Effect of vitamin D supplementation on Graves' disease: The DAGMAR trial

Final protocol, version 8, May 11, 2017 [Danish].

First approval January 25, 2013.

English translation August 16, 2022

ClinicalTrials.gov Identifier: NCT02384668

This supplement contains the following items:

1. Final protocol, summary of changes (Table 1+2).

2. Original statistical analysis plan, final statistical analysis plan.

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Table 1

Approved changes to original study protocol prior to study commencement		
November 27, 2014	Changes to study design From three to two study groups, equally randomized. Added exclusion criteria: pregnancy, abuse of alcohol or narcotics. The investigation program for a sub-cohort is extended with a visit at three months. Permission to expand planned biochemistry including flow-cytometry and to perform genetic investigations	
January 5, 2015	Homepage for study participants. Number of questionnaires during study period increased to nine	
January 7, 2015	Randomization by participating site. Collected blood volume with each sampling increased from 50 to 80 ml. HbA1c, glucose, and cholesterols analysed immediately and not as parts of batch analyses.	

Table 2

Overview of approved changes to study protocol after study commencement		
May 15, 2015	Case control study established with permission to recruit euthyroid controls (N=55) Frequency of questionnaires increased	No changes concerning the primary outcome of DAGMAR
July 31, 2015	Permission to recruit euthyroid controls through social media Homepage created explaining about the DAGMAR study	No changes concerning the primary outcome of DAGMAR
August 5, 2015	Permission to increase sampled blood volume at baseline, three, and nine months with 50 ml.	No changes concerning the primary outcome of DAGMAR
May, 11, 2017	For a sub group of participants, extensive physical examination was performed at baseline, three, and nine months. Permission obtained to repeat examination at the end-of-study visit. Permission to replace participant drop-outs. Upon the inclusion of participant number 260, drop-outs will be counted and recruitment will continue until a similar number of new participants has been	No changes concerning the primary outcome of DAGMAR Ensure sufficient power in primary analysis.

	enrolled or until the end of 2017. Replacement-participants will receive same randomization as drop-outs	

Importance of vitamin D supplementation in patients with Graves' disease: a randomized controlled trial

Introduction

Graves' disease is one of the most frequent causes of hyperthyroidism. The disease is characterized as an autoimmune disorder, caused by stimulating antibodies directed against the thyroid gland's TSH receptor. The disease is preferably treated medically, but with a high risk of relapse, as approx. 50% of patients experience a relapse of the disease, often within one year after the end of treatment.
Vitamin D has been found to be important for the immune system and to be associated with other autoimmune disorders. The vitamin D level in the blood has been shown to be lower in patients with Graves' disease than in healthy individuals. With this study, we want to investigate how supplementation with vitamin D affects the prognosis in patients with Graves' disease.

Background

Graves' disease

Hyperthyroidism is a frequent disease in Denmark with approx. 5000 new cases per year(1). Approximately 40% of cases is caused by Graves' disease (GS), an autoimmune disease with potential reversibility. GS is characterized by the presence of autoantibodies (thyroid-stimulating immunoglobulin (TRAb)) directed against the TSH receptor, which cause increased production of thyroid hormones. Triiodothyronine (T3) is the active hormone. All the body's cells have nuclear receptors T3, and T3 regulates the transcription of many different genes. Hyperthyroidism therefore affects all organs in the body.

T3 increases beta-adrenergic receptor activity, and cardiovascular symptoms are often predominant. Atrial fibrillation is one of the most serious complications of the disease, and is predominantly seen in patients over 40 years of age (2). Hyperthyroidism in general is associated with increased total mortality and cardiovascular mortality (3,4).

Bone turnover is increased in hyperthyroidism, although the bone loss assessed by bone mass density measurement has been shown to be recoverable within a shorter number of years(5,6). During the hyperthyroid phase and for a number of years afterwards, the fracture risk in hyperthyroidism is increased (6).
1.25 OH2 vitamin D is reduced as part of hyperthyroidism (7).
Whether bone recovery can be accelerated by vitamin D treatment of GS is unknown. A newer and more advanced method for assessing bone density is high resolution peripheral quantitative

computed tomography. There are no studies yet that use this method to describe the bone turnover in GS.

In GS, muscle function is affected, described as proximal myopathy (8). The patients' catabolic state is important. A possible significance of the underlying immunological disorder and of a low vitamin D level is unknown. Myopathy symptoms may resemble the symptoms of vitamin D deficiency. The degree of this muscle weakening and its possible normalization are sparsely described in the literature. There are a number of case series where the patients' muscle weakness improves over 4-5 months after starting anti-thyroid medication, and then reaches a plateau. How pronounced the degree of muscle weakness is compared to healthy people has not previously been described.
Applanation tonometry is a method used to assess vascular stiffness (9), which has been shown to be predict total and cardiac mortality and cardiac morbidity in an unselected population group (10). The method is well validated. Applanation tonometry has been used in very few studies on patients with GS and the studies suggest that vessel stiffness is increased in the hyperthyroid phase, but decreases again as the euthyroidism is restored.
Generally, GS is treated with anti-thyroid drugs for 1.5 years, after which the treatment is discontinued. However, recurrence is frequent and occurs in approx. 50% (11).

D vitamin
Vitamin D deficiency occurs with high frequency in Denmark. In patients with Graves' disease, lower vitamin D levels have been found compared to the background population (12) and compared to patients with other causes of hyperthyroidism (13). Both in vitro studies and animal experimental studies suggest that vitamin D affects the course of GS.

The effect can be caused by both a direct effect on gld. thyroid as of an effect on the autoimmune response in GS.

Active vitamin D (1,25(OH)2D) exerts its effect through a binding to the vitamin D receptor (VDR), which belongs to the group of nuclear hormone receptors. Binding of the ligand to VDR leads to conformational changes in VDR which facilitate a heterodimerization with the retinoid X receptor (RXR), after which the complex can bind to vitamin D responsive elements (VDRE) on the DNA strand and thereby affect gene transcription (14).

The vitamin D receptor is identified in thyroid follicular cells and 1,25(OD)2D has been shown to dose-dependently inhibit both TSH-stimulated iodine uptake and growth of follicular cells from rats (15,16).
Hyperthyroidism is associated with symptoms and findings that also occur with vitamin D deficiency, including decreased muscle strength and decreased bone mineral density. It is

unknown to what extent this can be attributed to the fact that vitamin D levels are often reduced in this group of patients.
Vitamin D has also been shown to be important for the development of autoimmune disease. In clinical epidemiological studies, a lack of vitamin D has been shown to be associated with an increased incidence of various autoimmune diseases, such as multiple sclerosis and type I diabetes mellitus (17).
VDR has been detected in most of the cell types of the immune system, including in antigen presenting cells such as macrophages, dendritic cells and CD4+ and CD8+ T-lymphocytes (18-20). Both in vivo and in vitro studies have shown that 1,25(OH)2D through its binding to VDR is able to affect the function of antigen presenting cells (21), including inhibiting differentiation, inhibiting IL12 synthesis, increasing IL10 synthesis, downregulating the synthesis of co-stimulatory cytokines, increasing the apoptosis tendency and inducing immunological tolerance (21-26). Of particular importance is probably the ability of 1,25(OH)2D to inhibit the synthesis of IL-12 simultaneously with an increased synthesis of IL-10, thereby reducing T-cell activation (27,28). In addition, 1,25(OH)2D has been shown to affect the differentiation and proliferation of B lymphocytes (29). In addition to counteracting the activation of T cells, studies suggest that vitamin D can downregulate the expression of HLA-II molecules on the follicle cells in gld. thyroid (30) and thereby possibly reduce the risk of developing an autoimmune reaction.

