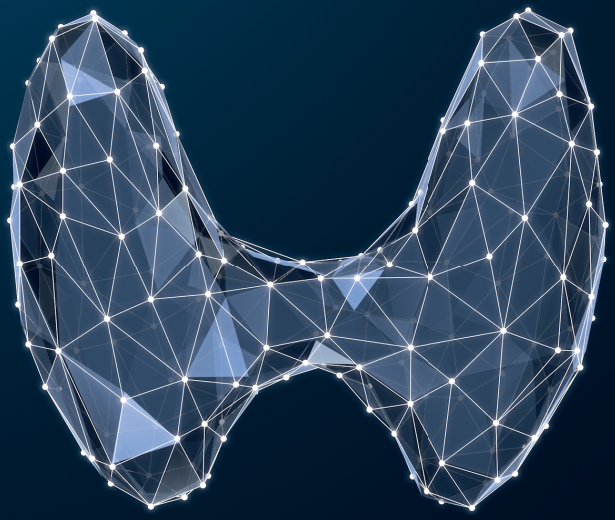
Subclinical hypothyroidism in community-dwelling older people: consequences and treatment outcomes

Du Puy, R.S.



Chapter 8

The effect of levothyroxine treatment on haemoglobin levels in older adults with subclinical hypothyroidism: pooled individual results from two randomized controlled trials

Robert S Du Puy
Rosalinde KE Poortvliet
Simon P Mooijaart
David J Stott
Terry Quinn
Naveed Sattar
Rudi GJ Westendorp
Patricia M Kearney
Vera JC McCarthy
Stephen Byrne
Nicolas Rodondi
Oliver Baretella
Tinh-Hai Collet
Diana van Heemst
Olaf M Dekkers
J Wouter Jukema
Jan WA Smit
Jacobijn Gussekloo
Wendy PJ den Elzen

Submitted

ABSTRACT

Background

Subclinical thyroid dysfunction and anaemia are common disorders, and both have increasing prevalence numbers with advancing age. The aim of this study was to assess whether levothyroxine treatment leads to a rise in haemoglobin levels in older persons with subclinical hypothyroidism.

Methods

In a pre-planned combined analysis of two randomised controlled trials of community-dwelling persons with subclinical hypothyroidism, we included participants aged 65 years and older and randomly assigned them to levothyroxine or placebo treatment. The dose of levothyroxine was periodically titrated aiming at thyroid stimulating hormone (TSH) level within the reference range and mock titrations in the placebo group. The outcome of the present analysis was the change in haemoglobin level after 12 months.

Results

Analyses included 669 participants (placebo n=337, levothyroxine n=332) with a median age of 75 years (range 65 to 97) and mean baseline haemoglobin of 13.8 g/dL (standard deviation 1.3). Although levothyroxine treatment resulted in a reduction in TSH from baseline after 12 months of follow-up compared to placebo (treatment effect -1.98 mIU/L, 95%CI -2.30 to -1.66), the change in haemoglobin level was not different between the levothyroxine and the placebo groups (-0.03 g/dL [95%CI -0.16 to 0.11). Similar results were found in stratified analyses including sex, age or TSH levels. No difference in change of haemoglobin levels after 12 months was identified in 69 participants with anaemia at baseline (-0.33 g/dL [95% CI -0.87 to 0.21]).

Conclusions

In persons aged 65 years and older with subclinical hypothyroidism, treatment with levothyroxine does not lead to a rise in haemoglobin levels, regardless of the presence of anaemia.

Trial registration

TRUST: Clinicaltrials.gov: NCT01660126, https://clinicaltrials.gov/ct2/show/NCT01660126

IEMO: Netherlands Trial Register: NTR3851, https://www.trialregister.nl/trial/3681

INTRODUCTION

Subclinical thyroid dysfunction and anaemia are common disorders, and both have increasing prevalence numbers with advancing age.[1-4] The symptoms of both subclinical hypothyroidism and anaemia are frequently non-specific and of similar nature (e.g. fatigue, malaise, shortness of breath and exercise intolerance).

A number of studies have suggested a potential causal relationship between thyroid dysfunction and haematopoiesis.[5-8] Lower haemoglobin levels have been observed in persons with (subclinical) hypothyroidism compared to their euthyroid counterparts and small cohort studies have shown increases in haemoglobin and erythropoietin levels after treatment of overt hypothyroidism.[5,9-18]

We reported earlier the results of an individual participant data meta-analysis using data from 16 independent observational cohort studies including more than 42,000 participants. In participants with both overt and subclinical hypothyroidism, we demonstrated a higher anaemia prevalence.[19] In addition, reduced thyroid function at baseline was associated with an increased risk of anaemia during follow-up. To date, however, there is a lack of experimental evidence of the effect of levothyroxine treatment on haemoglobin levels in older persons with subclinical hypothyroidism.

