Vitamin D supplementation does not improve CVD risk factors in vitamin D insufficient subjects

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Abstract

Objective: Low serum 25(OH)D levels are associated with cardiovascular disease (CVD) and some of its risk factors. However, in interventional studies the effects of vitamin D supplementation have been uncertain, possibly due to inclusion of vitamin D sufficient subjects. Our aim was therefore to examine effects of vitamin D supplementation on CVD risk factors in vitamin D insufficient subjects.

Design: Double-blinded randomized controlled trial.

Methods: A four months interventional study with high dose vitamin D (100 000 IU loading dose, followed by 20 000 IU/week) or placebo with measurements of blood pressure, lipids (total-, LDL- and HDL-cholesterol, triglycerides, apolipoproteins A1 and B), and glucose metabolism parameters (blood glucose, HbA_{1c}, sRAGE, insulin, C-peptide, and HOMA-IR).

Results: 422 subjects with mean serum 25(OH)D level 34 nmol/L were included, with 411 subjects completing the study. Serum 25(OH)D levels increased with 56 nmol/L and decreased with 4 nmol/L in the vitamin D and placebo group, respectively. We found no statistically significant differences between the two groups in any of the measured CVD risk factors, except for a minor increase in sRAGE in the vitamin D group. Stratified analyses of subjects with low baseline serum 25(OH)D levels alone, or combined with blood pressure, lipid and HOMA-IR values above the median for the cohort, did not skew the results in favour of vitamin D supplementation.

Conclusion: Supplementation with vitamin D in subjects with baseline vitamin D insufficiency does not improve CVD risk factors profile.

Introduction

It is widely acknowledged that the vitamin D receptor (VDR), and the enzymes necessary for the hydroxylation of vitamin D to 25-hydroxyvitamin D (25(OH)D) and to the active form 1,25-dihydroxyvitamin D (1,25(OH)$_2$D) are located in most tissues of the body (1). Initially related to rickets in children, vitamin D is nowadays considered as a vitamin/hormone with multiple functions, such as intestinal calcium absorption, involvement in cell proliferation and inflammation processes (1). Low serum 25(OH)D levels have additionally been related to numerous diseases, including cardiovascular disease (CVD) and their risk factors (2). Thus, a meta-analysis of 19 prospective studies focusing on the relation between vitamin D status and CVD found a 52 % risk increase for future CVD in subjects in the lowest vitamin D category (as defined by tertiles, quartiles or arbitrary cut-offs) when compared to those in the highest category) (3).

Possible explanatory factors might be the effects of vitamin D on CVD risk factors. There are associations pointing to an increase in systolic, and to a lesser degree diastolic blood pressure with decreasing serum 25(OH)D levels (4, 5). This may be due to activation of the renin-angiotensin system (RAS) as observed in vitamin D deficiency (6). Additionally, low serum 25(OH)D is associated with increased serum total cholesterol, low density lipoprotein cholesterol (LDL-cholesterol), decreased high density lipoprotein cholesterol (HDL-cholesterol), high apolipoprotein B/apolipoprotein A1 ratio (7, 8), increased serum triglycerides (7, 8), as well as reduced insulin sensitivity (9, 10). One mechanism for these effects could be vitamin D receptor activation related increase in CYP7A1 resulting in lowering of cholesterol levels, as observed in studies on mice (11).

However, interventional studies have yet to demonstrate with certainty effects of vitamin D supplementation on CVD risk factors, even when examined as meta-analyses. Beveridge et al. included 4541 participants from 46 RCTs using individual patient data, with no effect on systolic or diastolic blood pressure by vitamin D supplementation (12). In a meta-analysis by

Wang et al. that included 12 clinical trials consisting of 1346 participants, no beneficial effect on total-cholesterol, HDL-cholesterol and triglycerides were found, whereas an increase in LDL-cholesterol was seen (13). In Challoumas' review of 19 relevant RCTs, only one reported benefits of vitamin D supplementation on lipid profile parameters, while the rest showed no effects or even adverse outcomes (14). In a meta-analysis by Poolsup et al. including 10 trials, no effect of vitamin D on insulin resistance was found in subjects with pre-diabetes (15). And finally, in a study from 2017, Seibert et al. found no effect on CVD risk factors with 800 IU vitamin D/day in 105 vitamin D insufficient subjects (16).

However, many of these negative results might be explained by inclusion of subjects that were vitamin D sufficient, and accordingly, no effect of additional vitamin D were to be expected (16, 17). Furthermore, most of the studies included in these meta-analyses have been grossly underpowered and also complicated by differences in vitamin D doses and duration of trials.