Several studies have looked at the association between polymorphisms in VDR and the risk of developing GS. The results from most of these studies have recently been combined in a meta-analysis which showed that among Asians the risk of developing GS is significantly associated with the ApaI, BsmI and FokI polymorphisms, while a similar association did not occur in Caucasians (31). In an animal experimental study in which hyperthyroidism was induced in mice fed either a vitamin D-poor or vitamin D-rich diet, no effect was found on plasma levels of TSI, but vitamin D deficiency led to persistent hyperthyroidism (32).
There are very few studies of the possible clinical significance of vitamin D on the course of GS. In an unblinded study, 30 patients with GS, in addition to conventional anti-thyroid treatment with methimazole, were randomized to a daily supplement of 1.5 micrograms alfacalcidol or no alfacalcidol (33). After 24 weeks of treatment, the mean dose of methimazole was similar in the two groups. Treatment with alfacalcidol led to a significantly lower plasma concentration of total and free T3 and thyroxin (T4), while TSH was significantly higher in those who had received treatment with alfacalcidol. There was no difference in the plasma level of TRAb. Treatment with alfacalcidol led to a significant decrease in the plasma concentration of alkaline phosphatase (33). However, the clinical use of 1,25(OH)2D has been limited by hypercalcemic side effects. The fact that the activation of vitamin D from 25-hydroxyvitamin D to 1,25(OH)2D has now been shown to take place not only in the kidneys but also locally in a number of tissues, including the cells of the immune system, has increased interest in vitamin D's possible immunomodulatory properties (34). In an unpublished review of 90 medical charts from patients with GS treated at the Regional Hospital Silkeborg or the Department of Internal Medical and Endocrinology, Aarhus University

Hospital, a low plasma concentrations of 25OHD are associated with a significantly increased risk of recurrence of GS after cessation of anti-thyroid treatment. Compared to P-25OHD < 45nmol/l, a concentration > 45nmol/l was associated with a 60% reduced recurrence risk (RR 0.40; 95% CI, 0.22-0.71), while a P-25OHD concentration above compared to below 60 nmol/l was associated with a 21% (RR 0.79; 95% CI, 0.50-0.99) decreased recurrence risk. In contrast, there was no significant effect at a threshold value of 80 nmol/l (RR 1.08; 95%CI, 0.46 – 2.32). The study also showed a non-significantly reduced relapse risk in patients who used vitamin D supplements compared to those who did not take such a supplement (RR 0.71; 95%CI, 0.42 – 1.21). In the population, the overall recurrence rate was 47%.

The following describes the randomized study i).
The controlled cohort study ii) is described separately on page 18)

Purpose
To clarify the importance of vitamin D for the course of GS, as we want to test the following hypotheses:

Primary hypothesis.
Treatment with vitamin D supplementation will reduce the number of treatment failure of Graves' disease in the intervention group by 33% compared to the placebo group.

Secondary hypotheses.
Treatment with vitamin D supplements will accelerate the normalization of bone turnover and muscle balance function and shorten the time to symptom freedom.
Vitamin D supplementation reduces vascular stiffness in patients with GS.

Design
The study is conducted as a double-blind, randomized and placebo-controlled trial with a clinical follow-up time of up to three years. The participants are patients with newly diagnosed Graves' disease recruited from the hospitals in the Central Denmark Region. The study proceeds with two parallel study groups. The participants in each of the study groups take 1 tablet daily. Each tablet is either placebo or 70 micrograms of cholecalciferol. The placebo and cholecalciferol tablets are identical in appearance.
All subjects are randomized to one of the following two groups:
Group 1: 1 placebo tablet daily (50% of participants)
Group 2: 1 tablet of cholecalciferol of 70 micrograms (50% of the participants)
The study proceeds as shown in Figure 1.
After randomisation, a smaller cohort will also undergo a study program as outlined below.
Register-based follow-up studies will be carried out after 5 and 10 years on the entire cohort.

When 260 subject have been randomized, drop-outs will be replaced until the end of 2017 when the inclusion period ends. Study closure is when the last participant has completed the study program as described in figure 1, alternatively has stopped participation in the study.
The individual participant stops taking the project medication at the latest 12 months +/- 1 month after the end of anti-thyroid treatment - possibly earlier (cf. section on withdrawal). For the individual participant, the maximum length of treatment will be 36 months.
A control cohort of 55 healthy sex- and age-matched individuals is established.

Primary outcome measures:
The primary effect measure is assessed based on whether the participants have benefited from the treatment as assessed by:
• Treatment success: No signs of hyperthyroidism 12±1 months after the end of anti-thyroid treatment. Criteria for treatment failure not met.
• Treatment failure is defined as: referral to radioiodine therapy, surgery, failure to taper off anti-thyroid treatment after a maximum of 24 months of treatment, or recurrence of hyperthyroidism within one year after cessation of anti-thyroid treatment.

Secondary outcome measures:

• Relapse after ending anti-thyroid treatment, defined as recurrence of hyperthyroidism within one year after cessation of anti-thyroid treatment (TSH<0.1) or new prescription of anti-thyroid drugs in the same period.
• Plasma TRAb level
• Biochemical vitamin D status
• Dose-response relationship with supplementation with vitamin D
• Occurrence of TAO/GO
• Occurrence of side effects to anti-thyroid treatment (agranulocytosis, drug rash, hepatitis, vasculitis)
• Weight changes
• Quality of life (SF36v2) + ThyPRO.
• Effects on muscle and balance function
• Effects on calcium homeostasis and bone turnover
• Effects on blood pressure and arterial stiffness measures
• Heart rate variability
• Frequency of infection (incl. consumption of antibiotics)
• Hospital admissions / number of doctor visits

Statistical analysis plan (Original)
The data analysis will be performed in accordance with "guidance on Statistical Principles for Clinical Trials" (IHC guidance E9). Primarily, differences between the placebo and vitamin D groups

are analysed. The primary effect measure is assessed based on whether the participants have benefited from the treatment as assessed by:
• Treatment success: No signs of hyperthyroidism 12±1 months after the end of anti-thyroid treatment.
• Treatment failure is defined as: referral to radioiodine therapy, surgery, failure to taper off anti-thyroid treatment after a maximum of 24 months of treatment, or recurrence of hyperthyroidism within one year after cessation of anti-thyroid treatment (TSH<0.1 or new prescription of anti-thyroid drugs).
Predefined subanalyses:
We will investigate the effect of vitamin D supplementation in relation to the vitamin D level at inclusion. Furthermore, we will examine the effect of vitamin D supplements on the group of participants who have not used supplements with vitamin D on their own initiative.
In a pre-defined subanalysis, subjects who continue anti-thyroid medication beyond 24 months due to Graves' orbitopathy will be excluded.
Differences between groups will be assessed with Fisher's Exact test or chi square test.
Other effect measures are calculated with t-test for two independent groups or a corresponding non-parametric test, depending on the distribution of the material. A significance level of 0.05 will be used. Primarily, data will be analyzed according to the "intention-to-treat" principle, although secondarily an "on treatment" analysis will be carried out, where the degree of compliance is corrected.
The intention-to-treat population consists of all participants who have been randomized to the study and who have received at least one dose of the study medication. In the event of missing data in the Intention-to-treat analysis, "last value carried forward" will be used. In the final protocol, there will be an account of unused or omission of inauthentic data, as well as an account of reasons for any deviations from the originally planned statistical analyses.
It is planned to carry out interim analyzes when the sub-cohort of 100 subjects (which includes mainly from Aarhus University Hospital and Regional Hospital Silkeborg) has completed the entire study program. This is to ensure that this population can be included in a data analysis which will form the basis of a PhD thesis

Study population
260 patients, men and women, with newly diagnosed GS where anti-thyroid treatment is planned. Potential study participants are recruited from hospitals in Central Denmark Region in connection with patients' attendance at the outpatient clinic.

