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Effect of levothyroxine on the progression of carotid intima-media thickness in subclinical hypothyroidism patients: A meta-analysis

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Abstract

Background and aims: Subclinical hypothyroidism (SCH) has been associated with increased carotid intima-media thickness (C-IMT) in recent studies, but the effects of levothyroxine (L-T4) on C-IMT in SCH patients are still controversial. The aim of the current study was to evaluate the effect of L-T4 therapy on endothelial function as determined by C-IMT in patients with SCH.

Methods: Prior to July 2016, we searched the PUBMED, EMBASE, COCHRANE LIBRARY and GOOGLE SCHOLAR databases we selected published randomized controlled trials (RCTs) and self-controlled trials.

Results: Three RCTs with 117 patients were considered suitable for the meta-analysis. The results of the meta-analysis indicated that L-T4 significantly decreased the development of C-IMT [weighted mean difference (WMD), −0.05 mm, 95% CI −0.08, −0.01; p = 0.025]. We also analysed 9 studies (self-controlled trials) with 247 patients and extracted the IMT of SCH patients before and after L-T4 treatment. After L-T4 therapy, the pooled estimate of the WMD of decreased C-IMT was −0.03 mm (95% CI −0.05, −0.01; p = 0.001). Subgroup analysis showed that L-T4 therapy was associated with a decrease in C-IMT among patients of mixed gender (WMD, -0.03 mm, 95% CI -0.05, -0.01; p = 0.002). Longer treatment (>6

months) also resulted in a significant decrease in C-IMT (WMD, -0.05 mm, 95% CI -0.08, -0.02; p=0.001).

Conclusion: This meta-analysis indicates that L-T4 treatment of SCH patients can reduce C-IMT, possibly as a result of the reduction of TC, TG, LDL-C, SBP, DBP, LP(a), and FMD. Decreased C-IMT was observed in SCH patients after long-term (>6 months) L-T4 treatment. RCTs with large samples are needed to verify these observations.

Strengths and limitations of this study:

Strengths

A strong quality evidence on the effect of L-T4 therapy in SCH patients is preformed

Long-term treatment significantly improves C-IMT compared with short-term treatment.

We performed meta-analysis of RCTs.

We also performed meta-analysis of self-controlled trials.

We analysis other metabolic parameters

Limitations

The number of including studies was small, and the overall population of these trials was not large. Additionally, we did not perform subgroup analysis according to the level of TSH (the cutoff value is 10 mU/ml to distinguish mild SCH and severe SCH) because the original studies did not perform analyses according to the level of TSH.

Finally, we did not perform subgroup analysis by mean age (>65 or <65 years) because all participants' mean age was below 65 years.

Keywords: subclinical hypothyroidism; carotid intima-media thickness; thyroxin; cardiovascular risk

Introduction

Subclinical hypothyroidism (SCH) is characterized by a normal range of free thyroxin concentrations together with increased serum thyroxin (TSH) levels. Recently, SCH was found in 5% to 10% of the general population and 6% to 10% of women (approximately 15% was found in women more than 60 years old), while the incidence in males is 2.4 to 3% [1]. Although SCH is frequently asymptomatic, approximately 30% of patients have symptoms indicative of thyroid hormone deficiency [2, 3]. However, the clinical significance and therapeutic strategies of SCH are controversial .

Overt hypothyroidism is a well-known risk factor for cardiovascular disease and atherosclerosis [4]. A large-scale population-based study also concluded that SCH was an independent risk factor for atherosclerosis among elderly women [5]. We have proposed

several mechanisms for this association, including dyslipidaemia [6]; high blood pressure [7]; higher prevalence of metabolic syndrome [8]; endothelial dysfunction [9]; insulin resistance [10]; increased carotid intima-media thickness (C-IMT) [11]; hypercoagulability [12] and oxidative stress [13]. C-IMT is a generally acknowledged measure of subclinical atherosclerotic alterations. It is increasingly used to evaluate vascular function in clinical analyses assessing the efficacy of interventions that reduce atherosclerosis and related diseases [14]. This parameter is listed in the European guidelines for prophylaxis of cardiovascular disease, and 0.9 mm is the threshold value for C-IMT. Progression of atherosclerosis is indicated when the value of C-IMT is over the threshold. Several studies have demonstrated the relationship of C-IMT to primary cardiovascular risk factors [15, 16]. For a 0.1 mm difference in C-IMT, the prospective risk of myocardial infarction increased from 10% to 15%, and the stroke risk was increased from 13% to 18% [17].

