Serum PTH is not a good marker for defining a threshold for vitamin D deficiency

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Abstract

Objective: In addition to its skeletal effects, vitamin D may also be important for health in general. It is uncertain what level of serum 25-hydroxyvitamin D (25(OH)D), marker of vitamin D status, is sufficient for these effects. With decreasing serum 25(OH)D levels there is an increase in serum PTH. The point at which this occurs has been considered as a threshold for vitamin D sufficiency. The thresholds found have varied widely and have mainly been based on observational studies. However, to truly establish a threshold for vitamin D effects, this has to be based on randomized controlled trials (RCTs).

Methods: 2803 subjects from a general health survey, the Tromsø study, and pooled individual person data from five vitamin D intervention studies (n = 1544) were included. Serum parathyroid hormone (PTH) and change in PTH after vitamin D supplementation was related to serum 25(OH)D levels in steps of 25 nmol/L (< 24, 25-49, 50-74, 75-99, > 99 nmol/L).

Results: In the Tromsø study there was in the females a gradual decrease in serum PTH with increasing serum 25(OH)D with no apparent plateau, whereas in the males the decrease in PTH in subjects with serum 25(OH)D > 74 nmol/l was marginal. In pooled RCTs, there was a significant reduction in serum PTH by vitamin D supplementation regardless of baseline serum 25(OH)D level.

Conclusions: The use of the serum PTH – 25(OH)D relation from observations studies to determine a threshold for vitamin D sufficiency is highly questionable.
Introduction

Vitamin D is essential for intestinal calcium absorption and skeletal health. Vitamin D deficiency in children may lead to rickets, which can be prevented and/or treated by vitamin D supplementation (1). In addition to its importance in calcium metabolism and bone health, vitamin D may potentially also have a number of other health effects. Thus, low serum levels of 25-hydroxyvitamin D (25(OH)D), which is the currently accepted marker of vitamin D status, are associated with development of cancer, cardio-vascular diseases (CVD), diabetes, infections and immunological diseases (2). However, most intervention studies have so far not been able to prove positive effects of vitamin D supplementation (3, 4), except perhaps in those with very low baseline serum 25(OH)D levels (5).

During the last decade there has been considerable debate as to which serum 25(OH)D level is sufficient or optimal. The institute of medicine (IOM) has recommended that a level of 50 nmol/L is sufficient (6), whereas a level of 75 nmol/L has been recommended by the Endocrine Society (7). These recommendations are partly based on calcium absorption, presence of signs of osteomalacia, as well suppression of serum parathyroid hormone (PTH) by vitamin D (6-8).

Since the serum PTH secretion is mainly regulated by the serum calcium level, where a low calcium level simulates PTH secretion and a high level inhibits, vitamin D may suppress serum PTH levels indirectly by its effect on intestinal calcium absorption. Vitamin D may also have a direct effect on PTH synthesis and secretion since the active form of vitamin D, 1,25-dihydroxyvitamin D (1,25(OH)D), represses the transcription of PTH as well as the expression of the calcium-sensing receptor (9). Accordingly, a reverse relation between serum PTH and 25(OH)D has been repeatedly demonstrated, and most studies find a sharp increase in serum PTH in the lowest serum 25(OH)D ranges (10-12). The serum 25(OH)D level where
this PTH increase starts has by many been taken as a threshold level for sufficient serum 25(OH)D.

This threshold level has been reported as low as 30 nmol/L and as high as 100 nmol/L (4), and there are even studies where such a threshold or plateau has not been found at all (13). Most of these studies have been cross-sectional, and to truly define a threshold where a higher serum 25(OH)D level will not lead to further PTH suppression, this has to be based on randomized controlled trials (RCTs) (4). Numerous vitamin D RCTs have included serum 25(OH)D and PTH measurements (14), but to our knowledge, none that has specifically evaluated the effect of vitamin D supplementation on serum PTH in subjects with high baseline serum 25(OH)D levels.

At the Clinical Research Unit, University Hospital of North Norway, we have performed a number of vitamin D RCTs and thus have a huge database on PTH responses to vitamin D supplementation. Furthermore, the seventh survey of the Tromsø study was recently performed where > 3000 subjects had both serum 25(OH)D and PTH measured. We therefore had the opportunity to evaluate the PTH – 25(OH)D relation both from a cross-sectional and interventional point of view.

Materials and methods

Subjects

Individual participant data from the seventh survey of the Tromsø study and six intervention studies performed in Tromsø, Northern Norway, are used in the present analyses. These studies have been described in detail before and are summarized in short below.
The Tromsø study is a population-based health survey performed for the first time in 1974 in Tromsø, Northern Norway at 69° North. The seventh survey was performed in 2015/2016. All citizens aged 40 years and above and living in the municipality of Tromsø were invited, and 21,083 (64.7%) attended (15, 16). Serum PTH was measured in a subgroup of 3,769 subjects who also had attended the fourth survey in 1994/1995.

