**Supplemental Materials and Methods**

*Procedures*

Standard anthropometric data (height, weight) were obtained in all subjects. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of height in meters. Sexual, physical, and psychological symptoms were assessed using the International Index of Erectile Function-Erectile Function (IIEF-EF) questionnaire ([1](#_ENREF_1)) (0-6=severe dysfunction, 7-12=moderate dysfunction, 13-18=mild to moderate dysfunction, 19-24=mild dysfunction, 25-30=no dysfunction) and the Aging Male’s Symptoms (AMS) scale ([2](#_ENREF_2)) (level of complaints: 17-26=no/little, 27-36=mild, 37-49=moderate, ≥50=severe).

*Mass Spectrometry (MS) measurements*

25(OH)D measurements by Isotope-Dilution Liquid Chromatography Tandem Mass Spectrometry (ID-XLC-MS/MS) were performed at the Endocrine Laboratory of the VU University Medical Center (Amsterdam, the Netherlands) as described before ([3](#_ENREF_3)). In short, internal standards 13C5–25(OH)D3 and 2H6–25(OH)D2 were added to the samples and 25(OH)D was released from its binding proteins with acetonitrile. After a liquid-liquid extraction using hexane, samples were analysed by LC-MS/MS (Acquity UPLC coupled to a Quattro Premier XE MS/MS, Waters Corp., Milford, MA, USA)). The limit of quantitation (LOQ) was 4.0 nmol/L; intraassay CV was <4.5%, and interassay CV was <5.5% for concentrations between 17 and 160 nmol/L. 25(OH)D2 and 25(OH)D3 were measured separately, however, in the current article we used the sum of both.

TT measurements by ID-XLS-MS/MS were performed at the Endocrine Laboratory of the VU University Medical Center (Amsterdam, the Netherlands) as described before ([4](#_ENREF_4)). In short, an internal standard ([13C3]-testosterone) was added to the samples and testosterone was released from its binding proteins with acetonitrile. After a liquid-liquid extraction using hexane:ether, samples were analysed by LC-MS/MS (Acquity 2D-UPLC

Coupled to a Xevo TQ-S tandem mass spectrometer (Waters Corp., Milford, MA, USA)). The LOQ was 0.10 nmol/L (2.88 ng/dL); inter-assay variation at 0.1 nmol/L was 10.6% and between 0.9 and 14 nmol/L <6%.

Both the 25(OH)D and the testosterone method were standardized well ([3](#_ENREF_3), [5](#_ENREF_5)).

*Biochemical analyses*

Routine laboratory parameters were immediately measured on a daily basis. For evaluation of inclusion criteria, 25(OH)D was measured using a commercially available enzyme immunoassay (IDS, Boldon, UK) with intra- and interassay coefficients of variation (CV) of 5.6 and 6.4%, respectively, and TT was measured by luminescence immunoassay (Siemens, Erlangen, Germany; intra- and interassay CVs of <10%). SHBG was measured by luminescence immunoassay (Cobas, Roche, Basel, Switzerland) with an intra- and interassay CV of 1.3% and 2.1%, respectively. LH and FSH were measured by enzyme immunoassay (DiaMetra S.r.l., Segrate (MI), Italy; intra- and interassay CVs of <10%) and estradiol was measured by chemiluminescent immunoassay (Immulite, Siemens Healthcare, Germany; intra- and interassay CVs of 15 and 16%, respectively). Insulin (Siemens, Germany) was measured by luminescence immunoassay (intra- and interassay CVs of 4% and 2.6 %, respectively). PTH was measured with ElectroChemiLuminescence Immunoassay (ECLIA) (Roche Diagnostics, Mannheim, Germany) with an intra- and inter-assay CV of 1.5% to 2.7% and 3.0% to 6.5%, respectively. All other parameters (total cholesterol [TC], HDL, LDL, triglycerides, serum and urine calcium) were determined by routine laboratory procedures.

*Dual-energy X-ray absorptiometry (DXA)*

Body fat and lean mass was measured at baseline and at study end. Fat mass was assessed using DXA scans (iDXA, GE Lunar, Madison, WI) and lean mass was calculated as weight (kg) - fat mass (kg).Two investigators performed all analyses. DXAhas been validated previously in children, adults, and the elderlyand has been found to be a reliable and valid method for measuringfat mass ([6](#_ENREF_6), [7](#_ENREF_7)). The CV (*i.e.* SDfrom the mean) was evaluated in our laboratory by scanning oneperson (a female, 30 years of age, 30% body fat, with normal weightand height) seven times in the same day, with repositioningbetween each scan. For this individual, the CVwas 2% for abdominal fat mass and total fat mass. The equipmentwas calibrated each day using a standardized phantom to detectdrifts in measurements, and equipment servicing was performedregularly.

**References**

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**Supplemental table legends**

**Table 2:** Seasonal variation of 25-hydroxyvitamin D, PTH, total testosterone, free testosterone, and free androgen index at baseline.

Comparison of biochemical characteristics were performed using ANOVA. Season 1: January-March, season 2: April-June, season 3: July-September, season 4: October-December

**Supplemental table 1**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Season 1** | | **Season 2** | | **Season 3** | | **Season 4** | |  |
|  | Median | IQR | Median | IQR | Median | IQR | Median | IQR | p-value |
| 25-hydroxyvitamin D (nmol/l) | 48 | 37-61 | 52.5 | 44-68 | 73.5 | 60-84 | 49 | 37-58 | <0.001 |
| PTH (pg/ml) | 46.5 | 40.0-62.8 | 44.2 | 36.0-53.9 | 51.5 | 40.1-62 | 44.5 | 25.5-50.1 | 0.056 |
| Total testosterone (nmol/l) | 18.5 | 15.8-21.8 | 18.0 | 15.7-22.0 | 16.8 | 15.5-18.8 | 18.1 | 16.7-20.5 | 0.988 |
| Free testosterone (ng/ml) | 0.095 | 0.073-0.117 | 0.090 | 0.076--0.102 | 0.101 | 0.088-0.108 | 0.108 | 0.102-0.132 | 0.324 |
| Free androgen index | 8.4 | 5.6-11.5 | 7.6 | 5.8-13.2 | 6.1 | 4.7-7.1 | 4.9 | 4.3-8.6 | 0.258 |