Inclusion criteria
• Newly diagnosed Graves' disease verified by TSH below the reference range and thyroxin and/or triiodothyronine (T3 and/or T4) above the upper reference range.
• Age 18 years or older
• Elevated plasma level of thyroid stimulating immunoglobulin (TRAb)

• Planned or started (< 3 months) medical anti-thyroid treatment
• Speaks and reads Danish.
• Written consent after oral and written information

Exclusion criteria
• Daily intake of vitamin D supplements of more than 10 micrograms, which the participant wishes to continue.
• A previously diagnose of hyperthyroidism
• Planned treatment with radioactive iodine or thyroidectomy
• Chronic granulomatous disease
• Significant hypercalcemia (ionized plasma calcium > 1.40 mmol/l)
• Reduced kidney function (eGFR < 45 ml/min)
• Regular intake of medication with known immuno-modulating effects, including systemic glucocorticoids (>5mg/day for 3 months), imurel, methotrexate, TNFα-inhibiting agents.
• Active malignant disease
• Alcohol abuse resulting in the need for treatment or hospitalization
• Lack of willingness or desire to participate in the study
• Abuse of euphoric substances
• Pregnancy at the time of inclusion

Patients who meet all inclusion criteria and none of the exclusion criteria will be invited to participate in the study.
Withdrawal
Participants will be asked to cease treatment with the project medicine when an exclusion criteria is met:
• Changed treatment strategy for hyperthyroidism (radioiodine therapy, surgery).
• Hypercalcemia or reduced kidney function
• Onset of diseases or initiation of medication intake as listed under exclusion criteria (except systemic glucocorticoid treatment due to thyroid-associated ophthalmopathy).
• Recurrence of hyperthyroidism after discontinuation of anti-thyroid treatment.

The investigator can stop the treatment for a participant if this is deemed necessary for the participant's safety.
In case of pregnancy, the participant can continue with the project medication. Participants in the intensively studied sub-cohort (n=100) will be excluded from further studies during pregnancy, due to the radiation risk associated with the scans. However, the participants can continue in the main study (n=260).

Population size
Vitamin D and relapse risk in Graves' disease

There are no previous human studies with treatment with vitamin D3 supplements. In a medical record review of 90 medical records from patients with GS treated at Region Hospital Silkeborg or Aarhus University Hospital, it was found that P-25OHD > 45 nmol/L was associated with a 60% reduced recurrence risk compared to P-25OHD < 45 nmol/L (RR 0.40, 95% CI 0.22–0.71). The study also showed a non-significantly reduced recurrence risk in patients who used vitamin D supplements compared to those who did not take such a supplement (RR 0.71; 95% CI 0.42-1.21).
Power calculation: In order to show a difference in relapse risk for intervention with vitamin D compared to placebo of 33% (from 50% to 33%), a total of 260 participants must be included in the study, with 50% randomized to placebo and 50% to supplementation with vitamin D. (α=0.05; β=0.20). Drop-outs will be replaced by the inclusion of new participants: Upon the inclusion of participant no. 260, drop-outs will be counted and replaced by the inclusion of new participant until the end of year 2017 when the Inclusion Period ends.

Vitamin D and bone turnover, muscle function tests and cardiovascular indices
Bone turnover.
Based on the experience from other intervention studies with vitamin D, it is considered reasonable to expect that treatment with vitamin D will cause at least a 2% difference between the intervention and placebo groups. With measurements carried out on the lumbar spine (L1-L4), where it can be assumed that the mean BMD is 0.920 g/cm2 (SD 0.03 g/cm2), the required number of participants can be calculated - as it is desired to be able to demonstrate a 2% difference between the 2 groups - based on the following formula (α= 0.05; C2α = 1.96. β= 0.20; Cβ = 0.84):
N = (C2α + Cβ)2 x (2x SD/ (Mean x 0.02))2 = 72 participants.
Since 100% compliance cannot be expected and since some of the participants may drop out during the study period, it is reasonable to increase the number of participants. Thus, a total of 100 participants will be included, randomized equally to either treatment with placebo or vitamin D supplementation.
There are no applicable data for strength calculation for the effect of vitamin D supplementation on muscle function tests and cardiovascular indices in patients with Graves' disease.
Pulse wave velocity. A recent meta-analysis has shown that an increase in pulse wave velocity of 1 m/s leads to a 15% increase in total and cardiovascular mortality41. With 50 patients in each group, and given that the standard deviation in each group is 1.5 m/s, it will be possible to detect a difference of 1 m/s with a power of 92% (α=0.05).

Intervention and randomization

Vitamin D supplements
In the study, vitamin D tablets of 70 µg and a corresponding placebo are used, which are supplied free of charge by Orkla Health.

The project medication is packaged and labeled according to the Danish Medicines Agency's regulations by the Hospital Pharmacy at Aarhus University Hospital.
Vitamin D supplements and placebo tablets will be packed in quantities corresponding to 3-9 months' consumption and handed out to the participants upon attendance or forwarded by agreement at the regular contacts. (figure 1)
Procedures for randomization
The hospital pharmacy, Aarhus University Hospital, will carry out block-randomization, so that for every 4-16 participants included, there will be an equal number in each of those treated with either placebo or vitamin D supplementation. The exact size of the blocks will be unknown to the investigator. The participants are allocated consecutively. Site-randomization will be used to ensure equal randomization of study subjects at each participating department.

For each randomization number, the pharmacy supplies an opaque, sealed envelope numbered with the study's randomization number. The envelope can be opened if there is an urgent need to obtain information about which study medication the participant in question is receiving, as the envelope contains information about the contents of the tablets that the subject is taking. In this case, this is noted in the CRF, including the reason why the randomization code for the patient in question has been broken. The envelopes will be stored at Aarhus University Hospital.
The hospital pharmacy keeps a randomization list indicating the treatment group for all randomization numbers. The license is handed over to the Investigator when the experiment has been completed.
Data collection
The studies are carried out as outlined in Figure 1.
Baseline information, randomization and initiation of project medicine must begin within three months of anti-thyroid drug treatment.
For a sub-cohort of 100 patients (the first 100 subjects recruited from the outpatient clinics at the Medical Endocrinology Department, Aarhus University Hospital as well as interested participants from the regional hospitals in the central region) baseline information and the study program will be collected within four weeks after diagnosis of GS. Randomization and start-up of the project medication will take place immediately afterwards for this group.
In accordance with section 43, subsection 1 of the Health Act, participants' consent will be obtained to, via the medical charts, to collect information about treatment choice and course of GS, duration of treatment, occurrence of complications and side effects, including Graves' orbitopathy, side effects to the anti-thyroid treatment, as well as information about infections, other medical treatment, new illnesses including contact with a doctor and admissions.