A meta-analysis involving 8 studies with 3602 participants found a relationship between SCH and C-IMT, especially when TSH > 10.0 mIU/l [11].L-T4 replacement should be used to treat patients with clinical hypothyroidism; however, the treatment of SCH is complex. Several placebo-controlled studies have shown the beneficial effects of L-T4 therapy on early

atherosclerotic alterations and cardiovascular hazard in patients with SCH [16, 18]. Recently, in a large-scale retrospective cohort study examining the efficacy of L-T4 replacement on cardiovascular mortality, myocardial infarction and all-cause death, no beneficial effects in patients with SCH were found, except for patients under 65 years old [19]. The endothelial function in SCH and the effect of L-T4 treatment in ameliorating endothelial dysfunction are still unclear. The purpose of the current study was to evaluate the effect of L-T4 therapy on endothelial function as evaluated by C-IMT in patients with SCH.

Methods

Search strategy

Studies published in English were found in the EMBASE, PUBMED, GOOGLE SCHOLAR and COCHRANE LIBRARY electronic databanks prior to June 2016. We identified studies that measured IMT in patients with SCH before and after treatment with L-T4. Studies were obtained using three series of key items as subject headings: 1) hypothyroidism, thyroid disease, subclinical hypothyroid, subclinical thyroid dysfunction and thyroid-stimulating hormone; 2) intima media thickness, IMT, carotid wall thickness, C-IMT and carotid atherosclerosis; 3) levothyroxine, thyroxin, L-T4, replacement, therapy and treatment. For a

thorough review of the literature, we searched more studies by browsing the references of the identified reports and review articles.

Criteria for study selection

Subjects with SCH have standard free thyroxin levels and increased TSH levels. There is still debate about the TSH reference interval; several reviews have proposed a TSH upper cutoff between 4.5 and 5.0 mIU/l [20, 21], but some experts suggest that the peak TSH should be decreased to 2.5-3.0 mIU/l. Because there are no consistent conclusions, no specific TSH cutoff was used to define SCH, but all articles had a cutoff point from 3.6 to 5.5 mIU/l. To be included, a study had to satisfy the following conditions: 1) reported SCH defined based on a thyroid function test (normal free T4 and increased TSH); 2) defined as a RCT, which compared C-IMT in SCH patients in a L-T4 treatment and control group, or a self-controlled trial, which compared C-IMT value of a patient with SCH before and after L-T4 replacement.

Study selection

Two investigators independently screened both abstracts and titles of the studies, and the studies were excluded when they did not conform to the inclusion criteria or they accorded

with the exclusion criteria. Two investigators independently extracted data from the papers. If there was disagreement, a third reviewer was asked for advice. Discrepancies were solved by unanimous agreement.

Data extraction

The following information is contained in the data extraction table: 1) author 2) year of publication 3) region 4) sample size 5) sample gender 6) mean age of the cohort 7) definition of SCH 8) duration of SCH 9) SCH detection method 10) mean L-T4 dosage 11) duration of treatment 12) thyroid function after treatment 13) IMT assessment 14) IMT values (mean with SD) before and after treatment with the corresponding treatment method.

Assessment of study quality

The quality of non-randomized studies was evaluated using the MINORS method [22], which is based on the following items: a stated aim of the study, inclusion of consecutive patients, prospective collection of data, endpoint appropriate to the study aim, unbiased evaluation of endpoints, follow-up period appropriate to the major endpoint, and loss to follow-up not exceeding 5%.

