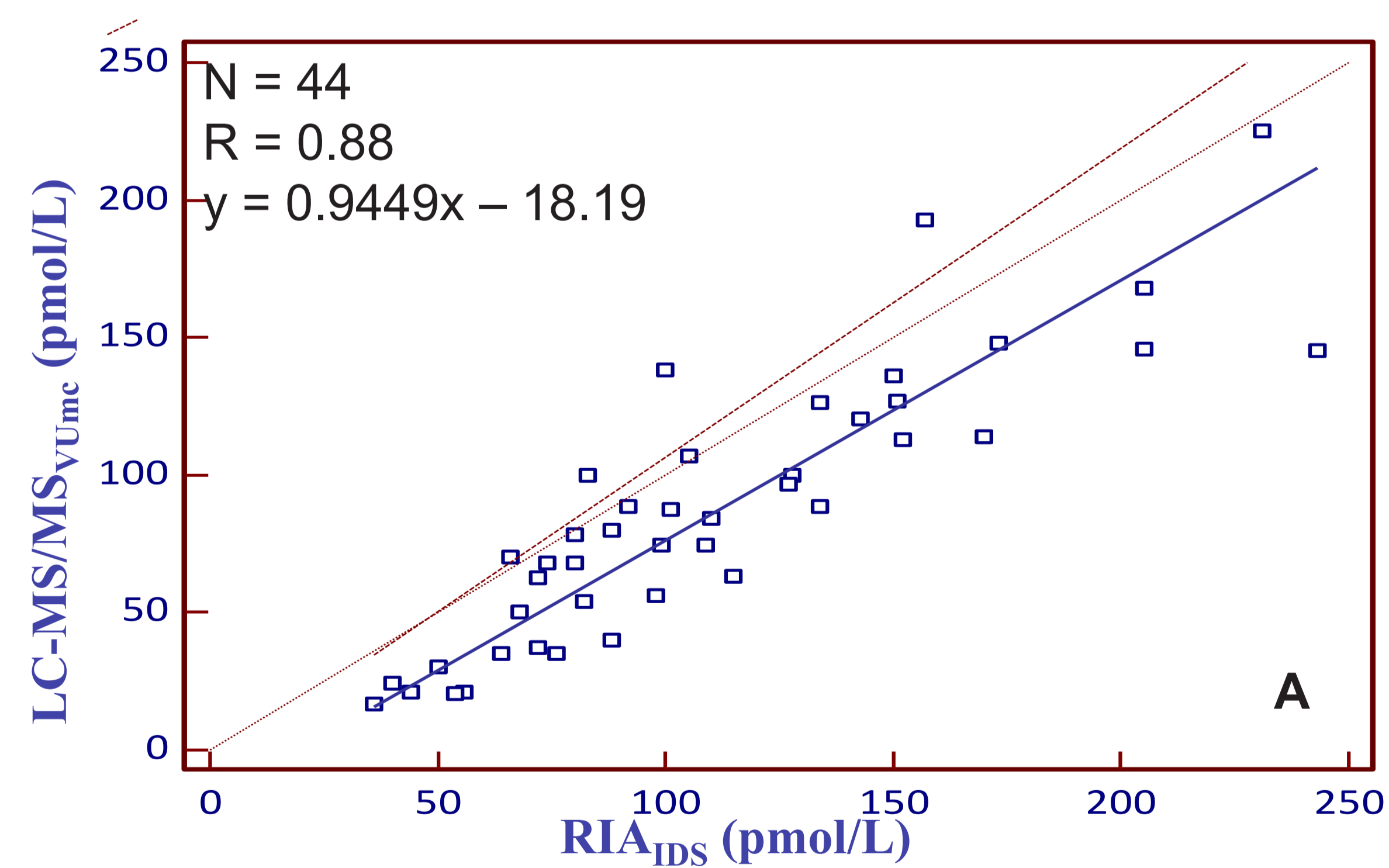
## Methods

Human reference values for serum total 1,25-dihydroxyvitamin D<sub>3</sub> and D<sub>2</sub> 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 immunaffinity columns and derivatization using PTAD. Intra- and inter-assay CVs for 1,25-dihydroxyvitamin D<sub>3</sub> 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 D<sub>3</sub>.

