TITLE	The effect of vitamin D supplementation on hypothyroidism		
Date of plan	**21/2/2023**		
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Background and overview			
Background	Hypothyroidsm occurs commonly; globally, the prevalence of hypothyroidism ranges from 1% to 2% in iodine-sufficient countries such as Australia and the United States [1]. The prevalence of hypothyroidism increases with age, rising to 7% for people aged 85 and above [1], and is approximately ten times more common in women than in men. In iodine-sufficient regions, the most common cause of hypothyroidism is autoimmune thyroid disease. Other factors associated with hypothyroidism include low iodine intake, obesity [2], and history of other autoimmune diseases [3]. Vitamin D, most well-known for its role in musculoskeletal health, may play an important role in other health outcomes. There is some suggestion of a link between vitamin D and autoimmune thyroid diseases; however, an underlying mechanism remains elusive. Vitamin D and thyroid hormone bind to similar nuclear receptors, and some specific vitamin D receptor gene polymorphisms have been associated with higher risk of autoimmune thyroid diseases [4]. *In vitro* and *in vivo* studies have also suggested a role for vitamin D in autoimmune diseases through downregulation of the production of inflammatory cytokines, B cell differentiation, and antibody production [5]. A recent systematic review and meta-analysis of observational studies found inverse associations between serum 25-hydroxyvitamin D (25(OH)D) concentration and hypothyroidism (9 studies, N=1,174), autoimmune thyroid diseases (13 studies, N=12,916), and Hashimoto's thyroiditis (12 studies, N=2440) [6]. However, there was significant heterogeneity and only six studies (five included in the meta-analysis of autoimmune thyroid diseases and one included in the meta-analysis of hypothyroidism) were of high quality. In addition, observational studies may be limited by the potential for reverse causality and residual confounding. A Mendelian randomisation study, where bias due to confounding is less likely, did not find an association between genetically predicted 25(OH)D concentration and hypothyroidism [7]. There is limited evidence from randomised controlled trials (RCTs) regarding the effect of vitamin D supplementation on thyroid diseases. Results from the large, double-blind, placebo-controlled VITamin D and OmegA-3 TriaL (VITAL, N=25,871) from the United States suggested a statistically significant beneficial effect of 2000 international units (IU) of supplemental vitamin D taken daily for approximately five years on the incidence of a combined group of autoimmune diseases, including rheumatoid arthritis, polymyalgia, autoimmune thyroid		

	diseases, psoriasis, and inflammatory bowel disease (hazard ratio 0.78; 95% confidence interval (CI) 0.61 to 0.99) [8]. The effect on autoimmune thyroid diseases was not reported separately. Some small RCTs suggested that vitamin D supplementation reduced thyroid antibodies in people with thyroid disease [9, 10]. Two meta-analyses of RCTs, one among patients with Hashimoto's thyroiditis (6 studies, N=258) [9] and the other in patients with autoimmune thyroiditis (6 studies, N=344) [10], found that vitamin D supplementation reduced the level of thyroid peroxidase antibodies, suggesting a role for vitamin D in the treatment of autoimmune thyroiditis. We will analyse data from the D-Health Trial to assess the effect of monthly doses of 60,000 IU supplemental vitamin D on incidence of hypothyroidism among older Australians, using prescription of levothyroxine as a surrogate for diagnosis of hypothyroidism.
Overview of current study	This study will investigate the effect of vitamin D supplementation on hypothyroidism, using prescription of levothyroxine as a surrogate for diagnosis.
Aims	The aim of the study is to investigate whether vitamin D supplementation influences the incidence of hypothyroidism. We also aim to evaluate whether vitamin D supplementation interacts with age, sex, body mass index (BMI), and predicted baseline 25(OH)D concentration to influence the incidence of hypothyroidism.
Instrument	Prescription of levothyroxine will be obtained from linked Pharmaceutical Benefits Scheme (PBS) data. The PBS data provides information related to prescribed drugs including date of prescribing, drug name, item number, and Anatomical Therapeutic Chemical (ATC) code. The ATC code for levothyroxine starts with "H03A".
Outcomes and hypotheses	
Outcome	The outcome is time to first prescription of levothyroxine.
Specific hypotheses	**Hypotheses** The incidence of hypothyroidism will differ between the two groups. There will be significant interactions between treatment allocation and: • Sex (male vs female) • Age at randomisation (<70 years vs ≥70 years) • BMI at randomisation (<25, 25 to <30, ≥30 kg/m^2) • Predicted baseline 25(OH)D concentration (<50 vs ≥50 nmol/L)
Data details	
Analysis package	SAS 9.4, R version 3.6.3, and Stata version 17
Datasets used to create "analysis dataset"	The SAS data used to generate published results will be stored in L:\Lab_RachelN\DHealthDataAnalysis\Projects\Thyroid\Dataset R:\Lab_RachelN\Dhealth\Thyroid\Dataset

Participants	In the analysis of the effect of vitamin D supplementation on the incidence of hypothyroidism, we will exclude people who: 1. did not give consent to both PBS and Medicare Benefits Schedule (MBS) linkage (n=1967); 2. did not have hospital data (n=7[a]); 3. were diagnosed with thyroid cancer prior to randomisation (n=35[a]); 4. had pre-existing hypothyroidism (defined as having at least one prescription of levothyroxine dispensed within **12 months** after randomisation, n= 1367[b]); 5. had a thyroidectomy and/or thyroid cancer within **12 months** after randomisation (n=8[b]); or 6. whose last known date alive was within **12 months** of randomisation (n=73[b]). [a] Restricted to participants who consented to both PBS and MBS linkage. [b] Restricted to participants who did not satifsy any of the first three criteria. If a participant satisfied more than one of the last three criteria, we assigned them to the exclusion criterion with the earliest date.
Competing risks	In our main analyses, the following will be treated as competing risks if experienced without prior prescription of levothyroxine: • Death; and • Thyroidectomy and/or thyroid cancer. All-cause mortality was the primary outcome of the D-Health Trial.[11] Deaths were ascertained primarily via linkage to state death registries; a small number of deaths included in the analysis of all-cause mortality were reported to the trial and did not appear in death registers (e.g., because they occurred outside Australia or in the Northern Territory or Australian Capital Territory for which linked data were unavailable). We will ascertain the date of thyroidectomy using hospital and MBS data. • Hospital procedure codes pertaining to thyroidectomy: 90046-02, 90046-00, 30310-00, 30309-00, 30308-00, 30306-01, 30297-02, 30297-01, 30296-01 • MBS item codes pertaining to thyroidectomy: 30296 (Total thyroidectomy) and 30310 (Partial thyroidectomy) We will ascertain thyroid cancer using hospital and cancer registry data. Participants who had a hospitalisation for thyroid cancer (ICD-10 AM principal diagnosis code starts with "C73") or a diagnosis of thyroid cancer in the cancer registry data (PSite starts with "C73"), but for whom we have no record of thyroidectomy, will be censored at the ***date of admission*** or ***date of diagnosis***. Note: Cancer registry data are incomplete due to delays in cancer registration. However, only 2 (20%) of the 10 participants with a post-randomisation thyroid cancer diagnosis in the registry data did not have any record of thyroidectomy and/or thyroid cancer in the hospital and/or MBS datasets. The combined use of registry, hospital, and MBS data should, therefore, capture almost all instances of thyroid cancer.