The Obesity study (performed 2005 – 2007) included 445 overweight subjects, randomized to vitamin D 40,000 IU per week, 20,000 IU per week or placebo for one year. All subjects were also given 500 mg calcium daily. The primary end point was weight reduction after one year. The subjects met to visits every third month (17).

The Depression study (performed 2008 – 2010) included 243 healthy subjects randomized to vitamin D 40,000 IU per week versus placebo for six months. The main endpoint was reduction in symptoms of depression evaluated with a range of depression inventories and scales (18). The study was registered at ClinicalTrials.gov (NCT00960232).

The Glucose clamp study (performed 2008 – 2010) included 108 healthy subjects randomized to vitamin D 40,000 IU per week versus placebo for six months. The main endpoint was change in insulin sensitivity and secretion as evaluate by a hyperglycemic glucose clamp technique (19). The study was registered at ClinicalTrial.gov (NCT00809744).

The Osteoporosis study (performed 2007 – 2010) included 297 postmenopausal women with bone mass density (BMD) T-score ≤ -2.0 in either lumbar spine or total hip. They were randomized to 6,500 IU vitamin D per day (20,000 IU twice per week + 800 IU per day) or 800 IU vitamin D per day (placebo + 800 IU per day) for one year and met to visits every third month. Both groups were given 1,000 mg elemental calcium per day. The primary endpoint was change in BMD (20).

The CVD risk factor study (performed 2015 – 2017) included 422 healthy subjects randomized to vitamin D (100,000 IU loading dose, followed by 20,000 IU per week) or
placebo for four months. The main endpoints were change in CVD risk factors (21). The study was registered at ClinicalTrials.gov (NCT02750293).

The Prevention of T2DM study (performed 2008 – 2015) included 511 subjects with prediabetes who were randomized to vitamin D 20,000 IU per week versus placebo for five years. The subjects met to visits annually. The main endpoint was progression to type 2 diabetes (T2DM) (22). The study was registered at ClinicalTrials.gov (NCT00685594).

**Measurements**

Serum 25(OH)D was determined in the Depression, Glucose clamp and Osteoporosis studies at the Hormone Laboratory, Haukeland University Hospital, Bergen, using an in-house-developed liquid chromatography double mass spectrometry (LC/MS-MS) method. All other biochemical analyses were performed at the Department of Medical Biochemistry, University Hospital of North Norway, Tromsø. Both laboratories take part in the external quality program DEQAS. Serum 25(OH)D in the Obesity study was determined by immunometry (electrochemiluminescence: ECLIA) using an automated clinical chemistry analyser (Modular E170, Roche Diagnostics), and in the three other studies by an in-house-developed LC/MS-MS method. Serum PTH in the Tromsø study and the CVD risk factor study was analysed with an electrochemiluminescence immunoassay (ECLIA) using an automated clinical chemistry analyser (Cobas 6000, Roche), and serum calcium by an automated analyser (Modular P, Roche Diagnostics, Mannheim, Germany) with reagents from Boehringer Mannheim. In the five other studies serum PTH was measured using an automated clinical chemistry analyzer (Immulite 2000, Siemens Healthcare Diagnostics, Los Angeles, CA, USA), and serum calcium with an automated analyser (Hitachi 917) with reagents from Boehringer Mannheim. Reference ranges for serum PTH were 1.1–6.8 pmol/L for those < 51 years and 1.1–7.5 pmol/L for those > 50 years, and for serum calcium 2.20 – 2.50 mmol/L.
Height and weight were measured wearing light clothing, and body mass index (BMI) calculated as kg/m².

**Statistical analyses**

Normal distribution was evaluated for skewness and kurtsosis and by visual inspection of histograms and found normal except for serum PTH which was normalized by log transformation before use as dependent variable. Comparisons between groups were performed with Student’s t-test or with a general linear model with adjustments as indicated in the text. Linear trends were evaluated with linear regression with adjustments as indicated in the tables. Only subjects who completed the studies and had valid serum 25(OH)D and PTH measurements were included. In the Prevention of T2DM two cohorts were analysed, all those who completed one year and those who completed five years.

The data are shown as mean ± SD. All tests were done two-sided, and a P-value < 0.05 was considered statically significant. The P-values are shown without corrections for multiple comparisons.

**Ethics**

All studies were approved by the Regional Committee for Medical and Health Research Ethics (REK Nord) and only participants with valid written consent were included.