Baseline
Based on medical record information, interview and questionnaire, the following will be registered in the CRF:

Age, conditions mentioned under criteria for participation in the study, current and previous illness, history of fractures, occurrence of fractures and illness in 1st generation relatives, consumption of dairy products, coffee, tea, tobacco and alcohol. Diet and sun-bathing habits. Medical history, including use of calcium and/or vitamin D supplements, vitamin supplements, dietary supplements and use of natural preparations. Use of hormonal contraceptives. The participants will be requested that the Investigator may obtain information from any previous admissions. Participants who have the opportunity to do so will be asked to complete an electronic version of the questionnaires via the internet (the website will be created in relation to the project being launched).

Follow-up

Up to nine times during the course of study, as indicated in figure 1, questionnaires on health- and disease-specific quality of life will be repeated (appendices 9, 10 and 11). Changes in treatment strategy, occurrence of side effects, complications and unintended events will be recorded based on interview and medical record information. Information on other illness during the study period is obtained from patients' medical chart. After five and ten years, register-based follow-up studies are carried out including vital status and discharge diagnoses based on data from the CPR register and the National Patient and Cause of Death Register.

Biochemistry

Blood samples will be taken as shown in Figure 1. At each blood sample, approx. 80 ml will be taken. For participants recruited from Aarhus University Hospital and for participants in the intensive examination program, 130 ml of blood will be taken during the first three of a total of four blood samplings. This will apply to 50 participants. The plasma concentration is determined immediately for the following parameter: albumin, ion calcium, phosphate, creatinine, magnesium, thyrotropin (TSH), T3, and T4. HbA1c, glucose and cholesterols. As part of the initial clinical investigation, TRAb is also determined.

Plasma will be frozen (-80 °C) at each blood sampling for later batch analysis of: TRAb, PTH, 25OHD, 1,25(OH)2D, vitamin D binding protein (DBP), bone-specific alkaline phosphatase (BSAP), osteocalcin and N-terminal extension peptide of procollagen (P1NP), CTX and antibodies. Furthermore, adiponectin, leptin, insulin. Markers of the renin-angiotensin-aldosterone system. Organ-specific antibodies, including TPO antibody, transglutaminase antibody IgA, total IgA, deamidated gliadin peptide antibody IgG, and markers of immune system activity, including high resolution CRP and immunoglobulins.

Furthermore, selected inflammatory and pro-inflammatory markers as well as adipokines. DNA is taken from leukocytes for genetic studies, see below.

Flow cytometry will be performed on fresh blood at the Department of Clinical Immunology, Aarhus University hospital, where lymphocyte subpopulations and cell differentiation will be examined. In cell culture studies, dendritic cells and their co-stimulatory factors will be examined. In addition, measurement of soluble HLA-G as well as functional studies of the regulatory T cells' suppressor function will be performed.

Surveys
A sub-cohort of 100 test subjects will complete the study program below, see figure 2. Separate information material has been prepared for this, see appendix 5.
Baseline examinations will be carried out within 14 days of referral.
Examinations at three and nine months will be carried out with a precision of +/- four weeks.
The examinations (including biopsies) will be repeated in connection with the end-of-study blood test after a renewed information interview and informed consent. Only participants who have previously participated in this research program and who continue to participate in the DAGMAR study will be able to participate. Pregnancy is an exclusion criterion. The background for the repeated examinations is that at the nine month investigations, especially the muscle strength testing, there are signs of continued improvement. Long-term clinical studies in Graves' disease on these aspects are currently limited or non-existent. It is therefore unclear when final restitution will be achieved, which these studies will be able to help shed light on.

Osteodensitometry
Bone scans will be performed as a "Dual-Energy X-ray absorptiometry" (DEXA) scan with a Hologic QDR Discovery scanner. BMC and BMD will be measured in the lumbar spine (L1-L4), the hip region (femoral neck, trochanter, Ward's triangle and intertrochanteric region), the forearm (proximal, middle 1/3 and ultra-distal) and the whole body. Furthermore, body composition will be determined.

HRpQCT scan
High resolution peripheral quantitative computed tomography (HRpQCT) scan of the distal radius and distal tibia (Xtreme CT, Scanco Medical AG, Bruüttisellen, Switzerland). The scans are performed on the non-dominant side. For a person who is right-handed, the scan is performed on the left arm and leg and vice versa. In the case of a metal implant or previous fracture in an area that is desired to be scanned, the scan is performed on the opposite side.
Muscle function and balance studies
Isometric voluntary muscle strength is measured on the dominant leg with a dynamometer (Meititur Ltd, Finland) that can measure isometric muscle strength in flexion and extension with the knee bent at an angle of 60° and 90°. In a similar way, muscle strength in the hand (grip strength) is measured.
Balance function is determined by measuring dynamic stability on a stadiometer (Meititur Ltd, Finland). In addition, two smaller physical tests are carried out, the "Get up and sit down test" (where the participants have to get up and sit on a chair ten times) (35) and the "Timed Up and Go test" (where the participants have to get up from a chair, walk three meters and turn around to go back and sit on the chair again) (36).

Arterial stiffness and blood pressure

Arterial stiffness is investigated with the commercially available tonometry-based equipment SphygmoCor® and Arteriograph24, determining aortic pulse wave velocity (APWV) and central aortic pressure (CAP). The tests are non-invasive, well-validated and without risks or discomfort. Expected time consumption is approx. 45 min. With the Arteriograph24, 24-hour blood pressure and 24-hour tonometry measurements are also determined.

Muscle and fat biopsies

Thirty participants are requested to have a muscle and fat biopsy performed at the time 0+3+9 months with a precision of +/- 4 weeks.

A biopsy will be taken laterally from the thigh, as local anesthesia with lidocaine and adrenaline is applied to the skin, subcutaneous tissue and muscle fascia under a sterile procedure. A minimal incision is then made in the skin and fascia, after which 100 mg of muscle tissue can be taken from the vastus lateralis muscle with a special needle (Bergstrøm's needle). The tissue is subsequently frozen in liquid nitrogen. The muscle biopsy is analyzed for protein expression using western blotting and subjected to gene expression analysis using quantitative PCR (qPCR). Furthermore, fiber type and cross-sectional area, myosin heavy chains including their relative distribution, various markers for and actors in muscle contractility are investigated.

The fat biopsy is taken after disinfection and local infiltration analgesia from the abdominal subcutaneous fat depot lateral to the umbilicus. Using a suction needle, approx. 1 gram of adipose tissue. The tissue is immediately frozen in liquid nitrogen and stored at -80 C until analyses. The adipose tissue will be examined for adipocytes and their content of vitamin D metabolites and vitamin D receptors as well as selected markers of inflammation and proinflammation and other markers of immunological activity.

Genetic determinants ALL

Polymorphisms in genes of importance for the risk of developing GS / the risk of relapse after treatment will be investigated. This will be carried out as a "genome wide search" where all polymorphisms are identified including polymorphisms in the vitamin D receptor (VDR), Group-specific component (vitamin D binding protein; GC), CTLA4 (CytotoxicT-lymphocyte-associated protein 4), Interferon inducedwithhelicase C domain 1 (IFHI1), Thyroglobulin gene (TG), Thyroid stimulating hormone receptor (TSHR).

