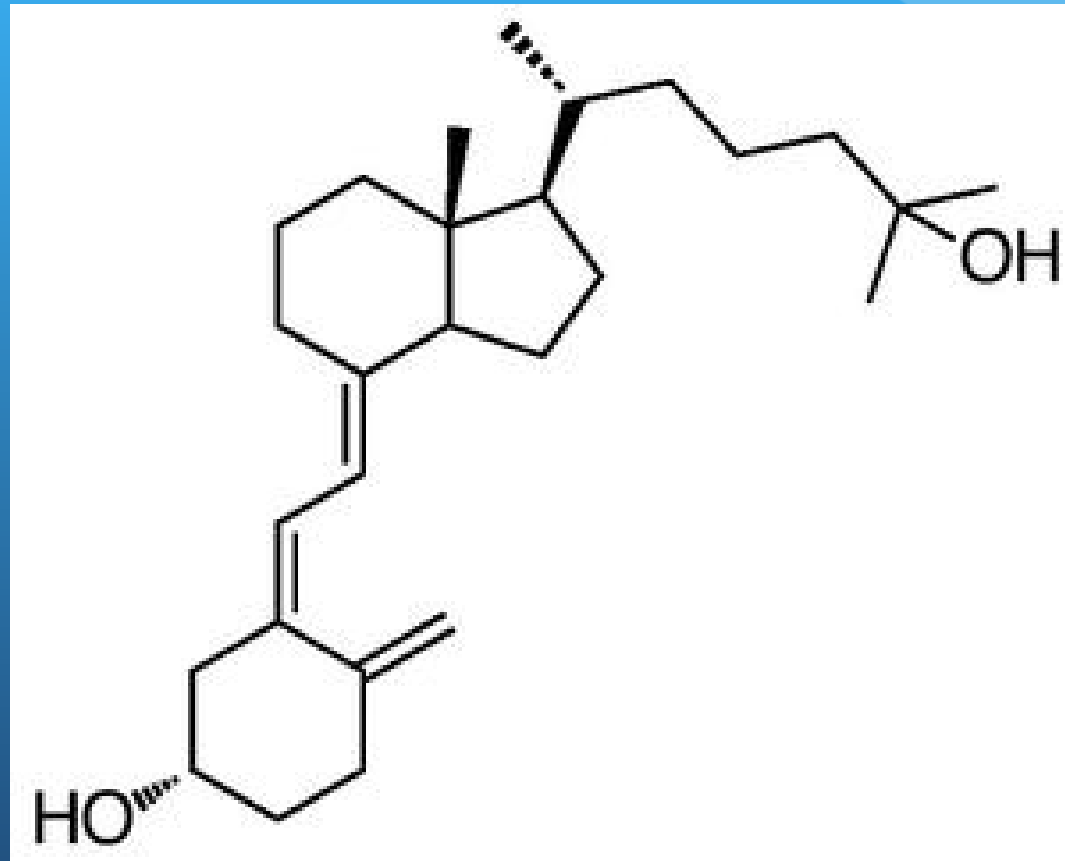


Free Vitamin D3 Analysis by State of the Art LC-MS/MS Technology

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25 Hydroxyvitamin D3



Hypothesis:

- Free 25OHD3 is representative of the effect of vitamin D on the human body.
- Free 25OHD3 plays a vital role in human health and disease, and its quantification will enable connections to be made between free concentrations and certain diseases and conditions.
- The measurement of total 25OHD, the current standard, is not as reliable an indicator of health and will not necessarily correspond to the level of free 25OHD3.

Our Goals:

- To employ state of the art LC- MS/MS technology in free 25OHD3 analysis
- To develop a tentative reference range for free 25OHD3
- To compare total 25OHD measurements to free and percent free 25OHD3 measurements of patient samples

Results:

Figure 1: Total Ion Chromatogram Showing Patient Sample (MRM: 383.3/229.2)

