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Evaluation of vitamin D3 intakes up to 15,000 international units/day and serum 25-hydroxyvitamin D concentrations up to 300 nmol/L on calcium metabolism in a community setting

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ABSTRACT

Supplementation by the general public with vitamin D at doses above the Tolerable Upper Level of Intake (UL) is becoming quite common. The objective of the current analysis was to characterize the effect of vitamin D supplementation at doses up to 15,000 IU/d in a community-based program on vitamin D status, calcium homeostasis as well as on kidney, liver and immune function. We evaluated data collected for 3,882 participants in a community program for whom there were blood measurements at program entry and at follow-up within 6–18 months between 2013 and 2015. Participants were supplemented with a wide range of vitamin D doses (1,000 – 15,000 IU/d) aimed at achieving serum 25-hydroxyvitamin D [25(OH)D] levels of at least 100 nmol/L. Serum 25(OH)D concentrations up to 300 nmol/L were achieved without perturbation of calcium homeostasis or incidence of toxicity. Hypercalcemia and hypercalciuria were not related to an increase in 25(OH)D concentrations nor vitamin D dose. To achieve serum 25(OH)D levels >100 nmol/L on average, required vitamin D intakes of 6,000 IU/d for normal Body Mass Index (BMI), 7,000 IU/d for overweight and 8,000 IU/d for obese. Doses of vitamin D in excess of 6,000 IU/d were required to achieve serum 25(OH)D concentrations above 100 nmol/L, especially in individuals who were overweight or obese without any evidence of toxicity. Serum 25(OH)D concentrations up to 300 nmol/L were found to be safe.

Introduction

Evidence suggests that optimal vitamin D status reduces the risk for a long list of chronic health conditions. However, the composite literature is often inconsistent and confusing and has led to heated debates about optimal vitamin D status. To confuse matters more, there is a wealth of expert opinions to support both sides of the argument.[1-4]

Nearly every cell in the body has a vitamin D receptor and vitamin D is necessary for a myriad of cellular functions.[5] In fact, low vitamin D status reduces the capacity of most tissues to carry out normal physiologic functions. Vitamin D is necessary for skeletal, health, immune, developmental, and cardiovascular health and to protect against cancer. As a result, low vitamin D status increases the risk of several diseases including autoimmune disorders, diabetes, cardiovascular disease and cancer.[6]

Logically, the criteria for determining nutrient intake requirements should be based on the actual function of the nutrient, not disease prevention. The challenges for setting an intake requirement for vitamin D are based in physiology. Three separate lines of evidence, encompassing i) the compensatory mechanism for vitamin D's role in calcium homeostasis, ii) natural ancestral levels that can be obtained through unhindered sun exposure and iii) levels required for breastmilk to contain adequate vitamin D for the nursing infant, converge to establish an optimal vitamin D status.[7,8] Heaney concluded that a 25(OH)D level of 100 to 130 nmol/L is the status best suited for normal physiology.[4] The safety of serum 25(OH)D

levels as high as 500 nmol/L has been reported[9-11] and recently confirmed in large community-based samples.[12,13]

An adult in a bathing suit exposed to an amount of sunlight that causes a slight pinkness to the skin 24 hours later (1 minimal erythemal dose; MED) is equivalent to ingesting approximately 15,000 IUs of vitamin D.[14-16] However physicians remain concerned with intakes above 4,000 IU/d. The Institute of Medicine (IOM) established 4,000 IU/d as the tolerable upper level of intake (the level unlikely to cause harm in almost all adults). Recent studies demonstrate that vitamin D supplement use has increased, whether due to self-selected or physician-directed dosing, and 25(OH)D levels above 150 nmol/L have increased by 200% over 10 y.[12-13] In addition, the amount of

vitamin D_3 supplementation required to achieve a serum 25(OH)D above 100 nmol/L is on average 5,000 IU/d[9,17-19] and 2–3 times more for overweight and obese individuals.[11,18]