The aim of this study was to assess whether levothyroxine treatment for subclinical hypothyroidism leads to a rise in haemoglobin levels in older persons. We performed a pre-planned secondary outcome analysis of two randomised controlled trials on levothyroxine treatment for subclinical hypothyroidism in older persons.

MATERIALS AND METHODS

The current pre-planned combined analysis uses data from the TRUST trial [20,21] and the IEMO 80-plus Thyroid trial.[22] Both trials were randomised, double-blind placebo-controlled parallel group trials investigating the potential multi-modal effects of levothyroxine treatment for persons with subclinical hypothyroidism aged 65 years and older, and aged 80 years and older, respectively. The identical design, study processes and infrastructure allowed for a pre-planned combined analysis. Consequently, the combined cohorts are presented and analysed as a single study group throughout the manuscript.

The full study protocols and statistical analysis plans for the TRUST Thyroid trial [20,21] and the IEMO 80-plus Thyroid trial [22] have been published previously. In short, persons aged

65 years and older (TRUST Thyroid Trial) or 80 years and older (IEMO 80-plus Thyroid Trial) with a diagnosis of subclinical hypothyroidism, defined as elevated TSH levels (≥ 4.6 and ≤ 19.9 mIU/L), measured on at least two occasions with an interval between > three months and three years apart, and free thyroxine (fT4) within normal local laboratory ranges, were enrolled. Exclusion criteria included, but were not limited to, the use of levothyroxine, antithyroid medication, clinical diagnosis of dementia, recent hospitalization for major illness and terminal illness.[20] Participants were randomised in a 1:1 ratio to levothyroxine or placebo.

The study medication consisted of levothyroxine sodium tablets and matching placebo tablets taken orally daily. The intervention group started with 50 micrograms daily (or 25 micrograms for participants with body weight < 50 kg or a history of coronary heart disease) and the control group with a matching placebo for six to eight weeks. Two optional up- or down-titrations after six-to-eight-week intervals, and one at 12 months of follow-up ensured adequate treatment while avoiding potential over-treatment, mirroring routine clinical practice. An adaptive mock titration schedule was applied for the placebo group. Participants, General Practitioners (GPs) and study personnel remained blinded to treatment allocation and thyroid function test results throughout the study. Ethics approval was obtained from the medical ethics committees and competent authorities in the Netherlands, United Kingdom, Switzerland, and Ireland. Written informed consent was obtained from all participants.

Study outcome

The predetermined outcome of the present analysis was the change from baseline haemoglobin levels after 12 months of treatment. Haemoglobin levels were measured as part of a complete blood count in automated analysis systems in EDTA anticoagulated blood samples and measured within four hours of sample collection (the Netherlands: Sysmex XN10/20; Scotland: Sysmex XN10; Switzerland: Siemens Advia; Ireland: Advia 2120i until October 2015, and afterwards Sysmex XN-9000), at baseline and after 12 months. Anaemia was defined according to the World Health Organization criteria (Hb < 12 g/dL for women and Hb < 13 g/dL for men) and anaemia severity as mild (women: 11.0 ≤ Hb ≤ 11.9 g/dL, men: 11.0 ≤ Hb ≤ 12.9 g/dL), moderate (all: 8.0 ≤ Hb ≤ 10.9 g/dL) or severe (all: Hb < 8.0 g/dL).[23]

Additional variables

Thyroid function tests (TSH and fT4 levels) were performed by certified medical laboratories at each site (the Netherlands: Elecsys 2010 (Roche), Architect (Abbott), UniCel DxL (Beckman), Immulite 2000XPI (Siemens) or Vitros ECoQ (Ortho Clinical Diagnostics); United Kingdom: Architect (Abbott); Switzerland: Elecsys 2010 (Roche) and Ireland: Cobas 8001 or E601 (Roche) or Architect (Abbott)).[20,22] fT4 levels at 12 months were available for a subsample of participants (total n=115, United Kingdom n=75, the Netherlands n=37, Ireland n=3). Socio-demographic data (age, sex and race), information on lifestyle factors (smoking

and alcohol intake), the presence of prior conditions and medication use (prescribed and over-the-counter) were recorded by research nurses during baseline and follow-up visits. Comorbidity was defined as having a history of one or more of the following diagnoses: myocardial infarction, angina, stroke, transient ischemic attacks, heart failure, peripheral vascular disease, revascularization, atrial fibrillation, hypertension, diabetes mellitus, epilepsy, dementia, osteoporosis or other disease.[20,22]

Statistical methods

To investigate cross-sectional associations between thyroid hormones and haemoglobin or anaemia status, linear mixed effect regression models and logistic regression models were used, adjusting for possible confounders age and sex at baseline. Treatment effects were calculated using linear mixed effect regression models (differences in the change of haemoglobin levels between baseline and at 12 months) and logistic regression models (odds of developing anaemia or resolving anaemia during follow-up), adjusting for study site, study, treatment dose at randomization, age, sex, smoking status and alcohol consumption, with treatment (levothyroxine or placebo) as the independent variable, haemoglobin level or anaemia (stratified for sex) as the dependent variable, age and alcohol as covariates and sex and smoking status as fixed factors. All confounding variables were assessed for statistical interaction with all p-for-interactions above the 0.05 significance threshold.

