CLINICAL STUDY

Association of hypogonadism with vitamin D status: the European Male Ageing Study

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Abstract

Objective: Interrelationships between hormones of the hypothalamic–pituitary–testicular (HPT) axis, hypogonadism, vitamin D and seasonality remain poorly defined. We investigated whether HPT axis hormones and hypogonadism are associated with serum levels of 25-hydroxyvitamin D (25(OH)D) in men.

Design and methods: Cross-sectional survey of 3369 community-dwelling men aged 40–79 years in eight European centres. Testosterone (T), oestradiol (E₂) and dihydrotestosterone were measured by gas chromatography–mass spectrometry; LH, FSH, sex hormone binding globulin (SHBG), 25(OH)D and parathyroid hormone by immunoassay. Free T was calculated from total T, SHBG and albumin. Gonadal status was categorised as eugonadal (normal T/LH), secondary (low T, low/normal LH), primary (low T, elevated LH) and compensated (normal T, elevated LH) hypogonadism. Associations of HPT axis hormones with 25(OH)D were examined using linear regression and hypogonadism with vitamin D using multinomial logistic regression.

Results: In univariate analyses, free T levels were lower ($P=0.02$) and E₂ and LH levels were higher ($P<0.05$) in men with vitamin D deficiency (25(OH)D <50 nmol/l). 25(OH)D was positively associated with total and free T and negatively with E₂ and LH in age- and centre-adjusted linear regressions. After adjusting for health and lifestyle factors, no significant associations were observed between 25(OH)D and individual hormones of the HPT axis. However, vitamin D deficiency was significantly associated with compensated (relative risk ratio (RRR)=1.52, $P=0.03$) and secondary hypogonadism (RRR=1.16, $P=0.05$). Seasonal variation was only observed for 25(OH)D ($P<0.001$). **Conclusions:** Secondary and compensated hypogonadism were associated with vitamin D deficiency and the clinical significance of this relationship warrants further investigation.

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Introduction

The classical role played by vitamin D and parathyroid hormone (PTH) in maintaining bone health and controlling calcium metabolism is well documented (1). Whether synthesised in the skin or derived from

dietary sources, vitamin D is first hydroxylated in the liver to produce 25-hydroxyvitamin D (25(OH)D), which is in turn further hydroxylated (primarily in the kidneys) to yield the active molecule, 1,25(OH)₂D (2). Serum levels of 25(OH)D, the major circulating form of the vitamin, are typically measured to determine

an individual's vitamin D status. An increasing body of observational data has linked low serum levels of vitamin D to a variety of chronic diseases related to ageing, including diabetes (3, 4) and cardiovascular disease (5). However, the nature of these associations is poorly defined and our understanding of the pathophysiological role(s) of vitamin D other than in calcium homeostasis remains rudimentary.

Age-related declines in testosterone (T) and other anabolic hormones have been well documented in men from the age of 40 years onwards (6–8), with low T levels suggested to be a risk factor for diabetes (9) and cardiovascular disease (10). However, the degree to which these changes in hypothalamic–pituitary–testicular (HPT) axis function directly or indirectly influence age-related declines in physical (frailty), cardiovascular (atherosclerosis, erectile dysfunction) and psychological health (cognitive function) remains contentious. Recently, Wehr *et al.* (11) observed a positive, cross-sectional association between T and 25(OH)D together with a concordant pattern of seasonal variation for both hormones. The authors hypothesise that serum vitamin D levels may impact directly on gonadal functioning, with biological plausibility stemming from the presence of vitamin D receptor (VDR) in the testis (12), hypothalamus (13) and pituitary gland (14). Previous work in our group has shown that multilevel functional alterations in the HPT axis are linked to distinct risk factors, such as obesity and comorbidity that interact with age to contribute to declining T levels (8). Since serum concentrations of vitamin D have also been linked to a number of other adverse health and lifestyle factors, it is important to investigate in more detail how 25(OH)D and also seasonality are associated with hormones of the HPT axis in men.

