

## CLINICAL STUDY

# Vitamin D increases circulating IGF1 in adults: potential implication for the treatment of GH deficiency

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## Abstract

**Objectives:** Previous studies suggested that vitamin D modulates circulating IGF1. We investigated this effect in adults and its clinical relevance in the management of GH deficiency (GHD).

**Design and methods:** IGF1 levels were prospectively measured before and after 12 weeks of treatment with oral vitamin D<sub>3</sub> (5000 or 7000 IU/week) vs no intervention in 39 subjects 61.9 ± 7.9 years old. The frequency of IGF1 values ≥ 50th age- and sex-specific percentile in relation to vitamin D status, as determined by the concentration of 25-hydroxyvitamin D (25(OH)D), was retrospectively assessed in 69 GHD patients (57.4 ± 16.6 years) on stable hormone replacement and with 25(OH)D and IGF1 concurrently measured.

**Results:** Treatment with 5000 and 7000 IU vitamin D<sub>3</sub>/week significantly raised 25(OH)D by 12.7 ± 8.4 and 13.1 ± 6.5 ng/ml respectively (both  $P < 0.001$  vs baseline). In the 7000 IU group, IGF1 levels also significantly increased by 31.3 ± 36.7 ng/ml ( $P = 0.01$ ). Neither 25(OH)D nor IGF1 significantly varied in controls. IGF1 was ≥ 50th percentile more frequently in GHD patients with 25(OH)D levels ≥ 15 than < 15 ng/ml (65.9 vs 40.0%,  $P < 0.05$ ). Logistic regression with adjustment for recombinant human GH (rhGH) dose, vitamin D supplements, gender, use of thyroid hormones, corticosteroids or estrogen/testosterone, and season revealed a significant positive association between ≥ 15 ng/ml 25(OH)D and IGF1 ≥ 50th percentile (OR 4.4, 95% CI 1.0–18.8,  $P < 0.05$ ). A significant negative correlation between 25(OH)D concentrations and rhGH dose was found after correcting for age and IGF1 ( $\beta -0.042$ ,  $P < 0.01$ ), but not after further adjusting for sex, thyroid, adrenal or gonadal replacement, and season ( $\beta -0.037$ ,  $P = 0.06$ ).

**Conclusions:** Vitamin D increases circulating IGF1 in adults. As a result, a better vitamin D status may ease the achievement of normal IGF1 values in GHD.

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## Introduction

It has long been known that insulin-like growth factor 1 (IGF1) causes an increase in the circulating levels of 1,25-dihydroxyvitamin D (1,25(OH)<sub>2</sub>D), the hormonally active vitamin D metabolite, by stimulating the expression and activity of the 1 $\alpha$ -hydroxylase that produces 1,25(OH)<sub>2</sub>D in the kidney (1, 2). Indeed, plasma 1,25(OH)<sub>2</sub>D is significantly higher in active than controlled acromegaly, with a tendency to hypercalcemia, hyperphosphatemia, and hypercalciuria (3). On the other hand, more recent data has suggested that vitamin D may contribute to determining IGF1 concentrations. Mice knockout for the vitamin D receptor (VDR) exhibit 30% lower IGF1

levels compared with WT animals (4) and a significant increase in serum IGF1 was noted in response to vitamin D in two small cohorts of children (5, 6). Furthermore, cross-sectional analyses of community-based cohorts disclosed a positive correlation between the concentrations of 25-hydroxyvitamin D (25(OH)D), the marker of vitamin D status, and IGF1 (7, 8, 9, 10).

Here we first conducted an open-label, controlled study of the changes in circulating IGF1 following vitamin D supplementation in adults. Then, we asked whether the influence of vitamin D on IGF1 might be clinically relevant for the management of growth hormone deficiency (GHD), which relies on measurement of IGF1 levels.

