CLINICAL STUDY

The serum 25-hydroxyvitamin D response to vitamin D supplementation is related to genetic factors, BMI, and baseline levels

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Abstract

Objective: The serum 25-hydroxyvitamin D (25(OH)D) level is not only dependent on vitamin D intake and production in the skin but also dependent on genetic factors. Thus, in large genome-wide association studies, it has been shown that single nucleotide polymorphisms (SNPs) in the vitamin D binding protein (DBP), as well as in enzymes related to activation or degradation of vitamin D and its metabolites, are as important for the serum 25(OH)D level as the effect of season. How these SNPs affect the serum 25(OH)D response to vitamin D supplementation is uncertain.

Design and methods: Data were pooled from three randomized controlled trials where 40 000 IU vitamin D/week was given for 6 months. Serum 25(OH)D was measured before and at the end of the intervention, and the subjects were genotyped for SNPs related to the serum 25(OH)D level.

Results: Baseline 25(OH)D levels were significantly related to SNPs in the *DBP* and *CYP2R1* genes. Those with SNPs associated with the lowest baseline 25(OH)D levels also had the smallest increase (delta) after supplementation. Those with the lowest baseline serum 25(OH)D (without regard to genotypes) had the highest increase (delta) after supplementation. Subjects with high BMI had lowest baseline 25(OH)D levels and also the smallest increase (delta) after supplementation.

Conclusions: The serum 25(OH)D response to supplementation depends on genes, baseline level, and BMI. However, whether this is clinically important or not depends on the therapeutic window of vitamin D, an issue that is still not settled.

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Introduction

Vitamin D is produced in the skin after u.v. exposure or obtained through the diet, particularly oily fish, such as salmon and sardines (1). For vitamin D to become biologically active, it first has to be hydroxylated in the liver to 25-hydroxyvitamin D (25(OH)D), which is the metabolite used to evaluate a subject's vitamin D status, and then in the kidneys to 1,25-dihydroxyvitamin D (1,25(OH)₂D) (1). Accordingly, the serum 25(OH)D level is classically thought of as a result of intake of vitamin D and the sun-induced production of vitamin D in the skin.

In recent years, there has been a great focus on vitamin D and its possible extraskelatal effects (2, 3). The vitamin D receptor is located in numerous tissues (1), and associations between low serum 25(OH)D levels and risk of diseases like cardiovascular disease (4), diabetes (5), and cancer as well as mortality (6) have been reported. Although there is no consensus on what

is the optimal serum 25(OH)D level, there is a general agreement that it should be at least 50 nmol/l (7, 8, 9). Accordingly, subjects with lower serum levels need vitamin D supplementation if the deficiency cannot be sufficiently corrected by changes in lifestyle with more outdoor activities or a vitamin D rich diet.

However, it has been shown that the serum 25(OH)D level is not only dependent on vitamin D intake and production in the skin but also dependent on genetic factors (10, 11, 12). In large genome-wide association studies (GWAS), it has been shown that single nucleotide polymorphisms (SNPs) in the vitamin D binding protein (DBP), as well as in enzymes related to activation or degradation of vitamin D and its metabolites, are as important for the serum 25(OH)D level as the effect of season (10, 11, 13, 14). As these SNPs affect the serum 25(OH)D levels based on vitamin D produced in the skin or obtained from the diet, it is plausible that it should also affect the part of the 25(OH)D level that is produced from vitamin D taken as

supplements. If so, it might be necessary to take genetic factors into account when recommending vitamin D supplementation. Obese subjects as a group have lower serum 25(OH)D levels than non-obese subjects, possibly due to a larger volume of distribution or degradation of vitamin D in adipose tissue (15, 16, 17). Obesity may therefore also be a factor to consider when vitamin D supplementation is needed.

We have recently performed three intervention studies with high-dose vitamin D supplementation and also genotyped the subjects with regard to vitamin D SNPs. We therefore had the opportunity to test the influence of these SNPs as well as obesity on the serum 25(OH)D response to vitamin D supplementation.