**Results**

**The seventh survey of the Tromsø study**
Three thousand seven hundred sixty-nine subjects had valid serum 25(OH)D and PTH measurements. Among these, 2,803 (1,682 females and 1,121 males) had serum calcium in the range 2.20 – 2.50 mmol/L and serum creatinine < 100 µmol/L and were included in the analyses. When all subjects were analysed together, as well as in females and males separately, there was with increasing serum 25(OH)D level (in steps of 25 nmol/L) a significant increase in age and serum calcium, and a significant decrease in serum PTH and BMI (Table 1). The decrease in serum PTH was attenuated with higher serum 25(OH)D levels. However, when comparing those in the serum 25(OH)D group of 74 – 99 nmol/L with those in the group with serum 25(OH)D > 99 nmol/L and adjusting for age and BMI, the serum PTH levels were significantly (P < 0.01) lower in the latter group. This was also seen when analysing the females separately, whereas the difference in serum PTH between these two serum 25(OH)D groups was not statistically significant for males. However, when in males comparing the serum 25(OH)D 50 – 74 nmol/L group with the serum 25(OH)D 75 – 99 nmol/L group, the difference in serum PTH was statistically significant (P < 0.05) (Table 1). This apparent difference between females and males, as well as the increasingly higher PTH levels in the lower serum 25(OH)D ranges, are further illustrated in Figures 1 and 2 where the serum 25(OH)D levels between 20 and 100 nmol/L are divided in steps of 10 nmol/L. In the females there appeared to be a gradual decrease in serum PTH also with serum 25(OH)D > 50 nmol/L, whereas the corresponding decrease in the males was marginal.

Pooled intervention studies

The baseline characteristics of the subjects who completed the intervention and had valid serum 25(OH)D and PTH measurements are shown for each of the intervention studies in Table 2. When the Obesity (n = 332), Depression (n = 228), Glucose clamp (n = 93), CVD risk factors (n = 411) and Prevention of T2DM (n = 480 (subjects who completed 1 year of
the intervention)) studies were pooled together, and the subjects grouped according to baseline serum 25(OH)D level (< 25, 25-49, 50-74, 75-99, > 99 nmol/L), there was with increasing baseline serum 25(OH)D a significant decrease in baseline serum PTH and an increase in serum calcium (Table 3).

In those given vitamin D, the increase in mean serum 25(OH)D ranged from 70 nmol/L in those with baseline values < 25 nmol/L, to 32 nmol/L in those with baseline values > 99 nmol/L. In the placebo group, there was an increase in serum 25(OH)D in those with baseline values < 50 nmol/L and a decrease in those with higher baseline values (Table 3). As compared to those given placebo, there was an increase in serum calcium in those given vitamin D, which was statistically significant for those with baseline serum 25(OH)D 25 – 49 nmol/L (Table 3). In all the baseline serum 25(OH)D groups (< 25, 25-49, 50-74, 75-99, > 99 nmol/L), those given vitamin D had a significant reduction in serum PTH compared to those given placebo. This reduction was most pronounced in those with baseline serum 25(OH)D < 25 nmol/L (Table 3). To examine if this reduction in serum PTH by vitamin D supplementation was a result of the increase in serum calcium, the above analyses were also done with adjustment for change in serum calcium. This made the difference in delta PTH for those in the serum 25(OH)D group 75 – 99 nmol/L non-significant (P = 0.058), whereas the other significance levels did not change.

**Effects of time and vitamin D dose on the PTH suppression**

In the Prevention of T2DM study 227 subjects completed the five years intervention with valid serum 25(OH)D and PTH measurements. In the vitamin D group there was an increase in mean serum 25(OH)D of 50 – 60 nmol/L, whereas the serum 25(OH)D levels in the placebo group remained stable at baseline levels. There was a decrease in serum PTH in the vitamin D group compared to the placebo group, which was statistically significant after 1, 3,
4 and 5 years. After the decrease in serum PTH during the first year, the serum PTH levels in the vitamin D group remained stable till the end of the study (Table 4).

In the Obesity study 332 subjects completed the 1-year intervention with valid serum 25(OH)D and PTH measurements. In the group given vitamin D 40,000 IU per week mean serum 25(OH)D increased ~ 60 nmol/L, in the group given 20,000 IU per week the increase in serum 25(OH)D was~ 40 nmol/L, and remained stable at baseline levels in the placebo group. In both vitamin D groups, the maximal decrease in serum PTH occurred already after three months. The decrease in serum PTH was throughout the intervention significantly larger in the 40,000 IU per week as compared to the placebo group, but not as compared to the group given 20,000 IU per week (Table 5).