Natural levels of 25(OH)D achieved through sun exposure in Maasai herdsman that is in the range of 100–150 nmol/l can also be achieved with oral intake of 5,000–10,000 IU/d.[4,17] The Endocrine Society Practice Guidelines recommend that up to 10,000 IUs daily was safe for adults.[8,14] This is in contrast to the recommended UL at 4,000 IU/d from the IOM. Thus the safety profile for supplemental intakes above 4,000 IU/d remains uncertain. For an individual with a high body mass index (BMI), doses over 10,000 IU/d may be necessary to achieve a 25(OH)D of at least 100 nmol/L. The present analysis evaluated vitamin D
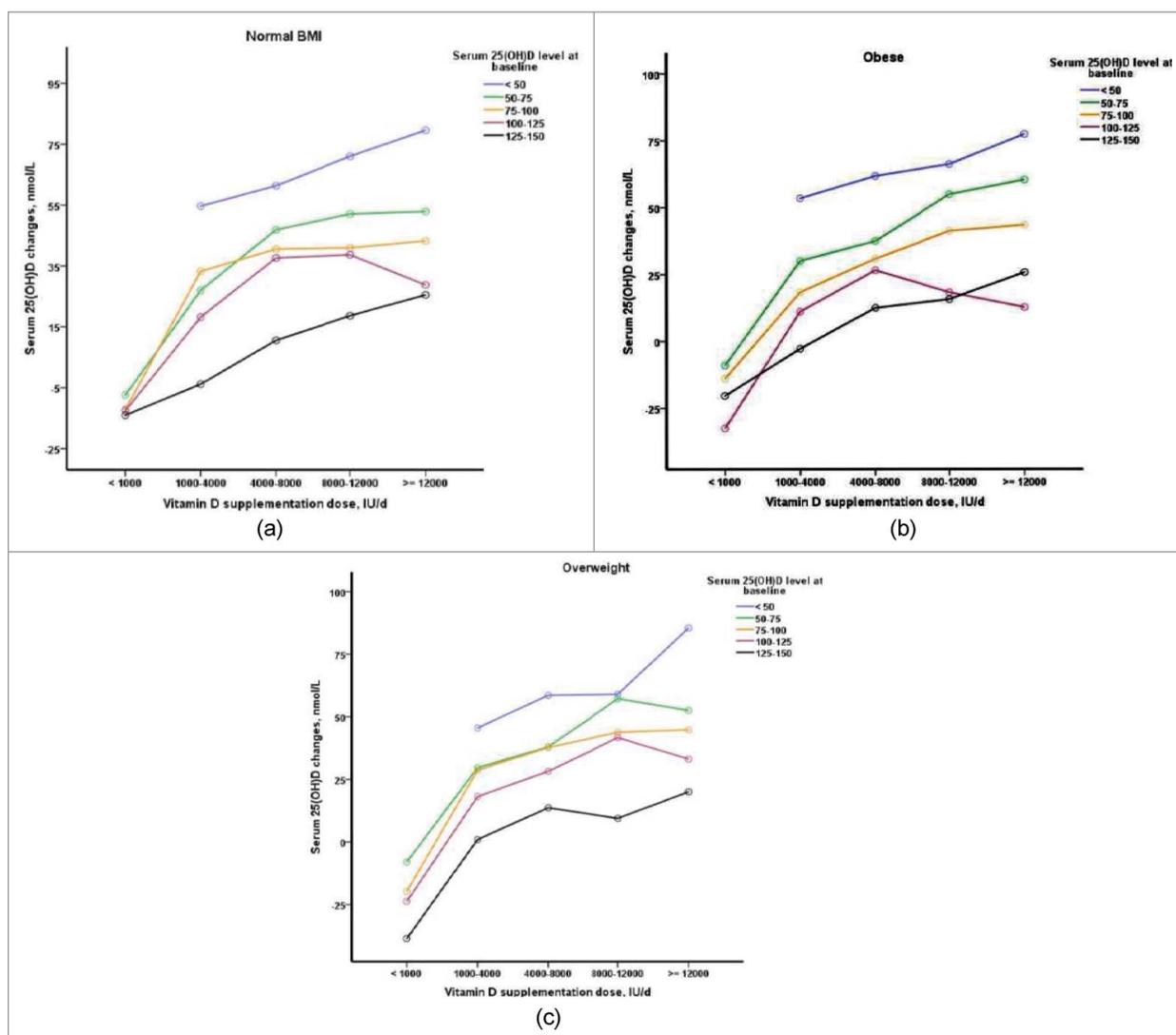

Figure 1. Response to vitamin D supplementation based on baseline 25(OH)D concentrations and BMI (a) Normal BMI, (b) Overweight; and (c) Obese.

supplementation at intakes up to 15,000 IU/d in a community setting on various parameters of calcium metabolism and potential toxicity.