## Subjects and methods

### Intervention study

The effect of vitamin D on IGF1 concentrations was investigated in 39 asymptomatic outpatients, who were consecutively selected among those referred to an Internal Medicine Clinic for cardiovascular risk assessment because of one or more established risk factors among familial history, smoking, arterial hypertension, and hypercholesterolemia. The following inclusion criteria were applied: absence of acute or uncontrolled chronic diseases; normal dietary habits; no evidence of intestinal malabsorption or hepatic disease; glomerular filtration rate  $> 60$  ml/min per  $1.73$  m<sup>2</sup>, as estimated by the MDRD equation; no use of medications known to affect vitamin D metabolism, nor of vitamin D supplements for the previous 6 months; and no evidence of pituitary disease. All subjects were Caucasian but one, who was Indian, and lived an active life, with variable time spent outdoors. The study was located in Genova, Italy (latitude 44° N). It was approved by the Local Ethic Committee and performed in accordance to the guidelines in the Declaration of Helsinki. Participants gave their written informed consent.

During the same visit, patients were screened, recruited if eligible, and randomized to treatment with oral vitamin D<sub>3</sub> (Abiogen Pharma, Pisa, Italy) or no intervention. Since we had preliminarily observed a comparable 25(OH)D increment in response to 5000 and 7000 IU vitamin D<sub>3</sub> once a week (11), we tested these two doses to determine whether they would also affect IGF1 levels in a similar way. All subjects were instructed not to take any additional vitamin D and left on a free diet. Nineteen participants were studied between June and November and another 20 between December and May, when 25(OH)D concentrations are highest and lowest, respectively, in northern Italian outpatients (12). During each time period, one out of every three participants was assigned to 5000 IU vitamin D<sub>3</sub>, another one to no treatment, and the last one to 7000 IU vitamin D<sub>3</sub>.

Total 25(OH)D, 1,25(OH)<sub>2</sub>D, and IGF1 were measured on fasted serum samples collected at baseline and 12 weeks later. Serum 25(OH)D was assayed by chemiluminescent immunoassay on the DiaSorin Liaison System (DiaSorin, Saluggia, Italy) (13); within-assay and between-assay variability (coefficients of variation (CV)) were 2.9–5.5 and 6.9–12.7% respectively. Concentrations of IGF1 were determined by RIA (DIASource ImmunoAssays, Nivelles, Belgium) after acid ethanol extraction, as previously described (14); within-assay and between-assay CV were 1.7–9.1 and 4.1–9.0% respectively. Serum 1,25(OH)<sub>2</sub>D was measured by RIA (Immunodiagnostic System, Tyne and Wear, UK), within-assay and total CV being 8.6–16.6 and 11.9–20% respectively. Pre- and post-samples from the same individual were run in the same assay.

Creatinine, calcium, and phosphate levels were also assessed by automated assay in the central laboratory of the hospital where the study was performed.

### Retrospective analysis

Data were obtained by reviewing the medical records of adult GHD patients followed by a tertiary center in Genova, who i) were on stable treatment with recombinant human GH (rhGH) and other hormones if indicated and ii) had undergone measurement of 25(OH)D and IGF1 concentrations on the same occasion. The following information was retrieved: weekly dose of rhGH (mg) and, if any, of vitamin D (IU) at the time of biochemical assessment; season of the year when 25(OH)D and IGF1 had been assessed (June–November or December–May); and use or not of thyroid hormones, corticosteroids, or estrogen/testosterone. To account for the possibility that some young women were sufficiently estrogenized despite not being on gonadal replacement therapy, gonadal hormone status of female patients was also categorized by dividing them into those older than 50 years (considered as menopausal or post-menopausal) and not given estrogen vs those younger than 50 years or on estrogen regardless of their age.