Follow up	In the main analysis, the follow up period will begin from 1 year post-randomisation and end at the earliest of: (i) first prescription of levothyroxine;[a] (ii) thyroidectomy;[a] (iii) thryoid cancer diagnosis;[b] (iv) date last known to be alive; (v) 5 years and 1 month after randomisation. [a] If first prescription of levothyoxine and thyroidectomy occurred on the same date (and this is the earliest date) we will censor the participant (i.e., treat the thyroidectomy as a competing risk). [b] If the participant had a thyroidectomy after the diagnosis of thryoid cancer, we will use date of the thyroidectomy.
Codebook	L:\Lab_RachelN\DHealthDataAnalysis\Projects\Thyroid\Codebook
Exposure variable(s)	Randomisation group
Covariates	<u>Adjustment variables:</u> • Age at randomisation: 60-64; 65-69; 70-74; 75+ • Sex: male; female • State of residence at randomisation: NSW; QLD; SA; TAS; VIC; WA <u>Variables that will be used to derive interaction terms:</u> • Sex (male vs female) • Age at randomisation (<70 years vs ≥70 years) • BMI at randomisation (<25, 25 to <30, ≥30 kg/m^2) • Predicted baseline 25(OH)D concentration (<50 vs ≥50 nmol/L)
Maintaining blinding	The investigators will remain blinded during the analysis of the study. Code will be written and tested using a dataset in which the randomisation group allocation and the participants' identification code (study code) are removed. Participants are randomly assigned to two equal groups; these will have no relationship to the true study group allocation. When all investigators have approved the analysis and the format of the pre-specified tables and figures, the true randomisation allocation will be returned to the dataset for the generation of final results. Any analysis performed after un-blinding will be declared as exploratory.
Power calculation	The sample size was primarily selected for analysis of the mortality outcome. For this analysis, we will include a total of 17853 participants in the main analysis. With this sample size, assuming that the incidence rate is 2.5 per 1000 person-years-at-risk (PYAR) for males and 8.2 per 1000 PYAR for females, we have 80% power to detect a difference of 28% in the incidence of hypothyroidism.
Handling missing data	***Missing covariate data*** BMI is the only variable (used in subgroup analyses) with missing data. People with missing BMI (n=75, 0.4%) will be excluded from the relevant stratified analyses.

Proposed sequence of analyses	
File management	The code used to generate the results will be stored in: L:\Lab_RachelN\DHealthDataAnalysis\Projects\Thyroid\Code R:\Lab_RachelN\Dhealth\Thyroid\Code
Main analysis	Analyses of the effect of vitamin D supplementation on incidence of hypothyroidism will follow an intention-to-treat approach. **Figure 1. CONSORT flow diagram** Flow of the participants included in the main analyses of the incidence of hypothyroidism will be presented using a CONSORT diagram. **Table 1. Baseline characteristics of participants included in the analysis according to randomisation group** For participants included in the main analysis, we will compare selected baseline characteristics between the two study groups. **Figure 2. Effect of vitamin D supplementation on incidence of hypothyroidism. Panel A shows the cause-specific cumulative probability of hypothyroidism according to follow-up time and panel B shows the time-varying hazard ratio.** **Table 2. The effect of vitamin D supplementation on the incidence of hypothyroidism** **Figure S1. Effect of vitamin D supplementation on the percentage difference in cause-specific standardised cumulative incidence functions** We will plot the cause-specific cumulative probability of hypothyroidism for each randomisation group, using Aalen-Johansen methods (Figure 2, panel A). To assess the effect of vitamin D supplementation on hazard of hypothyroidism, we will fit two flexible parametric survival models (FPSMs).[12, 13] When fitting these we will model the baseline log cumulative hazard function using a restricted cubic spline with two internal knots, placed at the 33rd and 67th percentiles of the uncensored log survival times. Both models will include randomisation group and the randomisation stratification variables of age, sex, and state of residence at baseline. • Model 1 will assume proportional hazards; it will be used to estimate an "overall" hazard ratio (HR) and 95% confidence interval (CI). We will report the number and percentage of people who developed hypothyroidism within each randomisation group, and the overall HR (95% CI) (Table 2). • Model 2 will include an interaction between randomisation group and follow-up time, fitted as a restricted cubic spline with one internal knot (placed at the median of uncensored log survival times), thereby allowing the HR for randomisation group to vary with time. We will report the p-value from the likelihood ratio test comparing Models 1 and 2 (i.e. testing the effect of including the interaction between time and randomisation group). Using Model 2, we will plot the estimated HR (95% CI) as a

	function of follow-up time (Figure 2, panel B), and report values at 2 and 4 years of follow-up (Table 2). We will estimate the cause-specific standardised cumulative incidence of hypothyroidism for each randomisation group, treating death (without prior hypothyroidism) and thyroidectomy and/or thyroid cancer (without prior hypothyroidism) as competing risks. We will use the user-written standsurv command in Stata with the competing risks models option. The analysis will use estimates from FPSMs that include randomisation group, age, sex, and state of residence at baseline, and an interaction between randomisation group and time since randomisation; we will model the baseline log cumulative hazard function and the interaction as described previously. The cause-specific cumulative incidence functions will be standardised to the distribution of age, sex, and state of residence at baseline for all participants included in this study. We will report the cause-specific standardised cumulative incidence (95% CI) of hypothyroidism for each randomisation group at 2 and 4 years of follow-up (Table 2), and plot the percentage difference in cause-specific standardised cumulative incidence functions (Figure S1). **Figure 3**. **The effect of vitamin D supplementation on the incidence of hypothyroidism overall and in subgroups of participants.** We will investigate whether the effect of supplementation on hypothyroidism is modified by the following baseline characteristics: • Age at baseline (< 70 years, ≥ 70 years); • Sex (men, women); • BMI at baseline (< 25 kg/m^2, 25 to <30 kg/m^2, ≥30 kg/m^2); • Predicted deseasonalised 25(OH)D concentration (< 50 nmol/L, ≥ 50 nmol/L). For each characteristic we will fit an FPSM that includes randomisation group, age, sex, state, the baseline characteristic of interest, and an interaction between randomisation group and the baseline characteristic. The baseline hazard will be modelled as described above. We will assume proportional hazards for all covariates included in the model. We will report the "overall" HR for each level of the baseline characteristic. The interaction between randomisation group and the baseline characteristic will be assessed using a likelihood ratio test that compares models with and without the interaction term.
Descriptive analysis	**Table S1. Baseline characteristics according to those included and excluded from the main analysis** We will use chi-squared tests to compare selected baseline characteristics of participants according to inclusion or exclusion from the main analysis. **Table S2. Associations between selected baseline characteristics and prevalent and incident use of levothyroxine** We will use logistic regression to estimate associations between prevalent use of levothyroxine (defined at having at least 1 dispense of levothyroxine within 12 months of randomisation) and selected baseline characteristics. We will use FPSMs to estimate associations between incident levothyroxine prescription and selected

	baseline characteristics. All models will include randomisation group, age at randomisation, and sex.
Overall Significance level	We will use a significance level of 0.05. There will be no adjustment for multiple testing.
Sensitivity analyses	**<u>Sensitivity analyses</u>** For each sensitivity analysis below, we will reproduce Table 2 and Figures 2 and S1. **Sensitivity analysis 1: Follow up starts from 7 months post randomisation** • Table S3 • Figures S2 and S3 The rationale for this analysis is that levothyroxine is usually dispensed 6 monthly. If a participant was dispensed a script just before randomisation, the next script dispensed should be dispensed within the first 6 months after randomisation, but we are allowing one month to account for delayed script supply. The only difference between this analysis and the main analysis is that the follow up period will begin from 7 months post-randomisation (instead of 12 months post-randomisation). **Sensitivity analysis 2: Accounting for medically treated hyperthyroidism** • Table S4 • Figures S4 and S5 For this analysis, the follow up period will begin from 1 year post-randomisation and end at the earliest of: (i) first prescription of levothyroxine; (ii) thyroidectomy; (iii) thyroid cancer diagnosis; (iv) first prescription of carbimazole or propylthiouracil (as a surrogate for hyperthyroidism, PBS ATC codes "H03BB01" and "H03BA02"); (v) date last known to be alive; (vi) 5 years and 1 month after randomisation. For this analysis, we will also exclude people with pre-existing hyperthyroidism (defined as having at least one prescription of carbimazole or propylthiouracil dispensed within **12 months** after randomisation, N=33)

REFERENCES

1. Taylor, P.N., et al., *Global epidemiology of hyperthyroidism and hypothyroidism.* Nat Rev Endocrinol, 2018. **14**(5): p. 301-316.
2. Song, R.H., et al., *The Impact of Obesity on Thyroid Autoimmunity and Dysfunction: A Systematic Review and Meta-Analysis.* Front Immunol, 2019. **10**: p. 2349.
3. Chaker, L., et al., *Hypothyroidism.* Lancet, 2017. **390**(10101): p. 1550-1562.

4. Feng, M., et al., *Polymorphisms in the vitamin D receptor gene and risk of autoimmune thyroid diseases: a meta-analysis.* Endocrine, 2013. **43**(2): p. 318-26.