Results

Anonymized data from 3,882 new participants were available with follow-up, on average one year later, between 2012 and 2015 and included in this analysis. The mean age of participants was 59.6 ± 14.8 y and 59.5% were female. Less than 1% of the participants had a BMI in the underweight range, 35.5% had a normal BMI, 37.0% were overweight and 27.5% were obese (18.2% obese I, 5.8% obese II, 3.5% extreme obese).

At entry to the program (baseline) 55% of participants reported taking some vitamin D. The average dose of vitamin D supplements increased from $2,106 \pm 2,471$ IU/d at baseline to $6,767 \pm 3,588$ IU/d at follow-up (n = 2,339). Overall, mean serum 25(OH)D concentrations increased from 87 ± 28 nmol/L to 126 ± 39 nmol/L (paired t, $p < 0.001$).

Serum 25(OH)D concentrations were influenced by vitamin D dose in a BMI-dependent manner. Participants with normal BMI and an average vitamin D intake of 6,100 IU/d had a mean increase in serum 25(OH)D levels from 92 ± 29 to 131 ± 40 nmol/L ($p < 0.001$). Dose-response was also influenced by baseline 25(OH)D concentrations for all BMI groups such that individuals with higher baseline 25(OH)D concentrations experienced a blunted response to the same vitamin D dose as compared with someone with lower baseline 25(OH)D concentrations (Fig. 1). In normal weight participants who were vitamin D-deficient at baseline, <50 nmol/L, the response to an average intake of 7,670 IU/d was substantially greater with serum 25(OH)D concentrations increased from 38 ± 8 nmol/L to 103 ± 37 nmol/L ($p < 0.001$). The response to vitamin D supplementation was less with increased BMI in a step-wise manner such that obese individuals had lower mean 25(OH)D concentrations (118 ± 38 nmol/L) than overweight (126 ± 38 nmol/L) who had lower levels than normal BMI (131 ± 40 nmol/L) at follow-up, despite higher vitamin D intakes (Table 1).

A goal of the community-based program was to achieve a 25(OH)D concentration of at least 100 nmol/L. At baseline 18.4% of participants met this target and at follow-up 76.3% of normal BMI, 74.5%

of overweight, and 65.5% of obese participants achieved levels above 100 nmol/L. Vitamin D_3 intakes of at least 6,000 IU/d were required for those with a normal BMI to achieve serum 25(OH)D concentrations above 100 nmol/L, or 7,000 IU/d and 8,000 IU/d for overweight and obese, respectively. Seventy percent of participants reached serum 25(OH)D levels above 100 nmol/L at follow-up and 45% above 125 nmol/L.

There was a subgroup of participants (n = 285) that reported substantial intakes of vitamin D supplements ($> 4,000$ IU/d) that did not experience an increase in serum 25(OH)D concentrations. We investigated whether BMI was a contributor: 36.5% were within a normal BMI, 34.0% were overweight, and 27.9% were obese. Conditions that could cause intestinal absorption [including Crohn's disease, celiac disease, inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), ulcerative colitis, gastrointestinal esophageal reflux disease (GERD)] were present in 38% of these participants, which increased to 60% of the 285 participants when we included whether participants reported stomach issues (including bloating, stomach pain, indigestion, upset stomach).

Mean serum calcium concentrations did not differ between baseline and follow-up (Table 1). There were 47 participants (1.2%) who presented with hypercalcemia at baseline and 41 participants (1.3%) at follow-up. Of those, 20 participants were new cases of hypercalcemia at follow-up (Supplemental Table 1). The majority of participants found to have a mildly elevated serum calcium level were those who had serum 25(OH)D concentrations of 50–100 nmol/L (Table 2). Furthermore it was found that hypercalcemia or hypercalciuria was more often observed in participants who had a serum 25(OH)D level (< 100 nmol/L) (Table 2).

Regression analyses revealed that when other variables were taken into account (including age, sex, BMI and baseline levels) serum 25(OH)D and vitamin D dose were negative predictors of serum calcium (Table 3).