### Statistical analysis

Categorical data are presented as relative and absolute frequencies and continuous variables as mean  $\pm$  s.d. or median (interquartile range), depending on the distribution. Comparisons were drawn by  $\chi^2$ -, *t*-test, Mann–Whitney *U* test, and one-way ANOVA, as appropriate. To correct for the differences in female:male ratio and BMI among the three groups of the intervention study, the 12-week changes in 25(OH)D and IGF1 were compared by analysis of covariance with adjustment for gender and BMI.

For each GHD case, we assessed whether serum IGF1 was at least equal to the 50th percentile for age and gender, based on reference values for the Italian population (14). The percentage of patients with IGF1  $\geq$  50th percentile was then examined across different vitamin D categories, defined as follows: i)  $< 15$  vs  $\geq 15$  ng/ml 25(OH)D, since the positive association between 25(OH)D and IGF1 was reported to be present only when 25(OH)D concentrations were above 15 ng/ml (7); ii)  $< 10$  ng/ml 25(OH)D, which identifies severe vitamin D deficiency,  $\geq 20$  ng/ml 25(OH)D, which meets the requirements of most individuals according to a recent position statement of the Institute of Medicine (15), and the intermediate class  $\geq 10$  ng/ml but  $< 20$  ng/ml 25(OH)D; and (iii)  $< 30$  vs  $\geq 30$  ng/ml 25(OH)D, which identify vitamin D deficiency/insufficiency and sufficiency, respectively, according to the Endocrine Society (16). These cutoffs were also entered as independent variables into logistic and linear

regression models in which IGF1 levels and rhGH dose were the outcomes. Statistical significance was set at  $P < 0.05$ . Analyses were performed with Statview version 5.0.1 for Windows (SAS Institute, Inc., Cary, NC, USA).

## Results

### Increase in circulating IGF1 following vitamin D<sub>3</sub> treatment

Baseline characteristics were similar between the intervention study groups, except for BMI, which was significantly higher in subjects allocated to 7000 IU vitamin D<sub>3</sub>/week than in those given no supplementation (Table 1). Mean 25(OH)D was nonsignificantly higher in untreated than treated patients and the prevalence of vitamin D deficiency, as defined by  $< 20$  ng/ml 25(OH)D, was 69.2% in controls, 78.6% in the 5000 IU group, and 83.3% in the 7000 IU one (NS). Serum IGF1 was nonsignificantly lower in subjects receiving 7000 IU vitamin D<sub>3</sub>/week than in the others (Table 1). Ongoing chronic therapies included anti-hypertensive drugs and statins, with no significant differences among the three groups (data not shown).

Vitamin D<sub>3</sub> supplementation significantly raised 25(OH)D levels over 12 weeks, with the values achieved using 5000 or 7000 IU/week being very similar (Table 1). Supplementation with 7000 IU/week but not 5000 IU/week, also significantly increased circulating IGF1. No significant variation in the concentrations of 1,25(OH)<sub>2</sub>D was observed in treated patients, nor of 25(OH)D, 1,25(OH)<sub>2</sub>D, and IGF1 in controls (Table 1). The 12-week changes in 25(OH)D and IGF1 levels remained significantly different among the study groups after correcting for gender and BMI (Table 1).

### Positive association of vitamin D status with circulating IGF1 in GHD

Sixty-nine GHD patients ( $57.4 \pm 16.6$  years old, 33 women) were on stable rhGH replacement therapy (mean dose  $2.2 \pm 1.4$  mg/week) and had undergone measurement of 25(OH)D and IGF1 at the same time. Most had postsurgical hypopituitarism and none had clinical or biochemical signs of malnutrition. The percentage of subjects with serum IGF1  $\geq 50$ th age- and sex-specific percentile nonsignificantly increased across the 25(OH)D categories  $< 10$  ng/ml, between 10 and 20 ng/ml, and  $\geq 20$  ng/ml (50, 57.1, and 60%

**Table 1** Patients' characteristics at baseline and after 12 weeks of treatment with vitamin D<sub>3</sub> vs no intervention. Data are presented as mean  $\pm$  s.d. unless otherwise indicated.