Using baseline data from the European Male Ageing Study (EMAS), we aimed to determine whether 25(OH)D levels were associated with the key hormonal components of the HPT axis, to evaluate the influence of season on vitamin D and individual HPT axis hormone levels, and to investigate whether biochemical hypogonadism, based on combined T/LH levels, was associated with low vitamin D status.

Materials and methods

Participants and study design

Our analyses are based on the baseline data from EMAS, a prospective, non-interventional cohort study on male ageing in Europe. Details regarding recruitment, response rates and assessments have been described elsewhere (15). Briefly, non-institutionalised men aged 40–79 years were recruited from municipal or population registers in eight centres: Florence (Italy), Leuven (Belgium), Łódź (Poland), Malmö (Sweden), Manchester (UK), Santiago de Compostela (Spain),

Szeged (Hungary) and Tartu (Estonia). For the baseline survey, stratified random sampling was used with the aim of recruiting equal numbers of men into each of the four age bands (40–49, 50–59, 60–69 and 70–79 years). Subjects were invited by letter to complete a short postal questionnaire and to attend for screening at a local clinic. Overall, the mean response rate for participation in the study was 41%. All participants provided written informed consent with ethical approval obtained in accordance with local institutional requirements in each centre.

Assessments

The short postal questionnaire included items concerning demographic, health and lifestyle information. Subjects were asked about tobacco use (response set = current/past per non-smoker) and typical alcohol consumption during the preceding month (response set = every day/5–6 days per week; 3–4 days per week; 1–2 days per week; <once per week; not at all). They were also asked to report any morbidities they were currently being treated for, including heart conditions, high blood pressure and diabetes. The details of questionnaire standardisation and validation have been described previously (15).

Those who agreed to participate subsequently attended a research clinic to complete an interviewer-assisted questionnaire and undergo clinical assessments. The questionnaire included Beck's Depression Inventory-II (BDI-II) (16) and the Physical Activity Scale for the Elderly (PASE) (17). Physical function was assessed using Reuben's Physical Performance Test (PPT) (18). Height and weight were measured using standard procedures and body mass index (BMI) defined as weight (kg) divided by the square of height (m). Current use of prescription and non-prescription drugs was corroborated by examination of medications and prescriptions brought into the clinic for that purpose.

Biochemistry

Phlebotomy was performed before 1000 h to obtain a fasting blood sample from all participants. Isolated serum was stored protected from light at -80°C prior to analysis and shipped on dry-ice to single laboratories for measurement of T, oestradiol (E_2) and dihydrotestosterone (DHT; Laval University, QC, USA); LH, FSH and sex hormone binding globulin (SHBG; University of Florence); 25(OH)D (University of Leuven) and PTH (Santiago de Compostela University). A validated gas chromatography–mass spectrometry system (19) was used to analyse T (lower limit of quantification (LLQ), 0.17 nmol/l; intra-assay coefficient of variation (CV), 2.9%; inter-assay CV 3.4%), E_2 (LLQ, 7.3 pmol/l; intra-assay CV, 3.5%; inter-assay CV 3.7%) and DHT (LLQ, 0.07 nmol/l; intra-assay CV, 3.1%; inter-assay CV

4.1%). The Modular E170 platform electrochemiluminescence immunoassay (Roche Diagnostics) was used to assay SHBG (detection limit, 0.35 nmol/l; intra-assay CV, 1.7%; inter-assay CV 3.2%) and LH (detection limit, 0.1 U/l; intra-assay CV, 1.9%; inter-assay CV 3.0%) as described previously (8). Free T levels were calculated from total T, SHBG and albumin concentrations using mass action equations and association constants from Vermeulen *et al.* (20). Serum 25(OH)D levels were determined using RIA (RIA kit: DiaSorin, Stillwater, MN, USA) with intra- and inter-assay CVs of 11 and 8%, respectively, and a detection limit of 5.0 nmol/l. PTH was assayed using a chemiluminescence immunoassay (Nichols Advantage Bio-Intact PTH assay, Quest Diagnostics, Madison, NJ, USA), with intra- and inter-assay CVs of 6 and 2.8%, respectively, and a detection limit of 0.16 pmol/l.