Subgroup analyses were performed, stratified by sex, age (< 80 vs. ≥ 80 years), TSH level by quartiles (4.6 to 5.10 mIU/L, 5.11 to 5.76 mIU/L, 5.77 to 6.99 mIU/L and >7 mIU/L), haemoglobin level by quartiles (men: 9.35 to 13.70, 13.71 to 14.50, 14.51 to 15.20 and >15.21 g/dL; women: 9.67 to 12.73, 12.74 to 13.40, 13.41 to 14.02 and >14.02 g/dL) and the presence of anaemia at baseline. Differences in median haemoglobin levels at baseline were analysed across quartiles of TSH and fT4 using Kruskall-Wallis tests. Analyses of the treatment effects were performed in the modified intention to treat (ITT) population, based on participants with data available for haemoglobin levels. A per protocol analysis in participants with uninterrupted use of study medication throughout the studies was included as a sensitivity analysis. Analyses were repeated while excluding participants using concomitant anti-anaemic medications (iron supplements, parenteral iron preparations, vitamin B12, folic acid, erythropoietin, or other anti-anaemic drug, n=47). All data analyses were performed with SPSS Statistics Software version 22.0 for Windows (IBM, Armond, NY, USA). A p-value less than 0.05 was considered statistically significant.

RESULTS

For both studies, 2,989 individuals were assessed for eligibility and a total of 842 participants (737 in TRUST, 105 in IEMO) were randomised in the main studies. For the present analysis, 669 participants (592 in TRUST, 77 in IEMO) whose haemoglobin levels were measured at baseline were included (placebo n=337, levothyroxine n=332, Figure 1). Table 1 presents the baseline characteristics of the study participants. The median age of the population was 75 years (range 65 to 97) in both treatment groups. Men constituted 47.5% and 49.1% of all participants in the placebo and levothyroxine groups, respectively. More than 83% of the study population had one or more comorbid condition. The mean haemoglobin level in the placebo group was 13.8 g/dL (standard deviation [SD] 1.3; mean in women 13.4 g/dL [SD 1.1], mean in men 14.3 g/dL [SD 1.3]) and in the levothyroxine group 13.8 g/dL (SD 1.3; mean in women 13.4 g/dL [SD 1.1]; mean in men 14.3 g/dL [SD 1.4]). At baseline, 13.1% of the participants had anaemia in the placebo group; 11.4% had anaemia in the levothyroxine group.