In Tromsø, Northern Norway, large population-based health surveys have been performed with 7-8 years intervals since 1974 (18). In the seventh survey in 2015/2016, more than 21 000 subjects were included and serum 25(OH)D measured. We therefore had the opportunity to invite a large group of subjects known to have low serum 25(OH)D levels. The aim of the present study was to investigate the effect of vitamin D supplementation on cardio-vascular risk factors in people with known vitamin D insufficiency.

Methods

Subjects

In the seventh survey of the Tromsø study conducted in 2015/2016, all citizens aged 40 years and above (n = 32 591) living in the municipality of Tromsø in northern Norway were invited

to participate and 21 083 attended (64.7%). With serum 25(OH)D successfully measured in 20 922 participants, 1489 males and females aged < 80 years with serum values below the 10th percentile (< 42 nmol/L) were invited by mail to participate in the present study. This serum 25(OH)D cut-off was estimated to result in sufficient number of participant for the study. A written reminder was sent out to non-responders after two-three weeks' time. All 639 responders were screened by phone by one of the study nurses at the Clinical Research Unit at the University Hospital of North Norway. This screening included questions about medical history to exclude subjects with known granulomatous disease, diabetes, renal stones last five years, or serious diseases that would make the subject unfit for participation, use of vitamin D supplements exceeding 800 IU vitamin D per day, use of solarium on a regular basis, and subjects who were planning holiday(s) in tropical areas during the study period. Women of childbearing potential (below the age of 50 years) without use of acceptable contraception (hormonal, IUD) were excluded. Information about weight, height and smoking status were sent to the hospital's research department for use in the randomization procedure.

Study design

Four hundred and fifty-five subjects passed the above telephone screening and came to the first visit (V1) at the Clinical Research Unit at the University Hospital of North Norway. The subjects were asked to be fasting overnight, and after signing the informed consent, medical history was taken, blood samples were drawn, followed by measurements of height and weight (wearing light clothing and no shoes) and blood pressure. These examinations revealed that 33 subjects were unfit for further participation. The remaining 422 subjects then came to the next visit (V2) within 2-5 days. The flow of inclusion is shown in Figure 1. At this second (non-fasting) visit, blood pressure was again measured and the study drugs

(cholecalciferol capsules (20 000 IU (500 ug) Dekristol, Mibe, Jena, Germany) or identical looking placebo capsules containing arachis oil (Ayanda GmbH & CoKG, Falkenhagen, Germany)) were dispensed. As it takes time for serum 25(OH)D levels to increase, five capsules were given as a loading dose. The weekly dose was based on our previous intervention studies where 20 000 IU vitamin D (cholecalciferol) per week was sufficient to raise mean serum 25(OH)D levels to ~ 80 nmol/L, whilst not displaying any side effects or health risks (19, 20).

The randomization was stratified according to gender, vitamin D status in the Tromsø study (serum 25(OH)D above/below 25 nmol/L), smoking status and body mass index (BMI) above/below 27 kg/m^2 (self-reported height and weight). Age was not used in the randomization process. Based on this, the randomization unit assigned the subject a randomization number using a block randomization procedure. This randomization number was sent to the Clinical Research Unit for registration and to the hospital's pharmacy who dispensed the medication accordingly. Except for the pharmacy, which had no contact with the study participants, all nurses, doctors and other study personnel were blinded to the randomization throughout the study.

Two months after the first visit the subjects were contacted by one of the study nurses and asked for adverse events and reminded to take the study medication. Two months thereafter the third visit (V3) was performed with examinations identical to V1. The fourth visit (V4) followed a few days later, with return of study medication and the additional blood pressure measurements. Compliance was calculated as the ratio between capsules used (capsules supplied minus capsules returned) and number of weeks between V2 and V4.

The subjects were asked not to take any vitamin D supplements (including cod liver oil) during the four months intervention period. At the end of the study all subjects (regardless of randomization) were advised vitamin D supplementation 800 IU per day.

Measurements

BMI was calculated as weight (kg) divided by squared height (m²). Blood pressure was measured after 15 min of rest with an automatic device (AND, A & D Medical, Tokyo, Japan) three times with two minutes apart. The baseline (and similarly the end of study) blood pressure was calculated as ((median BP from V1) + (median BP from V2))/2. Serum calcium was analyzed by an automated analyzer (Modular P, Roche Diagnostics, Mannheim, Germany) with reagents from Boehringer Mannheim and a modified Jaffe reaction (Roche Hitachi 911) was used for measurement of serum creatinin. Intact parathyroidea hormon (PTH) was analyzed with an electrochemiluminescence immunoassay (ECLIA) using an automated clinical chemistry analyzer (Cobas 6000, Roche). Serum cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides were analyzed with enzymatic colorimetric methods using an automated analyzer (Cobas 8000, Roche), and apolipoprotein A1, apolipoprotein B were analyzed by immune turbidimetry (Cobas 8000, Roche). Insulin and C-peptide were analyzed by immunometry (Cobas 8000, Roche), whereas glucose was measured by photometry (Cobas 8000, Roche). HbA_{1c} was measured by high performance liquid chromatography using a Tosho G8, Tokyo, Japan. Serum concentrations of 25(OH)D were measured by an in-house liquid chromatography–tandem mass spectrometry method that detects both $25(OH)D_3$ and $25(OH)D_2$ and the sum of these presented as 25(OH)D in the results (21). The CV for both assays are < 2%. Serum human receptors for advanced glycation end products (extracellular domain) (sRAGE) was measured using enzyme-linked immunosorbent assay (ELISA) kits (Quantikine, R&D Systems, Minneapolis, MN, USA) according to the manufacturer instructions at the Hormone Laboratory, Oslo University Hospital. The inter-assay coefficients of variation (CV) in our laboratory was 5.8% (22).