Methodological quality of the included RCTs was assessed using the Cochrane Handbook for Systematic Review of Interventions by several domains: generation of random sequence; allocation concealment; blinding; incomplete outcome date addressed; free of selective reporting; free of other bias.

Statistical analysis

We represented the results as weighted mean differences (WMDs) with 95% confidence intervals (CIs) for continuous variables. For continuous outcomes, if all reports had uniform units of measurement (expressed as mm), we showed the WMD and 95% CIs as the results. We evaluated statistical heterogeneity across the studies by Cochrane's Q test ($p < 0.1$ was statistically significant) and the I^2 test ($I^2 > 50\%$: high heterogeneity; $I^2 = 25\%$-50%: moderate heterogeneity; $I^2 < 25\%$: low heterogeneity) [23]. The pooled effect size was assessed with a random-effect model when significant heterogeneity was present; in contrast, we also selected a fixed-effect model. $p < 0.05$ was considered significant. We assessed publication bias with Begg's correlation test and Egger's regression method [24]. Sensitivity analysis was carried out for all papers in addition to the studies with boundary qualification. Pre-specified subgroup meta-analyses were performed to assess the between-study

heterogeneity on the basis of the gender of participants (female or mix) and duration of treatment (>6 months or ≤6 months). Among 9 including studies, 3 out of them are RCTs and the other of them are self-controlled trials. We performed Statistical analysis of three RCTs. SCH treatment group of three RCTs also have the information on C-IMT values between pre-treatment and after-treatment in SCH patients. We combined the SCH treatment group of RCTs with the other self-controlled trials to performed Statistical analysis for fully revealing the effect of L-T4 treatment in SCH patients. Statistical analyses were performed with STATA 11 for Windows.

Results

Study selection

Three RCTs [16, 25, 26] (57 patients treated with L-T4 and 60 included in the control group) were selected for the meta-analysis, with a total of 117 patients. A total of 9 publications [16, 25-32] with 247 SCH patients measured the C-IMT before and after L-T4. We selected these studies among 107 potentially related articles (Fig. 1).

Baseline characteristics and study quality

The baseline characteristics of the included trials are shown in Table 1(A)(B). The duration of therapy with L–T4 ranged from 2 to 18 months, the patient count varied from 14 to 56 subjects, and the year of the publication was from 2004 to 2016. Six trials included female and male patients, and the other three only examined female patients. Based on the type of original research, the methodological evaluation of study quality was different. We used MINORS to assess the quality of the self-controlled trials. In general, the included self-controlled trials were of moderate quality (Table1) .The results of RCT quality are shown in Fig. 2.

Progression of C-IMT in the follow-up and subgroup analysis

A total of 3 RCTs involving 117 patients were analysed. L-T4 treatment significantly decreased C-IMT progression in SCH patients. (WMD, −0.05 mm, 95% CI −0.08, −0.01; p = 0.025). The heterogeneity was very low (I^2=22.2%) (Fig. 3)

The nine self-controlled experiments compared the value of C-IMT before replacement and after treatment. After L-T4 therapy, the thyroid function of all patients was normalized from SCH, and then, patient IMT was measured again. L-T4 therapy was linked to a mild but significant decrease in the development of C-IMT (WMD, −0.03 mm, 95% CI −0.05, −0.01; p = 0.001). The heterogeneity was very low (I2=21.8%) (Fig. 4)

Subgroup analyses stratified by gender revealed that L–T4 therapy caused a significant decrease in C-IMT among patients of mixed gender (WMD, -0.03 mm, 95% CI -0.05, -0.01; p = 0.002). The outcomes showed low heterogeneity (I2= 39.2%). After L-T4 treatment,