Gene analyzes will be made that can reflect specific up- and down-regulation of different parts of the inflammatory response.

Data processing

Case record forms (CRF)

For each participant, a case record form (CRF) is kept, on which the date of the examinations that the participant has undergone, medication that has been dispensed/returned, deviations in the treatment, side effects and incidents are stated. In the case of "drop-outs" and "withdrawals", this

will be noted and explained in the CRF, including the time of cessation of intake of study medication.
All subsequent data processing takes place at the study site.
Investigator allows direct access to source data/documents (including patient records) during monitoring, auditing and inspection from the Research Ethics Committee for Central Region and the GCP unit for Aarhus University Hospital and its partners. The participants are made aware of this and specific consent is obtained in the form of a power of attorney.
All information obtained will be handled in accordance with the Act on the Processing of Personal Data.

Research biobank
A research biobank will be set up where plasma taken from each subject will be stored at -80 degrees Celsius. The material will be analyzed as described under "biochemistry".
After the end of the experiment, remaining material will be transferred to a biobank, where it will be stored for up to 15 years before being destroyed. Participants will be asked to consent to this. The biobank will be notified to the Danish Data Protection Authority in accordance with applicable legislation.

Medicine accounting
The investigator will keep careful records of who the study medication has been delivered to, incl. date and amount of medicine dispensed. In addition, the amount of returned medication will be recorded. In each participant's CRF, the quantity, batch number, and date of dispensing of study medication are noted.
For each participant, treatment compliance will be calculated as the difference between the number of dispensed and returned doses. Regardless of degree of compliance, participants will be encouraged to continue in the trial. If a participant stops taking the project medicine during the course of the study, the time of cessation of taking the study medicine will be noted together with the reason why the participant has stopped taking the medicine. However, the participants will be asked to continue to come to planned control visits, which will improve the quality of subsequent intention-to-treat analyses.

Risks, side effects and inconvenience

Risks
In several studies, supplementation with vitamin D has been shown to be largely harmless. In one study, it was thus found that a daily supplement of 250 micrograms over 3 months did not lead to side effects, including that no signs of hypercalcemia were found as a result of the treatment [33]. There are no epidemiological data for treatment with high doses of vitamin D in patients with Graves' disease. In the literature, hypercalcaemia has been described in relation to supplementation with vitamin D in granulomatous diseases such as sarcoidosis, tuberculosis and

silicosis, which is why patients with these diseases cannot participate in the trial. Symptoms of hypercalcemia include constipation, headache, arrhythmia, confusion, and dehydration with polyuria. Hypertension can also occur with overtreatment.
During scanning, a total radiation dose of 0.9 mSV is applied to each patient, which corresponds to approx. 90-120 days of normal background radiation. Exposure to this amount of radiation can theoretically be assumed to increase the lifetime risk of dying from cancer by 0.01%; from approx. 25% to 25.01%.
When participating in the additional examination program in connection with the end-of-study, each patient will be exposed to a total radiation dose of 0.45 mSv, which corresponds to approx. 45-60 days of normal background radiation. Theoretically, this radiation dose must be assumed to increase the lifetime risk of dying from cancer by 0.005%.
Pregnancy and breastfeeding
It has been shown in a recent intervention study that vitamin D supplements in doses up to 4000 IU/day (100 micrograms) are non-toxic for mother and fetus (37). The pregnant women simultaneously receive regular vitamin supplements including 10 micrograms of vitamin D. No hypervitaminosis D was found, and no adverse events or other events were recorded. In breastfeeding mothers, doses up to 164 micrograms/day have been shown to be safe for the breast-fed child (38).
Pregnant and breastfeeding women will therefore be able to continue treatment with project medicine. However, pregnancy and breastfeeding will result in exclusion from the subcohort study due to the radiation risk associated with DXA and HRpQCT scanning.

Safety measures
Upon inclusion, all test subjects will be informed orally and in writing about any symptoms of hypercalcaemia, and will be asked to contact the investigator immediately. The investigator will, by continuous contact with the test subjects, every 3-6 months, inquire about side effects and possible symptoms of hypercalcaemia. If this is suspected, the subject will be requested to have a blood test carried out as soon as possible to determine serum creatinine and serum ionized calcium. Persistently elevated s-calcium or s-creatinine, which does not decrease on repeated checks, when other possible calcium and vitamin D are discontinued and the patient is biochemically well treated for his Grave's disease, will lead to discontinuation of study medication and exclusion from the study. If the hypercalcemia persists thereafter, the patient will be offered a referral for investigation for hypercalcemia if necessary.
In the toxic phase of Graves' disease, mild hypercalcemia is a frequent and transient phenomenon and in this phase, elevated values will be acceptable if they show a stagnant/declining trend in a repeated control blood test: The subject will be asked to continue the project medication and simply repeat ionized calcium and p-creatinine after another week and as needed.
No biochemical abnormalities are expected as a result of the treatment. Should participants develop symptoms compatible with hypercalcemia (thirst, polyuria, dehydration, confusion), they

will be examined for this in accordance with current clinical guidelines. If hypercalcemia is found to be the cause of the symptoms, the treatment will be discontinued.
Participants are advised not to start supplemental treatment with vitamin D, in addition to the supplement they may receive through the project medication. Participants who, despite this, start taking a supplement of more than 10 micrograms/day will be excluded. Participants will be asked to inform the Investigator if they are put on medical treatment or start taking nutritional supplements or using herbal remedies.
In relation to the subjects' participation in the study, the Investigator will ensure that the participants are informed about and receive/are referred to relevant treatment for diseases/conditions that arise during the study period, including that any side effects to the treatment are handled/treated in the best interest of the trial participant.

Disadvantage
Some of the participants may find it uncomfortable to have a blood test. There is a minimal risk of infection at the injection site. During blood sampling, a total of around 320 ml of blood will be drawn for participants from the regional hospitals and 470 ml of blood for participants in the intensive examination program and for participants recruited from Aarhus University Hospital, which is not expected to cause discomfort.
The local anesthetic for the fat and muscle biopsy may be associated with pain, and there may be tenderness in the area afterwards. The muscle test will leave a small scar on the thigh and the fat test may cause a bruise. There is a small risk of infection and, in rare cases, a small collection of blood may occur, causing additional soreness for a few days.
For the sub-cohort of 100 subjects, a time consumption of approx. three hours for each of the three study days. The time consumption for the planned forth examination is also approximately three hours.
This examination does not require additional attendance, as the examinations will be able to be done in connection with the participant being in for the planned end-of-study blood test anyway. Completing questionnaires is assumed to take 30 minutes.

Side effects and events
No side effects are expected for the project drug.
At the start of the study, the participants will be informed orally and in writing to contact the Investigator if they experience incidents and side effects, including serious ones during the treatment. At each control visit, the participants will be asked about this. In the specific situation, it will be assessed whether the incident / side effect in question is of such a nature that the participant must withdraw from the study. In each case, this must be explained in the CRF. To ensure that all events / side effects are reported to the relevant authorities, a list will also be kept on which the randomization number and date of registration of the event / side effect are stated, each time such an event is registered. In addition, it is recorded on the list whether the incident / side effect in question has been immediately reported.