Statistical analysis

Statistical analyses were performed using Stata SE version 10.1 (StataCorp, College Station, TX, USA) and SPSS version 16.0 (SPSS, Inc., Chicago, IL, USA). Subjects with missing T or 25(OH)D data, prevalent pituitary or testicular disease, or those using medications likely to affect HPT function (anabolic-androgenic steroids, DHEA, anti-androgens, GnRH agonists, and psycholeptic agents) or clearance of sex steroids (e.g. anti-convulsants) were excluded (8), leaving 3051 men in the main analysis. 25(OH)D, reproductive hormones and SHBG were initially examined as continuous variables. 25(OH)D levels were also classified into sufficient (≥ 75 nmol/l (30 ng/ml)), sub-optimal (50–74.9 nmol/l (20–29.9 ng/ml)) and deficient (< 50 nmol/l (< 20 ng/ml)) categories broadly based on previously recommended cut-off points (21, 22). Age, physical activity (PASE), BMI and physical function (PPT) were treated as continuous variables, while the occurrence of heart conditions, hypertension, diabetes and depression (BDI-II) were dichotomised (absent vs present) in both linear and logistic regression models. Hypertension was defined from self-reported high blood pressure or if the subjects were using anti-hypertensive medication, diabetes from self-report or if using anti-diabetic medications and depression from a BDI-II score ≥ 14 (16) or using anti-depressants. The association of 25(OH)D (dependent variable) with T, E₂, DHT, LH, FSH and SHBG (independent variables) was determined using separate linear regressions. Adjustments were made for age and centre and additionally for BMI, smoking, alcohol consumption, physical activity, physical function, heart conditions, hypertension, diabetes and depression. Results are expressed as unstandardised beta coefficients (β) and 95% confidence intervals (CIs). As the distribution of both LH and FSH was positively skewed, these variables were transformed using the natural logarithm prior to multivariable linear regression analysis. Post-analysis of the regression

models where LH and FSH were log transformed confirmed that the residuals approximated a normal distribution (data not shown). In order to interpret the results as an average percentage change in LH or FSH for a one unit change in 25(OH)D, the regression coefficients may be expressed as $100 \times (\exp(\beta \text{ coefficient}) - 1)$.

Seasonality in 25(OH)D, PTH, reproductive hormones and SHBG levels were assessed by monthly variations using linear regression through the general linear model (GLM) procedure in SPSS to derive estimated marginal means (adjusting for age and centre), with Bonferroni correction to the CIs (95%), to identify monthly values significantly different from the lowest value.

A four-category variable of gonadal status was constructed from the baseline data of EMAS using two thresholds: a T level of 10.5 nmol/l and LH level of 9.4 U/l, as described previously (23). The four categories were normal or eugonadal (T ≥ 10.5 nmol/l and LH ≤ 9.4 U/l), secondary hypogonadism (T < 10.5 nmol/l and LH ≤ 9.4 U/l), primary hypogonadism (T < 10.5 nmol/l and LH > 9.4 U/l) and compensated hypogonadism (T ≥ 10.5 nmol/l and LH > 9.4 U/l). The chosen T cut-off point of 10.5 nmol/l was similar to that used in previous studies (24, 25). The LH threshold corresponded to the 97.5th centile (the upper limit of normal) value in the youngest group (40–44 years) in our analysis cohort (after exclusions; see Tajar *et al.* (23)). Retaining gonadal status as a four-level categorical outcome variable, multinomial logistic regression was used to examine associations between 25(OH)D status and gonadal group membership. Relative risk ratios (RRR) and 95% CI were estimated for the three hypogonadism groups with respect to the eugonadal group. Multinomial logistic regression models were adjusted for the same factors as in the multiple linear regressions.