Insulin sensitivity was assessed with the homeostasis model (HOMA-IR) [(insulin (pmol L^{-1}) x (glucose (mmol L^{-1}))/135] (23).

Statistical analysis

All variables were examined for normality of distribution, with analysis of skewness and kurtosis, visual inspection of histograms, P-P and Q-Q plots. Of the dependent variables HDL-cholesterol, triglycerides, PTH, insulin, C-peptide and HOMA-IR were not normally distributed at baseline, but were successfully corrected with log transformation and used as such in the statistical analyses. For delta values (value at end of study minus value at baseline), triglycerides, HOMA-IR and insulin had non-normal distribution and could not be normalized by log transformation.

At baseline, comparison between the vitamin D and placebo groups were performed by Student´s t-test for independent samples. The effect of the intervention was analysed per-protocol by a general linear regression model with the delta value as dependent variable, age, gender, baseline value and randomization status as covariates. For delta triglycerides, insulin and HOMA-IR the delta values were compared with Mann-Whitney U test. Interactions between baseline values and randomization status were tested by including the baseline values (dichotomous, median-split) in the unadjusted regression models. P-values < 0.10 of the interaction term were considered statistically significant.

Normally distributed data are presented as mean (SD), non-normally distributed values as median (5th, 95th percentiles). P < 0.05 was considered statistically significant and all tests were done two-sided. IBM SPSS Statistics version 24 was used for the statistical analysis.

Power calculation

The main endpoints in the study were effects on the cardio-vascular risk factors blood pressure, serum lipids and insulin resistance. The power calculation was made on the assumption that those included would have a mean serum 25(OH)D of 30 nmol/L and that those given vitamin D would have an increase in serum 25(OH)D to a mean level of 80 nmol/L. We assumed that the maximal, realistic effect of the vitamin D supplementation would be 2/3 of the difference in systolic and diastolic blood pressure, serum triglycerides and HDL-cholesterol and insulin resistance (as evaluated by HOMA-IR) between those with serum 25(OH)D levels of ~ 30 pmol/L and ~ 80 nmol/L found in cross-sectional studies (4, 7). Thus, if wanting a power of 0.8 and P < 0.05, we would need 450 subjects to show an effect of 6.7 mm Hg on systolic blood pressure, 410 subjects for 3.3 mm Hg on diastolic blood pressure, 300 subjects for 0.26 mmol/L on triglycerides, 490 subjects for 0.09 mmol/L on HDL-cholesterol, and 400 subjects for 0.57 on HOMA-IR. Based on this we would need ~ 500 subjects to complete the intervention to have a reasonable chance of demonstrating the anticipated effect on the primary endpoints. We assumed a dropout rate of 16 %, and therefore aimed to include 600 subjects in the study.

Ethics

All participants signed an informed consent form after receiving oral and written information on the study design, aim and duration. The subjects received a gift card value 200 NKR (20 Euro) to cover travel expenses in the study. The study was approved by the Regional Committee for Medical Research Ethics (REK NORD 2013/1464) and by the Norwegian Medicines Agency (2013-003514-40). The study is registered at ClinicalTrials.gov NCT02750293.

Results

The characteristics of the 422 subjects (224 men and 198 women) who fulfilled all inclusion

criteria and came to V2 are shown in Table 1; mean age of 50 years, BMI 28 kg/m^2 and

serum 25(OH)D 34 nmol/L. One hundred and ten subjects were included during the summer

months May – August and 312 subjects during September – March (when there is no

effective UV radiation at our latitude). At baseline the vitamin D group (n = 211) and the

placebo group (n= 211) did not differ significantly except for a slight difference in serum

creatinine (Table 1).

Two hundred and eight subjects in the vitamin D group and 203 in the placebo group

completed the study. The compliance rate was very high; 15 % of the subjects had a

compliance rate between 84 and 100 %, and the rest a compliance rate of 100% or higher.