Serious incidents and serious side effects related to the treatment will be immediately reported to the Scientific Ethics Committee for Central Denmark Region. All events and side effects will also be reported on a form at the end of the trial.

Time and place

The participants are recruited from the medical departments in the Central Denmark Region, as randomization, handling of study drug and specific examinations as described above will take place at the Department of Endocrinology and internal Medicine, Aarhus University Hospital. As a starting point, the inclusion interview will take place at the patients' home hospital. Blood sampling as well. In cases where the patients have not planned attendance at the home hospital (have switched to "letter responses"), the patients will be invited to an inclusion interview at Aarhus University Hospital. In selected cases, ie. patients who come from afar, the inclusion interview will be able to take place via Skype (video transmitted telephone conversation over the Internet with image and sound).

It is planned that the study will start in early 2015. The inclusion is expected to be completed during 2017, so that the entire study is expected to be completed during 2021.

Confidentiality, access to documents, right of appeal, and possibility of compensation

All information about the subjects' health conditions, other purely private matters and other confidential information is subject to confidentiality. The test subjects are protected - and all personal data, including tissue, blood samples etc. are stored - in accordance with the Act on the processing of personal data and in accordance with the Health Act. The participants have the opportunity to inspect the trial protocol in accordance with the provisions of the Public Disclosure Act.

In connection with participation in the study, in the event of damage related to errors/negligence, the participants have the opportunity to complain and seek compensation in accordance with the Act on Access to Complaints and Compensation within the Health Service. Furthermore, the participants are covered by the patient insurance of the participating hospitals. In the event of harmful effects related to medicines, the participants are covered according to the Law on Compensation for Medicine Damages.

Recruitment and consent

According to the Health Act section 46, subsection 1, potential test subjects will be sought based on a review of referrals and patient records, obtaining information on referral diagnosis (thyrotoxicosis without specification, thyrotoxicosis, and Graves' disease), biochemical results for p-thyrotropin (TSH) and total or free triiodothyronine , total or free thyroxine, and thyroid stimulating immunoglobulin, TRAB.

Potential participants will be informed about the study by contacting the patient in writing and making inquiries in connection with attending an outpatient check-up. The letter sent will contain participant information (appendix 5 or 6) and the pamphlet "Subject's rights in a biomedical research project" (appendix 7). In addition, a cover letter (appendix 3 or 4). It will appear from the

written information material that further information about the project can be obtained by contacting the investigator whose telephone number is given.
A brochure about the project will be available in the waiting rooms of the participating endocrinology clinics. This is to make potential participants aware of the project. The brochure contains relevant contact information for the project coordinator, and information is provided about the project's website address (www.dagmar.auh.dk). On this website you can read more about the project and see all relevant documents that will be available online. You will also be able to see copies of the questionnaires and supplementary information about the examination program (DXA).
Patients indicate an interest in the project by contacting the investigator directly by telephone or by informing the attending physician about this, after which the investigator contacts the patient by telephone. A time for oral information is agreed here. The participant is informed about the possibility of bringing a companion. The information interview will take place at Aarhus University Hospital or at the treating hospital and will be conducted by the investigator or someone else from the project group. The conversation will take place in an undisturbed setting behind closed doors and without interruptions. Sufficient time is set aside for conducting the interview (minimum 30 min).
From both written and oral information, it will appear that this is a scientific experiment. The design of the survey will be carefully reviewed. The project's purpose, potential risks and discomfort as well as expected benefits will also be stated. Furthermore, the participant will be made aware that there may be unpredictable risks and burdens linked to participation in a biomedical research project. It will be emphasized that participation is voluntary and that the person has the right to withdraw their commitment to participate in the study at any time without further justification and without this affecting the rest of the treatment at the hospital.
The project participant will be informed of the right to reflection time ("can go home and think about things") before giving a final answer. If the person concerned wishes to participate in the study, the person concerned can give oral and written consent to this at the end of the interview. If the patient asks for time to think, the person is requested to give feedback within 2 weeks, in the event that the person decides to participate in the study. In that case, a new time will be agreed for a repeated information interview under a similarly undisturbed framework and with the possibility of co-sitters. Verbal and written consent to participate in the study will then be obtained. The project participant receives a copy of this.
After obtaining consent, the investigator will inform each subject's general practitioner that he or she is participating in the study.

Especially regarding the controlled cohort study ii)

Purpose

Examining the muscle balance function in patients with newly diagnosed Graves' disease, etc. to clarify whether this has normalized three months after starting anti-thyroid treatment.

Hypothesis.
Muscle and balance function in newly diagnosed patients with Graves' disease will be reduced compared to healthy sex- and age-matched controls.
Three months after starting anti-thyroid treatment, there is no longer a difference between controls and the patients who have received vitamin D supplementation, whereas patients who have not received vitamin D supplementation continue to have reduced muscle and balance function compared to the controls.

Design
Controlled prospective cohort study of patients with newly diagnosed Graves' disease vs healthy sex- and age-matched controls.

Population
Fifty-five healthy controls, matched for sex and age (+/- 2 years) and menopause status.

Inclusion criteria
• Between 18 and 80 years old
• Euthyroidism
• Speaks and reads Danish
• Written consent after oral and written information

Exclusion criteria
• Current or previous metabolic disease
• Significant other disease, including renal impairment (eGFR <60 ml/min, hypercalcemia, treatment with the glucocorticoid system.
• Known neuromuscular disorders
• Pregnancy
• Alcohol abuse resulting in the need for treatment or hospitalization
• Abuse of euphoric substances
Statistics/strength calculation
In our research group, in a previous study on healthy adults, we have found that the mean value for maximum knee flexion at 90 degrees is 463N with a standard deviation of 170 N. To detect a difference between groups of 20% with a power of 0, 80 given a significance level of 0.05, 53 participants are required in each group.

During thyrotoxicosis, the loss of bone mass has been estimated at 9% per remodeling cycle. Assuming that the average lumbar bone mass density is 0.92 g/cm2 (SD 0.11 g/cm2), with the

stated population size we will be able to demonstrate a difference between groups of 10% at baseline with a power of 0.97 (α=0 .05).

A recent meta-analysis found that the risk of all-cause mortality and cardiovascular death increased by 15% for an increase in pulse wave velocity (PWV) of 1 m/s. Given a standard deviation of 1.5 m/s in each group, the study size will be able to demonstrate a difference between groups at a baseline of 1 m/s with a power of 0.94 (α=0.05).

Data collection
Survey program
The participants complete an examination program similar to the baseline examinations for the intensively examined cohort in the randomized clinical trial:

Baseline
Health information
Blood tests
Questionnaire about i.a. quality of life
DXA scan
HRpQCT scan
Arterial stiffness and blood pressure meassurement
Muscle and balance function examination

Follow-up, carried out with a precision of +/-4 weeks
Blood tests
questionnaire
Muscle and balance function
Arterial stiffness and blood pressure meassurement

Health information and questionnaire
Participants answer the same baseline, ThyPRO and follow-up questionnaire as approved for the main study.
Based on the interview and questionnaire, the following will be registered in the CRF:
Age, conditions mentioned under criteria for participation in the study, current and previous illness, fracture history, consumption of dairy products, coffee, tea, tobacco and alcohol. Diet and sunbathing habits. Medical history, including use of calcium and/or vitamin D supplements, vitamin supplements, dietary supplements and use of natural preparations. Use of hormonal contraceptives. Participants who have the opportunity to do so will be asked to complete an electronic version of the questionnaires via the internet (the website will be created in relation to the project being launched).