5. Dankers, W., et al., *Vitamin D in Autoimmunity: Molecular Mechanisms and Therapeutic Potential.* Front Immunol, 2016. **7**: p. 697.

6. Taheriniya, S., et al., *Vitamin D and thyroid disorders: a systematic review and Meta-analysis of observational studies.* BMC Endocr Disord, 2021. **21**(1): p. 171.

7. Ye, Y., et al., *A comprehensive genetic and epidemiological association analysis of vitamin D with common diseases/traits in the UK Biobank.* Genet Epidemiol, 2021. **45**(1): p. 24-35.

8. Hahn, J., et al., *Vitamin D and marine omega 3 fatty acid supplementation and incident autoimmune disease: VITAL randomized controlled trial.* Bmj, 2022. **376**: p. e066452.

9. Jiang, H., et al., *Effects of vitamin D treatment on thyroid function and autoimmunity markers in patients with Hashimoto's thyroiditis-A meta-analysis of randomized controlled trials.* J Clin Pharm Ther, 2022. **47**(6): p. 767-775.

10. Wang, S., et al., *The effect of vitamin D supplementation on thyroid autoantibody levels in the treatment of autoimmune thyroiditis: a systematic review and a meta-analysis.* Endocrine, 2018. **59**(3): p. 499-505.

11. Neale, R.E., et al., *The D-Health Trial: a randomised controlled trial of the effect of vitamin D on mortality.* Lancet Diabetes Endocrinol, 2022. **10**(2): p. 120-128.

12. Lambert, P.C. and P. Royston, *Further development of flexible parametric models for survival analysis.* The Stata Journal, 2009. **9**(2): p. 265-290.

13. Royston, P. and M.K. Parmar, *Flexible parametric proportional-hazards and proportional-odds models for censored survival data, with application to prognostic modelling and estimation of treatment effects.* Stat Med, 2002. **21**(15): p. 2175-97.

THE FOLLOWING APPENDICES CONTAIN SAMPLE TABLES AND FIGURES BASED ON "FAKE" DATA

To generate the "fake" data, we removed the true randomisation and participant identification codes from the original dataset, and then randomly assigned participants to two groups of equal size. There is no relationship between the new groups and the true treatment allocation.

APPENDICES. Pre-specified tables and figures

Table 1. Baseline characteristics of participants included in the analysis according to randomisation group

	N (%)	
Basleine Characteristic	**Vitamin D (N = 8,892)**	**Placebo (N = 8,961)**
Age (years)		
60-64	2202 (24.8)	2237 (25.0)
65-69	2450 (27.6)	2458 (27.4)
70-74	2392 (26.9)	2447 (27.3)
≥ 75	1848 (20.8)	1819 (20.3)
Sex		
Men	5155 (58.0)	5064 (56.5)
Women	3737 (42.0)	3897 (43.5)
Body mass index (kg/m²)		
< 25	2679 (30.3)	2721 (30.5)
25 to < 30	3852 (43.5)	3825 (42.9)
≥ 30	2322 (26.2)	2379 (26.7)
Missing	*39*	*36*
Smoking history		
Never	4755 (54.0)	4892 (55.0)
Ex-smoker	3671 (41.7)	3643 (40.9)
Current	383 (4.3)	362 (4.1)
Missing	*83*	*64*
Alcohol consumption (drinks/week)		
< 1	2036 (23.8)	2057 (23.8)
1 to 7	3742 (43.8)	3838 (44.4)
> 7 to 14	1644 (19.2)	1601 (18.5)
> 14	1131 (13.2)	1157 (13.4)
Missing	*339*	*308*
Self-rated overall health		
Excellent or very good	4924 (56.2)	5037 (57.1)
Good	3134 (35.8)	3055 (34.6)
Fair or poor	700 (8.0)	730 (8.3)
Missing	*134*	*139*
Self-rated quality of life		
Excellent or very good	5901 (67.9)	6026 (68.7)
Good	2336 (26.9)	2244 (25.6)
Fair or poor	456 (5.2)	505 (5.8)
Missing	*199*	*186*
Predicted 25(OH)D concentration (nmol/L)		
< 50	2106 (23.7)	2189 (24.4)
≥ 50	6786 (76.3)	6772 (75.6)

Table 2. The effect of vitamin D supplementation on the incidence of hypothyroidism*

	Cumulative incidence (%) (95% CI)[1,2]		
	Placebo (N = 8961)	**Vitamin D (N = 8892)**	**Hazard Ratio (95% CI)[1]**
At 2 years from start of follow-up[3]	0.8 (0.6 to 1.0)	1.0 (0.8 to 1.2)	0.98 (0.76, 1.26)
At 4 years from start of follow-up[3]	1.6 (1.3 to 1.8)	1.7 (1.4 to 2.0)	0.85 (0.59, 1.23)
Overall HR[4]			1.06 (0.85, 1.34)

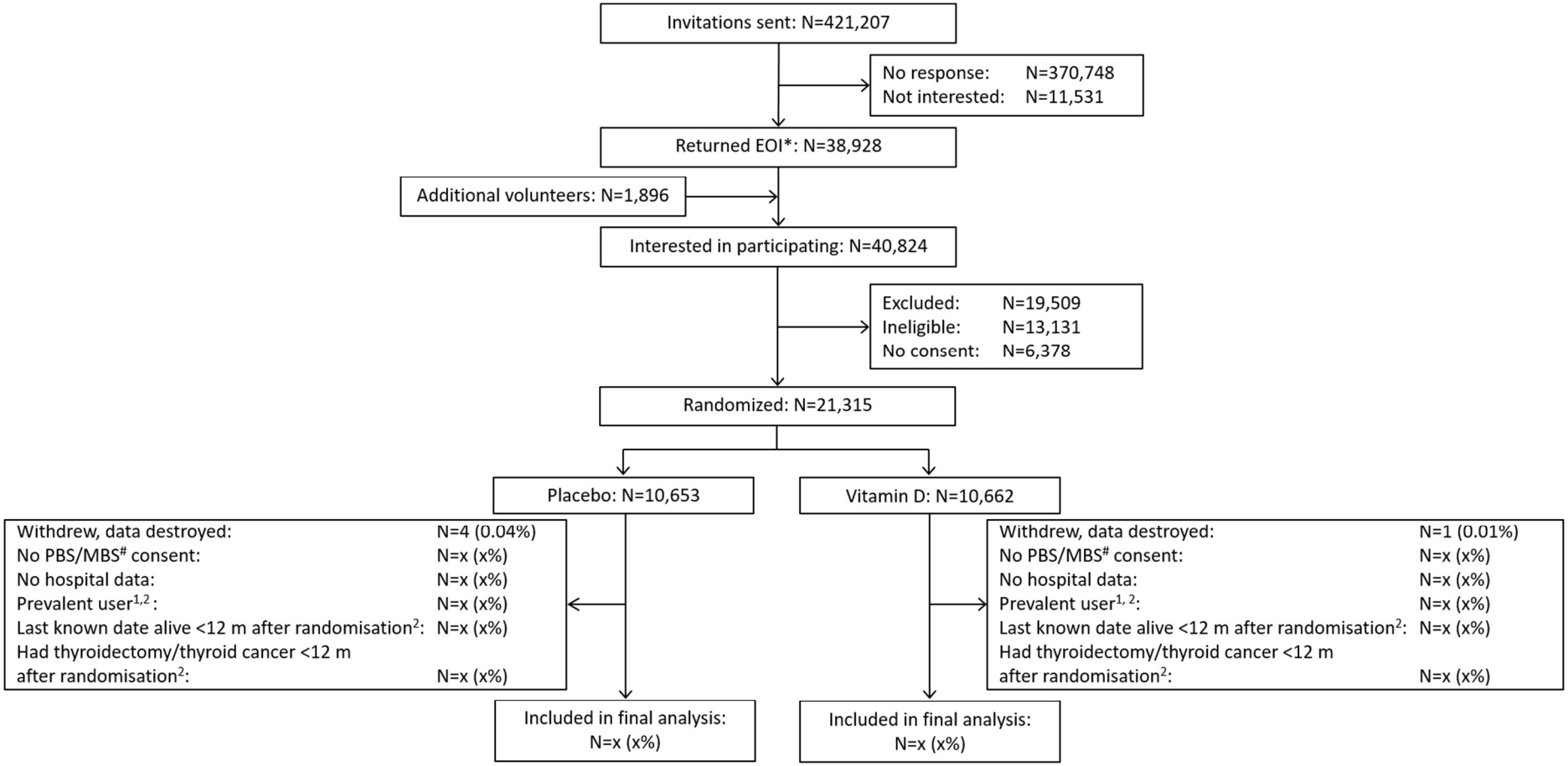
* Follow-up began 1 year post-randomisation and ended at the earliest of: (i) first prescription of levothyroxine (used as a surrogate for diagnosis of hypothyroidism); (ii) thyroidectomy; (iii) thryoid cancer diagnosis; (iv) date last known to be alive; (v) 5 years and 1 month after randomisation.