In the vitamin D group mean serum 25(OH)D levels increased to 88.7 nmol/L, whereas

there was a slight decrease in the placebo group to 30.6 nmol/L. Except for a decrease in

serum PTH and an increase in serum creatinine in the vitamin D group, there were no

significant changes in any of the measured parameters between the vitamin D and the placebo

groups at the end of the study (Table 2). Thirty subjects in the vitamin D group and 36

subjects in the placebo group used blood pressure lowering drugs, and 16 and 32 subjects

used lipid-lowering drugs, respectively. The lack of effect on blood pressure and lipids was

seen also when subjects using blood pressure or lipids lowering drugs were excluded (data

not shown).

Subgroup analyses

Effect of baseline and end of study serum 25(OH)D

Subgroup analyses on subjects with baseline serum 25(OH)D < 40 nmol/L, and for those in

the vitamin D group with end of study serum 25(OH)D > 70 nmol/L (n = 136) and those in

the placebo group with end of study 25(OH)D < 40 nmol/L (n = 127) were performed. In this subgroup there was again significant differences in delta values for serum PTH and creatinine. In addition, delta sRAGE was significantly (P < 0.05) higher in the vitamin D group, whereas no significant difference was found for the other delta values (Table 3). Further analysis with lowering the cut-off baseline value to 30 nmol/L were also performed (87 subjects in the vitamin D and 77 subjects in the placebo group), and yielded similar results (data not shown).

Effects of baseline blood pressure, baseline serum lipids and baseline HOMA-IR
There were no significant interactions between baseline levels of blood pressure, serum lipids and HOMA-IR and randomization status in regard to the delta values. To further explore if subjects more exposed to the risk factors would benefit from vitamin D supplementation, separate analyses were made in subjects with baseline level of the risk factor above the median for the cohort (or below for HDL-cholesterol and apolipoprotein A1) combined with baseline serum 25(OH)D < 40 nmol/L and the expected end of study response in serum 25(OH)D. However, this did not show any significant benefit from the vitamin D supplementation (Supplemental Table 1).

Side effects
No serious study-related side effects were recorded. Two subjects developed hypercalcemia (serum calcium = 2.57 mmol/L); one male whose serum calcium normalized upon re-testing, and one female who was found to have developed primary hyperparathyroidism.

Discussion

To summarize, we found no significant effect of four months vitamin D supplementation on blood pressure, serum lipids, insulin resistance or other cardiovascular risk factors, regardless of baseline serum 25(OH)D levels and risk factors profile. This is in agreement with the recently published study by Seibert et al. (16).

The indications for an association between vitamin D and CVDs and their risk factors are substantial. There are numerous prospective, observational studies showing these relations, and their corresponding meta-analyses and systematic reviews are all confirmative (3, 12-15, 24). Furthermore, the vitamin D receptor is found in blood vessels, as well as in beta-cells and adipocytes (1), and effect of vitamin D on the renin-angiotensin system has been demonstrated in experimental animals (6). However, showing a causal relationship in interventional trials has been difficult, which could be explained by underpowered studies and not including subjects with vitamin D insufficiency (serum 25(OH)D < 50 nmol/L) or deficiency (serum 25(OH)D < 30 nmol/L), as defined by the Institute of Medicine (19).

To account for this, we invited a large group of subjects with low serum 25(OH)D levels, and still the results were negative, with no improvement in the cardiovascular risk factors profile. Since we included subjects based on serum 25(OH)D levels measured up to two months prior to start of the study, some subjects had serum 25(OH)D levels > 40 nmol/L at baseline. However, even when restricting the analyses to subjects with baseline serum 25(OH)D levels < 40 nmol/L (or even < 30 nmol/L) and with the expected increase in serum 25(OH)D in the vitamin D group and lack of increase in the placebo group, there was no improvement in the results in favour of vitamin D.

If lack of vitamin D is causally related to a definite risk factor, it is reasonable to believe that supplementation would be most efficient in the subjects with the highest risk level. To explore this, we examined potential interactions between baseline level of the risk factors and randomization status regarding the outcomes; but found no such interactions. Furthermore,

we did stratified analyses of subjects with blood pressure, lipid and HOMA-IR values above the median for the cohort, combined with low baseline serum 25(OH)D; but again, no significant effects of vitamin D.

Previous studies on vitamin D and lipid and glucose metabolism have generally included standard measures like triglycerides and cholesterols, glucose, insulin and HbA_{1c}. Recently it has been shown that apolipoprotein A1 and apolipoprotein B might be as good or even better than the standard lipid parameters for the evaluation of the risk of future myocardial infarction (25). Similarly, sRAGE, which is the soluble receptor for advanced glycation end-products and may thus block/counteract their detrimental inflammatory effects (26), may reflect other aspects of glucose metabolism than HbA_{1c} and HOMA-IR, and has in one study been reported to increase with vitamin D supplementation (27). These measurements were therefore included in our study, and for sRAGE there was a significant increase in the vitamin D compared to the placebo group when the analysis was restricted to those with low baseline serum 25(OH)D. The difference was modest and should be interpreted accordingly. However, if confirmed in other studies, a relation between vitamin D and sRAGE could potentially be of importance for prevention of CVD diseases.