Biochemistry
At each blood sample, approx. 80 ml will be taken. Immediate analyzes as mentioned under "Biochemistry" p. 12.
Plasma will be frozen (-80 °C) at each blood sampling for later batch analysis as stated under "Biochemistry" on p. 12.
Osteodensitometry
Bone scans will be performed as a "Dual-Energy X-ray absorptiometry" (DEXA) scan with a Hologic QDR Discovery scanner. BMC and BMD will be measured in the lumbar spine (L1-L4), the hip region (femoral neck, trochanter, Ward's triangle and intertrochanteric region), the forearm (proximal, middle 1/3 and ultra-distal) and the whole body. Furthermore, body composition will be determined.

Muscle function and balance studies
Isometric voluntary muscle strength is measured on the dominant leg with a dynamometer (Meititur Ltd, Finland), which can measure isometric muscle strength in flexion and extension with the knee bent at an angle of 60° and 90° as well as flexion and extension above the elbow joint at 90°. In a similar way, muscle strength in the hand (grip strength) is measured.
Balance function is determined by measuring dynamic stability on a stadiometer (Meititur Ltd, Finland). In addition, two smaller physical tests are carried out, the "Get up and sit down test" (where the participants have to get up and sit on a chair ten times) (35) and the "Timed Up and Go test" (where the participants have to get up from a chair, walk three meters and turn around to go back and sit on the chair again) (36).

Arterial stiffness and blood pressure
Arterial stiffness is investigated with the commercially available tonometry-based equipment (SphygmoCor® and Arteriograph24), determining aortic pulse wave velocity (APWV) and central aortic pressure (CAP). The tests are non-invasive, well-validated and without risks or discomfort. Expected time consumption approx. 45 min. With Arteriograph24, 24-hour blood pressure and 24-hour tonometry measurements are also determined.
Data processing
Case record forms (CRF)
Detailed CRFs are kept for all participants, in which the results of the above examinations are stated.
Investigator allows direct access to source data/documents (including patient records) during monitoring, auditing and inspection from the Research Ethics Committee for Central Region and the GCP unit for Aarhus University Hospital and its partners. The participants are made aware of this and specific consent is obtained in the form of a power of attorney.
All information obtained will be handled in accordance with the Act on the Processing of Personal Data.

Risks, side effects and inconvenience
The blood test may be associated with discomfort. There is a minimal risk of infection at the injection site. When taking a blood sample, 80 ml will be drawn each time, i.e. around 160 ml of blood in total. This is not expected to cause discomfort.
During the scans, a total so-called effective radiation dose of an estimated 26 microSv is applied to each patient, which corresponds to approx. four days of normal background radiation.

The total time consumption is estimated to be four hours.
It is the investigator's belief that any disadvantages, risks and side effects are minimal and are out-weighed by the new knowledge that the study will be able to provide.

Time and place
Participants are recruited by letter from the Aarhus area, see under recruitment. Inclusion interviews and examinations take place at the Department of Endocrinology and Internal Medicine, The Osteoporosis Clinic, Aarhus University Hospital, Tage-Hansens Gade 2, 8000 Aarhus C.
It is planned that inclusion will begin in August 2015. The inclusion is expected to take place over 1 year, so that the entire study is completed within 1.5 years.
Confidentiality, access to documents, right of appeal and possibility of compensation
All information about the subjects' health conditions, other purely private matters and other confidential information is subject to confidentiality. The test subjects are protected - and all personal data, including tissue, blood samples etc. are stored - in accordance with the Act on the processing of personal data and in accordance with the Health Act. The participants have the opportunity to inspect the trial protocol in accordance with the provisions of the Public Disclosure Act.
In connection with participation in the study, in the event of damage related to errors/negligence, the participants have the opportunity to complain and seek compensation in accordance with the Act on Access to Complaints and Compensation within the Health Service. Furthermore, the participants are covered by the patient insurance of the participating hospitals. In the event of harmful effects related to medicines, the participants are covered according to the Act on Compensation for Medicine Damages.

Recruitment
There will be an advertisement for test subjects via the daily press as well as via notice boards at e.g. educational institutions and larger workplaces (see appendix 15), as well as via the social media: Facebook. Also via www.forsøgspersonner.dk (see appendix 16).
A Facebook page called "Subjects for the "DAGMAR project" will be created. On this page, under the "About" tab, there will be more specific information about the project, see appendix 17. There will be a fixed notice on the page, see appendix 18, and there will be more specific notices on age and gender, see appendix 19. There advertising for both the Facebook page and for small postings

(appendix 20) on the Facebook page (not for app. 19) is purchased. The announcement will contain the text in appendices 18 and 20 respectively and will be targeted at gender, age and place of residence corresponding to the inclusion criteria for the project (age, sex, place of residence in the Aarhus area). These ads will be visible on the target group's Facebook home page and will be listed as "sponsored ad". If you choose to follow the project's Facebook page (by "liking" it), future posts on the Facebook page (both appendices 19 and 20) will be displayed on your Facebook homepage as a regular post, i.e. not as a sponsored ad. In connection with all posts and announcements as stated, the project will be called "DAGMAR euthyroid" corresponding to the name on the Facebook page.
If you contact us regarding the above advertisements, you will be sent written information about the project before any informational interview.
“Forskerservice” will be requested a data file with name, address, age, and gender, and social security number for 25,200 people aged 18-80 from the Aarhus area (200 per age per gender). Every time a patient with Graves' disease is included in the intensive study cohort, a letter will be sent via e-box to 20 gender- and age-matched (+/- 2 years) people from this data extract.
The letter sent will contain a cover letter (appendix 12) as well as participant information (appendix 13) and the pamphlet "Subject's rights in a biomedical research project" (appendix 7). In the cover letter, there will be a link to an internet-based mixed response (appendix 14), in which the participant can state whether he or she is interested in participating in the survey. If interest is expressed, the participant will be asked to answer a short questionnaire regarding menopause status (women only) and exclusion criteria. The questionnaire is answered pseudo-anonymized without directly identifiable information using a username and codes in the sent covering letter. It will appear from the written information material that further information about the project can be obtained by contacting the investigator whose telephone number is given. If there is no response, information about the project will be re-sent after 1 week. If no response is obtained from the potential trial participants, a letter is sent out to another 20 people who are matched in the same way on age and gender. The above procedure is repeated if there is no response.
Interested potential participants are requested to come to an interview about the project. The participant is informed about the possibility of bringing a companion. The information interview will take place at Aarhus University Hospital and will be handled by the investigator or someone else from the project group. The conversation will take place in an undisturbed setting behind closed doors and without interruptions. Sufficient time is set aside for conducting the interview (minimum 30 min). The procedure for this information interview will follow the guidelines as stated under the randomized study i) above.
Participant information (appendix 13) and the pamphlet "Subject's rights in a biomedical research project" (appendix 7) will be reviewed and handed out. From both written and oral information, it will appear that this is a scientific experiment. The design of the survey will be carefully reviewed. The project's purpose, potential risks and discomfort as well as expected benefits will also be stated. Furthermore, the participant will be made aware that there may be unpredictable risks and burdens linked to participation in a research project. It will be emphasized that participation is

voluntary and that the person has the right to withdraw their commitment to participate in the study at any time without further justification and without this affecting future treatment at the hospital.
The project participant will be informed of the right to reflection time ("can go home and think about things") before giving a final answer. If the person concerned wishes to participate in the study, the person concerned can give oral and written consent to this at the end of the interview. If the person asks for time to think, the person is requested to give feedback within 1 week in the event that the person decides to participate in the investigation. In that case, a new time will be agreed for a repeated information interview under a similarly undisturbed framework and with the possibility of co-sitters. Verbal and written consent to participate in the study will then be obtained. The project participant receives a copy of this.
After obtaining consent, the investigator, with the patient's consent, will inform each subject's general practitioner that he or she is participating in the study.
Regarding both study i) and ii)