Subjects and methods

Study population

In this study, we have pooled data from three intervention studies:

- The vitamin D and bone density study including postmenopausal women aged 50–80 years old, with a *T*-score in total hip or lumbar spine (L2–L4) ≤ -2.0 , and randomized to vitamin D 20 000 IU twice/week vs placebo for 1 year. In addition, all subjects were given daily supplements with 1 g calcium and 800 IU vitamin D (ClinicalTrials.gov NCT00491920) (18).
- The vitamin D and insulin sensitivity study where subjects aged 30–75 years old with serum 25(OH)D < 50 nmol/l were included and randomized to vitamin D 20 000 IU twice/week vs placebo for 6 months (ClinicalTrials.gov NCT00809744) (19).
- The vitamin D and depression study comprising subjects aged 30–75 years old, with serum 25(OH)D ≤ 55 nmol/l measured in the sixth Tromsø study, and randomized to vitamin D 20 000 IU twice/week vs placebo for 6 months (ClinicalTrials.gov NCT00960232) (20).

Measurements

Height and weight were measured wearing light clothing and no shoes. BMI was calculated as weight divided by height squared. Serum PTH was measured as described previously (18). Serum 25(OH)D was measured with an LC-MSMS method (19). This assay has a within-day precision (coefficient of variation (CV)) $< 3.2\%$ and between-day precision (CV) $< 8.8\%$. The laboratory takes part in the external quality program DEQAS. All genotyping was performed by KBioscience (<http://www.kbioscience.co.uk>) using KASP SNP genotyping system. KASP is a competitive allele-specific PCR incorporating a FRET quencher cassette (21).

Selection of SNPs for analysis

Based on the reports from GWAS where several SNPs have been related to the serum 25(OH)D level (10, 11, 12), we have in a previous study selected within each gene the one SNP with the highest difference in serum 25(OH)D between major and minor homozygotes: rs2282679 located at the DBP gene (*DBP* or *GC*); rs10741657 at the 25-hydroxylase gene (*CYP2R1*) involved in the conversion of vitamin D into 25(OH)D; rs3829251 at the 7-dehydrocholesterol (7-DHC) reductase/NAD synthetase 1 gene (*DHCR7/NADSYN1*) responsible for the availability of 7-DHC in the skin; and rs6013897 in the 24-hydroxylase gene (*CYP24A1*) involved in the degradation of 25(OH)D (21). In addition to these four SNPs, rs7041 and rs4588 (at the *DBP/GC* gene) were included in the study as the effect of these two SNPs on the serum 25(OH)D response to vitamin D substitution has previously been published (22).

Statistical analyses

Distribution of the dependent variables serum 25(OH)D, delta 25(OH)D (serum 25(OH)D value at 6 months minus value at baseline), and BMI was evaluated for skewness and kurtosis and by visual inspection of histograms and found normal. Trends across the genotypes were evaluated with linear regression with age, gender, BMI, and season (summer/winter) as covariates where appropriate. The summer months were defined as May through September. Correlations were tested with Pearson's correlation coefficient *r*. The genotype frequencies were examined for compliance with Hardy–Weinberg equilibrium using χ^2 analysis (23). Linkage disequilibrium (LD) between SNPs was evaluated with r^2 and Lewontin's *D'* statistics (24). Based on the β coefficients from regression analyses with serum 25(OH)D as dependent variable, the risk alleles (the ones associated with low serum 25(OH)D levels) were weighted and a genetic risk score constructed (10, 21). A *P* value < 0.05 was considered statically significant. The data are presented as mean (s.d.) unless otherwise specified.

Ethics

The study was approved by the Regional Committee for Medical and Health Research Ethics. Only participants with valid written consent were included.

Results

rs4588 was in LD with rs2282679 ($r^2 = 0.98$) and therefore excluded. The other SNPs were not in LD and were also in Hardy–Weinberg equilibrium (χ^2 testing $P > 0.05$). In the bone density, insulin sensitivity, and

depression studies, 258, 91, and 219 subjects respectively completed the intervention and had complete data sets including genotyping for the three principal SNPs (see below). All but five subjects were of Northern European ancestry. In the bone density study and the insulin sensitivity study, ~2/3 of the subjects were included during the winter months, whereas in the depression study, all subjects were included during the winter. As expected, the serum 25(OH)D levels were higher in samples drawn during the summer than in the winter months in both the bone density and the insulin sensitivity studies (the two studies with inclusion throughout the year), but the mean difference was only 5 nmol/l (NS; Table 1).