The main source of vitamin D is solar UV-radiation, and select dietary sources like fatty fish, but the serum level of 25(OH)D is also affected by genetic factors (28). There are several single nucleotide polymorphisms (SNPs) related to the serum 25(OH)D level, and if these SNPs are also related to the CVD risk factors, that would indicate a causal relationship. Another approach for evaluating the effect of vitamin D on the risk factors would therefore be Mendelian randomization analyses. For vitamin D and blood pressure, Vimaleswaran et al. used a genetic 25(OH)D synthesis score in a combined cohort of 146 581 subjects and found that for each risk allele (out of four) there was a slight, but significant increase of 0.10 mm Hg in systolic blood pressure and 0.08 mm Hg in diastolic blood pressure (29). On the other

hand, low 25(OH)D levels did not associate genetically with levels of remnant and low-density lipoprotein-cholesterol in a Danish study on 31 345 subjects (30). However, even results from Mendelian randomization analyses should be viewed with caution as the genes involved may not only affect serum 25(OH)D, but the production of other metabolites as well. The relation to serum 25(OH)D could also simply be co-variation since the vitamin D SNPs could be linked to other SNPs that truly have the effects in question. In spite of these limitations, the lack of/or minor effect in Mendelian randomization studies give strong support to the results from the clinical intervention studies.

CVD risk factors are surrogate end-points for clinical disease, and the true test of benefit from vitamin D supplementation is of course prevention of disease and reduction in mortality. One large RCT testing this, the ViDa study from New Zealand that included 5110 subjects, has recently been published and found no effect on cardio-vascular events (31). Several other large vitamin D RCTs are ongoing, with results expected within a few years (32, 33). Unfortunately, even these large studies might not give the definite answer as most of the subjects included probably are vitamin D sufficient (34). Therefore, large RCTs with inclusion of vitamin D deficient subjects and surrogate end-point, like our study, are of importance.

Our study has several limitations. We aimed to include 600 subjects with a mean serum 25(OH)D of 30 nmol/L, but were only able to include 422 subjects with a mean serum 25(OH)D level of 34 nmol/L. However, we find it unlikely that inclusion of 200 more subjects would have changed the results in favors of the vitamin D supplementation as none of the observations were even close to reach statistical significance. Similarly, we find it unlikely that our negative results were due to the inclusion of subjects with slightly higher serum 25(OH)D levels than anticipated, as sub-group analyses based on baseline as well as on final serum 25(OH)D levels did not change the results in favor of vitamin D.

Although we could invite the subjects with the lowest serum 25(OH)D levels in a population-based study including more than 21 000 subjects, only 12 of our subjects had serum 25(OH)D < 15 nmol/L. We therefore cannot exclude effects on the risk factors in subjects with more profound vitamin D deficiency. We did stratified analyses on the subjects with high risk factors combined with low serum 25(OH)D levels (which is the ideal groups to study), but such stratification reduced the number of subjects considerably and accordingly, the statistical power was limited. The intervention time was only four months, and we cannot rule out that a longer intervention (in particular for insulin resistance that is strongly related to body weight) might have given different results. We used weekly doses, where daily doses that result in not only elevated serum 25(OH)D levels, but also increased serum cholecalciferol levels, would possibly be more biologically efficient (35). Furthermore, it might have been better if the entire study had been performed during the winter months to avoid endogenous synthesis of vitamin D in the skin. Additionally, there are findings that suggest people may have varying molecular responses to vitamin D and therefore may need different supplementation/intervention doses. This has been conceptualized as a personal vitamin D response index, based on the epigenetic response and gene transcription following vitamin D supplementation (36). We did not have that information available for our participants, but might be relevant to include in future studies.

On the other hand, our study has also several strengths. The expected fall in serum PTH in the vitamin D group provided our study an intern validity. Our RCT is to our knowledge the largest conducted RCT so far in vitamin D insufficient and deficient subjects with change in CVD risk factors profile as primary endpoint. The study was performed with strict adherence to RCT rules, and the vitamin D dose given was sufficient to increase serum 25(OH)D levels adequately. Moreover, we used blood pressure measurements from two days

both at start and end of the study, we included a wide range of lipid and glucose metabolism parameters, and the dropout rate was low and the compliance high.