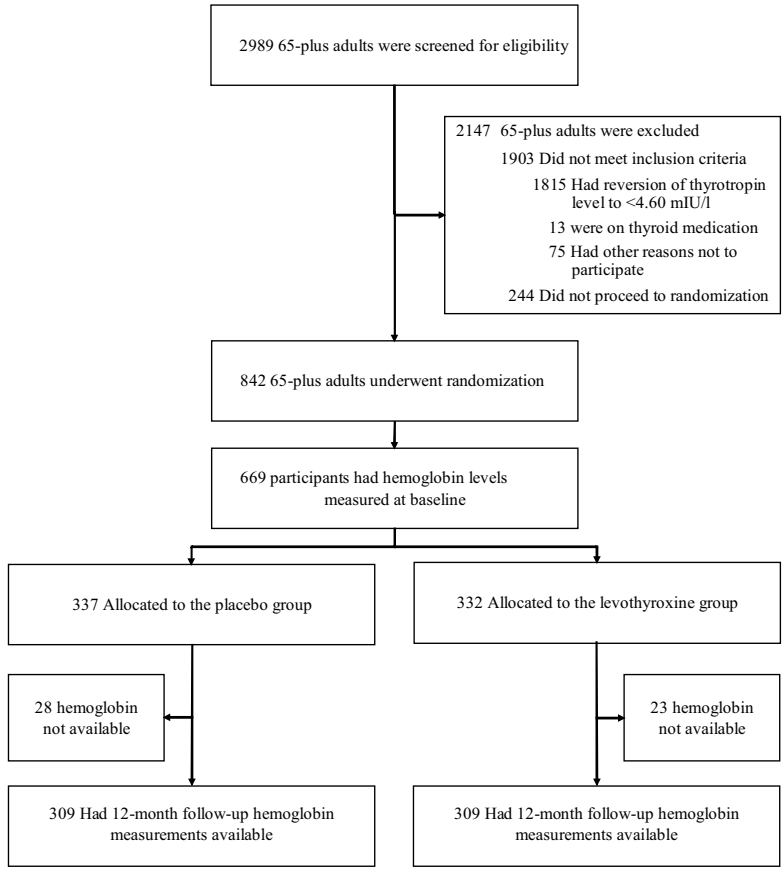


Figure 1. Recruitment, Randomization, and Patient Flow of the Participants

Table 1. Baseline characteristics of the study participants

Characteristic	Placebo group	Levothyroxine group
	n=337	n=332
Socio-demographic characteristics:		
Age, median (range)	75 (65 - 97)	75 (65 - 93)
Male, no. (%)	160 (47.5)	163 (49.1)
MMSE, median (IQR)	29 (28 - 30)	29 (27 - 29)
Barthel Index, mean (SD)	19.6 (0.9)	19.7 (0.9)
Instrumental activities of daily living score, mean (SD)	13.6 (1.1)	13.7 (0.8)
Handgrip strength, kg	27.6 (11.1)	28.3 (10.6)
Body mass index, kg/m^2	27.6 (4.4)	28.1 (5.2)
Systolic blood pressure, mmHg	143.3 (19.3)	141.6 (19.0)
Diastolic blood pressure, mmHg	75.4 (11.9)	74.1 (11.6)
Current smoker, no. (%)	24 (7.1)	25 (7.5)
Current alcohol user, no. (%)[a]	213 (63.2)	187 (56.3)
Comorbidities, no. (%)[b]	280 (83.1)	282 (84.9)
0-1	161 (47.8)	144 (43.4)
2-4	160 (47.5)	167 (50.3)
4+	16 (4.7)	21 (6.3)
Caucasian, no. (%)	331 (98.2)	328 (98.8)
Concurrent anti-anaemic medication use, no. (%)[c]	21 (6.2)	26 (7.8)
Thyroid function tests		
TSH, median (IQR), mIU/L	5.8 (5.1 - 6.8)	5.8 (5.1 - 7.1)
fT4, pmol/L	13.4 (1.9)	13.4 (2.0)
Haematological parameters		
Haemoglobin, g/dL[d]	13.8 (1.3)	13.8 (1.3)
Men	14.3 (1.3)	14.3 (1.4)
Women	13.4 (1.1)	13.4 (1.1)
Anaemia status, no. (%)[e]		
No anaemia	293 (86.9)	294 (88.6)
Mild anaemia	36 (10.7)	31 (9.3)
Moderate anaemia	8 (2.4)	7 (2.1)
Severe anaemia	0 (0)	0 (0)

Continuous data are presented as mean (standard deviation) and, if stated otherwise, as median (interquartile range); categorical data are presented as number (percentage)

[a] Defined as an average of one or more self-reported consumed units of alcohol per week.

[b] Comorbidity was defined as having a history of one or more of the following: myocardial infarction, angina, stroke, transient ischemic attacks, heart failure, peripheral vascular disease, revascularisation, atrial fibrillation, hypertension, diabetes mellitus, epilepsy, dementia, osteoporosis or other.

[c] Concurrent medication use was defined as using one or more of the following medications: iron supplements, parenteral iron preparations, vitamin B12, folic acid, erythropoietin, or other anti-anaemic drugs.

[d] Conversion factor of haemoglobin from g/dL to mmol/L = value*0.6206.

[e] Anaemia defined by WHO categories: women haemoglobin [Hb] < 12.0 g/dL, men Hb < 13.0 g/dL. Anaemia severity was graded as: mild (women: 11.0 ≤ Hb ≤ 11.9 g/dL, men: 11.0 ≤ Hb ≤ 12.9 g/dL), moderate (all: 8.0 ≤ Hb ≤ 10.9 g/dL), severe (all: Hb < 8.0 g/dL).

Table 2 shows the relation between thyroid function and haematological parameters at baseline. No association was observed between TSH or fT4 levels and haemoglobin levels in univariable and multivariable regression models. Although a small association between higher fT4 and the odds of having anaemia was observed at baseline (odds ratio [OR] 1.13, 95%CI 1.01 to 1.26, p=0.041), in the multivariable models higher levels of TSH or fT4 were not associated with increased odds of having anaemia. Median haemoglobin levels at baseline were comparable across quartiles of TSH and fT4 (Figure 2).

Table 2. Baseline associations between thyroid function and hematologic parameters in 669 older participants with subclinical hypothyroidism.

	Estimate	95% CI	p-value
Thyroid function and haemoglobin	**Beta**		
TSH			
Univariable	-0.01	-0.06 to 0.04	0.649
Multivariable	-0.01	-0.06 to 0.04	0.736
fT4			
Univariable	-0.05	-0.10 to 0.00	0.065
Multivariable	-0.03	-0.07 to 0.02	0.267
Thyroid function and anaemia (n=82)	**Odds Ratio**		
TSH			
Univariable	1.04	0.93 to 1.16	0.523
Multivariable	1.03	0.91 to 1.17	0.595
fT4			
Univariable	1.13	1.01 to 1.26	0.041
Multivariable	1.08	0.96 to 1.22	0.200

Betas and corresponding confidence intervals were calculated using linear mixed effect regression models and represent the difference in haemoglobin level (g/dL) per unit increase in TSH (mIU/L) or fT4 (pmol/L), Odds ratios with corresponding confidence intervals were calculated using logistic regression models, per unit increase in TSH or fT4. Multivariable models were adjusted for possible confounders age and sex.
Units: TSH mIU/L; fT4 pmol/L.