	No treatment	5000 IU D <sub>3</sub> /week	7000 IU D <sub>3</sub> /week	P for trend
<i>n</i>	13	14	12	
June–November	6	7	6	
December–May	7	7	6	0.61
Women ( <i>n</i> , %)	9 (69.2)	11 (78.6)	7 (58.3)	0.76
Age (years)	61.9 $\pm$ 7.5	62.7 $\pm$ 6.4	61.1 $\pm$ 10.2	0.99
BMI (kg/m <sup>2</sup> )	25.9 $\pm$ 5.4	29.6 $\pm$ 4.1	32.1 $\pm$ 6.1 <sup>a</sup>	0.02
eGFR (ml/min per 1.73 m <sup>2</sup> )	77 $\pm$ 10.7	82.5 $\pm$ 18.4	83.4 $\pm$ 11.9	0.48
Serum creatinine (mg/dl)				
Baseline	0.8 $\pm$ 0.1	0.8 $\pm$ 0.2	0.8 $\pm$ 0.1	0.70
12 weeks	0.8 $\pm$ 0.1	0.8 $\pm$ 0.1	0.8 $\pm$ 0.2	0.75
Serum calcium (mg/dl)				
Baseline	9.4 $\pm$ 0.7	9.5 $\pm$ 0.4	9.4 $\pm$ 0.7	0.90
12 weeks	9.2 $\pm$ 0.7	9.6 $\pm$ 0.4	9.6 $\pm$ 0.6	0.16
Serum phosphate (mg/dl)				
Baseline	3.2 $\pm$ 0.7	3.2 $\pm$ 0.5	3.0 $\pm$ 0.4	0.50
12 weeks	3.2 $\pm$ 0.6	3.4 $\pm$ 0.6	2.8 $\pm$ 0.6	0.11
Serum 25(OH)D (ng/ml)				
Baseline	16 $\pm$ 8.8	14.3 $\pm$ 6.5	12.2 $\pm$ 6.6	0.44
12 weeks	19.5 $\pm$ 10	27 $\pm$ 10.4 <sup>†</sup>	25.3 $\pm$ 7.9 <sup>†</sup>	0.12
12-week change	3.6 $\pm$ 7.3	12.7 $\pm$ 8.4	13.1 $\pm$ 6.5 <sup>b</sup>	0.008
Serum 1,25(OH) <sub>2</sub> D (ng/ml)				
Baseline	92 $\pm$ 34.5	77.8 $\pm$ 25.5	92.1 $\pm$ 38.8	0.47
12 weeks	85.3 $\pm$ 30	92.4 $\pm$ 34.4	104.6 $\pm$ 36.7	0.55
12-week change	-5.3 $\pm$ 30.5	13.5 $\pm$ 16.6	12.6 $\pm$ 30.8	0.48
Serum IGF1 (ng/ml)				
Baseline	183.9 $\pm$ 59	184.5 $\pm$ 79.1	157.5 $\pm$ 39.8	0.47
12 weeks	173.3 $\pm$ 60.7	188.7 $\pm$ 83.1	188.8 $\pm$ 60.3 <sup>*</sup>	0.81
12-week change	-10.6 $\pm$ 17.8	4.15 $\pm$ 26.1	31.3 $\pm$ 36.7 <sup>c</sup>	0.007

eGFR, estimated glomerular filtration rate. \* $P = 0.01$  vs baseline and <sup>†</sup> $P < 0.001$  vs baseline.

<sup>a</sup>Fisher's *post-hoc* test,  $P < 0.05$  for 7000 IU vitamin D<sub>3</sub>/week vs no treatment.

<sup>b</sup>Fisher's *post-hoc* test,  $P < 0.01$  for both 5000 and 7000 IU/week vs no treatment.

<sup>c</sup>Fisher's *post-hoc* test,  $P < 0.01$  for 7000 IU/week vs both no treatment and 5000 IU/week.