To investigate the effect of vitamin D supplementation on the development of hypercalcemia, we investigated the probable cause of the newly developed hypercalcemia on a case-by-case basis. Of the 20 cases, 6 were no longer hypercalcemic upon re-testing. There were 2 cases of hyperparathyroidism, one who was being treated by an endocrinologist and one who was

Table 1. Comparison of measurements between baseline and follow-up within categories of Body Mass Index (BMI).

	Normal (BMI < 25)				Overweight (BMI = 25–30)				Obese (BMI ≥ 30)			
	N	Baseline	Follow-up	Baseline vs. Follow-up	N	Baseline	Follow-up	Baseline vs. Follow-up	N	Baseline	Follow-up	Baseline vs. Follow-up
BMI (kg/m²)	1398	22.4 ± 1.9	22.9 ± 2.5	<0.001	1437	27.3 ± 1.4	27.6 ± 1.9	<0.001	1047	34.4 ± 4	34.5 ± 4.4	0.02
Vitamin D dose (IU/d)	1225	2232 ± 2463	6090 ± 3221	<0.001	1246	2111 ± 2391	6751 ± 3520	<0.001	928	1932 ± 2578	7652 ± 4026	<0.001
Serum 25(OH)D (nmol/L)	1366	91.5 ± 29	131 ± 40	<0.001	1446	87.2 ± 28	126 ± 38	<0.001	1053	80 ± 26	118 ± 38	<0.001
Serum Calcium (mmol/L)	1350	2.40 ± 0.08	2.39 ± 0.08	0.08	1413	2.40 ± 0.09	2.39 ± 0.09	0.3	1045	2.39 ± 0.09	2.39 ± 0.09	0.2
Serum PTH (ng/L)	453	35.7 ± 14	35.2 ± 14	0.4	494	37.8 ± 14	36.6 ± 14.5	0.06	327	41.9 ± 16.4	39.3 ± 15	0.001
Serum hs-CRP (mg/L)	1122	1.10 ± 1.4	1.33 ± 1.4	<0.001	1155	1.63 ± 1.7	1.86 ± 1.6	<0.001	773	2.68 ± 2.2	2.86 ± 2.2	<0.001
Serum Creatinine (µmol/L)	1193	77.6 ± 18.7	78.2 ± 16.7	<0.001	1243	82 ± 19	82.8 ± 20	0.01	915	82.5 ± 32	81.9 ± 23	0.8
Estimated GFR (mL/min)	1193	78.5 ± 20	76.7 ± 18	<0.001	1243	75.6 ± 16.7	74.5 ± 17.3	<0.001	915	75 ± 17.5	74.7 ± 17.5	0.1
Serum ALT (U/L)	1191	20.6 ± 16.6	18.3 ± 11	<0.001	1246	24.7 ± 19.3	22.2 ± 15.8	<0.001	907	27.3 ± 22	24.5 ± 16	<0.001
Serum GGT (U/L)	1191	24.7 ± 37.6	20.6 ± 20.8	<0.001	1246	30.7 ± 36	27.5 ± 31.6	<0.001	907	35.4 ± 28	31.6 ± 29	<0.001
Urine Calcium: Creatinine Ratio (mg/mg)	190	0.124 ± 0.08	0.131 ± 0.08	0.2	193	0.108 ± 0.07	0.129 ± 0.07	<0.001	135	0.096 ± 0.07	0.111 ± 0.07	0.01

PTH: Parathyroid hormone; hs-CRP: high-sensitivity C reactive protein; GFR: Glomerular filtration rate; ALT: Alanine aminotransferase; GGT: Gamma glutamyltransferase

Table 2. Calcium measures, serum PTH and vitamin D supplementation dose at follow-up based on categories of serum 25(OH)D concentration.