[1]Estimates produced using flexible parametric survival models that included randomisation group, age, sex, and state of residence at baseline.

[2]Cause-specific standardised cumulative incidence estimated treating death (without prior hypothyroidism) and thyroidectomy and/or thyroid cancer (without prior hypothyroidism) as competing risks. Incidence, expressed as a percentage, is standardised to the distribution of age, sex, and state of residence at baseline for all participants included in this study.

[3]Estimates from a model that also included an interaction between randomisation group and time since randomisation.

[4]Overall estimate from a model that assumed proportional hazards.



*EOI=expression of interest; #PBS=Pharmaceutical Benefits Scheme, MBS=Medicare Benefits Schedule

[1]Defined as having at least 1 dispense of levothyroxine within 12 months of randomisation.
[2]Participants satisfying multiple exclusion criteria were assigned to the criterion that they satisfied first.

Figure 1. Participant CONSORT flow diagram

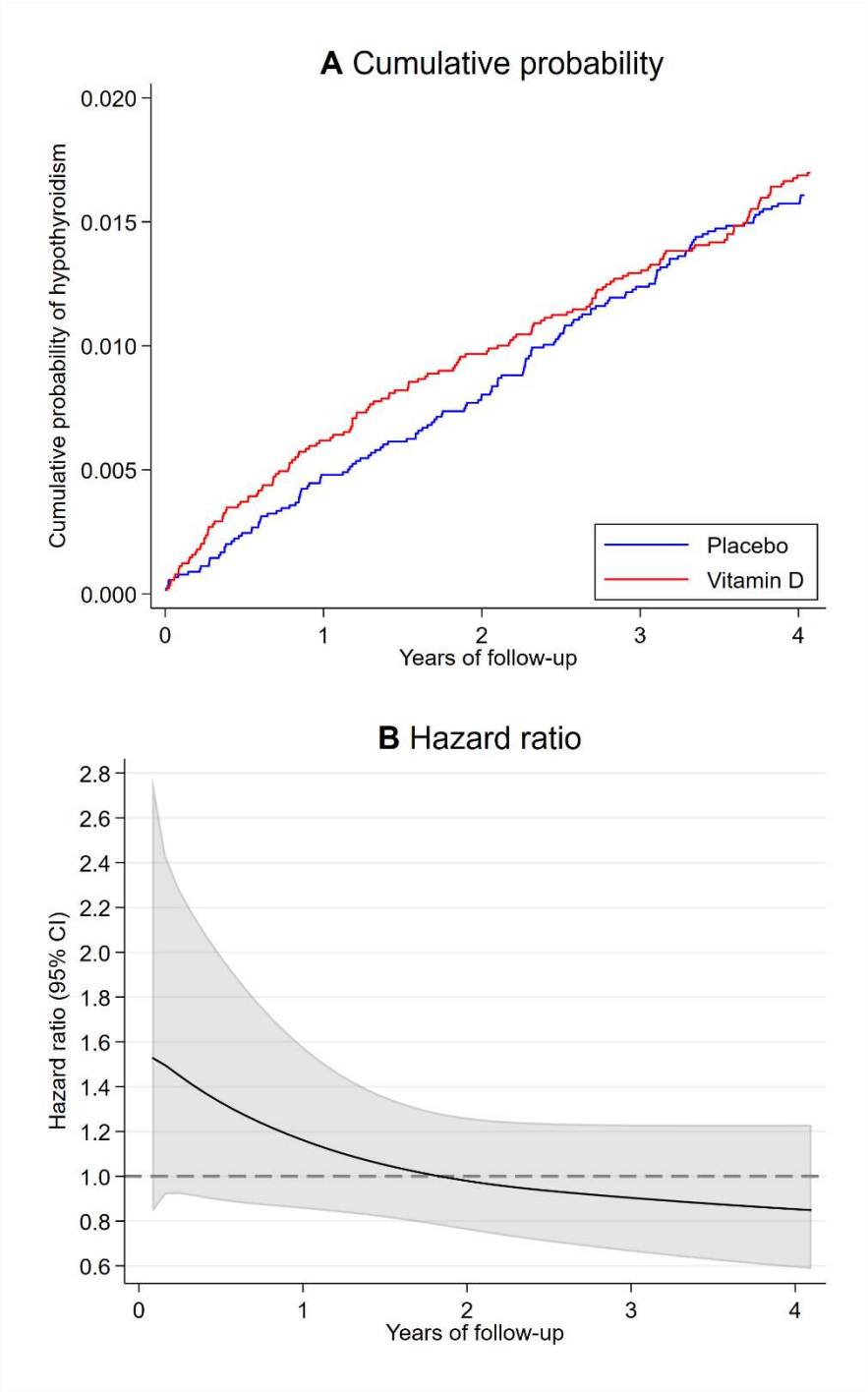


Figure 2. Effect of vitamin D supplementation on incidence of hypothyroidism. Panel A shows the cause-specific cumulative probability of hypothyroidism according to follow-up time, and panel B shows the time-varying hazard ratio

Follow-up began 1 year post-randomisation and ended at the earliest of: (i) first prescription of levothyroxine (used as a surrogate for diagnosis of hypothyroidism); (ii) thyroidectomy; (iii) thyroid cancer diagnosis; (iv) date last known to be alive; (v) 5 years and 1 month after randomisation. Panel A: Curves estimated using Aalen-Johansen methods, treating death (without prior hypothyroidism) and thryoidectomy and/or thryoid cancer (without prior hypothyroidism) as competing risks. Panel B: Estimates (vitamin D versus placebo) from a flexible parametric survival model that included randomisation group, age, sex, and state of residence at baseline, and an interaction between randomisation group and years of follow up, fitted as a restricted cubic spline with one internal knot (placed at the median of uncensored log survival times).
Abbreviation: CI = confidence interval