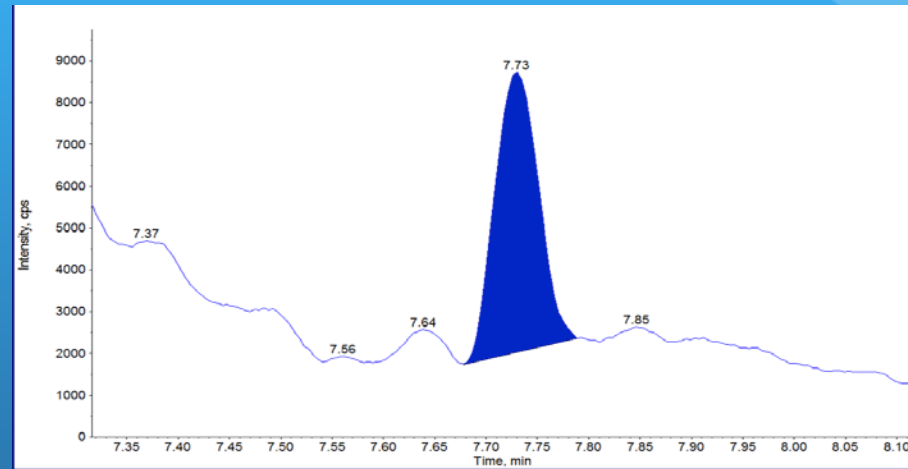
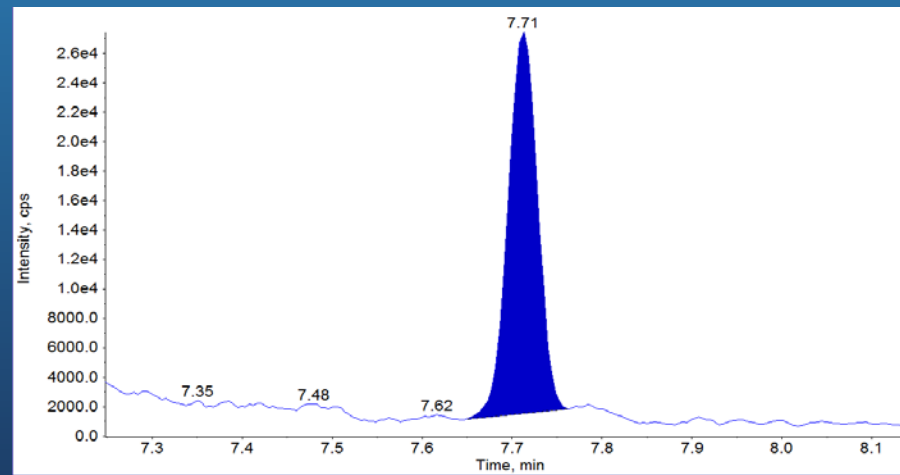
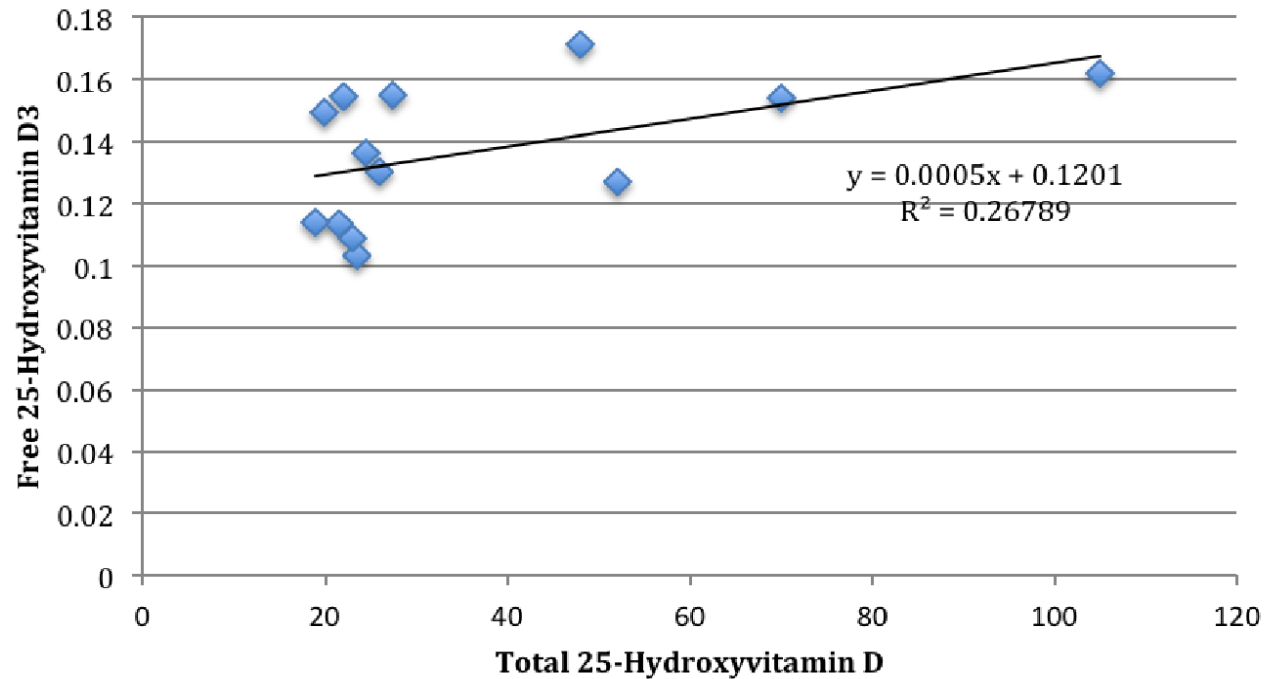