There was no statistically significant relationship between the genotypes and age, BMI (except for BMI and rs3829251), and serum PTH in the three studies together, or between the genotypes and gender in the insulin sensitivity and depression studies. However, there was a significant effect of genotype for rs2282679, rs10741657, and rs7041 on serum 25(OH)D with a difference between the major and minor homozygote genotypes for rs2282679 of 15.4 nmol/l, for rs7041 of 13.7 nmol/l, and for rs10741657 of 5.8 nmol/l. Similar results were seen when samples drawn during the winter or summer were analyzed separately. For the other SNPs, no such significant trend was seen. As expected, there was a clear difference between the genotype risk quartiles regarding serum 25(OH)D but not regarding age or BMI (Table 1). As previously published, the intake of vitamin D in the three studies was safe with no serious side effects, and in all three studies, there was a significant decrease in serum PTH after vitamin D supplementation (18, 19, 20).

Effect of genotype on serum 25(OH)D and PTH after vitamin D supplementation

In the bone density, insulin sensitivity, and depression studies, 127, 47, and 111 subjects respectively were randomized to vitamin D and these subjects were included in the following. In Table 2, the baseline, 6 months, and delta serum 25(OH)D levels (value at 6 months minus baseline) are shown for the three SNPs rs2282679, rs10741657, and rs7041 in relation to genotypes and also for the genotype score quartiles. For all these SNPs, the genotype with the highest baseline 25(OH)D level had the highest increase (delta) in serum 25(OH)D after vitamin D substitution. This was statistically significant for rs2282679 and rs10741657 with a difference in delta value between the genotype with the highest and lowest baseline 25(OH)D level of 15.5 nmol/l for rs2282679 and 15.9 nmol/l for rs10741657. Similar results were seen for the genotype score quartiles with a difference in delta values between quartile 1 (the quartile with the highest baseline serum 25(OH)D) and quartile 4 of 21.4 nmol/l (Table 2).

There was for all genotypes a decrease in serum PTH after vitamin D supplementation. For rs2282679, the decrease in serum PTH was significantly related to genotype, with the major homozygote (which was associated with the highest increase (delta) in serum 25(OH)D) having the largest decrease (delta) in serum PTH (Table 3).

Effect of baseline level on serum 25(OH)D after vitamin D supplementation

There was a highly significant negative correlation between baseline serum 25(OH)D and increase (delta) in serum 25(OH)D ($r = -0.25$, $P < 0.001$). In Table 4, serum 25(OH)D after 6 months and delta 25(OH)D values are shown according to baseline 25(OH)D levels. Those with lowest baseline levels had the highest increase (delta) in serum 25(OH)D. However, the 6-month 25(OH)D values were still substantially lower in those starting < 40 nmol/l when compared with those starting > 69 nmol/l. In trying to eliminate the effect of genetics on baseline values, we selected a group that was heterozygote for both rs2282679 and rs7041 (the two SNPs with the largest difference in 25(OH)D between the genotypes at baseline). In this cohort, the difference in delta values between those with low and high baseline 25(OH)D was even higher than when all genotypes were analyzed together, but the 6-month values were still lower in those with low baseline values (Table 4).

Effect of BMI on serum 25(OH)D after vitamin D supplementation

At baseline, there was a negative association between serum 25(OH)D and BMI, which became statistically significant after 6 months with vitamin D supplementation ($P < 0.001$; Table 4). There was a highly significant negative correlation between increase (delta) in serum 25(OH)D after 6 months and baseline BMI ($r = -0.30$, $P < 0.001$) and a significant decrease in delta 25(OH)D with increasing BMI quartiles ($P < 0.001$) (Table 4). In the bone density study that lasted for 1 year, the difference in serum 25(OH)D between the BMI quartiles after vitamin D supplementation was statistically significant after 3 months and increased slightly thereafter till the end of the intervention (Table 5).