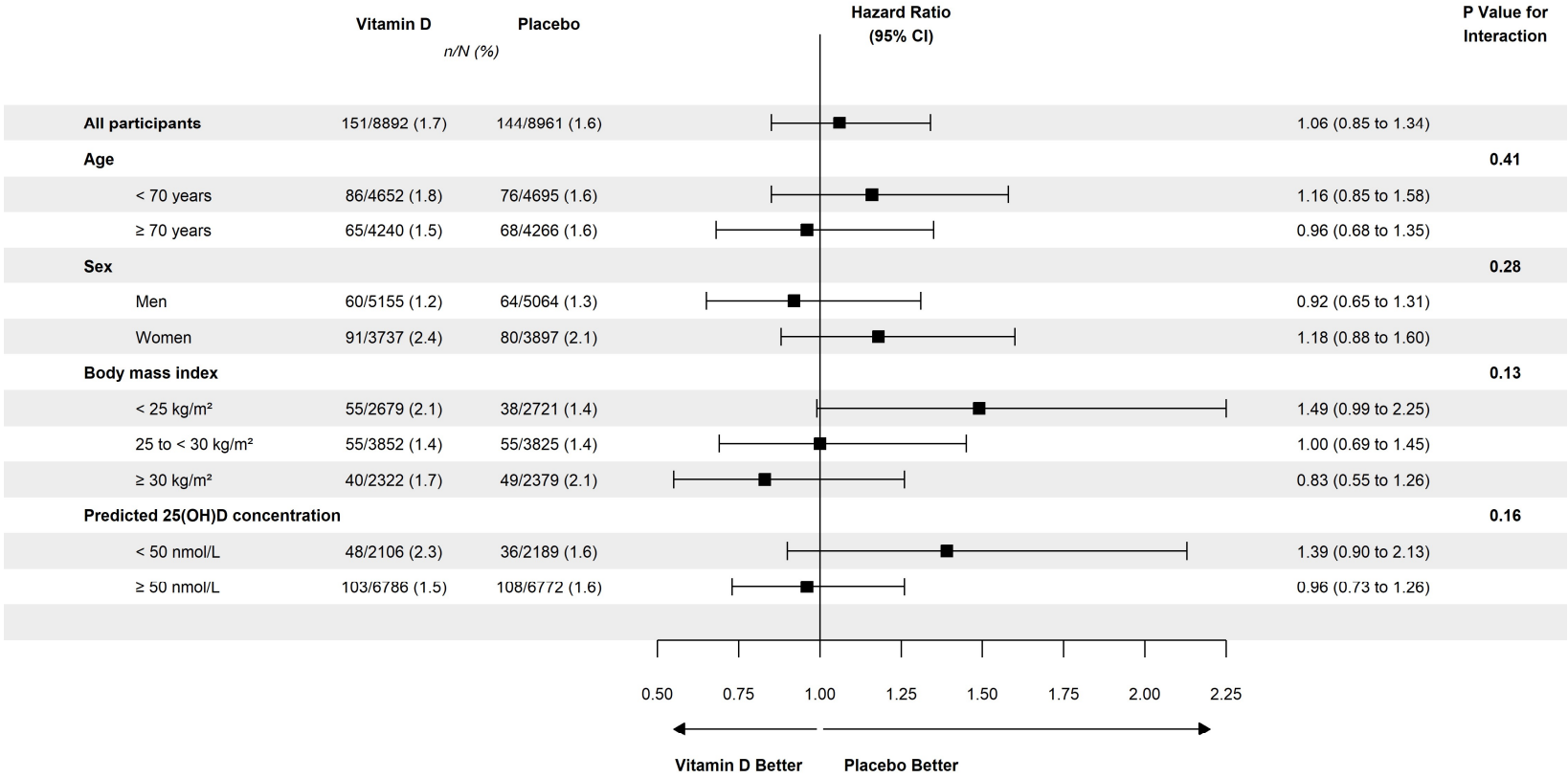


Figure 3. The effect of vitamin D supplementation on incidence of hypothyroidism overall and in subgroups of participants.

Hazard ratios (95% CI) comparing vitamin D to placebo are from flexible parametric survival models. All models include randomisation group, age, sex, and state of residence at baseline. Models producing estimates by levels of age, sex, BMI, and predicted baseline 25(OH)D concentration, include the characteristic of interest and an interaction between randomisation group and the characteristic of interest. P-values were from likelihood ratio tests that compared models with and without the interaction term. Follow-up began 1 year post-randomisation and ended at the earliest of: (i) first prescription of levothyroxine (used as a surrogate for diagnosis of hypothyroidism); (ii) thyroidectomy; (iii) thyroid cancer diagnosis; (iv) date last known to be alive; (v) 5 years and 1 month after randomisation.

Abbreviation: BMI, body mass index; CI = confidence interval

Table S1. Baseline characteristics of participants included versus excluded from the main analysis

Baseline characteristic	N (%) Included (N = 17,853)	Excluded (N = 3,457)	P-value[1]
Randomisation Group			
Placebo	8961 (50.2)	1694 (49.0)	0.20
Vitamin D	8892 (49.8)	1763 (51.0)	
Age (years)			
60-64	4439 (24.9)	813 (23.5)	0.11
65-69	4908 (27.5)	926 (26.8)	
70-74	4839 (27.1)	957 (27.7)	
≥ 75	3667 (20.5)	761 (22.0)	
Sex			
Men	10219 (57.2)	1311 (37.9)	<0.0001
Women	7634 (42.8)	2146 (62.1)	
Body mass index (kg/m²)			
< 25	5400 (30.4)	1017 (29.8)	<0.0001
25 to < 30	7677 (43.2)	1352 (39.6)	
≥ 30	4701 (26.4)	1044 (30.6)	
Missing	*75*	*44*	
Smoking history			
Never	9647 (54.5)	1945 (56.9)	0.02
Ex-smoker	7314 (41.3)	1323 (38.7)	
Current	745 (4.2)	151 (4.4)	
Missing	*147*	*38*	
Alcohol consumption (drinks/week)			
< 1	4093 (23.8)	954 (29.1)	<0.0001
1 to 7	7580 (44.1)	1524 (46.5)	
> 7 to 14	3245 (18.9)	509 (15.5)	
> 14	2288 (13.3)	292 (8.9)	
Missing	*647*	*178*	
Self-rated overall health			
Excellent or very good	9961 (56.7)	1686 (49.8)	<0.0001
Good	6189 (35.2)	1329 (39.3)	
Fair or poor	1430 (8.1)	370 (10.9)	
Missing	*273*	*72*	
Self-rated quality of life			
Excellent or very good	11927 (68.3)	2033 (60.7)	<0.0001
Good	4580 (26.2)	1051 (31.4)	
Fair or poor	961 (5.5)	263 (7.9)	
Missing	*385*	*110*	
Predicted 25(OH)D concentration (nmol/L)			
< 50	4295 (24.1)	905 (26.2)	0.01
≥ 50	13558 (75.9)	2552 (73.8)	

[1] *P* value from chi-squared test

Table S2. Associations between selected baseline characteristics and prevalent and incident use of levothyroxine

	Prevalent use of levothyroxine[1]	Incidence use of levothyroxine[2]
n cases/N participants (%)	**1367/19301 (7.1)**	**295/17853 (1.7)**
	OR (95% CI)[3]	**HR (95% CI)[4]**
Age (years)		
60-64	ref.	ref.
65-69	1.19 (1.01 to 1.39)	1.35 (0.98 to 1.84)
70-74	1.38 (1.18 to 1.61)	1.07 (0.76 to 1.49)
≥ 75	1.52 (1.28 to 1.80)	1.31 (0.92 to 1.86)
Sex		
Men	ref.	ref.
Women	5.02 (4.39 to 5.73)	1.86 (1.47 to 2.35)
Body mass index (kg/m²)		
< 25	ref.	ref.
25 to < 30	1.15 (1.00 to 1.32)	0.90 (0.68 to 1.19)
≥ 30	1.52 (1.32 to 1.76)	1.13 (0.85 to 1.51)
Predicted 25(OH)D concentration (nmol/L)		
< 50	ref.	ref.
≥ 50	1.02 (0.90 to 1.16)	0.82 (0.64 to 1.06)
Self-rated overall health		
Excellent or very good	ref.	ref.
Good	1.48 (1.31 to 1.68)	1.62 (1.27 to 2.08)
Fair or poor	1.96 (1.62 to 2.36)	2.04 (1.40 to 2.97)
Self-rated quality of life		
Excellent or very good	ref.	ref.
Good	1.30 (1.14 to 1.47)	1.26 (0.97 to 1.63)
Fair or poor	1.54 (1.23 to 1.93)	2.02 (1.35 to 3.03)

[1]Prevalent user is defined as having at least 1 prescription of levothyroxine dispensed within 12 months after randomisation.
[2]Follow-up began 1 year post-randomisation and ended at the earliest of: (i) first prescription of levothyroxine (used as a surrogate for diagnosis of hypothyroidism); (ii) thyroidectomy; (iii) thyroid cancer diagnosis; (iv) date last known to be alive; (v) 5 years and 1 month after randomisation.
[3]OR from logistic regression
[4]HR from flexible parametric survival models where proportional hazards assumed for all covariates included in the model.
Note: All estimates were adjusted for age and sex.
Abbreviations: CI = confidence interval; HR = hazard ratio; OR = odds ratio.