	Serum 25(OH)D (nmol/L)				
	50–100	100–150	150–200	200–250	250–300
N (blood)	973	1744	673	98	14
Vitamin D dose (IU/d)	6086 ± 4002	7016 ± 3670	7228 ± 3315	8310 ± 3505	7533 ± 3136
25(OH)D (nmol/L)	80 ± 13	123 ± 14	169 ± 14	218 ± 14	264 ± 12
PTH (ng/L)	40.8 ± 16	37.7 ± 15	34.9 ± 14	31.5 ± 12	33.3 ± 13
Albumin-corrected calcium (mmol/L)	2.32 ± 0.08	2.34 ± 0.08	2.34 ± 0.08	2.33 ± 0.08	2.36 ± 0.06
Hypercalcemia (%)*	1.7	1.4	0.8	1	0
N of Hypercalcemia	12	20	5	1	0
N (urine)	97	257	117	23	3
Urinary calcium:creatinine ratio (mg/mg)	0.099 ± 0.06	0.132 ± 0.07	0.129 ± 0.07	0.133 ± 0.08	0.106 ± 0.02
Hypercalciuria (%)*	1.2	10.9	4.4	1.2	0
N of Hypercalciuria	6	54	22	6	0

*% of total

referred to their general physician for care. Four cases involved participants with suboptimal 25(OH)D values at baseline that were increased significantly at follow-up (55 ± 26 to 135 ± 52 nmol/L) in whom PTH levels were decreased. In one participant serum 25 (OH)D concentrations rose from 115 nmol/L at baseline to 185 nmol/L at follow-up with a serum calcium of 2.56 or 0.01 mmol/L outside the reference range. In this participant PTH values did not change between baseline and follow-up and were within reference ranges (41 and 42 ng/L, respectively). In one participant serum 25(OH)D concentrations increased from 125 to 258 nmol/L with serum calcium levels increasing from 2.48 to 2.59 mmol/L and PTH levels decreased from 54 to 47 ng/L. Two participants who had serum calcium levels within the reference range at baseline experienced decreases in 25(OH)D concentrations (from 85 and 150 nmol/L to 73 and 108 nmol/L, respectively) and both had a follow-up serum calcium of 2.67 mmol/L. For the remaining 4

participants, PTH levels were not obtained on both visits and participants did not return to the clinic for further follow-up by the time this analyses was conducted. Overall, serum 25(OH)D concentrations increased modestly (from 94 ± 4 to 119 ± 18 nmol/L) with an increase in serum calcium levels (from 2.48 ± 0.04 to 2.58 ± 0.02 mmol/L).

Urine calcium and creatinine measurements were available for 521 participants. Hypercalciuria (urine calcium:creatinine ≥ 0.2 mg/mg) was detected in 17.6% of participants at baseline. There was virtually no increase in the prevalence of hypercalciuria at follow-up (17.7%). Regression analysis revealed no effect of serum 25(OH)D or vitamin D dose on urine calcium:creatinine ratios when age, sex, BMI and baseline levels were accounted for (Table 3).

We examined the 52 new cases of hypercalciuria at follow-up in detail (Supplemental Table 2). There were 9 cases in which serum 25(OH)D concentrations decreased between baseline and follow-up. None of

Table 3. Predictors of Serum Calcium and Urinary Calcium: Creatinine Ratio, using Multiple Linear Regression Model.

Dependent variable at follow-up	Predictors	B	Standardized Coefficients (β)	P-Value	95% Confidence Interval
Serum calcium (mmol/L)	Model ($R^2 = 0.35$)				
	Age	0.001	0.11	<0.001*	0–0.001
	Gender	−0.006	−0.04	0.01*	−0.011–0.001
	Calcium baseline	0.49	0.53	<0.001*	0.458–0.517
	Serum 25(OH)D (nmol/L)	−9.07E-5	−0.03	0.04*	0.000–0.000
	Vitamin D dose (IU/d)	−8.36E-7	−0.04	0.02*	0.000–0.000
	BMI (kg/m^2)	0.001	0.04	0.01*	0–0.001
Urine calcium: creatinine ratio (mg/mg)	Model ($R^2 = 0.16$)				
	Age	0	0.09	0.05*	0–0.001
	Gender	−0.01	−0.08	0.07	−0.024–0.001
	Calcium baseline (mmol/L)	0.06	0.07	0.1	−0.013–0.135
	Serum 25(OH)D (nmol/L)	0	0.07	0.09	0.000–0.000
	Vitamin D dose (IU/d)	1.35E-7	0.006	0.8	0.000–0.000
	BMI (kg/m^2)	−0.001	−0.07	0.1	−0.002–0.000
	Urine calcium:creatinine Baseline	0.35	0.34	<0.001*	0.261–0.433

the cases were associated with hypercalcemia or a reduction in PTH values.