Effects in the control groups

In the placebo groups in the insulin sensitivity and depression studies, no significant changes were seen in serum 25(OH)D (data not shown). In the control group in the bone density study where 800 IU vitamin D were given per day, similar but smaller changes in serum 25(OH)D were seen as in those given 6800 IU/day

Table 1 Baseline characteristics in relation to study and SNP genotypes.

	Baseline all subjects					Baseline winter		Baseline summer	
	<i>n</i>	Females (%)	Age (years)	BMI (kg/m ²)	Serum PTH (pmol/l)	Serum 25(OH)D (nmol/l)	<i>n</i>	Serum 25(OH)D (nmol/l)	<i>n</i>
Study									
All studies	568	74.5	57.0 (10.4)	26.2 (3.9)	5.18 (1.78)	56.9 (22.7)	447	54.2 (22.3)	121
Bone density study	258	100	63.0 (7.2)	24.7 (3.4)	5.08 (1.67)	70.5 (22.7)	165	68.7 (24.6)	93
Insulin sensitivity study	91	47.3	51.9 (9.1)	26.8 (3.0)	5.96 (1.58)	41.0 (13.1)	63	39.4 (12.8)	28
Depression study	219	55.7	51.9 (10.3)	27.7 (4.1)	4.98 (1.90)	47.5 (15.7)	219	47.5 (15.7)	
Vitamin D SNPs									
rs2282679 (GC)									
Major homozygote	270	80.7	57.0 (10.0)	25.9 (3.7)	5.19 (1.80)	61.9 (24.5)	209	58.7 (24.3)	61
Heterozygote	254	70.1	56.9 (10.7)	26.5 (4.0)	5.18 (1.75)	53.4 (20.3)	202	51.3 (20.0)	52
Minor homozygote	44	61.4	57.3 (11.1)	26.4 (4.2)	5.10 (1.88)	46.5 (16.4) [†]	36	43.3 (15.3)*	8
rs7041 (GC)									
Major homozygote	128	82.0	57.6 (10.2)	25.7 (3.7)	4.97 (1.42)	63.7 (24.8)	96	62.2 (25.4)	32
Heterozygote	302	73.8	56.8 (10.6)	26.5 (3.8)	5.29 (1.91)	57.2 (22.4)	235	54.1 (21.8)	67
Minor homozygote	138	68.8	56.7 (10.2)	26.0 (4.3)	5.12 (1.79)	50.0 (19.2) [†]	116	47.6 (18.0) [†]	22
rs10741657 (CYP2R1)									
Major homozygote	191	74.9	56.2 (9.9)	26.3 (4.1)	5.30 (1.82)	55.6 (21.9)	148	52.4 (20.9)	43
Heterozygote	278	73.8	57.7 (10.9)	26.0 (3.8)	5.14 (1.80)	56.2 (22.5)	217	53.2 (22.1)	61
Minor homozygote	99	75.8	56.3 (9.9)	26.3 (3.8)	5.04 (1.64)	61.4 (24.4)*	82	59.9 (24.2)*	17
rs3829251 (DHCR7/MADSYN) ^a									
Major homozygote	292	76.7	57.4 (10.0)	25.8 (4.0)	5.27 (1.84)	57.4 (22.6)	224	54.4 (22.2)	68
Heterozygote	227	71.8	56.1 (10.8)	26.5 (3.9)	5.06 (1.71)	55.3 (21.9)	183	52.3 (21.2)	44
Minor homozygote	44	75.0	58.3 (10.5)	27.2 (3.2)*	5.24 (1.79)	63.7 (26.2)	35	63.8 (26.0)	9
rs6013897 (CYP24A1) ^a									
Major homozygote	300	75.0	56.8 (10.6)	26.2 (3.8)	5.00 (1.75)	57.3 (22.3)	237	54.7 (21.8)	63
Heterozygote	226	73.0	57.2 (10.3)	26.1 (4.0)	5.37 (1.87)	56.1 (23.0)	176	52.6 (22.7)	50
Minor homozygote	37	75.7	56.1 (10.4)	26.2 (3.6)	5.38 (1.39)	59.0 (25.0)	31	59.2 (23.8)	6
Genotype score quartile									
Quartile 1	155	83.2	57.5 (10.3)	25.8 (3.7)	5.05 (1.69)	65.2 (25.7)	118	62.8 (26.2)	37
Quartile 2	129	76.7	56.3 (10.0)	26.3 (3.6)	5.27 (1.88)	57.4 (21.9)	101	54.1 (20.8)	28
Quartile 3	158	70.3	56.8 (10.8)	26.4 (4.0)	5.22 (1.83)	53.1 (19.9)	131	50.9 (19.0)	27
Quartile 4	126	66.7	57.1 (10.5)	26.5 (4.2)	5.17 (1.73)	51.1 (19.9) [†]	97	48.2 (19.5)	29