Table S3. Sensitivity analysis 1: The effect of vitamin D supplementation on the incidence of hypothyroidism*

	Cumulative incidence (%) (95% CI)[1,2]		
	Placebo (N = 9029)	Vitamin D (N = 8955)	Hazard Ratio[1] (95% CI)
At 2 years from start of follow-up[3]	1.2 (1.0 to 1.5)	1.3 (1.1 to 1.6)	1.01 (0.80, 1.28)
At 4 years from start of follow-up[3]	2.0 (1.7 to 2.3)	2.0 (1.8 to 2.3)	0.93 (0.69, 1.25)
Overall HR[4]			1.01 (0.83, 1.23)

[*] ***Follow-up began 7 months*** post-randomisation and ended at the earliest of: (i) first prescription of levothyroxine (used as a surrogate for diagnosis of hypothyroidism); (ii) thyroidectomy; (iii) thryoid cancer diagnosis; (iv) date last known to be alive; (v) 5 years and 1 month after randomisation.
[1]Estimates produced using flexible parametric survival models that included randomisation group, age, sex, and state of residence at baseline.
[2]Cause-specific standardised cumulative incidence estimated treating death (without prior hypothyroidism) and thyroidectomy and/or thyroid cancer (without prior hypothyroidism) as competing risks. Incidence, expressed as a percentage, is standardised to the distribution of age, sex, and state of residence at baseline for all participants included in this study.
[3]Estimates from a model that also included an interaction between randomisation group and time since randomisation.
[4]Overall estimate from a model that assumed proportional hazards.

Table S4. Sensitivity analysis 2: The effect of vitamin D supplementation on the incidence of hypothyroidism*

	Cumulative incidence (%) (95% CI)[1,2]		
	Placebo (N = 8932)	Vitamin D (N = 8877)	Hazard Ratio[1] (95% CI)
At 2 years from start of follow-up[3]	0.8 (0.6 to 1.0)	1.0 (0.8 to 1.2)	0.97 (0.75, 1.26)
At 4 years from start of follow-up[3]	1.5 (1.3 to 1.8)	1.6 (1.4 to 1.9)	0.85 (0.58, 1.23)
Overall HR[4]			1.06 (0.84, 1.34)

[*] Follow-up began 1 year post-randomisation and ended at the earliest of: (i) first prescription of levothyroxine (used as a surrogate for diagnosis of hypothyroidism); (ii) thyroidectomy; (iii) thryoid cancer diagnosis; (iv) ***first prescription of carbimazole or propylthiouracil (as a surrogate for hyperthyroidism)***; (v) date last known to be alive; (vi) 5 years and 1 month after randomisation.
[1]Estimates produced using flexible parametric survival models that included randomisation group, age, sex, and state of residence at baseline.
[2]Cause-specific standardised cumulative incidence estimated treating death (without prior hypothyroidism), thyroidectomy and/or thyroid cancer (without prior hypothyroidism), and ***prescription of carbimazole/propylthiouracil (as a surrogate for hyperthyroidism)*** (without prior hypothyroidism) as competing risks. Incidence, expressed as a percentage, is standardised to the distribution of age, sex, and state of residence at baseline for all participants included in this study.
[3]Estimates from a model that also included an interaction between randomisation group and time since randomisation.
[4]Overall estimate from a model that assumed proportional hazards.

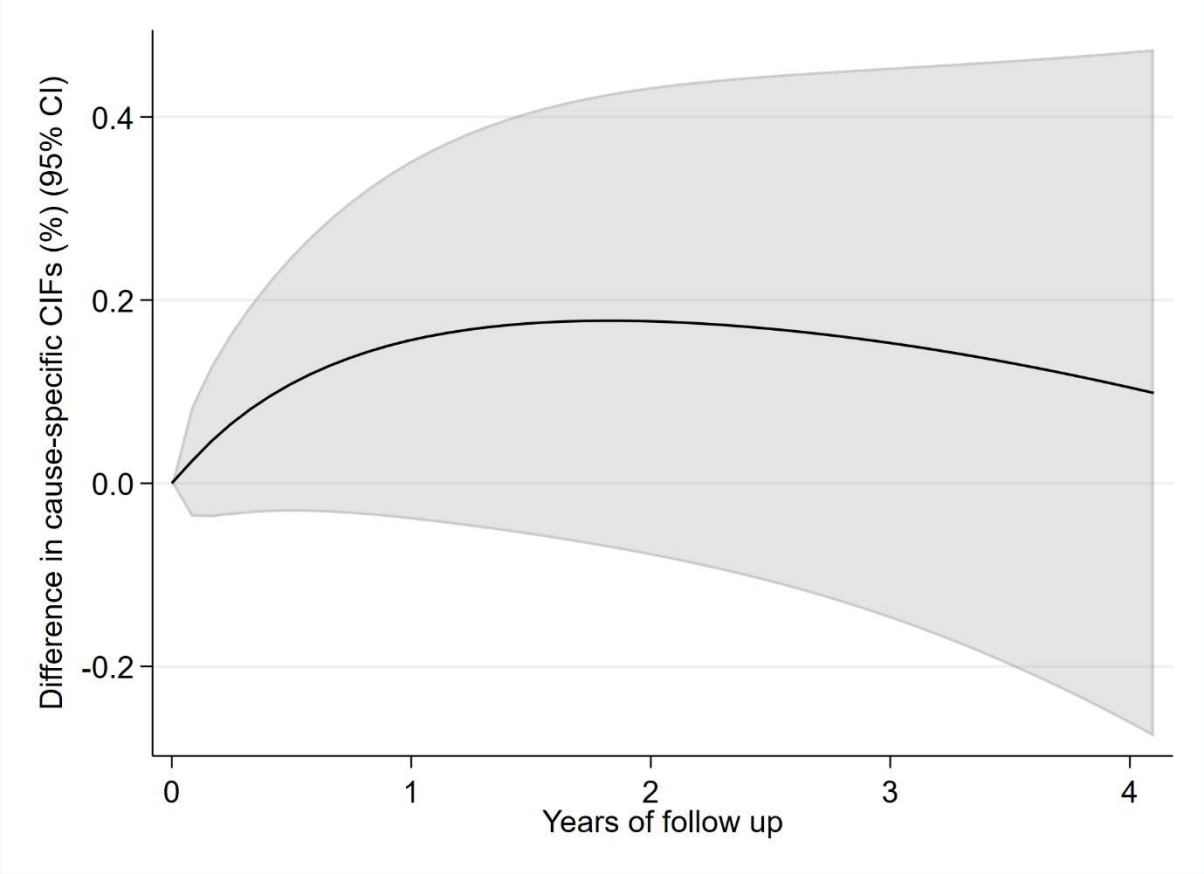


Figure S1. Effect of vitamin D supplementation on the percentage difference in cause-specific standardised cumulative incidence functions

Follow-up began 1 year post-randomisation and ended at the earliest of: (i) first prescription of levothyroxine (used as a surrogate for diagnosis of hypothyroidism); (ii) thyroidectomy; (iii) thryoid cancer diagnosis; (iv) date last known to be alive; (v) 5 years and 1 month after randomisation. Estimates (vitamin D versus placebo) from a flexible parametric survival model that included randomisation group, age, sex, and state of residence at baseline, and an interaction between randomisation group and years of follow up, fitted as a restricted cubic spline with one internal knot (placed at the median of uncensored log survival times). Death (without prior hypothyroidism) and thryoidectomy and/or thryoid cancer (without prior hypothyroidism) were treated as competing risks. Probabilities were standardised to the distribution of age, sex, and state of residence at baseline in the study cohort.
Abbreviation: CI = confidence interval