TABLE 1(A). Summary of the Characteristics of the Included Studies

Author/ Year	Sample Size	Region	Gender	Mean Age (year)	Definition of SCH	SCH Duration (month)	L-T4 Dosage (initial value)	Treatment Duration (month)	Thyroid function after treatment	CIMT(mm) (Before/After)	Quality Score
Monzani 2004	23	Italy	mix	37±11	TSH >3.6 mIU/L	at least 6 months	25 -75 ug/d	10.5[a]	normal	0.76±0.14 0.67±0.13	12
Soo-Kyung 2009	28	Korea	mix	36.0±6.2	TSH >5.5 mIU/L	newly diagnosed	67μg/d[b]	18[b]	normal	0.67±0.11 0.60±0.10	9
Monica 2011	14	Brazil	female	43.4±9.8	TSH >4.0 mIU/L	_	44.23±18.13mg/d[d]	12	normal	0.66±0.11 0.66±0.15	10
Levent 2010	38	Turkey	mix	49.8±10.0	TSH >5.0 mIU/L	at least 2 months	101±27.46μg/d[c]	6[b]	normal	0.64±0.13 0.63±0.12	11
Nasmi 2016	25	Iran	mix	35.9±7.6	TSH >4.0 mIU/L	_	50ug/d	2	normal	0.56±0.09 0.57±0.08	11
Ilknur 2014	56	Turkey	mix	41.3±14.5	TSH>4.2 mIU/L	_	25-50 mcg/d	_	normal	0.533±0.112 0.507±0.126	12
Dursun 2007	20	Turkey	female	36±11	TSH>4.2 mIU/L	newly diagnosed	25-100ug/d	5[b]	normal	0.65±0.99 0.55±0.08	12
Adrees 2009	20	Ireland	female	50±9	_	at least 6 months	100 ± 30 ug/d[c]	18	normal	0.82±0.2 0.71±0.2	10
Dilek 2015	23	Turkey	mix	35.2±10.7	TSH >4.0 mIU/L	at least 6 months	_	6	normal	0.51±0.09 0.46±0.07	11

Abbreviations: TSH, thyroid-stimulating hormone; CIMT, Carotid intima-media thickness; L-T4, levothyroxine.

Mix=including male and female. Age and IMT are expressed as mean± s.d.

[a] median ; [b] mean; [c] mean± s.d; [d] median± interquartile range

TABLE 1(B). Summary of the Characteristics of the Included Studies

Author	TSH assay	Carotid IMT assessment
Monzani	ultrasensitive immunoradiometric assay	both the near and far wall of the right and left common carotid artery and carotid bifurcation(high-resolution ultrasonography)
Soo-Kyung	chemiluminescent immunometric assay	20 mm proximal to the origin of the carotid bulb (high-resolution ultrasonographic system)
Monica	immunochemiluminescence	both the near and far wall of the right and left common artery and the carotid bifurcation.(high-resolution ultrasound imaging)
Levent	Chemiluminescence immunometric assay	approximately 1-cm segment from both the left and right CCA proximal and distal portions (Ultrasonographic images)
Nasmi	_	sonogram B-mode images
Ilknur	electrochemiluminescence immunoassays	Three arterial wall segments of the common carotid artery were measured bilaterally after imaging from a fixed lateral transducer angle and designated（high-resolution B-mode ultrasonography）
Dursun	immunoassay	right and left extracranial carotid arteries: the CCA (1 cm proximal to the dilation of the carotid bulb), the bifurcation (the 1-cm segment proximal to the flow divider), and the internal carotid artery (the 1-cm segment in the internal branch distal to the flow divider).(high-resolution ultrasonography)
Adrees	chemiluminescent immunometric assay	1 cm proximal to the carotid bulb when a satisfactory position was found.(calibrated ultrasound machine)
Dilek	_	ultrasound system

Abbreviations: CCA, common carotid artery.

female participants with SCH displayed a non-significant reduction in C-IMT (WMD, -0.04 mm, 95% CI -0.12, 0.03; p = 0.256). The heterogeneity was very low (I2= 0.0%) (Fig.5).