**P* < 0.05 and [†]*P* < 0.01; linear trend over SNP genotypes evaluated with age, gender, BMI, and season (summer/winter) as covariates.^aFive subjects had missing genotype data.

Table 2 Serum 25(OH)D at baseline, after 6 months, and delta values (value at 6 months minus baseline) in relation to vitamin D SNP genotypes in all subjects randomized to high-dose vitamin D supplementation.

Vitamin D SNP genotypes	<i>n</i>	Baseline serum 25(OH)D (nmol/l)	6-month serum 25(OH)D (nmol/l)	Delta serum 25(OH)D (nmol/l)
rs2282679 (<i>GC</i>)				
Major homozygote	138	61.3 (23.5)	169.9 (36.1)	108.6 (32.1)
Heterozygote	131	53.7 (22.1)	152.2 (28.3)	98.4 (29.3)
Minor homozygote	16	46.0 (15.5) [†]	139.2 (23.9) [†]	93.2 (21.6)*
rs7041 (<i>GC</i>)				
Major homozygote	70	62.5 (22.7)	169.9 (35.2)	107.5 (29.3)
Heterozygote	155	57.6 (24.1)	158.7 (32.2)	101.2 (31.7)
Minor homozygote	60	48.9 (17.3) [†]	151.9 (32.7)*	102.9 (30.1)
rs10741657 (<i>CYP2R1</i>)				
Major homozygote	96	55.9 (23.3)	152.8 (31.6)	96.9 (31.1)
Heterozygote	136	55.0 (20.5)	158.7 (31.2)	103.7 (28.9)
Minor homozygote	53	64.0 (26.6)	176.8 (37.4) [†]	112.8 (33.0) [†]
Genotype score quartile				
Quartile 1	78	64.5 (24.4)	175.4 (36.2)	110.9 (33.2)
Quartile 2	71	58.0 (22.2)	163.2 (33.6)	105.2 (29.6)
Quartile 3	80	52.4 (20.2)	155.6 (28.2)	103.1 (27.1)
Quartile 4	56	51.6 (22.6) [†]	141.1 (25.6) [†]	89.5 (30.0) [†]

* $P < 0.05$ and [†] $P < 0.01$; linear trend over SNP genotypes evaluated with age, gender, BMI, and season (summer/winter) as covariates.

regarding the effects of genotypes and BMI (data not shown). When dividing this control group according to the baseline serum 25(OH)D levels, the differences in 25(OH)D almost disappeared after the 800 IU supplementation, probably because many of the subjects with high baseline values were taking vitamin D supplements that were stopped at inclusion in the study (Table 6).

Discussion

In this study, we have shown that the serum 25(OH)D levels are genetically determined and that the increase in serum 25(OH)D after vitamin D supplementation is

related to genotype, baseline 25(OH)D level, and BMI. Furthermore, this was not restricted to a short 6-month intervention but persisted at least for 1 year as shown in the bone density study.

Our baseline results with a 15 nmol/l difference in serum 25(OH)D level between the major and minor homozygote genotypes for the *DBP* SNPs and a difference of 7.7 nmol/l for the *CYP2R1* SNP is in agreement with what have previously been published by other groups (10, 11, 12, 25), as is the observation that the serum 25(OH)D levels are inversely related to BMI (15, 26).

The serum 25(OH)D levels measured by immunoassays as well as LC-MSMS methods reflect the total

Table 3 Serum PTH at baseline, after 6 months, and delta values (value at 6 months minus baseline) in relation to vitamin D SNP genotypes in all subjects randomized to high-dose vitamin D supplementation.