Although significant reductions in TSH (-1.98 mIU/L, 95% CI -2.30 to -1.66, $p < 0.001$) and increases in fT4 (2.33, 95% CI 1.87 to 2.80, $p < 0.001$) over 12 months of follow-up were observed in the levothyroxine group compared to the placebo group, no differences between the groups were observed in the change in haemoglobin levels after 12 months of follow-up (between group difference -0.03 g/dL, 95% CI -0.16 to 0.11, p=0.703, Figure 3).

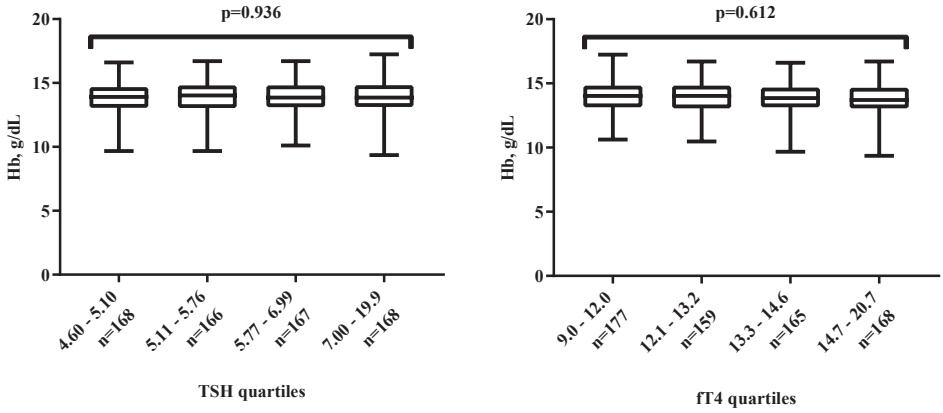


Figure 2. Baseline Hb levels per quartiles of TSH and fT4
Legend: Boxplots represent the median, interquartile range, minimum and maximum in Hb levels per quartile of TSH (mIU/L) and fT4 (pmol/L). Difference in medians were evaluated using Kruskall-Wallis test.

Results were not substantially different in the per protocol analysis for participants that remained on the assigned treatment after 12 months and had no major protocol violations (haemoglobin treatment effect -0.05 g/dL, 95% CI -0.19 to 0.10, p=0.530). Similar results were also found when subgroups were stratified by sex, age, quartiles of TSH levels, quartiles of haemoglobin levels in men and women, or the presence of anaemia at baseline (Table 3).

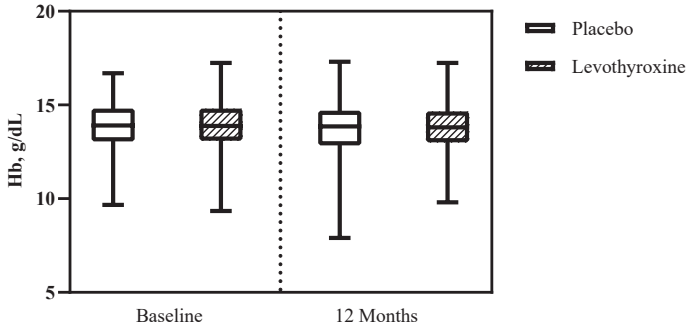


Figure 3. Levothyroxine treatment effect on Hb levels after 12 months of treatment
Legend: Boxplots represent the median, interquartile range, minimum and maximum in Hb levels. Treatment effect -0.03 g/dL (95%CI -0.16 to 0.11). Treatment effect (95% CI) was calculated using linear mixed effect regression models adjusting for study site, study, treatment dose at randomization, age and sex and represents the additional change in haemoglobin level after 12 months of treatment with levothyroxine, compared to placebo.

Table 3. Changes in TSH, fT4 and haemoglobin levels in relevant subgroups in older participants with subclinical hypothyroidism on levothyroxine treatment, compared to placebo.