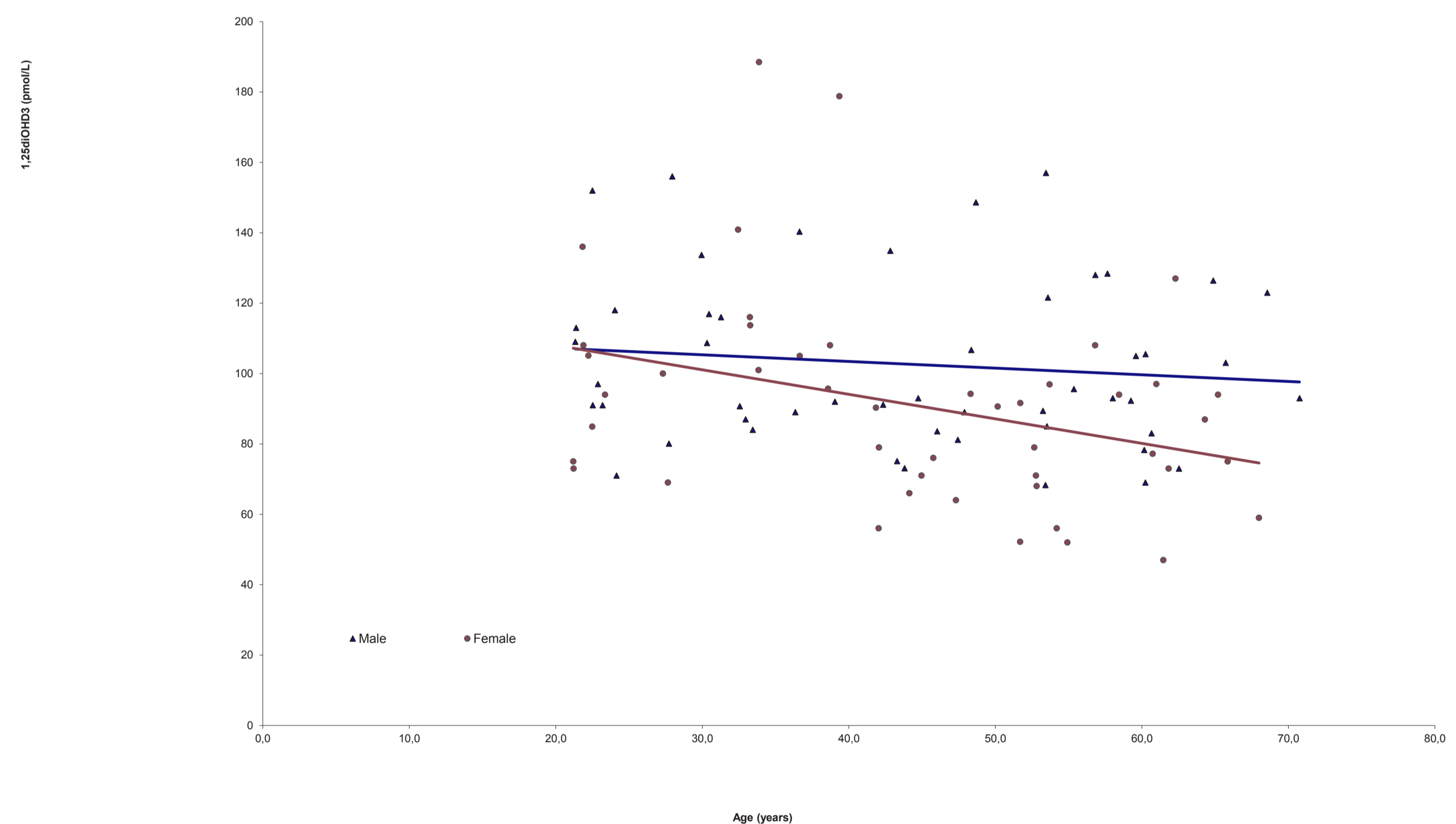
## Results



**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<sub>VUMC</sub>

1,25-dihydroxyvitamin D <sub>3</sub>	59 – 159 pmol/L
1,25-dihydroxyvitamin D <sub>2</sub>	< 7.2 pmol/L



**Fig. 3:** Linear regression analysis of 1,25-dihydroxyvitamin D<sub>3</sub> concentrations versus age in men and women.

## Conclusion

We have determined reference values for 1,25-dihydroxyvitamin D<sub>3</sub> in men and women.

A relationship between 1,25-dihydroxyvitamin D<sub>3</sub> 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.

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