Results

Cohort characteristics

The clinical characteristics of the 3051 men included in the analysis sample according to 25(OH)D status are shown in Table 1. Total T, DHT, FSH and SHBG levels did not differ significantly between 25(OH)D categories, while free T was lower ($P < 0.001$) and E₂ ($P = 0.04$) and LH ($P = 0.02$) were higher in the deficient and sub-optimal categories. As reported previously (26), PTH levels showed an inverse association with 25(OH)D ($P < 0.001$). Men with deficient or sub-optimal 25(OH)D levels had a higher BMI, were less physically active, had poorer physical function and depression scores, reported a higher frequency of cardiovascular disease and diabetes, were more likely smokers, drank alcohol less frequently and were more often hypogonadal compared with men who were 25(OH)D sufficient.

Table 1 Baseline characteristics by vitamin D status.

	25(OH)D categories			<i>P</i> value ^a
	Deficient (< 50 nmol/l)	Sub-optimal (50–74.9 nmol/l)	Sufficient (≥ 75 nmol/l)	
Characteristics (mean (s.d.))				
<i>n</i>	1251	928	872	
Age (years)	59.4 (11.0)	60.5 (11.0)	59.2 (10.8)	0.02
25(OH)D (nmol/l)	34.7 (10.0)	62.0 (7.2)	102 (23.1)	< 0.001
TT (nmol/l)	16.4 (6.1)	16.4 (6.1)	16.8 (5.6)	0.18
FT (pmol/l)	288 (89)	287 (87)	302 (86)	< 0.001
E ₂ (pmol/l)	74.8 (25.6)	73.7 (25.4)	72.0 (22.8)	0.04
DHT (nmol/l)	1.33 (0.65)	1.32 (0.61)	1.35 (0.56)	0.43
LH (IU/l)	6.48 (4.88)	6.15 (4.43)	5.75 (3.35)	0.01
FSH (IU/l)	8.92 (10.1)	8.71 (8.61)	7.80 (7.34)	0.31
SHBG (nmol/l)	43.1 (21.1)	43.2 (19.3)	42.0 (18.2)	0.33
PTH (pmol/l)	3.17 (1.68)	2.88 (1.37)	2.63 (1.06)	< 0.001
BMI (kg/m ²)	27.9 (4.5)	27.9 (3.9)	27.0 (3.5)	< 0.001
Physical activity	188 (92)	200 (90)	210 (91)	< 0.001
Physical performance	23.7 (3.0)	24.0 (2.7)	24.3 (2.3)	< 0.001
Depression score (BDI-II)	8.0 (7.0)	6.7 (6.2)	5.3 (5.3)	< 0.001
Hypogonadal group ^b (<i>n</i> (%))				
Eugonadal	912 (73)	709 (77)	713 (82)	< 0.001
Compensated	142 (11)	86 (9)	59 (7)	
Primary	28 (2)	21 (2)	11 (1)	
Secondary	167 (13)	110 (12)	88 (10)	
Obese (BMI ≥ 30; <i>n</i> (%))	334 (27)	237 (26)	155 (18)	< 0.001
Heart condition (<i>n</i> (%))	221 (18)	144 (16)	115 (13)	0.02
Hypertension ^c (<i>n</i> (%))	421 (34)	333 (36)	249 (29)	0.003
Diabetes ^d (<i>n</i> (%))	111 (9)	66 (7)	51 (6)	0.03
Depression ^e (<i>n</i> (%))	269 (22)	147 (16)	101 (12)	< 0.001
Current smoker (<i>n</i> (%))	357 (29)	168 (18)	123 (14)	< 0.001
Alcohol (≥ 1 day/week)	627 (51)	502 (54)	588 (68)	< 0.001

TT, total T; FT, free T; DHT, dihydrotestosterone; PTH, parathyroid hormone.

^aOne-way ANOVA or Kruskal–Wallis test for continuous variables, χ^2 test for categorical variables: between 25(OH)D categories.

^bDefined using total T (10.5 nmol/l) and LH (9.4 U/l) cut points.

^cSelf-reported high BP and/or using anti-hypertensives.

^dSelf-reported diabetes and/or using anti-diabetic drugs.

^eBDI-II ≥ 14 and/or using anti-depressants.