Figure 2: Total Ion Chromatogram of Deuterated Internal Standard (MRM: 389.3/211.2)

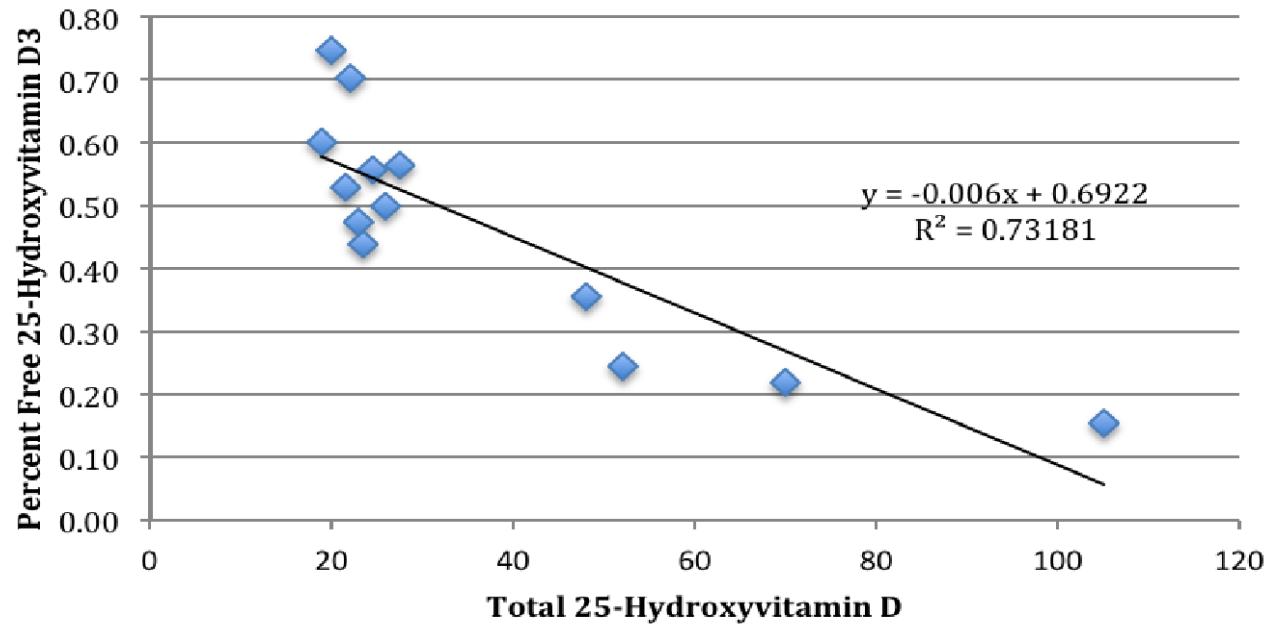


Sample	Total 25OHVit D (ng/mL)	Free 25OHVitD3 (ng/mL)	Percent Free OHVitD3
1	28	0.15	0.56
2	26	0.13	0.50
3	25	0.14	0.56
4	24	0.10	0.44
5	23	0.11	0.47
6	22	0.15	0.70
7	22	0.11	0.53
8	20	0.15	0.75
9	19	0.11	0.60
10	105	0.16	0.15
11	70	0.15	0.22
12	52	0.13	0.24
13	48	0.17	0.36
Range:	19-105	0.10-0.17	0.15-0.75

Total 25-Hydroxyvitamin D vs. Free 25-Hydroxyvitamin D3 in Patient Samples



Total 25-Hydroxyvitamin D vs. Percent Free 25-Hydroxyvitamin D3 in Patient Samples



Future Directions:

- Examine the role of free 25-Hydroxyvitamin D in bone and immune health
- Examine the relationship between free 25-Hydroxyvitamin D and parathyroid hormone in the different patient populations of the National Institutes of Health

Free Vitamin D3 Analysis Employing State of the Art LC-MS/MS Technology



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Background

Introduction:

The future belongs to the free and not to the bound. Free hormones are not bound to proteins in the blood, and they are the biologically active agents that are able to bind with receptors on specific target cells to initiate a response. The number of binding proteins and the concentration of free hormones vary between individuals and with clinical conditions. Measuring the free improves both diagnosis and treatment of disease. Free 25-Hydroxyvitamin D3 (25OHD3) is of particular interest because of its extensive role in both immune response and bone health. Reference intervals for the measurement of total 25-Hydroxyvitamin D (25OHD) are controversial, but 30-100 ng/mL is generally accepted in regards to optimal health. It may be that free 25OHD3 will give a lighter reference range for the optimal amount of vitamin D needed in various disease states. Yet, up until this point, no method has been able to accurately and consistently measure the analyte. In this study, free 25OHD3 has been measured via state of the art LC-MS/MS. A tentative reference range, based on thirteen patient samples, was found to be between 100 and 171 pg/mL. This opens the door to a wide array of clinical applications and permits the optimization of treatment in various bone diseases, cancers etc.

Hypothesis:

- Free 25OHD3 is representative of the effect of vitamin D on the human body.
- Free 25OHD3 plays a vital role in human health and disease, and its quantification will enable connections to be made between free concentrations and certain diseases and conditions.
- The measurement of total 25OHD, the current standard, is not as reliable an indicator of health and will not necessarily correspond to the level of free 25OHD3.

Our Goals:

- To employ state of the art LC-MS/MS technology in free 25OHD3 analysis
- To develop a tentative reference range for free 25OHD3
- To compare total 25OHD measurements to free and percent free 25OHD3 measurements of patient samples

Method

Samples:

- 13 serum samples were obtained from in and out patient leftover samples after all identifiers has been removed. These samples were stored at -20 °C until analysis.

Total 25-Hydroxyvitamin D Analysis:

- Diasorin Liaison XL Chemiluminescent Immunoassay

Free 25-Hydroxyvitamin D3 Sample Prep:

- Patent Pending

Free 25-Hydroxyvitamin D Analysis:

- Patent Pending

Results

Figure 1: Total Ion Chromatogram Showing Patient Sample (MRM: 383.3/229.2)



Figure 2: Total Ion Chromatogram of Deuterated Internal Standard (MRM: 389.3/211.2)

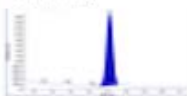


Table One: Comparison of Total 25OHD vs. Free 25OHD3

Sample	Total 25OHD (ng/mL)	Free 25OHD3 (ng/mL)	Percent Free 25OHD3
1	26	0.15	0.56
2	26	0.13	0.50
3	25	0.14	0.56
4	24	0.20	0.84
5	23	0.21	0.91
6	22	0.18	0.79
7	22	0.21	0.95
8	20	0.15	0.75
9	19	0.21	0.80
10	105	0.16	0.15
11	76	0.18	0.22
12	52	0.13	0.24
13	48	0.17	0.35

Range: 19-105 0.15-0.17 0.15-0.75

Figure 3: Linear Regression of Total 25OHD vs. Free 25OHD3

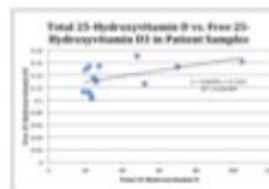
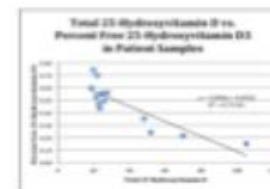


Figure 4: Linear Regression of Total 25OHD vs. Percent Free 25OHD3



Discussion

This study successfully quantified levels of free 25OHD3 in patient samples. A preliminary reference range, based on the thirteen samples run, was found to be between 0.10 and 0.17 ng/mL. This is a much lighter range than that of the total 25OHD, given at 19-105 ng/mL for this study. An important point is the lack of significant correlation between the total 25OHD and the free 25OHD3. With a correlation coefficient of 0.27, the total is not a reliable marker of free levels. In addition, the higher percent free 25OHD3 levels are not linked to higher total 25OHD levels. This can be seen by examining Samples 8 and 10. Sample 8, having a total 25OHD level of 20 ng/mL displayed a 0.75% free 25OHD3 while Sample 10, which had a total 25OHD level of 105 ng/mL displayed a 0.15% free 25OHD3.

Future Directions:

- Examine the role of free 25OHD3 in bone and immune health
- Examine the relationship between free 25OHD3 and parathyroid hormone in the different patient populations of the National Institutes of Health

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