Ethical considerations
The study is carried out in accordance with the conditions mentioned in the Helsinki II declaration regarding biomedical research involving humans.
The guidelines in accordance with GCP (International Conference on Harmonization (ICH): guidance E6) will be followed. The GCP unit at Aarhus University Hospital has been requested to monitor the study.
The project has been notified to the Danish Data Protection Authority via the region's joint notification.
The trial will be registered at www.clinicaltrial.gov.
Informed consent will be obtained from all participants (Appendix 8), and the study will be carried out in accordance with the applicable recommendations. Participation in the study is voluntary, and any participant can withdraw from the study at any time if they wish to do so, and the participant cannot be required to provide any explanation in this regard. All trial participants will be given a copy of the consent form.

Ethical considerations
It is the investigator's belief that any risks and side effects are minimal and are outweighed by the new knowledge that the study will be able to provide. The individual participant has no immediate benefit from his participation. Should there be a demonstrable vitamin D deficiency at the end of the experiment, the participant will be informed and guided about this.
During the planned examinations, unexpected findings may arise, e.g. non-symptom-producing diseases and conditions. If diseases and conditions requiring treatment are observed during the examinations carried out, the project participant will be offered a referral to relevant treatment.

When the study is finally completed, the participants will be sent a brief description of the study's most important results with information on whether they were treated with active medication or placebo during the study period.
After the end of the experiment, the investigator will inform the Scientific Ethics Committee for Central Denmark Region about the results obtained, observed side effects, number of patients treated, doses used and duration of dosing.
Lack of vitamin D is widespread in Denmark. The present study will elucidate the importance of a vitamin D supplement for the prognosis and symptomatology of patients with Graves' disease. If the study shows a significant effect of intervention on the recurrence rate in the cohort, this may have an impact on the future treatment of patients with Graves' disease.

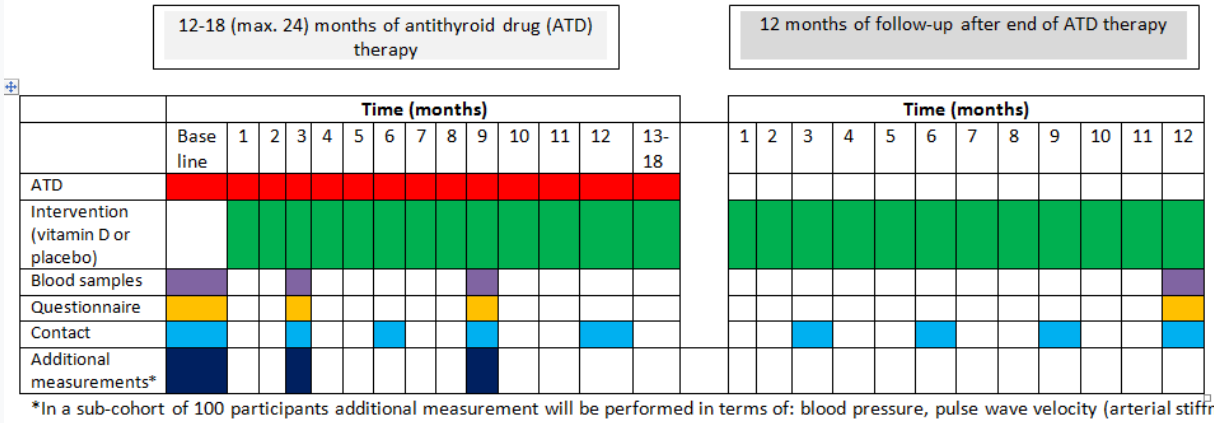
Publication

The results from the study will be sought for publication in international medical journals, as both positive, negative and inconclusive results will be published. The publications are designed exclusively by the trial manager and investigator together with the other co-investigators. There are no companies or individuals who may be presumed to have commercial interests in the interests of the study that influence the results statement or the interpretation of the trial results.

Economy

The study was created on the initiative of doctors from the Department of Medical Endocrinology, Aarhus University Hospital. No one from the project group has financial interests in the study. Expenses for the investigation will be financed by the departments involved, as fund support is also applied for.
No financial remuneration is provided to the test subjects, but possibly a transport allowance. Study medication in the form of vitamin D and placebo is provided free of charge by the food manufacturer and dietary supplement manufacturer Orkla Health.

Figure 1. Flow-chart showing the course of treatment and investigations in the individual participants (n=260) randomized to treatment with vitamin D or similar placebo.



*In a sub-cohort of 100 participants additional measurement will be performed in terms of: blood pressure, pulse wave velocity (arterial stiffness), muscle- and balance-measurements, bone mineral density (BMD) and body composition (by DXA-scans), 3D bone microstructure (by HR pQCT- scans), and immunological analysis. From this sub-cohort 30 participants will undertake muscle and fat biopsy at baseline, 3 and 9 months.

Statistical analysis plan, final

Analyses of treatment effects will be based on the intention-to-treat principle. The first analysis will compare baseline characteristics by randomized treatment assignment to ensure that balance was achieved by the randomization. Characteristics to be examined include known risk factors for treatment failure including age, sex, smoking habits, severity of thyrotoxicosis, former use of vitamin D and baseline vitamin D status.

Differences between groups will be evaluated using a Chi Square test for categorical variables and a two-sample *t* test or Wilcoxon ranksum test, as appropriate, for continuous variables. For the primary outcome, the risk of treatment failure will be calculated as risk ratio (RR) with 95% confidence interval (CI).

With the intention-to-treat (ITT) approach, all subject who had been randomized and had received at least one dose of study medication will be included in the analysis. If study outcome is unknown, e.g. study withdrawal or exclusion, participants will be included in the ITT assuming either a *worst-case scenario* (all had treatment failure) or a *best-case scenario* (all experienced a treatment success)

In per-protocol analyses, only participants in whom outcomes can be assessed will be included.

In a sub-analysis, the risk of relapse at any given time after ATD discontinuation until 12 ± 1 months will be examined using Cox regression.

In pre-specified analyses, the interaction between the intervention and baseline 25(OH)D status (insufficient (<50 mmol/L) or replete) and prior use of vitamin D supplementation (yes or no) will be assessed using logistic regression.

in addition to the original SAP, we will explore the interaction between the intervention and risk factors for relapse such as sex, age (above or below 40 year), time since diagnosis (above or below 35 days), and smoking status (current vs not). In case of significant interaction, the effect of intervention will be tested in each group separately.

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