Association of 25(OH)D with reproductive hormones and SHBG

The results from the linear regression models exploring the association of 25(OH)D with T, E₂, DHT, LH, FSH and SHBG are summarised in Table 2. Models are presented unadjusted; adjusted for age and centre; and adjusted for age, centre, BMI, smoking, alcohol consumption, physical activity, physical function, heart conditions, hypertension, diabetes and depression. Higher levels of 25(OH)D were associated with higher total T ($\beta=0.007$, $P=0.049$) and free T ($\beta=0.207$, $P<0.001$) and lower E₂ ($\beta=-0.045$, $P=0.002$) and log LH ($\beta=-0.001$, $P<0.001$) in unadjusted models. Following adjustment for age and centre, these associations were largely unchanged, aside from free T where the relationship was markedly attenuated ($\beta=0.113$, $P=0.02$). However, after full adjustment for age, centre and the other confounders, no independent associations were observed between 25(OH)D and any of the HPT axis hormones or SHBG (all $P>0.05$).

Monthly variability in vitamin D, hormone and SHBG levels

Figure 1 shows the adjusted mean levels and 95% CI derived from linear regressions (SPSS Univariate GLM procedure) for 25(OH)D, PTH, T, E₂, LH and SHBG for each month, adjusted for age and centre. An unambiguous seasonal variation was only seen for 25(OH)D (nadir in April (47.4 nmol/l), zenith in August (83.1 nmol/l)). The monthly variation in 25(OH)D was significant ($P_{\text{GLM}} < 0.001$) and persisted following additional adjustment for BMI, physical activity, depression and smoking (data not shown). No significant monthly variation in PTH, reproductive hormones (graphs not shown for free T, DHT and FSH) and SHBG levels was found in the age- and centre-adjusted models (all $P>0.05$).

Association between vitamin D status and gonadal group

The four groups of gonadal status, as defined by total T (10.5 nmol/l) and LH (9.4 U/l) thresholds (23), are

Table 2 Association of 25(OH)D with hormones and SHBG: linear regressions.

Dependent variable	25(OH)D (β coefficient (95% confidence interval))		
	Model 1	Model 2	Model 3
TT (nmol/l)	0.007 (0.001, 0.014)*	0.007 (0.001, 0.014)*	0.004 (−0.003, 0.010)
FT (pmol/l)	0.207 (0.108, 0.307) [†]	0.113 (0.019, 0.207)*	0.066 (−0.032, 0.163)
E ₂ (pmol/l)	−0.045 (−0.073, −0.016) [†]	−0.030 (−0.059, −0.001)*	−0.015 (−0.046, 0.016)
DHT (nmol/l)	0.000 (−0.001, 0.001)	0.001 (−0.001, 0.001)	0.000 (−0.001, 0.001)
Log LH (IU/l) ^a	−0.001 (−0.002, −0.000) [†]	−0.001 (−0.001, −0.000) [†]	−0.001 (−0.001, 0.001)
Log FSH (IU/l) ^a	−0.001 (−0.001, 0.000)	−0.000 (−0.001, 0.001)	0.000 (−0.001, 0.001)
SHBG (nmol/l)	−0.017 (−0.039, 0.006)	−0.001 (−0.022, 0.022)	−0.004 (−0.027, 0.018)

Model 1, unadjusted; model 2, adjusted for age and centre; model 3, adjusted for age, centre, body mass index, smoking, alcohol consumption, physical activity, physical function, heart conditions, hypertension, diabetes and depression; TT, total T; FT, free T; DHT, dihydrotestosterone. * $P < 0.05$, [†] $P < 0.01$, [‡] $P < 0.001$. Additional adjustment for season of attendance did not markedly change the association between 25(OH)D and LH ($\beta = -0.006$, $P < 0.05$).

^aTo derive average percentage change in LH or FSH from log hormone (dependent variable) = $100 \times (\exp(\beta \text{ coefficient}) - 1)$.

shown in Fig. 2. Over three quarters of men were eugonadal (77%), whereas 9, 2 and 12% had compensated, primary and secondary hypogonadism respectively. The proportion of subjects in sufficient, sub-optimal and deficient vitamin D categories differed significantly ($\chi^2 = 24.4$, $P < 0.001$) by gonadal group (Fig. 1, inset), with almost half of hypogonadal men having deficient levels of 25(OH)D (< 50 nmol/l) compared with 39% of eugonadal men.