In the Osteoporosis study 273 women completed the 1-year intervention with valid measurements. In the group given vitamin D 45,600 IU per week serum 25(OH)D increased 100 – 115 nmol/L, whereas the increase in the group given 5,600 IU per week was ~ 20 nmol/L. At all three months intervals the decrease in serum PTH was significantly higher in those given 45,600 IU per week, and as in the Obesity study, the lowest PTH levels were seen already after three months (Table 6).

**Discussion**

In the cross-sectional part of the present study we have confirmed the well-known inverse relationship between serum 25(OH)D and PTH, but could not find a 25(OH)D level where the serum PTH stabilized or reached a plateau. In the intervention studies, the decrease in serum PTH by vitamin D supplementation was dose dependent and occurred within three months with no further suppression thereafter. And most importantly, the serum PTH level was
significantly suppressed by vitamin D supplementation even in subjects with serum 25(OH)D levels > 100 nmol/L.

There are numerous cross-sectional reports on thresholds for serum 25(OH)D suppression of serum PTH (10-12, 23-25). This has been estimated by advanced statistical methods (10, 12) as well as with simple visual curve inspection (12). The cohorts included have differed widely as have the results. As examples can be mentioned the large study by Saliba et al. including 19,172 subjects from an Israeli clinical laboratory database, where a serum 25(OH)D threshold of 79 nmol/L was found. However, after excluding subjects with hypercalcemia and reduced kidney function, the threshold was reduced to 46 nmol/L (11). In a study by Sohl et al. including 1,164 elderly subjects, the threshold for women was 68 nmol/L, whereas in men the threshold was 45 nmol/L. In the same study the 25(OH)D threshold was also affected by BMI, being 47 nmol/L in those with BMI < 25 kg/m², and 62 nmol/L in those with higher BMI (12). Among 1,258 Caucasians Wright et al. found a threshold of approximately 75 nmol/L, whereas in 423 African Americans the threshold was approximately 50 nmol/L (24). In a study by Durazo-Arvizu et al. including 387 subjects from an osteoporosis treatment/prevention study and using a three-phase model, two thresholds (or significant change in the serum PTH – 25(OH)D curve) of 30 and 70 nmol/L could be identified (10). And finally, in 500 adults of African ancestry, there was an almost linear relation between serum PTH and 25(OH)D in the serum 25(OH)D 25 – 150 nmol/L range (23).

Our observational study adds to this multitude of thresholds, or lack of such. In the males the decrease in serum PTH in those with serum 25(OH)D > 50 – 75 nmol/L was marginal, whereas in the females no plateau was reached, at least not below 100 nmol/L. Even if the ideal observational study on the serum PTH – 25(OH)D relation was performed in a population-based cohort where adjustments could be made for relevant factors like calcium
and magnesium intake, and where the number of subjects were large enough to do sub-group analyses in males and females, in young and old, in low- normal- and over-weight subjects, one would not be able to reliably identify a threshold (or lack of such) where no additional, meaningful PTH suppression by vitamin D would occur. This is simply because one cannot imply causality from observational data, and the 25(OH)D threshold, similar to health effects of vitamin D, has to be tested in RCTs (4, 26).

The effect of vitamin D on serum PTH in RCTs was systematically reviewed by Björkman et al. where 41 clinical trials were included, and the results suggested that serum PTH would decrease quite linearly during vitamin D supplementation at any given serum 25(OH)D level (14). However, in the majority of the studies the mean baseline serum 25(OH)D level was < 50 nmol/L, and no study had mean baseline serum 25(OH)D > 85 nmol/L. Individual person data (IPD) were not used in the analyses, and accordingly, the PTH – 25(OH)D relation in subjects with serum 25(OH)D > 85 nmol/L not investigated.

In our intervention studies we found that the serum PTH suppression by vitamin D appeared to reach its full effect after three months as demonstrated in the Obesity and Osteoporosis studies, and that even five years with continuously high serum 25(OH)D levels had no additional effect in the Prevention of T2DM study. Furthermore, we confirmed the importance of the vitamin D dose given (27), as the suppression of serum PTH in the Osteoporosis study was considerably higher with vitamin D 45,600 IU per week versus the 5,600 IU per week. On the other hand, there was no significant difference in PTH suppression between 40,000 IU per week versus 20,000 IU per week in the Obesity study. We therefore found it relevant to pool the baseline and end of study (or one year) IPD from the Obesity, Depression, Glucose clamp, CVD risk factors, and the Prevention of T2DM studies, even though different vitamin D doses and intervention times were used. With this approach the
cohort became sufficiently large to allow meaningful analyses of PTH suppression in a broad range of baseline serum 25(OH)D levels.