		Baseline		12-month follow-up			
	N	Placebo	Levothyroxine	Placebo	Levothyroxine	Treatment effect (95%CI)[a]	p-value
Effect on thyroid hormone levels[b]							
TSH, mIU/L[b]	660	6.3 (1.7)	6.5 (2.1)	5.5 (2.5)	3.6 (2.1)	-1.98 (-2.30 to -1.66)	< 0.001
fT4, pmol/L [b]	177	13.4 (1.9)	13.4 (2.0)	12.4 (1.7)	14.7 (2.8)	2.33 (1.87 to 2.80)	< 0.001
Effect on haemoglobin levels							
Sex							
Men	298	14.3 (1.3)	14.3 (1.4)	14.2 (1.5)	14.2 (1.5)	-0.05 (-0.26 to 0.15)	0.599
Women	320	13.4 (1.1)	13.4 (1.1)	13.4 (1.3)	13.4 (1.1)	0.01 (-0.16 to 0.17)	0.950
Age							
< 80 years	466	14.0 (1.2)	13.9 (1.3)	14.0 (1.3)	13.8 (1.3)	-0.06 (-0.20 to 0.08)	0.412
≥ 80 years	152	13.4 (1.4)	13.6 (1.4)	13.3 (1.5)	13.5 (1.5)	0.10 (-0.23 to 0.44)	0.540
TSH							
4.60 – 5.10	157	13.8 (1.2)	13.8 (1.3)	13.6 (1.3)	13.6 (1.4)	-0.09 (-0.32 to 0.13)	0.418
5.11 – 5.76	152	13.8 (1.4)	13.9 (1.2)	13.8 (1.7)	13.9 (1.3)	-0.03 (-0.35 to 0.29)	0.863
5.77 – 6.99	158	13.9 (1.2)	13.8 (1.3)	13.8 (1.5)	13.9 (1.4)	0.16 (-0.08 to 0.41)	0.188
> 7.0	151	13.8 (1.3)	13.8 (1.5)	13.8 (1.2)	13.8 (1.3)	-0.13 (-0.40 to 0.14)	0.336
Haemoglobin quartiles split by sex							
Men							
9.35 – 13.70	67	12.4 (0.8)	12.3 (1.1)	12.5 (1.2)	12.5 (1.4)	-0.26 (-0.95 to 0.44)	0.455
13.71 – 14.50	93	14.2 (0.3)	14.1 (0.3)	14.3 (0.7)	14.0 (0.7)	-0.20 (-0.70 to 0.30)	0.419
14.51 – 15.20	60	15.0 (0.2)	14.9 (0.2)	14.4 (1.5)	14.8 (0.7)	-0.09 (-0.52 to 0.34)	0.680

Table 3. Changes in TSH, fT4 and haemoglobin levels in relevant subgroups in older participants with subclinical hypothyroidism on levothyroxine treatment, compared to placebo. (*continued*)

		Baseline		12-month follow-up			
15.21 – 17.24	73	15.8 (0.4)	15.8 (0.5)	15.5 (0.8)	15.4 (1.1)	0.03 (-0.30 to 0.35)	0.877
Women							
9.67 – 12.73	79	11.9 (0.8)	12.1 (0.5)	12.0 (1.3)	12.1 (0.9)	0.04 (-0.35 to 0.43)	0.842
12.74 – 13.40	84	13.1 (0.2)	13.2 (0.2)	13.0 (0.8)	13.3 (0.6)	0.02 (-0.21 to 0.25)	0.858
13.41 – 14.02	76	13.8 (0.2)	13.7 (0.2)	13.8 (0.5)	13.7 (0.7)	-0.19 (-0.55 to 0.18)	0.317
14.03 – 16.30	81	14.7 (0.4)	14.7 (0.6)	14.6 (0.9)	14.4 (0.8)	-0.13 (-0.55 to 0.29)	0.540
Anaemia at baseline							
No anaemia	549	14.1 (1.0)	14.1 (1.1)	14.0 (1.2)	14.0 (1.2)	0.01 (-0.12 to 0.15)	0.886
Anaemia	69	11.6 (0.8)	11.6 (0.8)	11.9 (1.3)	11.8 (1.2)	-0.33 (-0.87 to 0.21)	0.233

[a] Treatment effects were calculated using linear mixed effect regression models adjusting for study site, study, treatment dose at randomization, age and sex and represent the additional change in haemoglobin level (g/dL) after 12 months of treatment with levothyroxine, compared to placebo. In stratified analysis the stratifying variable was not an adjusting variable for that analysis.

[b] Treatment effects represent the additional change in TSH (mIU/L) or fT4 (pmol/L) after 12 months of treatment with levothyroxine, compared to placebo. Anaemia defined by WHO categories: Women haemoglobin (Hb) <12.0 g/dL, men Hb <13.0 g/dL.

In the 618 (92.4%) participants for whom haemoglobin measurements were available at 12 months, levothyroxine treatment was not associated with decreased odds of developing anaemia (placebo n=17/272, levothyroxine n=19/277, OR 1.16 [95% CI 0.59 to 2.29], p=0.675) or increased odds of resolution of anaemia (placebo n=11/37, levothyroxine n=7/32, OR 1.80 [95% CI 0.54 to 5.93], p=0.337). Similar results were found in sensitivity analyses restricted to participants with anaemia without anti-anaemic medication (data not shown).

No confounding variables in any of the models demonstrated significant statistical interaction (data not shown).

DISCUSSION

In this combined analysis of two randomised trials of older adults with a diagnosis of subclinical hypothyroidism, levothyroxine treatment for 12 months was not associated with an increase in haemoglobin levels. Additionally, no changes in haemoglobin levels were observed in relevant subgroup analyses including sex, age and baseline haemoglobin levels. No baseline associations were identified between TSH and haemoglobin levels or the presence of anaemia. A clinically insignificant increased odds of having anaemia at baseline was identified for fT4 in the univariable analysis, that was no longer present when correcting for the influence of sex and age, and is regarded as a chance finding.