Table 3 shows the results of the multinomial logistic regressions examining the association between vitamin D status and gonadal group membership. Age- and centre-adjusted models demonstrated a consistent association of deficient vitamin D status with an increased risk of being in one of the hypogonadal groups. The RRR of being in the compensated group was almost twice (1.92, $P < 0.001$) than that of being in the eugonadal group (base category) for men with deficient vitamin D vs those with sufficient vitamin D. The corresponding RRRs for being in the primary or secondary hypogonadism groups were 2.02 ($P < 0.001$) and 1.49 ($P = 0.007$) respectively. After full adjustment for BMI, smoking, alcohol consumption, physical activity, physical function, heart conditions, hypertension, diabetes and depression, these relationships were attenuated but remained significant for compensated (RRR of 1.52, $P = 0.034$) and secondary (RRR of 1.16, $P = 0.05$) hypogonadism.

Discussion

In this population-based study of middle-aged and older European men, we examined whether serum levels of the main hormones of the HPT axis were associated with the circulating form of vitamin D (25(OH)D). Lower levels of 25(OH)D were significantly associated with lower total and free T and higher E₂ and LH concentrations after adjustment for age and centre. However, following additional adjustment for health and lifestyle factors, these associations became

non-significant. While a significant seasonal variation was seen in 25(OH)D levels, no corresponding pattern was observed for the reproductive hormones, SHBG or PTH. The adjusted relative risk of secondary hypogonadism increased by 16% and compensated hypogonadism by 52% between the deficient and sufficient categories of serum 25(OH)D.

We are aware of only one other large observational study having specifically explored the association between 25(OH)D and T in men. Using data from the Ludwigshafen Risk and Cardiovascular Health (LURIC) study, Wehr *et al.* (11) found that serum 25(OH)D levels were independently associated with T, SHBG and calculated free androgen index in a sample of 2299 men with a mean $\pm$ s.d. age of 62 ± 11 years. In addition, the authors reported analogous patterns of seasonal variation in both 25(OH)D and T levels with peaks in late summer and nadirs in spring (11). These findings are in contrast to the lack of association we found between vitamin D and T and between hormones of the HPT axis and season. The men enrolled in the LURIC study consisted of a convenience, and hence biased, sample of patients with elevated cardiovascular risk specifically referred for coronary angiography. Indeed, around 70% of the LURIC men were hypertensive, 80% had pre-existing coronary artery disease and 30% were diabetic, compared with 33, 16 and 7% in the EMAS cohort respectively. It is, therefore, questionable whether the LURIC findings can be compared to our essentially healthy, community-dwelling sample and generalised to the male population. However, restricting our analyses to men with self-reported cardiovascular disease ($n = 1082$) did not change the observed lack of association of T with either 25(OH)D or season (all $P > 0.05$; data not shown). The LURIC study was also restricted to one geographical location and it is possible that seasonal variation in T levels may have differed between EMAS centres, although we attempted to control for this by adjusting for centre in the multi-variable models. Furthermore, when we stratified the GLM regression analyses by centre and adjusted the

Figure 1 Seasonal variation in mean vitamin D, hormone and SHBG levels: by month. Significant difference from the lowest value (†): general linear model adjusted for age and centre * $P < 0.05$; § $P < 0.01$. (A) 25-Hydroxyvitamin D, (B) parathyroid hormone, (C) T, (D) oestradiol, (E) LH, (F) SHBG. Adjusted means and 95% confidence intervals.

models for age, we found no evidence of significant monthly variation in T levels in any of the eight centres (data not shown).