As expected, the suppression was most pronounced in those with low baseline serum 25(OH)D, who also had the highest baseline PTH, and also as expected (28), the highest increase in serum 25(OH)D after supplementation. However, there was a substantial and statistically significant decrease in serum PTH even in those with baseline serum 25(OH)D > 100 nmol/L. This reduction in serum PTH was statistically significant also after adjusting for the concomitant changes in serum calcium, indicating a direct effect by vitamin D on the PTH synthesis/secretion. Accordingly, it is very difficult to justify using cross-sectional data to identify a 25(OH)D threshold at a serum 25(OH)D level of ~50 nmol/L. In addition, serum PTH suppression is for vitamin D supplementation only a surrogate marker for health effects and should not be used to define vitamin D sufficiency.

Even though there are strong indications for extra-skeletal effects of vitamin D, like the wide tissue distributing of the vitamin D receptor (VDR) and the enzymes necessary for activation of vitamin D (1), together with all the favourable observational studies, firm evidence from RCTs is lacking (3, 29). Estimates of vitamin D thresholds based on extra-skeletal effects of vitamin D are therefore at present not relevant. For fractures, on the other hand, there appears to be fairly good evidence for a protective effect of combined use of calcium and vitamin D, but this has so far not been definitely related to a serum 25(OH)D level (29).

Serum PTH is the parameter most consistently associated with serum 25(OH)D both in cross-sectional and interventional studies. In spite of this, a wide range of 25(OH)D thresholds have been reported, which probably also will be the case for skeletal effects of vitamin D. It is also likely that such a serum 25(OH)D threshold will be affected by calcium intake, similar to what is seen for the risk of rickets in children where a high calcium intake is
protective (29). A high calcium intake increases the half-life of 25(OH)D, and may thus be good for the calcium economy (30). This may influence the 25(OH)D – PTH relation, as demonstrated by Patel et al. who found lower serum PTH values for given 25(OH)D concentrations in subjects with high calcium intake (31). A low dietary magnesium intake may also alter the 25(OH)D – PTH relationship, at least in overweight or obese subjects (32). Differences in calcium and magnesium intakes may therefore partly explain the wide range in 25(OH)D thresholds reported. Additionally, other factors like BMI, age, ethnicity and gender may influence the threshold, which may even differ between the endpoints used for threshold identification (12, 24). Finding a clinically relevant and trustworthy threshold for vitamin D sufficiency may therefore prove difficult.

Our study has several weaknesses. Although we had large cohorts both in the observational and interventional parts of the study, relatively few subjects had very high baseline serum 25(OH)D levels. We can therefore not draw conclusions about the serum PTH – 25(OH)D relation in subjects with serum 25(OH)D > ~125 nmol/L. Although we feel we have justified pooling the five intervention studies together, they were still different studies with different inclusion criteria and interventions. Furthermore, different laboratories and assays were used for the 25(OH)D and PTH measurements, without any formal examination of consistency between the analytic methods. This could be of particular importance for PTH where there is no standardization program, and we cannot rule out that this may have affected the results. On the other hand, our study also has several strengths. In the observational part we only included subjects with normal serum calcium and creatinine levels, and in the interventional part we examined the PTH – 25(OH)D relation over a broad range of serum 25(OH)D levels.
In conclusion, we have found that serum PTH is suppressed by vitamin D supplementation even if the baseline serum 25(OH)D is > 100 nmol/L. This argues against using serum PTH suppression to define vitamin D sufficiency.

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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**Legends to figures**

**Figure 1.** Mean serum PTH in relation to serum 25(OH)D in 1695 females in the seventh survey of the Tromsø study

**Figure 2.** Mean serum PTH in relation to serum 25(OH)D in 1124 males in the seventh survey of the Tromsø study
Table 1. Age, BMI, serum PTH and calcium in relation serum 25(OH)D levels in 2,803 subjects in the seventh survey of The Tromsø study