These findings are in contrast with differences in haemoglobin levels between persons with different levels of thyroid function identified in earlier observational studies,[5,9-11,14,15,17-19] but in line with recent systematic review and cohort studies.[24,25] The addition of the experimental results from this manuscript underpin the proposition that subclinical hypothyroidism and anaemia may not be causally related. In line with earlier results from the TRUST and IEMO trials, in which no beneficial effects of levothyroxine treatment were demonstrated for a range of clinically relevant outcomes including thyroid-specific and generic quality of life, grip strength, blood pressure and body mass index,[21,26] the lack of a beneficial effect on haemoglobin levels is an added finding suggesting a limited clinical value of treating subclinical hypothyroidism with levothyroxine mono-therapy in older persons.

Interestingly, Christ-Crain and colleagues observed increases in erythropoietin levels upon levothyroxine treatment in a small placebo-controlled RCT of women (mean age 59 years [SD 1]) with subclinical hypothyroidism, while haemoglobin levels and haematocrit remained unchanged after 48 weeks of treatment.[27] One may hypothesize that effects of thyroid function on haemoglobin levels may only become apparent in those with overt hypothyroidism or severe anaemia.[6-8,15,28-30] Indeed, a number of studies have shown a beneficial

effect of thyroid hormone treatment in patients with overt hypothyroidism on erythropoietin levels.[9,14,17] Nevertheless, the absence of change in haemoglobin levels in our study – even in those with the lowest haemoglobin levels - may suggest that in older persons with subclinical hypothyroidism hematopoietic processes are quite robust to changes such as levothyroxine treatment.

The present study used data from the largest randomised controlled trials (RCTs) to date on levothyroxine treatment for subclinical hypothyroidism in community-dwelling older adults, with 12 months of follow-up, but some limitations must be acknowledged. First, despite the sample size of these RCTs, few participants (n=21) had a baseline TSH level of more than 10 mIU/L, i.e. the upper end of the subclinical hypothyroid spectrum, in which a few earlier studies identified additional risks of unwanted health effects.[4] The majority of participants in our RCTs had mild subclinical hypothyroidism (TSH between 4.6 and 10.0 mIU/L). Second, the number of participants with anaemia at baseline was rather low, leading to insufficient power to study the effects of levothyroxine on haemoglobin in those with anaemia. However, an absence of treatment effect was consistent across stratifications based on quartiles of haemoglobin levels at baseline. Third, fT4 levels were not routinely measured at 12 months and were only available in a subset of participants. Still, these results illustrate that, apart from persistently lowering TSH, levothyroxine treatment did result in a significant increase in fT4. The lack of effect of levothyroxine on haemoglobin levels in this study can therefore not be explained by a lack of increase in thyroid hormone function. Fourth, additional markers of erythropoiesis or other potential causes of anaemia such as ferritin, iron, folate, vitamin B12 or kidney function, were not available, restricting further exploration of underlying pathophysiological mechanisms. Fifth, although sensitivity analyses were performed excluding those using anti-anaemic medication such as iron, vitamin B12 or erythropoietin, no information was available on blood transfusions or venesection.

In conclusion, treatment with levothyroxine does not improve haemoglobin levels in individuals with a diagnosis of subclinical hypothyroidism aged 65 years and older. Whether anaemia in patients with more marked hypothyroidism is responsive to treatment with levothyroxine needs further experimental studies.