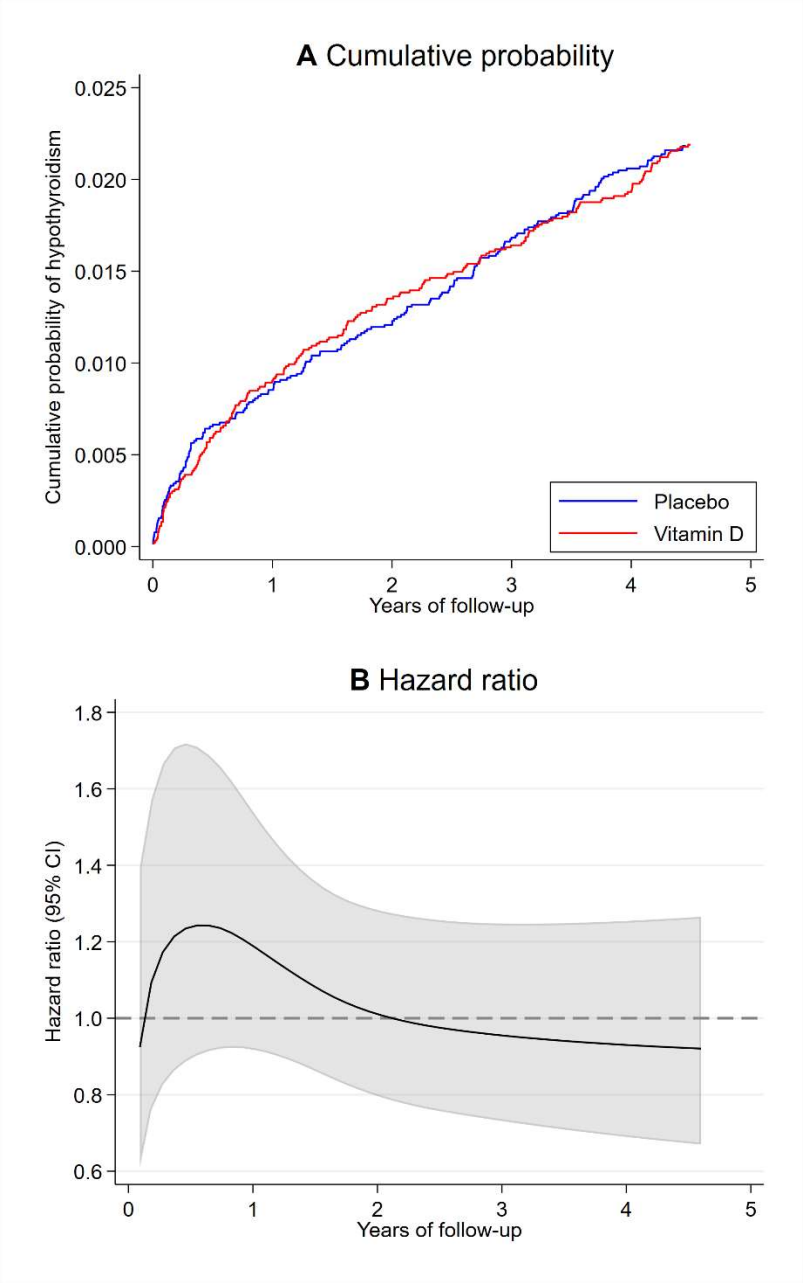


Figure S2. Sensitivity analysis 1: Effect of vitamin D supplementation on incidence of hypothyroidism. Panel A shows the cause-specific cumulative probability of hypothyroidism according to follow-up time, and panel B shows the time-varying hazard ratio

Follow-up began 7 months post-randomisation and ended at the earliest of: (i) first prescription of levothyroxine (used as a surrogate for diagnosis of hypothyroidism); (ii) date of thyroidectomy; (iii) date of thryoid cancer; (iv) date last known to be alive; (v) 5 years and 1 month after randomisation. Panel A: Curves estimated using Aalen-Johansen methods, treating death (without prior hypothyroidism) and thryoidectomy and/or thryoid cancer (without prior hypothyroidism) as competing risks. Panel B: Estimates (vitamin D versus placebo) from a flexible parametric survival model that included randomisation group, age, sex, and state of residence at baseline, and an interaction between randomisation group and years of follow up, fitted as a restricted cubic spline with one internal knot (placed at the median of uncensored log survival times). Abbreviation: CI = confidence interval

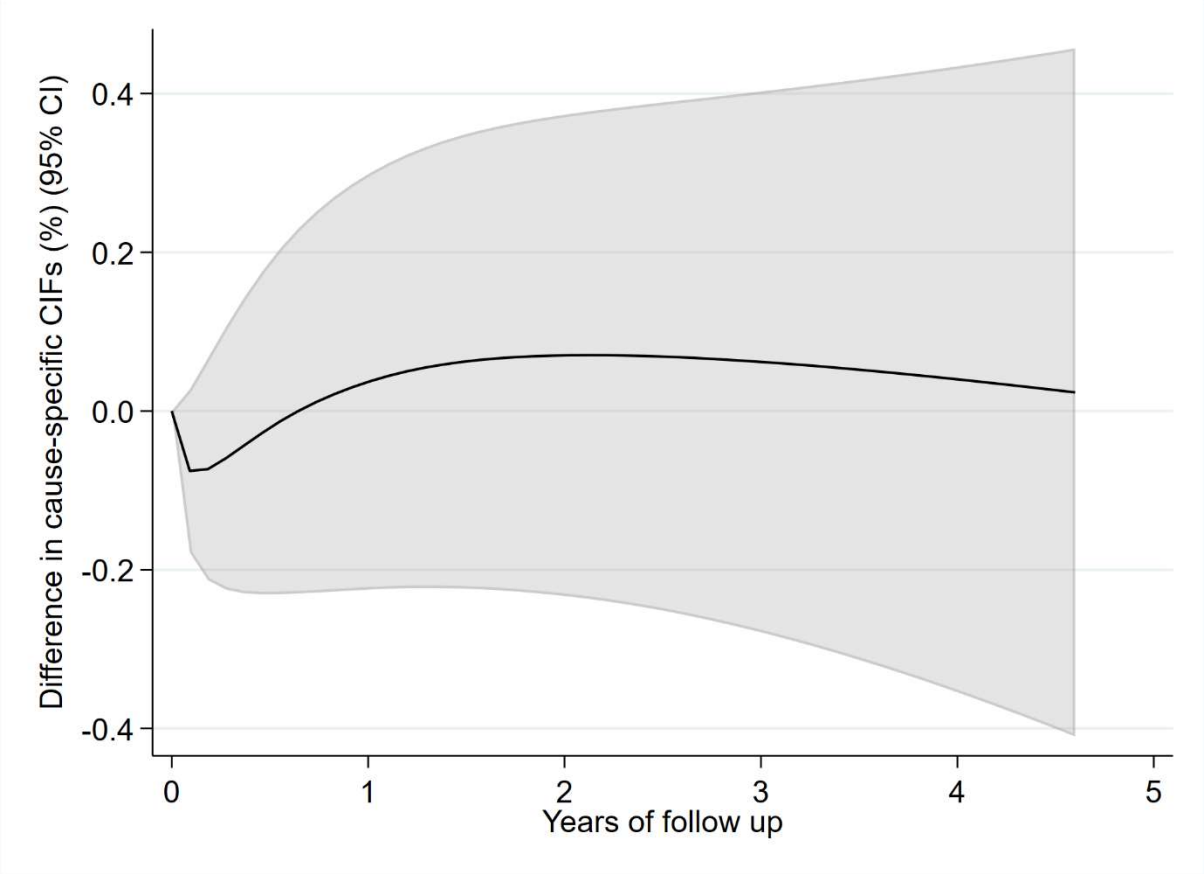


Figure S3. Sensitivity Analysis 1: Effect of vitamin D supplementation on the percentage difference in cause-specific standardised cumulative incidence functions

Follow-up began 7 months post-randomisation and ended at the earliest of: (i) first prescription of levothyroxine (used as a surrogate for diagnosis of hypothyroidism); (ii) thyroidectomy; (iii) thryoid cancer diagnosis; (iv) date last known to be alive; (v) 5 years and 1 month after randomisation. Estimates (vitamin D versus placebo) from a flexible parametric survival model that included randomisation group, age, sex, and state of residence at baseline, and an interaction between randomisation group and years of follow up, fitted as a restricted cubic spline with one internal knot (placed at the median of uncensored log survival times). Death (without prior hypothyroidism) and thryoidectomy and/or thryoid cancer (without prior hypothyroidism) were treated as competing risks. Probabilities were standardised to the distribution of age, sex, and state of residence at baseline in the study cohort. Abbreviation: CI = confidence interval