Vitamin D SNP genotypes	<i>n</i>	Baseline serum PTH (pmol/l)	6-month serum PTH (pmol/l)	Delta serum PTH (pmol/l)
rs2282679 (<i>GC</i>)				
Major homozygote	138	5.25 (1.61)	4.00 (1.35)	-1.25 (1.29)
Heterozygote	131	5.17 (1.81)	4.24 (1.38)	-0.94 (1.31)
Minor homozygote	16	4.91 (1.47)	4.40 (1.56)	-0.51 (1.48)*
rs7041 (<i>GC</i>)				
Major homozygote	70	5.10 (1.49)	3.87 (1.32)	-1.23 (1.19)
Heterozygote	155	5.30 (1.78)	4.26 (1.41)	-1.04 (1.30)
Minor homozygote	60	5.02 (1.70)	4.09 (1.36)	-0.94 (1.50)
rs10741657 (<i>CYP2R1</i>)				
Major homozygote	96	5.46 (1.76)	4.25 (1.34)	-1.21 (1.39)
Heterozygote	136	5.05 (1.68)	4.07 (1.40)	-0.98 (1.18)
Minor homozygote	53	5.08 (1.60)	4.06 (1.40)	-1.02 (1.52)
Genotype score quartile				
Quartile 1	78	4.97 (1.53)	3.86 (1.20)	-1.11 (1.33)
Quartile 2	71	5.50 (1.69)	4.17 (1.52)	-1.34 (1.28)
Quartile 3	80	5.13 (1.76)	4.18 (1.46)	-0.95 (1.12)
Quartile 4	56	5.21 (1.81)	4.39 (1.28)	-0.81 (1.57)

* $P < 0.05$; linear trend over SNP genotypes evaluated with age, gender, BMI, and season (summer/winter) as covariates.

Table 4 Serum 25(OH)D at baseline, after 6 months, and delta values (value at 6 months minus baseline) in relation to baseline serum 25(OH)D level in all subjects and in subjects heterozygote for rs2282679 and rs7041 randomized to high-dose vitamin D supplementation.

Subjects	n	Baseline serum 25(OH)D (nmol/l)	6-month serum 25(OH)D (nmol/l)	Delta serum 25(OH)D (nmol/l)
All subjects randomized to high-dose vitamin D				
Baseline serum 25(OH)D <40 nmol/l	70	32.3 (5.8)	145.1 (31.3)	112.9 (30.8)
Baseline serum 25(OH)D 40–54 nmol/l	82	46.8 (4.6)	151.8 (28.3)	105.1 (28.5)
Baseline serum 25(OH)D 55–69 nmol/l	60	61.7 (4.3)	160.0 (30.8)	98.3 (30.5)
Baseline serum 25(OH)D >69 nmol/l	73	88.2 (16.5)	183.5 (31.0)*	95.4 (31.3)*
Subjects heterozygote for rs2282679 and rs7041				
Baseline serum 25(OH)D <40 nmol/l	25	31.6 (6.9)	143.7 (27.9)	112.0 (26.3)
Baseline serum 25(OH)D 40–54 nmol/l	33	46.6 (4.6)	148.7 (28.7)	102.3 (28.8)
Baseline serum 25(OH)D 55–69 nmol/l	24	60.6 (3.7)	153.5 (24.5)	92.9 (24.2)
Baseline serum 25(OH)D >69 nmol/l	21	90.5 (19.8)	169.8 (28.2)	79.3 (34.4)*
All subjects randomized to vitamin D				
Baseline BMI <22.5 kg/m ²	49	63.2 (25.2)	181.4 (32.8)	118.2 (34.9)
Baseline BMI 22.5–24.9 kg/m ²	68	59.7 (26.1)	169.2 (34.2)	109.4 (31.2)
Baseline BMI 25.0–27.4 kg/m ²	64	57.9 (20.6)	156.5 (28.9)	98.6 (27.2)
Baseline BMI 27.5–29.9 kg/m ²	56	52.0 (20.3)	148.9 (29.5)	96.9 (29.0)
Baseline BMI >29.9 kg/m ²	48	51.2 (19.3)	143.1 (29.0)*	91.9 (25.2)*

* $P < 0.001$; linear trend over serum 25(OH)D and BMI groups with age, gender, and BMI (where appropriate) as covariates.