REFERENCES

1. Beghe C, Wilson A, Ershler WB. Prevalence and outcomes of anemia in geriatrics: a systematic review of the literature. *Am J Med.* 2004;116 Suppl 7A:3S-10S.
2. Cooper DS, Biondi B. Subclinical thyroid disease. *Lancet (London, England).* 2012;379(9821):1142-1154.
3. Gaskell H, Derry S, Andrew Moore R, McQuay HJ. Prevalence of anaemia in older persons: systematic review. *BMC Geriatr.* 2008;8:1.
4. Rodondi N, den Elzen WP, Bauer DC, et al. Subclinical hypothyroidism and the risk of coronary heart disease and mortality. *JAMA.* 2010;304(12):1365-1374.
5. Das KC, Mukherjee M, Sarkar TK, Dash RJ, Rastogi GK. Erythropoiesis and erythropoietin in hypo- and hyperthyroidism. *J Clin Endocrinol Metab.* 1975;40(2):211-220.
6. Fandrey J, Pagel H, Frede S, Wolff M, Jelkmann W. Thyroid hormones enhance hypoxia-induced erythropoietin production in vitro. *Experimental hematology.* 1994;22(3):272-277.
7. Fein HG, Rivlin RS. Anemia in thyroid diseases. *The Medical clinics of North America.* 1975;59(5):1133-1145.
8. Golde DW, Bersch N, Chopra IJ, Cline MJ. Thyroid hormones stimulate erythropoiesis in vitro. *Br J Haematol.* 1977;37(2):173-177.
9. Bremner AP, Feddema P, Joske DJ, et al. Significant association between thyroid hormones and erythrocyte indices in euthyroid subjects. *Clin Endocrinol (Oxf).* 2012;76(2):304-311.
10. den Elzen WP, de Craen AJ, Mooijaart SP, Gussekloo J. Low thyroid function and anemia in old age: the Leiden 85-plus study. *J Am Geriatr Soc.* 2015;63(2):407-409.
11. Evans ES, Rosenberg LL, Simpson ME. Erythropoietic response to calorigenic hormones. *Endocrinology.* 1961;68:517-532.
12. Gianoukakis AG, Leigh MJ, Richards P, et al. Characterization of the anaemia associated with Graves' disease. *Clin Endocrinol (Oxf).* 2009;70(5):781-787.
13. Horsley V. The Brown Lectures on Pathology. *Br Med J.* 1885;1(1261):419-423.
14. Horton L, Coburn RJ, England JM, Himsworth RL. The haematology of hypothyroidism. *The Quarterly journal of medicine.* 1976;45(177):101-123.
15. Kawa MP, Grymula K, Paczkowska E, et al. Clinical relevance of thyroid dysfunction in human haematopoiesis: biochemical and molecular studies. *European journal of endocrinology / European Federation of Endocrine Societies.* 2010;162(2):295-305.
16. Kendrick TS, Payne CJ, Epis MR, et al. Erythroid defects in TRalpha-/- mice. *Blood.* 2008;111(6):3245-3248.
17. Tudhope GR, Wilson GM. Anaemia in hypothyroidism. Incidence, pathogenesis, and response to treatment. *The Quarterly journal of medicine.* 1960;29:513-537.
18. Vitale G, Fatti LM, Prolo S, et al. Screening for hypothyroidism in older hospitalized patients with anemia: a new insight into an old disease. *J Am Geriatr Soc.* 2010;58(9):1825-1827.
19. Wopereis DM, Du Puy RS, van Heemst D, et al. The Relation Between Thyroid Function and Anemia: A Pooled Analysis of Individual Participant Data. *J Clin Endocrinol Metab.* 2018;103(10):3658-3667.
20. Stott DJ, Gussekloo J, Kearney PM, et al. Study protocol; Thyroid hormone Replacement for Untreated older adults with Subclinical hypothyroidism - a randomised placebo controlled Trial (TRUST). *BMC Endocr Disord.* 2017;17(1):6.
21. Stott DJ, Rodondi N, Kearney PM, et al. Thyroid Hormone Therapy for Older Adults with Subclinical Hypothyroidism. *The New England journal of medicine.* 2017;376(26):2534-2544.

22. Du Puy RS, Postmus I, Stott DJ, et al. Study protocol: a randomised controlled trial on the clinical effects of levothyroxine treatment for subclinical hypothyroidism in people aged 80 years and over. *BMC Endocr Disord.* 2018;18(1):67.
23. Nutritional anaemias. Report of a WHO scientific group. *World Health Organization technical report series.* 1968;405:5-37.
24. Floriani C, Feller M, Aubert CE, et al. Thyroid Dysfunction and Anemia: A Prospective Cohort Study and a Systematic Review. *Thyroid.* 2018;28(5):575-582.
25. M'Rabet-Bensalah K, Aubert CE, Coslovsky M, et al. Thyroid dysfunction and anaemia in a large population-based study. *Clin Endocrinol (Oxf).* 2016;84(4):627-631.
26. Mooijaart SP, Du Puy RS, Stott DJ, et al. Association Between Levothyroxine Treatment and Thyroid-Related Symptoms Among Adults Aged 80 Years and Older With Subclinical Hypothyroidism. *JAMA.* 2019;322(20):1-11.
27. Christ-Crain M, Meier C, Huber P, Zulewski H, Staub JJ, Muller B. Effect of restoration of euthyroidism on peripheral blood cells and erythropoietin in women with subclinical hypothyroidism. *Hormones (Athens, Greece).* 2003;2(4):237-242.
28. Maggio M, De Vita F, Fisichella A, et al. The Role of the Multiple Hormonal Dysregulation in the Onset of "Anemia of Aging": Focus on Testosterone, IGF-1, and Thyroid Hormones. *Int J Endocrinol.* 2015;2015:292574.
29. Perrin MC, Blanchet JP, Mouchiroud G. Modulation of human and mouse erythropoiesis by thyroid hormone and retinoic acid: evidence for specific effects at different steps of the erythroid pathway. *Hematol Cell Ther.* 1997;39(1):19-26.
30. Sullivan PS, McDonald TP. Thyroxine suppresses thrombocytopoiesis and stimulates erythropoiesis in mice. *Proceedings of the Society for Experimental Biology and Medicine Society for Experimental Biology and Medicine (New York, NY).* 1992;201(3):271-277.