| Serum 25(OH)D (nmol/L) | < 25 | 25 – 49 | 50 – 74 | 75 – 99 | > 99 |
|------------------------|------|---------|---------|---------|------|
| All subjects (n)       | 23   | 459     | 1296    | 788     | 237  |
| Age (years)            | 66.0 ± 13.4 | 67.5 ± 11.0 | 69.6 ± 9.9 | 70.5 ± 8.9 | 71.4 ± 8.4* |
| BMI (kg/m²)            | 29.4 ± 6.3  | 28.7 ± 5.1  | 27.5 ± 4.5  | 26.6 ± 4.2  | 25.7 ± 3.9*  |
| Serum 25(OH)D (nmol/L) | 22 ± 3 | 41 ± 6  | 63 ± 7  | 85 ± 7  | 113 ± 13  |
| Serum PTH (pmol/L)     | 6.8 ± 2.7 | 6.3 ± 2.0 | 5.5 ± 1.7 | 5.3 ± 1.6 | 4.9 ± 1.5* |
| Serum calcium (mmol/L) | 2.37 ± 0.08 | 2.35 ± 0.07 | 2.36 ± 0.07 | 2.37 ± 0.07 | 2.38 ± 0.07* |
| Females (n)            | 12   | 226     | 760     | 517     | 167  |
| Age (years)            | 66.3 ± 14.5 | 67.5 ± 11.5 | 69.4 ± 10.3 | 70.5 ± 8.7 | 71.9 ± 8.3* |
| BMI (kg/m²)            | 31.7 ± 7.2  | 28.9 ± 5.8  | 27.4 ± 4.8  | 26.4 ± 4.5  | 25.6 ± 4.2*  |
| Serum 25(OH)D (nmol/L) | 21 ± 2 | 41 ± 7  | 64 ± 7  | 85 ± 7  | 113 ± 14  |
| Serum PTH (pmol/L)     | 7.4 ± 3.0 | 6.5 ± 2.2 | 5.7 ± 1.8 | 5.3 ± 1.6 | 4.9 ± 1.5* |
| Serum calcium (mmol/L) | 2.38 ± 0.07 | 2.35 ± 0.07 | 2.36 ± 0.07 | 2.37 ± 0.07 | 2.39 ± 0.06* |
| Males (n)              | 11   | 233     | 536     | 271     | 70   |
| Age (years)            | 65.6 ± 12.6 | 67.5 ± 10.6 | 69.8 ± 9.5 | 70.5 ± 9.2 | 70.2 ± 8.5* |
| BMI (kg/m²)            | 26.9 ± 4.0  | 28.6 ± 4.3  | 27.7 ± 4.0  | 26.8 ± 3.6  | 26.1 ± 3.2*  |
| Serum 25(OH)D (nmol/L) | 22 ± 4 | 41 ± 6  | 63 ± 7  | 85 ± 7  | 113 ± 10  |
| Serum PTH (pmol/L)     | 6.2 ± 2.4 | 6.1 ± 1.9 | 5.3 ± 1.6 | 5.1 ± 1.7 | 5.1 ± 1.5* |
| Serum calcium (mmol/L) | 2.37 ± 0.08 | 2.35 ± 0.07 | 2.36 ± 0.07 | 2.36 ± 0.07 | 2.37 ± 0.07* |

*P < 0.01 (linear trend, adjusted for age and BMI)
Table 2. Baseline characteristics of the six intervention studies in those who completed the study and had valid serum 25(OH)D and PTH measurements

| Study                  | Females/Males (n/n) | Age (years) | BMI (kg/m²) | Serum 25(OH)D (nmol/L) | Serum PTH (pmol/L) | Serum calcium (mmol/L) |
|------------------------|---------------------|-------------|-------------|-------------------------|--------------------|------------------------|
| Obesity                | 203/129             | 49.2 ± 11.2 | 34.6 ± 3.9  | 54 ± 27                 | 5.4 ± 1.7          | 2.31 ± 0.10            |
| Depression             | 128/100             | 51.8 ± 10.2 | 27.7 ± 4.1  | 48 ± 16                 | 5.0 ± 1.9          | 2.28 ± 0.08            |
| Glucose clamp          | 45/48               | 52.2 ± 9.2  | 26.8 ± 3.0  | 41 ± 13                 | 5.2 ± 1.5          | 2.31 ± 0.09            |
| Osteoporosis           | 273/0               | 63.1 ± 7.1  | 24.7 ± 3.4  | 71 ± 23                 | 5.1 ± 1.7          | 2.36 ± 0.08            |
| CVD risk factors       | 192/219             | 52.0 ± 8.7  | 27.9 ± 4.9  | 34 ± 12                 | 6.7 ± 2.0          | 2.27 ± 0.07            |
| Prevention of T2DM*    | 183/297             | 62.0 ± 8.6  | 29.9 ± 4.3  | 61 ± 22                 | 5.7 ± 2.2          | 2.31 ± 0.08            |
| Prevention of T2DM**   | 82/145              | 62.2 ± 8.1  | 29.3 ± 3.8  | 63 ± 22                 | 5.6 ± 2.3          | 2.31 ± 0.08            |
| All studies            | 1,024/793           | 55.8 ± 10.8 | 29.1 ± 5.2  | 52 ± 22                 | 5.7 ± 2.0          | 2.31 ± 0.09            |

*Subjects who completed one year of the intervention
** Subjects who completed five years of the intervention
Table 3. Baseline and change (delta values) in serum 25(OH)D, PTH and calcium in relation to intervention and baseline serum 25(OH)D level, the Obesity, Depression, Glucose clamp, CVD risk factors and Prevention of T2DM studies pooled together.