amount of 25(OH)D consisting of the DBP bound, the albumin bound, and the free 25(OH)D fraction. As the part bound to DBP is by far the largest (27), it is no surprise that polymorphisms in the *DBP* could also affect the serum 25(OH)D level. Similarly, activating (or inactivating) polymorphisms in the *CYP2R1* gene, which is involved in the hydroxylation of vitamin D to 25(OH)D, are also easy to envision as of importance for the 25(OH)D level. On the other hand, there is no consensus why obesity should affect the serum 25(OH)D level. This could be due to reduced intake of vitamin D, reduced sun exposure, increased storage and/or sequestration of vitamin D in adipose tissue, or simply to an increased distribution volume for vitamin D (16, 28, 29).

Although there are several reports on the effect of SNPs on basal serum 25(OH)D levels, the importance of these SNPs for the response to vitamin D substitution is sparse. Fu *et al.* (22) included 98 adults in a 1-year vitamin D intervention and related the increase in serum 25(OH)D to the rs4588 SNP genotypes (which in our study was in LD with rs2282679). They found the proportional (percentage) as well as absolute increase

(delta) in mean 25(OH)D after vitamin D supplementation to be highest in those with the minor homozygote genotype, the group which also had the lowest baseline levels. In our study, the increase (delta) in serum 25(OH)D was highest in the *DBP* genotypes with the highest baseline levels, but there were only four subjects with this genotype in the study by Fu *et al.* (22), which may explain the opposing results. In a recent study by Engelman *et al.* (30), the association between serum 25(OH)D levels and SNPs in the *GC (DBP)* and *CYP2R1* genes were highly significant in samples drawn during the summer months and in subjects with high vitamin D intake (high exposure), but not in samples drawn during the winter and in subjects with low vitamin D intake (low exposure). This is in agreement with our results where the differences in serum 25(OH)D levels between the genotypes were most pronounced after 6 months with vitamin D supplementation.

It is unlikely that lifestyle factors (like diet or sun exposure) should be related to genotype, and the genetic effects on serum 25(OH)D are most likely due to differences in vitamin D metabolism. These metabolic

Table 5 Serum 25(OH)D at baseline and after 3, 6, 9, and 12 months in 127 postmenopausal women in the bone density study randomized to high-dose vitamin D supplementation.

Baseline BMI (kg/m ²)	n	Serum 25(OH)D (nmol/l)				
		Baseline	3 months	6 months	9 months	12 months
<22.5	33	73.5 (23.1)	178.0 (29.5)	186.4 (29.8)	199.1 (36.7)	197.8 (33.4)
22.5–24.9	36	73.8 (25.3)	173.6 (30.0)	184.9 (32.7)	194.5 (33.3)	192.0 (37.4)
25.0–27.4	30	68.2 (21.6)	164.0 (23.8)	168.3 (3.1)	176.5 (27.4)	177.1 (32.8)
27.5–29.9	16	69.1 (23.6)	155.8 (20.8)	164.2 (34.2)	174.7 (22.5)	174.9 (28.0)
>29.9	12	62.5 (20.2)	161.7 (22.0)*	171.3 (19.4)*	181.0 (28.7) [†]	172.9 (28.4) [†]

* $P < 0.05$ and [†] $P < 0.01$; linear trend over BMI groups with age as covariate.

Table 6 Serum 25(OH)D at baseline, after 6 months, and delta values (value at 6 months minus baseline) in relation to baseline serum 25(OH)D level in the subjects in the bone density study that were given 800 IU vitamin D/day.