Other studies examining the seasonal variation in T levels have yielded inconsistent results. Tancredi *et al.* (27) reported no major seasonal variation in calculated free T levels among a large sample ($n = 5028$) of community-dwelling men aged 50–70 years attending a clinic-based andropause assessment. Using data from the Tromsø study, Svartberg *et al.* (28) reported a bimodal seasonal variation in total T levels with a prominent peak in October and November and a nadir in June. They also observed a significant monthly variation in free T, with the peak in December and nadir in August. However, Tromsø's latitude is $69^{\circ} 40' N$ (the most northerly centre in EMAS is Tartu at $58^{\circ} 18' N$) and its wide annual disparity in both daylight and temperature may not relate to the geographical areas in our study with less extreme seasonal variation. Pilz *et al.* (29) recently reported that daily supplementation with $83 \mu\text{g}$ (3332 IU) vitamin D given to overweight men over a period of 12 months significantly elevated both serum 25(OH)D and T levels compared with placebo. However, the sample size was relatively small ($n = 54$) and given the patients were participating in a weight

reduction programme, the study's relevance to community-dwelling individuals is uncertain.

Data remain lacking with regard to the biological plausibility of serum vitamin D levels directly impacting the functioning of the HPT axis. While a recent genome-wide association study (GWAS) identified a number of genetic variants affecting vitamin D concentrations near genes involved in cholesterol synthesis, hydroxylation and vitamin D transport (30), parallel data on sex steroid levels were not included. The expression of VDR and vitamin D metabolising enzymes in human testis, ejaculatory tract and mature spermatozoa (12) suggests a potential role for vitamin D in spermatogenesis and spermatozoa maturation. Specifically, the observed cytoplasmic co-expression of the VDR and vitamin D metabolising enzymes (CYP2R1, CYP27A1 and CYP27B1) in Leydig cells suggests that vitamin D may be linked to male reproductive hormone functioning, although uncertainty remains as to the consistency of some of the immunohistochemistry data (12, 31). The observation that VDR knockout mice develop hypergonadotrophic hypogonadism (32) also advocates a potential link between the vitamin D and HPT axes. Bilateral orchiectomy has been shown to be associated with a significant reduction in 25(OH)D levels despite adequate T replacement therapy (33), leading to the suggestion that the microsomal form of 25-hydroxylase (CYP2R1) expressed in Leydig cells may play a physiologically important role in vitamin D activation. The VDR and 1α -hydroxylase (CYP27B1) have also been shown to be expressed in virtually all cell types within the anterior pituitary (14) and hypothalamus (13). Whether vitamin D plays any role in regulating

Figure 2 Relationship between biochemical gonadal group and vitamin D status. The vertical line corresponds to total T = 10.5 nmol/l , the horizontal line corresponds to LH = 9.4 U/l (23). The proportion of men who had a sufficient vitamin D status was highest among the eugonadal group (39%) and lowest among those with primary hypogonadism (18%).

Table 3 Association between hypogonadal group membership and vitamin D status: multinomial logistic regressions. The relative risk ratio (RRR) indicates the likelihood of being classified in one of the outcome categories of compensated, primary or secondary hypogonadism with reference to eugonadal (base category) in relation to the independent variable (i.e. vitamin D status). For example, the RRR of 2.02 indicates that men with a 25-hydroxyvitamin D (25(OH)D) level <50 nmol/l (deficient) are two times more likely to be in the 'primary hypogonadism' category vs the 'eugonadal category' than those with a 25(OH)D level of 75 nmol/l or higher (sufficient), while holding other variables in the model constant. Additional adjustment for season did not substantively change the magnitude or significance of the associations.

25(OH)D status	Relative risk ratios (95% confidence interval)		
	Compensated hypogonadism	Primary hypogonadism	Secondary hypogonadism
Model 1			
Sufficient	1.00 (reference)	1.00 (reference)	1.00 (reference)
Sub-optimal	1.35 (1.05, 1.75)*	1.73 (0.73, 4.11)	1.25 (0.96, 1.62)
Deficient	1.92 (1.33, 2.78) [‡]	2.02 (1.42, 2.87) [‡]	1.49 (1.12, 1.99) [‡]
Model 2			
Sufficient	1.00 (reference)	1.00 (reference)	1.00 (reference)
Sub-optimal	1.33 (1.01, 1.74)*	1.33 (0.59, 2.97)	0.96 (0.71, 1.30)
Deficient	1.52 (1.03, 2.25)*	1.43 (0.98, 2.10)	1.16 (1.00, 1.34)*

Model 1, adjusted for age and centre; model 2, adjusted for age, centre, body mass index, smoking, alcohol consumption, physical activity, physical function, heart conditions, hypertension, diabetes and depression. * $P < 0.05$, [†] $P < 0.01$, [‡] $P < 0.001$ (reference category = sufficient).

gene expression and hormone secretion in these component structures of the HPT axis remains unknown.