The association between serum 25(OH)D levels and CVD risk factors in observational studies is most likely due to confounding factors, as we have previously reported for serum lipids based on cross-sectional data (37). In addition, there is always a high probability of reverse causation for vitamin D and health; good health (which is associated with health predictors like blood pressure, lipids and glucose metabolism) probably allows people to stay more outdoors and get increased sun exposure, and is therefore the driver of the vitamin D association.

In conclusion, vitamin D supplementation does not improve the cardio-vascular risk factors blood pressure, lipids or insulin resistance, with the possible exception of serum sRAGE. This does not exclude an effect in those with severe vitamin D deficiency, but these subjects should be treated with vitamin D for skeletal health reasons regardless of CVD risk factors.

Declaration of interest

The authors report noting to declare.

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Figure 1. Flow chart for the inclusion and performance of the study
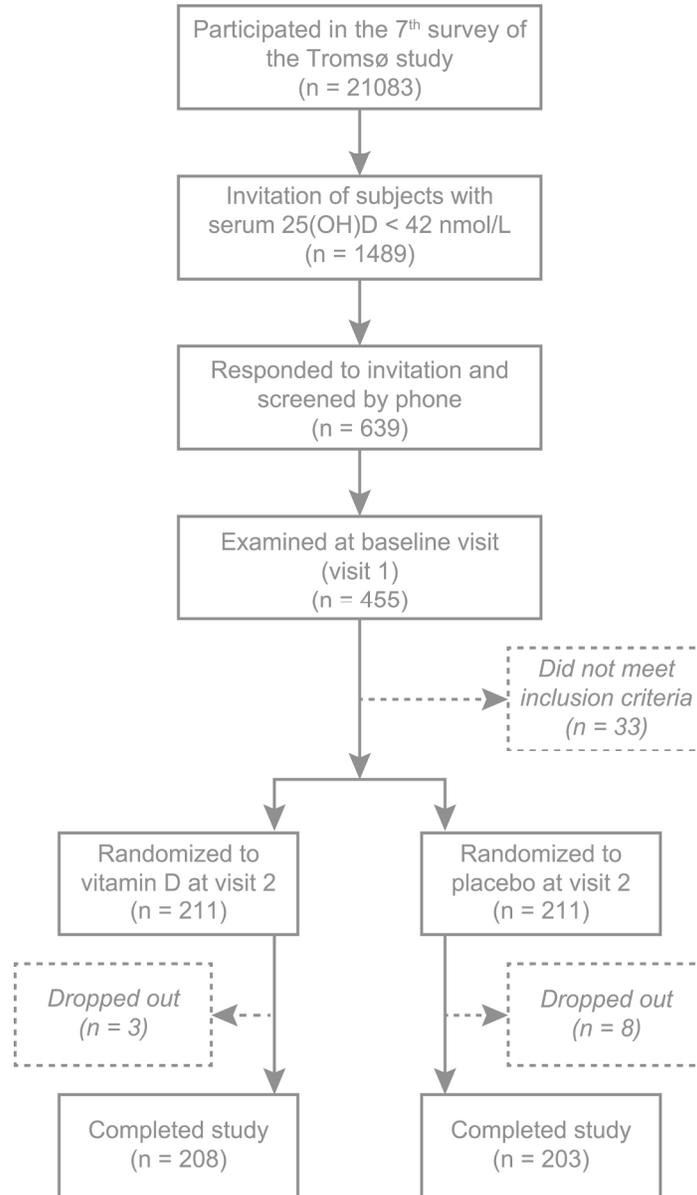
Figure 1. Flow chart for the inclusion and performance of the study

140x241mm (300 x 300 DPI)

Table 1. Baseline characteristics of all subjects randomized

	Vitamin D	Placebo
Count	211	211
Sex (male %)	53.1	53.1
Age (years)	50 (41, 68)	51 (41, 70)
Smoking (%)	21.8	21.3
Serum 25(OH)D (nmol/L)	32.9 (11.7)	35.4 (13.9)
BMI (kg/m^2)	27.9 (5.1)	27.8 (4.7)
Systolic BP (mmHg)	122 (13)	123 (12)
Diastolic BP (mmHg)	77 (9)	77 (9)
Serum creatinine (μmol/L)	70.5 (11.8)*	72.5 (13.1)
Serum calcium (mmol/L)	2.27 (0.07)	2.27 (0.07)
Serum PTH (pmol/L)	6.2 (4.1, 11.2)	6.6 (4.3, 10.4)
Serum triglycerides (mmol/L)	1.20 (0.60, 2.80)	1.10 (0.60, 2.50)
Serum cholesterol (mmol/L)	5.22 (0.97)	5.13 (0.96)
Serum LDL-cholesterol (mmol/L)	3.54 (0.90)	3.45 (0.91)
Serum HDL-cholesterol (mmol/L)	1.30 (0.90, 2.20)	1.40 (0.80, 2.20)
Serum apolipoprotein A1 (mmol/L)	1.50 (0.25)	1.50 (0.27)
Serum apolipoprotein B (mmol/L)	1.10 (0.28)	1.07 (0.27)
Blood glucose (mmol/L)	5.45 (0.52)	5.49 (0.56)
HbA$_{1c}$ (%)	5.49 (0.31)	5.49 (0.31)
Serum insulin (pmol/L)	67 (27, 207)	68 (23, 189)
Serum C-peptide (pmol/L)	836 (443, 1645)	819 (412, 1666)
Serum sRAGE (pg/ml)	1239 (469)	1169 (445)
HOMA-IR	2.70 (1.00, 8.48)	2.84 (0.86, 8.15)