Subjects	n	Baseline serum 25(OH)D (nmol/l)	6-month serum 25(OH)D (nmol/l)	Delta serum 25(OH)D (nmol/l)
Baseline serum 25(OH)D < 40 nmol/l	10	33.2 (5.1)	91.2 (11.5)	58.0 (13.5)
Baseline serum 25(OH)D 40–54 nmol/l	25	48.0 (4.1)	81.6 (16.3)	33.7 (16.3)
Baseline serum 25(OH)D 55–69 nmol/l	32	62.6 (4.0)	81.2 (15.8)	18.6 (15.7)
Baseline serum 25(OH)D > 69 nmol/l	64	88.8 (14.7)	103.7 (27.4)	14.9 (25.1)*

* $P < 0.001$; linear trend over serum 25(OH)D groups with age and BMI as covariates.

differences probably affect vitamin D coming from the skin and the ordinary diet in a similar way as vitamin D given as a supplement. Accordingly, subjects with a genetic potential for a high basal serum 25(OH)D level also have the greatest potential for increasing the serum 25(OH)D level after vitamin D supplementation. This is particularly so since the 25(OH)D level is mainly the result of vitamin D supply in contrast to the active form 1,25(OH)₂D, which is under strict regulation by PTH (1). This reasoning may not apply to differences in serum 25(OH)D due to the SNPs affecting the vitamin D production in the skin. However, polymorphisms in this system appears to affect the 25(OH)D level to a much lesser degree than the SNPs included in this study (10).

In addition to genotype, we confirmed previous observations that baseline serum 25(OH)D predicted the response to vitamin D supplementation (31, 32). Thus, those with lower baseline levels had the highest increase (delta). This relationship was to be expected as the main reason for low serum 25(OH)D levels is not genetic differences but differences in lifestyle (33). Therefore, those with low baseline levels have a greater potential for increase as the supplementation will make up for the effect of an unhealthy life style. This was further illustrated in the cohort where we tried to minimize the genetic effect by only including subjects heterozygote for two of the main SNPs affecting the serum 25(OH)D levels (rs2282679 and rs7041). And as expected, those with low baseline levels had a particularly high increase (delta) in serum 25(OH)D. However, it should be borne in mind that this observation could in part reflect a regression toward the mean.

And finally, we found BMI to affect the increase in serum 25(OH)D after supplementation as we and others have demonstrated before (15, 26). This was probably due to the same mechanism(s) causing low baseline 25(OH)D levels in obese subjects. Of particular note was that this effect of obesity did not diminish during a 12-month supplementation period, indicating that a fairly steady state with serum 25(OH)D levels lower than those in lean subjects is reached within 3–6 months of supplementation.

These findings implicate that in order to achieve a specific serum 25(OH)D target, the amount of vitamin D supplementation needed depends on genotype, basal level, as well as BMI. It is at present not certain what is the optimal 25(OH)D level nor how large the therapeutic window is. If this window is narrow, tailoring of supplementation will be of great importance and dose adjustments have to be made. However, it is more likely that the window is rather wide as it has been difficult to show a clinical effect of increasing the serum 25(OH)D level from 50–70 nmol/l to close to 150 nmol/l (18, 19, 20, 34, 35, 36). Furthermore, it should be considered that what we are presently measuring is the total serum 25(OH)D level, and it is possible that the free or biologically active fraction is what should be considered as demonstrated by Powe *et al.* (37) regarding the relationship between 25(OH)D and bone mass density. Whether the free fraction also differs between the different SNPs in the same way as total 25(OH)D differs remains to be settled.

This study has some weaknesses. We pooled data from three different studies, and due to different study profiles, some heterogeneity would be expected, and only for the bone density study had we more than one measurement after starting the vitamin D supplementation. We did not have DBP measurements and it would have been of interest to know to what extent the SNPs correlate with this parameter, and thereby the effects on free/bioavailable serum 25(OH)D. In this regard, it should also be noted that there was no relationship between the vitamin D SNPs and baseline serum PTH, which may indicate that the total serum 25(OH)D as measured in our study not necessarily reflects the biologically active part of 25(OH)D in serum. On the other hand, after vitamin D supplementation where the effect of genotype on serum 25(OH)D was more pronounced, there was at least for rs2282679 a relationship between genotype and decrease in serum PTH. Furthermore, the study was performed at a latitude of 69° north and the results are therefore primarily applicable to populations with very low sun exposure. Our study also has strength in that we included a large group of subjects and the results were

consistent and in accordance with present knowledge of vitamin D metabolism. It is also noteworthy that the high doses given by us appeared to be safe at least for a 6- to 12-month period.

In conclusion, we have found a strong association between genetic polymorphisms and basal levels of serum 25(OH)D and response to supplementation. However, the clinical importance of this observation remains to be determined.

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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