**Table 2** Characteristics of patients with GHD as divided by the 15 ng/ml 25(OH)D cutoff. Age and BMI are given as mean  $\pm$  s.d., rhGH dose as median with interquartile range; categorical variables are presented as absolute and relative frequencies.

	25(OH)D		P
	< 15 ng/ml	$\geq$ 15 ng/ml	
<i>n</i>	25	44	
Age (years)	54 $\pm$ 17	59.3 $\pm$ 16.2	0.21
Women ( <i>n</i> , %)	15 (60)	18 (40.9)	
Menopausal/post-menopausal and not on ERT	9 (60)	15 (83.3)	0.27
BMI (kg/m <sup>2</sup> )	29.6 $\pm$ 5.6	29.5 $\pm$ 6.2	0.96
Patients with serum IGF1 $\geq$ 50th percentile ( <i>n</i> , %)	10 (40)	29 (65.9)	0.04
rhGH dose (mg/week)	2.4 (1.3–2.7)	1.8 (1.2–2.4)	0.20

ERT, estrogen replacement therapy.

respectively). Only six cases (8.7%) had  $\geq$  30 ng/ml 25(OH)D, and IGF1 values were  $\geq$  50th percentile in three of them. In contrast, IGF1 levels  $\geq$  50th percentile were significantly more frequent in patients with  $\geq$  15 ng/ml 25(OH)D than in those with < 15 ng/ml 25(OH)D (Table 2). In logistic regression accounting for rhGH dose and use of vitamin D supplements, 25(OH)D concentrations  $\geq$  15 ng/ml were significantly associated with IGF1 levels  $\geq$  50th percentile (OR 3.5, 95% CI 1.1–10.6,  $P < 0.05$ ). This result was not substantially changed by further adjustment for gender, use of thyroid hormones, corticosteroids or estrogen/testosterone, and season (OR 4.4, 95% CI 1.0–18.8,  $P < 0.05$ ), nor was it modified when gonadal hormone status was taken into account instead of estrogen replacement in female patients (OR 4.6, 95% CI 1.0–20.6,  $P < 0.05$ ). Since there was a trend for use of higher rhGH doses in cases with < 15 ng/ml 25(OH)D (Table 2), we studied the relationship between vitamin D status and weekly amount of rhGH. Linear regression with adjustment for age and IGF1 concentrations revealed a significant negative correlation between 25(OH)D levels and rhGH dose ( $\beta = -0.042$ ,  $P < 0.01$ ). However, the strength of the association was attenuated to nonsignificance when gender, use of hormones, and season were corrected for ( $\beta = -0.037$ ,  $P = 0.06$ ). No significant results were obtained when the other 25(OH)D cutoffs were substituted for the 15 ng/ml one in the regression models.

## Discussion

This work consisted of two steps. First, we assessed the effects of vitamin D on circulating IGF1 in adults. Second, we explored the clinical impact of vitamin D modulation of IGF1 in GHD. To our knowledge, this latter issue has never been addressed before.

Concentrations of IGF1 were significantly higher following vitamin D supplementation in our sample.

Although the possibility of a type 1 error cannot be excluded given the small size of the treatment cohort, this finding is substantiated by previous studies in children with rickets, in which serum IGF1 also significantly increased after treatment with vitamin D (5, 6). Overall, these data indicate that vitamin D positively affects IGF1 levels. Consistent with these findings, circulating IGF1 is lower in *Vdr*<sup>-/-</sup> mice than in WT (4). Moreover, a positive association between 25(OH)D and IGF1 values has been repeatedly found in healthy subjects (7, 8, 9, 10).