| Group       | Baseline serum 25(OH)D (nmol/L) | < 25 | 25-49 | 50-74 | 75-99 | > 99 |
|-------------|---------------------------------|------|-------|-------|-------|------|
| Number of subjects ‡ | Vitamin D | 24/43 | 196/214 | 136/133 | 45/33 | 5/7 |
| (females/males) | Placebo | 28/34 | 162/168 | 115/130 | 33/24 | 7/7 |
| Baseline serum 25(OH)D (nmol/L) | Vitamin D | 20 ± 4 | 38 ± 7 | 60 ± 7 | 83 ± 7 | 122 ± 24 |
| | Placebo | 20 ± 4 | 38 ± 7 | 60 ± 7 | 84 ± 7 | 113 ± 15 |
| Delta serum 25(OH)D (nmol/L) | Vitamin D | 67 ± 26*** | 67 ± 33*** | 53 ± 29*** | 48 ± 29*** | 32 ± 32***†† |
| | Placebo | 7 ± 9 | 3 ± 13 | -1 ± 17 | -7 ± 17 | -16 ± 17†† |
| Baseline serum PTH (pmol/L) | Vitamin D | 7.1 ± 2.5 | 5.9 ± 2.1 | 5.5 ± 1.9 | 4.8 ± 1.6 | 5.5 ± 2.1†† |
| | Placebo | 7.2 ± 2.3 | 6.2 ± 2.2 | 5.3 ± 1.8 | 4.9 ± 1.7 | 4.2 ± 1.0†† |
| Delta serum PTH (pmol/L) | Vitamin D | -1.4 ± 1.8*** | -0.6 ± 1.4*** | -0.7 ± 1.5*** | -0.3 ± 1.5* | -0.8 ± 1.6† |
| | Placebo | 0.0 ± 1.5 | 0.3 ± 1.7 | 0.3 ± 1.6 | 0.2 ± 0.9 | 0.5 ± 1.0 |
| Baseline serum calcium (mmol/L) | Vitamin D | 2.28 ± 0.07 | 2.29 ± 0.08 | 2.30 ± 0.10 | 2.31 ± 0.08 | 2.34 ± 0.07† |
| | Placebo | 2.27 ± 0.07 | 2.28 ± 0.08 | 2.31 ± 0.09 | 2.31 ± 0.08 | 2.30 ± 0.06†† |
| Delta serum calcium (mmol/L) | Vitamin D | 0.01 ± 0.07 | 0.00 ± 0.09** | -0.02 ± 0.09 | -0.02 ± 0.10 | -0.07 ± 0.05*†† |
| | Placebo | 0.00 ± 0.07 | -0.02 ± 0.09 | -0.03 ± 0.08 | -0.04 ± 0.09 | -0.02 ± 0.07 |

Delta values are end of study values minus baseline (for the Prevention of T2DM study 1-year values minus baseline)

*P < 0.05; **P < 0.01; ***P < 0.001 (versus placebo group, Student’s t-test)

†P < 0.01; ††P < 0.001 (linear trend)

‡ Subjects from the Obesity, Depression, Glucose clamp, CVD risk factors and Prevention of T2DM pooled together. Only subjects who completed the intervention with valid serum 25(OH)D and PTH measurements are included

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Table 4. Serum 25(OH)D, PTH and delta PTH in 116 subjects given vitamin D and 111 placebo and who completed five years intervention in the prevention of T2DM study

|                | Serum 25(OH)D (nmol/L) | Serum PTH (pmol/L) | Delta serum PTH (pmol/L)† |
|----------------|------------------------|--------------------|---------------------------|
|                | Vitamin D              | Placebo            | Vitamin D                 | Placebo                   | Vitamin D | Placebo |
| Baseline       | 63 ± 23                | 63 ± 21            | 5.7 ± 2.1                 | 5.5 ± 2.4                 |           |         |
| 1 year         | 110 ± 30***            | 65 ± 22            | 5.2 ± 1.6                 | 5.7 ± 1.9                 | -0.6 ± 1.5* | 0.2 ± 1.7 |
| 2 years        | 114 ± 24**             | 64 ± 19            | 5.5 ± 2.0                 | 5.7 ± 1.7                 | -0.2 ± 1.5 | 0.2 ± 1.8 |
| 3 years        | 118 ± 26**             | 66 ± 20            | 5.4 ± 1.9                 | 6.0 ± 2.3                 | -0.3 ± 1.6** | 0.5 ± 1.5 |
| 4 years        | 120 ± 26**             | 65 ± 19            | 5.3 ± 2.1                 | 5.7 ± 1.9                 | -0.4 ± 1.6* | 0.3 ± 2.0 |
| 5 years        | 122 ± 25**             | 67 ± 19            | 5.2 ± 1.7                 | 5.7 ± 2.3                 | -0.5 ± 1.4** | 0.2 ± 1.4 |