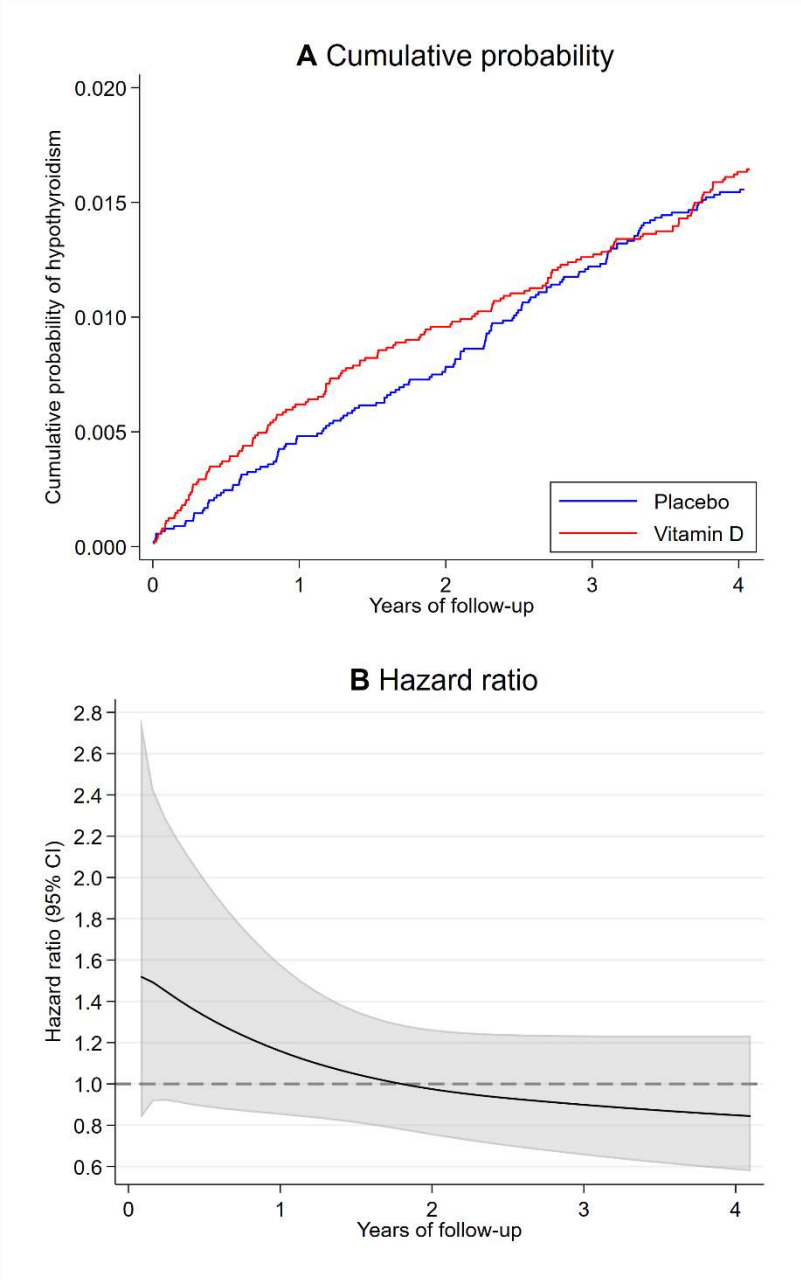


Figure S4. Sensitivity analysis 2: Effect of vitamin D supplementation on incidence of hypothyroidism. Panel A shows the cause-specific cumulative probability of hypothyroidism according to follow-up time, and panel B shows the time-varying hazard ratio

Follow-up began 1 year post-randomisation and ended at the earliest of: (i) first prescription of levothyroxine (used as a surrogate for diagnosis of hypothyroidism); (ii) thyroidectomy; (iii) thyroid cancer diagnosis; (iv) ***first prescription of carbimazole or propylthiouracil (as a surrogate for hyperthyroidism)***; (v) date last known to be alive; (vi) 5 years and 1 month after randomisation. Panel A: Curves estimated using Aalen-Johansen methods, treating death (without prior hypothyroidism), thryoidectomy and/or thryoid cancer (without prior hypothyroidism), and ***prescription of carbimazole/ propylthiouracil*** (without prior hypothyroidism) as competing risks. Panel B: Estimates (vitamin D versus placebo) from a flexible parametric survival model that included randomisation group, age, sex, and state of residence at baseline, and an interaction between randomisation group and years of follow up, fitted as a restricted cubic spline with one internal knot (placed at the median of uncensored log survival times).
Abbreviation: CI = confidence interval

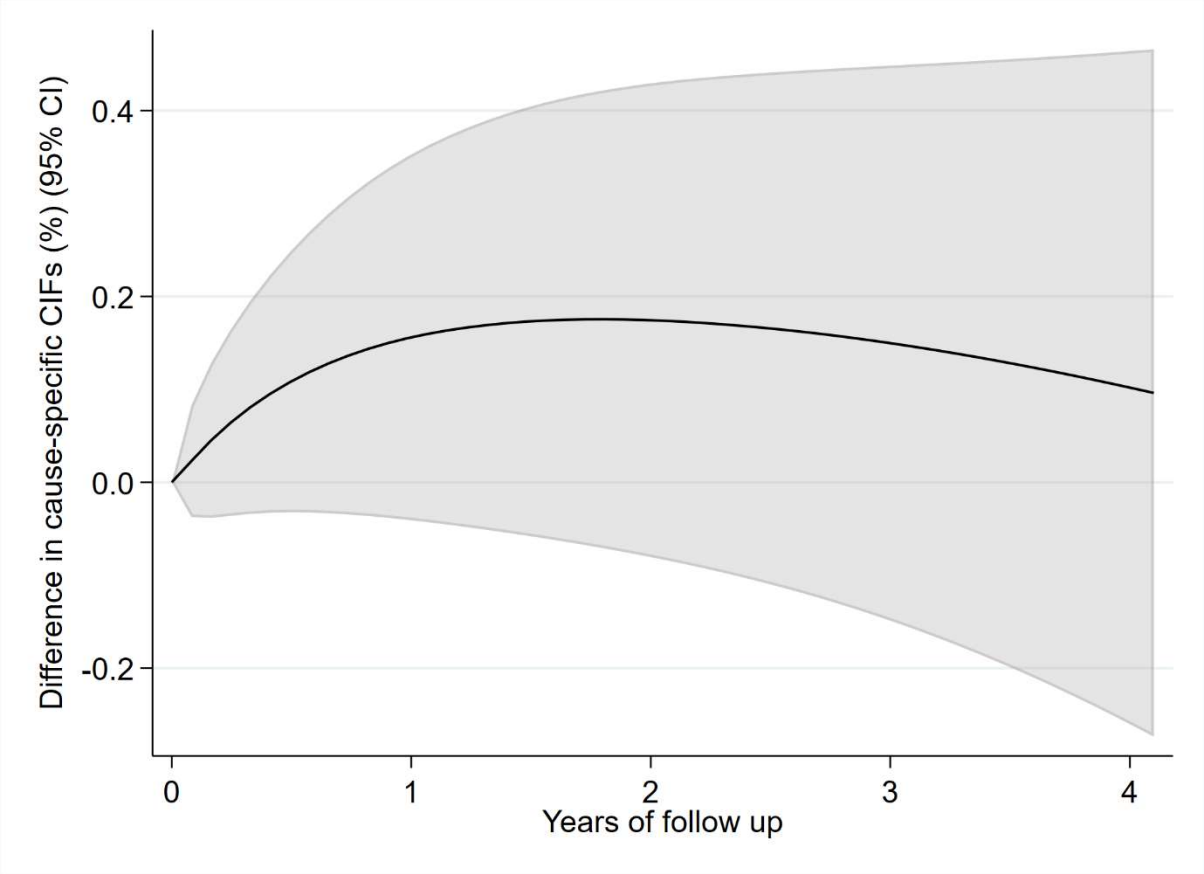


Figure S5. Sensitivity Analysis 2: Effect of vitamin D supplementation on the percentage difference in cause-specific standardised cumulative incidence functions

Follow-up began 1 year post-randomisation and ended at the earliest of: (i) first prescription of levothyroxine (used as a surrogate for diagnosis of hypothyroidism); (ii) thyroidectomy; (iii) thyroid cancer diagnosis; (iv) ***first prescription of carbimazole or propylthiouracil (as a surrogate for hyperthyroidism)***; (v) date last known to be alive; (vi) 5 years and 1 month after randomisation. Estimates (vitamin D versus placebo) from a flexible parametric survival model that included randomisation group, age, sex, and state of residence at baseline, and an interaction between randomisation group and years of follow up, fitted as a restricted cubic spline with one internal knot (placed at the median of uncensored log survival times). Death (without prior hypothyroidism), thryoidectomy and/or thryoid cancer (without prior hypothyroidism), and ***prescription of carbimazole/propylthiouracil*** (without prior hypothyroidism) were treated as competing risks. Probabilities were standardised to the distribution of age, sex, and state of residence at baseline in the study cohort. Abbreviation: CI = confidence interval