We also examined the participants who were hypercalciuric at baseline and who had a follow-up urine measurement (n = 67) to determine the effect of vitamin D supplementation on the incidence of hyper-calciuria. Urine calcium:creatinine ratios were decreased after vitamin D supplementation (from 0.255 ± 0.54 to 0.175 ± 0.077). At follow-up 67% were no longer hypercalciuric while on vitamin D supplementation.

None of the participants developed any biochemical evidence for vitamin D toxicity i.e. hypercalcemia associated with a suppressed PTH level.

Biochemical markers for kidney function (serum creatinine and eGFR) and liver function (ALT and GGT) remained within the reference ranges (Table 1). Kidney and liver function tests were not influenced by serum 25(OH)D concentrations (Supplemental Table 3).There was a weak negative correlation between 25(OH)D and hs-CRP (R^2 = 0.003, y = −0.002x + 2.29, p = 0.001).

Discussion

Naturally acquired vitamin D from whole body sun exposure (1 MED) is equivalent to ingesting ∼15,000 IU vitamin D supplement.[14,16] Equatorial tribes exposed to sunlight on a daily basis at the equator achieve an average 25(OH)D level of 115 nmol/L[8]. The IOM states that the safety profile for supplemen-tal intakes above 4,000 IUs daily and blood levels of 25(OH)D above 75 nmol/L is uncertain and above 125 nmol/L may increase mortality.[1] The Endocrine Society concluded that ingestion of up to 10,000 IUs daily was not associated with any significant alteration in calcium metabolism and recommended that circu-lating levels of 25(OH)D to be at least 75 nmol/L with a preferred range of 100–150 nmol/L for maximum bone and muscle health. We observed that the amount of vitamin D required to achieve serum 25(OH)D concentrations of at least 100 nmol/L, particularly in overweight and obese individuals was between 6,000–8,000 IU/d on average. Some of the participants were taking as much as 15,000 IUs of vitamin D daily with-out any untoward toxicity.

The Endocrine Society recommended that a blood level of 25(OH)D up to 250 nmol/L was not associated with toxicity and that vitamin D toxicity is usually observed when the blood level is above 375 nmol/L.[11] Our data are consistent with this recommendation. Some of the participants achieved serum 25(OH)D levels up to 300 nmol/L without any evidence of hypercalciuria or hypercalcemia. Suppression of the serum PTH concentration is the most sensitive indica-tor of a perturbation in calcium homeostasis, and thus an indication of an adverse effect of vitamin D supple-mentation. There was no significant reduction in the PTH levels in those participants who had the highest intakes of vitamin D and achieved blood levels of 25(OH)D above 250 nmol/L.

Higher serum 25(OH)D have been found in 2 other community-based studies.[12,13] Dudenkov et al. report an increase in the incidence of serum 25(OH)D con-centrations above 125 nmol/L (50 ng/mL) in Rochester over 10 y from 9 to 233 cases per 100,000 person-years. Of the 20,308 measurements they found 8.4% were above 125 nmol/L.[12] Similarly, we found that only 45% of participants achieved a serum 25(OH)D above 125 nmol/L despite average intakes of vitamin D at 7,000 IU/d. The serum 25(OH)D concentrations were not related with serum calcium or an increased risk of hypercalcemia,[12] similar to what we observed. Perez-Barrios et al. report that 11.1% of 25,567 measurements made over 6 y from hospital samples were found to have 25(OH)D concentrations over 160 nmol/L and that less than 4% of these cases were associated with hypercalcemia. Unfortunately, the authors did not report the prevalence of hypercalcemia in the samples with serum 25(OH)D below 160 nmol/L[13].

As expected,[20] when baseline serum 25(OH)D lev-els were below 50 nmol/L the increase in 25(OH)D was much more robust with the mean increase of 60 nmol/L for those participants who ingested 4,000–8,000 IU/d of vitamin D. In those with higher baseline values, the increase in 25(OH)D was much lower (mean increase of 8–15 nmol/L) for participants who took the same amount of vitamin D. The increase also depended on BMI as previously reported.[18,21] BMI was found to be the most significant factor for the vitamin D dose-response. The higher doses of vitamin D needed for overweight and obese to achieve the same serum 25(OH)D concentrations as normal BMI may be due to several factors in addition to the poten-tial for vitamin D to be distributed to adipose tissue.