Data are shown as prevalence or as mean (SD) for normally distributed data, and median (5th, 95th percentiles) for non-normal data.
*P< 0.05, Student's t-test

Abbreviations: 25(OH)D (cholecalciferol), BMI (Body Mass Index), BP (Blood Pressure), PTH (Parathyroid Hormone), LDL (Low Density Lipoprotein), HDL (High Density Lipoprotein), HbA1c (Glycosylated Hemoglobin), sRAGE (Serum Human Receptors for Advanced Glycation End Products), HOMA-IR (Homeostatic Model Assessment for Insulin Resistance)

Table 2. Baseline characteristics and delta values (end of study value minus baseline) in those the vitamin D and placebo group who completed the study

	Baseline		Delta values	
	Vitamin D	Placebo	Vitamin D	Placebo
Count	208	203	208	203
Sex (male %)	53.4	53.2		
Age (years)	50 (41, 67)	51 (41, 70)		
Smoking (%)	22.1	22.2		
Serum 25(OH)D (nmol/L)	32.6 (11.1)	35.1 (13.6)	56.2 (22.1)**	-4.5 (12.8)
BMI (kg/m^2)	27.9 (5.1)	27.8 (4.7)	0.18 (0.68)	0.12 (0.70)
Systolic BP (mmHg)***	122 (13)	123 (13)	0.2 (7.8)	-0.9 (7.2)
Diastolic BP (mmHg)***	77 (9)	77 (9)	0.4 (4.8)	-0.5 (4.6)
Serum creatinine (µmol/L)	70.5 (11.8)*	72.6 (13.3)	1.0 (5.8)*	-0.1 (5.1)
Serum calcium (mmol/L)	2.27 (0.07)	2.27 (0.07)	0.01 (0.07)	0.00 (0.06)
Serum PTH (pmol/L)	6.2 (4.1, 11.2)	6.68 (4.3, 10.4)	-0.76 (1.39)**	0.54 (1.49)
Serum triglycerides (mmol/L)****	1.20 (0.60, 2.60)	1.10 (0.60, 2.50)	0.00 (-0.50, 0.90)	0.00 (-0.60, 0.70)
Serum cholesterol (mmol/L)****	5.22 (0.95)	5.15 (0.95)	0.11 (0.52)	0.03 (0.58)
Serum LDL-cholesterol (mmol/L)****	3.55 (0.89)	3.46 (0.91)	0.11 (0.51)	0.04 (0.53)
Serum HDL-cholesterol (mmol/L)****	1.30 (0.90, 2.20)	1.35 (0.80, 2.20)	-0.02 (0.18)	-0.01 (0.16)
Serum apolipoprotein A1 (mmol/L)****	1.50 (0.25)	1.50 (0.28)	-0.03 (0.15)	-0.03 (0.15)
Serum apolipoprotein B (mmol/L)****	1.10 (0.28)	1.07 (0.27)	0.04 (0.15)	0.04 (0.15)
Blood glucose (mmol/L)	5.45 (0.51)	5.50 (0.56)	0.02 (0.41)	-0.02 (0.40)
HbA$_{1c}$ (%)	5.49 (0.31)	5.50 (0.32)	0.04 (0.15)	0.03 (0.15)
Serum insulin (pmol/L)	67 (27, 199)	69 (23, 189)	4 (-45, 59)	3 (-51, 78)
Serum C-peptide (pmol/L)	838 (450, 1644)	833 (412, 1666)	43 (205)	4 (216)
Serum sRAGE (pg/ml)	1230 (465)	1167 (451)	145 (267)	108 (289)

HOMA-IR	2.70 (1.00, 8.43)	2.89 (0.86, 8.15)	0.15 (-2.03, 2.61)	0.11 (-2.73, 3.30)

Data are shown as prevalence, mean (SD) for normally distributed data, and median (5th, 95th percentiles) for non-normal data.