The present study did not investigate the mechanism by which vitamin D may influence the amount of IGF1 in the bloodstream, nor has it been done by others. Vitamin D is most likely to regulate IGF1 concentrations by acting in the liver, since this organ is the main source of circulating IGF1 (17). In addition, hepatic cells secrete IGF1 and acid-labile subunit, which carry IGF1 in the blood within a ternary complex (18). Interestingly, transgenic mice deficient in steroid receptor coactivator-3, a co-activator of the VDR, have reduced IGF1 levels because of a decrease in liver expression of the *IGFBP3* gene and thereby in serum IGF1 (19).

The effect of vitamin D on IGF1 appeared to be dose dependent in our patients. While the change in 25(OH)D induced by 5000 or 7000 IU vitamin D<sub>3</sub>/week was very similar, only the highest dosage significantly raised IGF1 concentrations. Variations in both 25(OH)D and IGF1 were not significant in controls. It must be acknowledged that the untreated, 5000 and 7000 IU groups were significantly different in terms of BMI. This is a major shortcoming of our work, likely due to the small size and highly heterogeneous composition of the study sample, which randomization could not correct for. The lack of a higher increase in 25(OH)D in the 7000 group was most probably due to their higher BMI, since excess adiposity dilutes supplemental vitamin D (20). The amount of adipose tissue is also known to affect GH and IGF1 and, although the analysis of the changes in IGF1 was not substantially modified by adjustment for BMI, residual confounding may still exist. However, the possibility of a dose–response relationship between vitamin D and IGF1 should be taken into consideration and specifically addressed by future studies with far more different vitamin D<sub>3</sub> doses than those used here. Based on the evidence that hepatic cells i) not only hydroxylate vitamin D to 25(OH)D but can also further convert 25(OH)D to 1,25(OH)<sub>2</sub>D (21) and ii) express the VDR (22), we hypothesize that oral vitamin D<sub>3</sub> reaches the liver through the portal circulation, is sequentially converted to 25(OH)D and then 1,25(OH)<sub>2</sub>D, and stimulates the synthesis of IGF1/IGFBP3 in a paracrine/autocrine fashion. The fat mass would not interfere with this intra-hepatic activity of vitamin D, since it occurs before the passage into the systemic circulation which brings

vitamin D to the adipose tissue. Clearly, this interpretation is purely speculative and must be verified.

The induction of IGF1 by vitamin D may be relevant for human health, since epidemiological studies showed a positive interaction between vitamin D intake or levels and circulating IGF1 in diminishing the risk of adverse outcomes, such as metabolic syndrome (8) or mammographic breast density (a surrogate of breast cancer risk) (23). On the other hand, excess IGF1 may contribute to the development of several types of tumors (24), which raises the possibility that the increase in serum IGF1 following vitamin D treatment may be detrimental in some cases.

Out of the research context, however, IGF1 concentrations in the blood are measured in selected cases, among which is GHD. Low IGF1 levels are partly responsible for the morbidity and mortality associated with GHD and normalization of IGF1 is one of the goals of treatment with rhGH (25, 26). Factors such as age, gender, and estrogen status are known to impact the hepatic synthesis of IGF1 in response to rhGH and it is recommended that GH dosing take them into account (26). We reasoned that knowing whether vitamin D status affects circulating IGF1 would be of practical importance for the endocrinologist managing GHD. In our series, cases with serum 25(OH)D equal to or higher than 15 ng/ml were over four times more likely to have IGF1 values at least at the 50th percentile for age and sex. Therefore, it can be speculated that a better vitamin D status may favor the normalization of IGF1 in adults with treated GHD. Specifically designed and adequately powered studies are required to confirm this hypothesis.

In conclusion, the present work confirms that vitamin D increases circulating IGF1, for the first time demonstrating this effect specifically in adults. More importantly, it puts the influence of vitamin D on IGF1 in a clinical perspective, showing it may be relevant for the management of GHD. The interactions between vitamin D and IGF1 are certainly worth further investigation.

## Declaration of interest

A Giusti received lecture fees from Abiogen Pharma. D Ferone consults for Eli Lilly. The other authors have nothing to declare.

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