Obesity is associated with chronic inflammation in metabolic tissues.[22] Vitamin D is a potent immuno-modulator and anti-inflammatory agent. The constant

state of low-grade inflammation characteristic of obesity may increase requirements for vitamin D. Another consideration is the role of the gut microbiota. Vitamin D has been found to modulate the gut microbiome in the upper gastrointestinal tract. Disturbances in the microbiome have been suggested to play a role in the pathogenesis of metabolic disorders and the composition of gut microbes is strongly influenced by diet.[23] Further, gut microbes influence gut motility and absorption of nutrients, thus an unbalanced microbiome may result in reduced absorption of vitamin D. There is also the role of the microbiome in development and maintenance of the immune system.[24] A disruption of the microbiome that alters immune function may also alter immune cell requirements or use of vitamin D.

We found that 9% of participants reported an intake of vitamin D of at least 1,000 IU/d did not experience an increase in 25(OH)D concentrations; 90% of these were taking > 4,000 IU/d and up to 16,000 IU/d. There are several possible reasons for this. The most obvious is non-compliance. However, the effect of obesity must be considered with 62% of these participants being overweight and obese. The response to a given dose of vitamin D has been found to be 2–3 times less in overweight and obese individuals in comparison with individuals with a normal BMI.[18] Further, malabsorption may play a significant role. Sixty per cent of these participants reported stomach issues such as IBD and Crohn's disease in the present analysis. Twenty million Canadians have digestive disorders.[25] A recent meta-analysis reported that patients with IBD have a 64% higher odds of being vitamin D deficient.[26] In addition, bone disease is present in nearly half of patients studied with Celiac disease[27] and IBD.[28] In patients with Crohn's disease the ability to absorb a dose of vitamin D was found to be 30% lower than in healthy controls.[29] The role of gastrointestinal health in vitamin and mineral absorption is of concern.

This study has several limitations including the retrospective design, bias introduced from self-reported vitamin D intakes, and lack of complete data for all participants (e.g. urine calcium: creatinine ratios were available for half of participants). Another limitation includes the potential for selection bias – we were only able to assess participants that remained in the program and had follow-up visits. However, we would expect that those remaining in the program would have higher serum 25(OH)D values because of program adherence and thus would allow us a greater ability to detect hypercalcemia related to vitamin D intake. Among the strengths of the current analyses are the large community-based population, nearly 4,000 participants, and access to all follow-up assessments that were performed, including any related to probably vitamin D toxicity.

Vitamin D may play a key role in health optimization and prevention of chronic disease. A considerable amount of literature suggests that serum 25(OH)D concentrations around 100–150 nmol/L are ideal for physiology[4] and disease prevention.[30] A statistical error has been reported and confirmed independently that suggests the correct Recommended Daily Allowance (RDA) is ∼7,000 IU/d[4,19]. However, there remains considerable public debate among experts. Ultimately, the decision to take vitamin D supplements is up to the individual. While the present study does not address what is an optimal vitamin D status, it does confirm the safety of serum 25(OH)D concentrations up to 300 nmol/L and intakes of vitamin D up to 15,000 IU/d. Further, the results presented here demonstrate a variable response to vitamin D intake and suggest that intakes of 6,000–8,000 IU/d are required to achieve serum 25(OH)D above 100 nmol/L.

Methods and materials

Intervention

This study was a database analysis of a wellness program focused on the prevention of chronic diseases. The program provides lifestyle advice, education and optimization of nutrition through the use of research-based nutritional supplements, with a focus on achieving 25(OH)D levels above 100 nmol/L. Supplement recommendations are based on analysis of each participant's biometric measurements, blood results and clinical intake data. Health care professionals review and explain blood work results with the participant and, based on their clinical knowledge and nutrient expertise, make recommendations accordingly. Each participant is treated as an individual and a treatment plan developed to meet that individual's nutrient requirements. All participants were encouraged to achieve a 25(OH)D level of at least 100 nmol/L and individual vitamin D doses were adjusted accordingly. This clinical program has been registered with ISRCTN18397898. The ISRCTN is a registry and a

database containing essential information to describe a study deemed important by the World Health Organization (WHO), International Clinical Trials Registry Platform (ICTRP), and the International Committee of Medical Journal Editors (ICMJE).