*P< 0.05, **P< 0.01 versus placebo group. For baseline values Student's t-test, for delta values linear regression with age, gender and baseline value as covariates

*** Two subjects in the vitamin D and four subjects in the placebo group excluded due to change in use of blood pressure medication during the study

**** Three subjects in the vitamin D and one subject in the placebo group excluded due to change in use of lipid lowering medication during the study

Abbreviations: 25(OH)D (cholecalciferol), BMI (Body Mass Index), BP (Blood Pressure), PTH (Parathyroid Hormone), LDL (Low Density Lipoprotein), HDL (High Density Lipoprotein), HbA1c (Glycosylated Hemoglobin), sRAGE (Serum Human Receptors for Advanced Glycation End Products), HOMA-IR (Homeostatic Model Assessment for Insulin Resistance)

Table 3. Subgroup baseline and delta values for subjects with serum 25(OH)D baseline values <40 nmol/L and end of study values >70 nmol/L for the vitamin D group and <40 nmol/L for the placebo group

	Baseline		Delta values	
	Vitamin D	Placebo	Vitamin D	Placebo
Count	136	127	136	127
Sex (male %)	47.1	51.2		
Age (years)	49 (41, 66)	51 (41, 70)		
Smoking (%)	25.0	26.8		
Serum 25(OH)D (nmol/L)	28.0 (6.7)	27.9 (6.7)	65.9 (18.7)	-1.7 (7.0)
BMI (kg/m^2)	27.5 (4.9)	28.1 (4.9)	0.16 (0.69)	0.08 (0.69)
Systolic BP (mmHg)	121 (13)	124 (12)	-0.3 (7.6)	-0.6 (7.3)
Diastolic BP (mmHg)	76 (9)	77 (8)	0.15 (4.6)	-0.8 (4.3)
Serum creatinine (µmol/L)	70.8 (11.6)	71.6 (11.6)	1.0 (5.5)*	-0.3 (5.0)
Serum calcium (mmol/L)	2.27 (0.07)	2.27 (0.08)	0.01 (0.07)	0.0 (0.06)
Serum PTH (pmol/L)	6.4 (4.0, 10.7)	6.8 (4.3, 11.0)	-0.81 (1.34)**	0.50 (1.57)
Serum triglycerides (mmol/L)	1.20 (0.60, 2.60)	1.20 (0.60, 2.60)	0.00 (-0.60, 0.90)	0.00 (-0.80, 0.70)
Serum cholesterol (mmol/L)	5.27 (0.89)	5.08 (0.93)	0.05 (0.45)	0.05 (0.52)
Serum LDL-cholesterol (mmol/L)	3.57 (0.82)	3.42 (0.88)	0.06 (0.41)	0.04 (0.46)
Serum HDL-cholesterol (mmol/L)	1.40 (0.90, 2.30)	1.30 (0.80, 2.10)	-0.01 (0.19)	0.01 (0.15)
Serum apolipoprotein A1 (mmol/L)	1.54 (0.26)	1.47 (0.29)	-0.03 (0.15)	-0.02 (0.13)
Serum apolipoprotein B (mmol/L)	1.10 (0.25)	1.08 (0.27)	0.04 (0.13)	0.04 (0.13)
Blood glucose (mmol/L)	5.41 (0.47)	5.49 (0.58)	0.04 (0.41)	-0.01 (0.38)
HbA$_{1c}$ (%)	5.47 (0.28)	5.49 (0.32)	0.05 (0.15)	0.05 (0.15)
Serum insulin (pmol/L)	58 (24, 164)	71 (25, 189)	3.0 (-32, 54)	2.0 (-50, 96)
Serum C-peptide (pmol/L)	788 (433, 1477)	831 (410, 1681)	47 (198)	11 (201)
Serum sRAGE (pg/ml)	1217 (460)	1211 (482)	156 (274)*	80 (300)

HOMA-IR	2.31 (0.84, 7.41)	2.89 (0.96, 8.54)	0.15 (-1.45, 2.41)	0.11 (-2.93, 4.18)

Data are shown as prevalence, mean (SD) for normally distributed data, and median (5th, 95th percentiles) for non-normal data.
*P < 0.05, **P< 0.01 versus placebo group, linear regression with age, gender and baseline value as covariates
*** One subjects in the vitamin D and four subjects in the placebo group excluded due to change in use of blood pressure medication during the study
**** One subject in the vitamin D group excluded due to change in use of lipid lowering medication during the study

Abbreviations: 25(OH)D (cholecalciferol), BMI (Body Mass Index), BP (Blood Pressure), PTH (Parathyroid Hormone), LDL (Low Density Lipoprotein), HDL (High Density Lipoprotein), HbA1c (Glycosylated Hemoglobin), sRAGE (Serum Human Receptors for Advanced Glycation End Products), HOMA-IR (Homeostatic Model Assessment for Insulin Resistance)