Although we failed to observe any independent associations between 25(OH)D and the individual hormones of the HPT axis, a significant relationship was found between deficient levels of 25(OH)D (< 50 nmol/l) and biochemical hypogonadism based on combined cut-off levels of T and LH (Table 3). In the fully adjusted multinomial logistic regression models, hypovitaminosis D was associated with both secondary and compensated hypogonadism, while the association with primary hypogonadism just failed to reach statistical significance probably due to the small number of men in this category. Given the association of 25(OH)D with hypogonadism (combined T and LH), it is plausible that the link with low serum vitamin D levels affects multiple levels of the HPT axis. We have previously shown that primary hypogonadism (probably the genuine form of late-onset hypogonadism) is most strongly associated with age, secondary hypogonadism with obesity and compensated hypogonadism with age, smoking and physical function limitations (8). Our observation that secondary hypogonadism is linked predominately to obesity raises the possibility that increased aromatisation of T to E₂ in adipose tissue, increased insulin resistance and proinflammatory cytokine production (tumour necrosis factor α and interleukin 6) from adipocytes could impact negatively upon the vitamin D endocrine axis. Alternatively, the hypogonadal subjects, particularly those in the compensated group, may simply have received less sun exposure through lower levels of outdoor physical activity. Although we adjusted for physical activity levels in the multivariable models using the PASE score, this questionnaire instrument does not uniquely capture outdoor activity nor can we exclude the possibility of reverse causality. Overall, our data suggest that hypovitaminosis D is associated with

multi-level dysfunction in the HPT axis. While individual changes in the individual HPT hormones were too small to reach significance, when combined, as in the diagnosis of biochemical hypogonadism, the association was significant.

Our study has a number of strengths: it was based on a large population-based sample and used uniform methods to assess reproductive hormone, vitamin D and SHBG levels, and also potential confounders such as physical activity, physical function, depression and obesity. Although limitations of the EMAS study have been published previously (15), specific factors need to be considered when interpreting the results presented here. We enrolled non-institutionalised, primarily Caucasian men with a response rate of 41% and our data may not, therefore, be generalisable to other groups. It is also possible that hormone and vitamin D levels may not reflect those in the population from which the study sample was drawn. However, this should not affect the results of the analysis, which is based on an internal comparison of responders. Reproductive hormones SHBG and 25(OH)D were assayed from single measurements, we did not attempt to determine dietary intakes of vitamin D, and the intra- and inter-assay CVs for the 25(OH)D RIA were relatively large (11 and 8% respectively). In addition, the assessment of comorbidities (heart conditions, hypertension and diabetes) was largely by self-report and taken together all of these factors could have increased random measurement error, thereby reducing the strength of the associations reported here. It is possible that the observed associations between vitamin D and hypogonadism may reflect either unmeasured variables or residual confounding. Finally, the cross-sectional study design precludes any examination of the temporal nature of the observed relationships and may also have restricted our ability to detect seasonal variation in sex steroid levels. Longitudinal studies with systematic

sampling of subjects throughout the year are warranted to better elucidate this latter point.

Our findings demonstrate that among generally healthy, community-dwelling men, low vitamin D levels, as assessed by serum 25(OH)D (< 50 nmol/l), are significantly associated with biochemical hypogonadism based on combined T and LH measurements. These data suggest that both low vitamin D and hypogonadism are markers of poor health or increasing homeostatic disruption, perhaps sharing common underlying aetiologies. Seasonal variation was only observed for 25(OH)D levels, but not for T and the other HPT hormones. Further studies are needed to clarify the relationship between vitamin D status and function of the HPT axis before we can justify investigations to examine the effects of vitamin D supplementation in hypogonadal men.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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