† Annual serum PTH value minus baseline PTH
*P < 0.01; **P < 0.001 (versus placebo group, Student’s t-test)
Table 5. Serum 25(OH)D, PTH and delta PTH in the 332 subjects who completed one year intervention in the obesity study

|                              | Baseline | 3 months | 6 months | 9 months | 12 months |
|------------------------------|----------|----------|----------|----------|-----------|
| **Serum 25(OH)D (nmol/L)**   |          |          |          |          |           |
| Vitamin D 40,000 IU per week (n=114) | 56 ± 16  | 108 ± 22***† | 117 ± 23***† | 121 ± 28***† | 116 ± 27***† |
| Vitamin D 20,000 IU per week (n=106) | 53 ± 19  | 88 ± 20***  | 93 ± 21***  | 95 ± 21***  | 91 ± 22***  |
| Placebo (n=112)              | 53 ± 16  | 56 ± 17  | 59 ± 16  | 56 ± 15  | 51 ± 14  |

| **Serum PTH (pmol/L)**       |          |          |          |          |           |
| Vitamin D 40,000 IU per week (n=114) | 5.1 ± 1.6 | 3.4 ± 1.2 | 3.5 ± 1.3 | 3.6 ± 1.4 | 4.2 ± 1.5 |
| Vitamin D 20,000 IU per week (n=106) | 5.5 ± 1.8 | 4.0 ± 1.7 | 4.1 ± 1.6 | 4.2 ± 1.6 | 4.7 ± 1.7 |
| Placebo (n=112)              | 5.6 ± 1.6 | 4.4 ± 1.8 | 4.5 ± 1.8 | 4.6 ± 1.6 | 5.4 ± 1.9 |

| **Delta serum PTH (pmol/L)** |          |          |          |          |           |
| Vitamin D 40,000 IU per week (n=114) | -1.8 ± 1.5* | -1.7 ± 1.5** | -1.6 ± 1.5* | -0.9 ± 1.5** |
| Vitamin D 20,000 IU per week (n=106) | -1.5 ± 1.4 | -1.4 ± 1.6 | -1.3 ± 1.4 | -0.8 ± 1.4** |
| Placebo (n=112)               | -1.2 ± 1.6 | -1.1 ± 1.4 | -1.0 ± 1.7 | -0.3 ± 1.6 |

Delta values are the specific month value minus baseline. Only subjects who completed the intervention with valid serum 25(OH)D and PTH measurements are included.

*P < 0.05; **P < 0.01; ***P < 0.001 (vs placebo, Student’s t-test)
†P < 0.001 (vs the vitamin D 20,000 IU per week group, Student’s t-test)
Table 6. Serum 25(OH)D, PTH and delta PTH in the 273 postmenopausal women who completed one year intervention in the Osteoporosis study

|                      | Baseline | 3 months | 6 months | 9 months | 12 months |
|----------------------|----------|----------|----------|----------|-----------|
| Serum 25(OH)D (nmol/L) |          |          |          |          |           |
| Vitamin D 45,600 IU per week (n=135) | 71 ± 23  | 168 ± 27 | 177 ± 32 | 187 ± 32 | 186 ± 34  |
| Vitamin D 5,600 IU per week (n=140) | 71 ± 22  | 91 ± 19  | 93 ± 24  | 90 ± 18  | 89 ± 17   |
| Serum PTH (pmol/L)    |          |          |          |          |           |
| Vitamin D 45,600 IU per week (n=135) | 5.0 ± 1.6 | 3.5 ± 1.3 | 3.7 ± 1.2 | 3.7 ± 1.2 | 3.9 ± 1.1 |
| Vitamin D 5,600 IU per week (n=140) | 5.0 ± 1.6 | 3.9 ± 1.3 | 4.2 ± 1.5 | 4.2 ± 1.5 | 4.5 ± 1.5 |
| Delta serum PTH (pmol/L) |          |          |          |          |           |
| Vitamin D 45,600 IU per week (n=135) | -1.5 ± 1.2* | -1.4 ± 1.4** | -1.3 ± 1.5** | -1.2 ± 1.4** |
| Vitamin D 5,600 IU per week (n=140) | -1.1 ± 1.4 | -0.8 ± 1.5 | -0.8 ± 1.4 | -0.6 ± 1.5 |

Delta values are the specific month value minus baseline. Only subjects who completed the intervention with valid serum 25(OH)D and PTH measurements are included.

All subjects were given 1,000 mg calcium daily

*P <0.05; **P < 0.01 (vs vitamin D 5,600 IU per week, Student’s t-test)
Figure 2

150x150mm (600 x 600 DPI)