Database

Anonymized data from the program were assessed for all new participants who entered the program between 2012 and 2015 consented for the use of their anonymized data for research and who had follow-up within a 6–18 month period after their first visit (n = 3,882). We selected a period when all of the biochemical results were obtained from one laboratory, Doctors' Data (St. Charles, IL). The majority of participants in the program were healthy adults, without any history of hyperparathyroidism, granulomatous diseases, hypercalcemia and chronic kidney diseases or on any medications that could influence calcium and vitamin D metabolism. Parathyroid Hormone (PTH) concentrations were evaluated up until January of 2014, after which only participants that presented with high serum calcium values had PTH evaluated. All participants had provided written, informed consent to permit anonymous analysis of their data for research.

Measurements

Demographic information was obtained for all participants. Body mass index (BMI) was calculated dividing body weight (kg) by square height (m^2). All sample preparation and biochemical measurements were performed by Doctor's Data Laboratory (St. Charles, IL), a fully accredited laboratory. Four different categories of biochemical parameters involving vitamin D safety were evaluated, including; calcium homeostasis [serum calcium, 25(OH)D, PTH and urinary calcium: creatinine ratio], inflammation [high-sensitivity C-reactive protein (hs-CRP)], liver function [Alanine Amino-Transferase (ALT), Gamma Glutamyl Transferase (GGT)] and kidney function [estimated Glomerular Filtration Rate (eGFR), serum creatinine]. Serum 25(OH)D was measured using Liquid Chromatography and tandem Mass Spectrometry (LC/MS-MS) with the inter-assay CV of 2.4%. Serum calcium and albumin concentrations were measured using spectrophotometric method. To avoid any affect due to hemoconcentration, all calcium measures were

completed using the first tube of blood collected from each blood draw. Serum hs-CRP and PTH were measured with immuno-turbidimetric and ELISA methods, respectively (inter-assay CVs were both 2.5%). Serum ALT and GGT were measured on the Beckman Coulter, using enzymatic method with the inter-assay CVs of 4.3% and 2.9%, respectively. Serum creatinine was measured using the Jaffe method with the inter-assay CV of 1.1%. Estimated GFR was calculated using following equation: eGFR = 186 × (creatinine/88.4)$^{-1.154}$ × (age)$^{-0.203}$ × (0.742 if female) × (1.21 if black).[31] All laboratory testing was validated according to ongoing externally provided accreditation test samples. Values are presented as mean ± standard deviation.

Categories

Participants were categorized according to their gender, age, BMI and serum 25(OH)D status. Serum 25(OH)D categories were defined *a priori* in 50 nmol/L increments: < 50, 51–100, 101–150, 151–200, 201–250, 251–300, > 300 nmol/L. Vitamin D intake categories were defined as: <1,000 IU/d, 1,000-<4,000 IU/d, 4,000-<8,000 IU/d, 8,000-<12,000 IU/d and ≥ 12,000 IU/d. The reference range for serum calcium, 2.10 – 2.55 mmol/L (8.4 – 10.3 mg/dL), had been established by the laboratory using standard procedures.[32] Hypercalcemia was defined as serum calcium concentration ≥ 2.55 mmol/L (10.3 mg/dL). Hypercalciuria was defined as urine calcium: creatinine ratio above 0.2. The reference interval for urine calcium: creatinine was < 0.14 and between 0.14 and 0.2 was considered borderline.[33]

Statistical analyses

Data were analyzed using SPSS version 23 (SPSS Inc., Chicago, IL). Descriptive analysis was performed to establish the distribution of categorical data. Since the data was not normally distributed, non-parametric tests were applied. Wilcoxon Signed Rank Test was performed to evaluate changes in different variables over follow-up period. Mann Whitney U-test and Kruskal-Wallis test were done to compare means according to different categorical groups including age, gender, BMI and serum 25(OH)D status. Multiple linear regression was performed to determine the predictors of vitamin D status, PTH levels, hypercalcemia, hypercalciuria,

inflammation, liver and kidney function. Significance was defined as $p < 0.05$.

Ethics

This study was performed using secondary data that was anonymized (participants were identified by a randomly generated 36-digit αnumeric code only) and did not require ethics board approval.

Disclosure of potential conflicts of interest

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