Subgroup analyses were performed using the duration of L-T4 replacement. Except for one reports, all trials had information on the length of the therapy. There was a significant decrease in C-IMT in the patients with long-term (>6 months) treatment (WMD, -0.05 mm, 95% CI -0.08, -0.02; p=0.001). The outcome showed low heterogeneity (I^2= 12.3%). A non-significant reduction was found in C-IMT in the participants with short-term (≤6 months) replacement therapy (WMD, -0.02 mm, 95% CI -0.05, 0.01; p=0.214). The heterogeneity was low (I^2= 11.2%). (Fig. 6).

Changes in metabolic parameters

Table 2 indicates the changes in metabolic parameters from pre-replacement to post-treatment. L-T4 therapy was linked to significant reductions in TC, TG, LDL, SBP, DBP, and LP(a) and decreased FMD. There were no significant changes in BMI, waist circumference, HDL, ApoA, ApoB and glucose between the pre-treatment and post-therapy conditions.

Sensitivity and publication bias evaluation

We carried out sensitivity analyses by sequentially eliminating one study to probe the change in the total WMD and 95% CI of C-IMT. The results of three RCT sensitivity and publication

Table 2

The difference of the changes in metabolic parameters between pre-treatment and post-treatment.

Parameters	No. of patients	WMD (95% CI)	P-value
Waist circumference	66	-0.544(-4.041,2.954)	0.761
BMI	152	-0.231(-1.044,0.582)	0.577
Glucose	48	0.090(-0.109,0.289)	0.375
SBP	109	-9.391(-14.405,-4.377)	0
DBP	109	-5.693(-8.471,-2.916)	0
TC	166	-9.037(-16.635,-1.439)	0.02
TG	166	-9.842(-18.763,-0.921)	0.031
HDL	166	-1.032(-4.059,1.995)	0.504
LDL	166	-14.303(-19.133,-9.472)	0
FMD	39	3.962(2.636,5.289)	0
ApoA	37	-10.092(-23.173,2.989)	0.131
ApoB	37	-7.718(-21.723,6.287)	0.28
LP(a)	34	-9.974(-12.844,-7.103)	0

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; TC, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein; ApoA, Apolipoprotein A; ApoB, Apolipoprotein B; Lp(a), lipoprotein(a); WMD, weighted mean difference; CI: confidence interval;FMD, flow-mediated dilatation.

bias analyses indicated no variation in the direction of WMD when each publication was removed. Neither Egger's test (p=0.881) nor Begg's test (p=0.602) of C-IMT in SCH manifested publication biases. We also evaluate the sensitivity and publication bias of the nine self-controlled trial. The results showed that there was only a slight change in the WMD or 95% CI, and no change in the direction of WMD when any one study was omitted. Egger's test (p=0.378) and Begg's test (p=0.602) of C-IMT in SCH manifested publication biases.

Discussion

This meta-analysis indicated that L-T4 has beneficial effects on the development of C-IMT, particularly in patients of mixed gender. Decreased C-IMT was also observed with longer treatment (>6 months). L–T4 therapy of SCH participants also resulted in a significant reduction in TC, TG, LDL, SBP, DBP, LP(a), and FMD.

Recent epidemiological surveys found that SCH is an independent risk factors for cardiovascular disease and atherosclerosis [5, 33]. A meta-analysis of 8 studies including 37,197 subjects indicated that C-IMT is a powerful predictor of prospective vascular disorders [17]. The American Heart Association/American College of Cardiology guidelines designated C-IMT along with coronary artery calcium (CAC) score as a class IIa

recommendation for cardiovascular risk assessment in asymptomatic adults at intermediate risk of cardiovascular disease.

In the past decades, reports have indicated that SCH is linked to increased C-IMT, but the findings were inconsistent. Recently, a meta-analysis that incorporated eight observational trials with 3,602 SCH participants found a strong correlation between SCH and C-IMT.

Many studies reported L-T4 therapy had beneficial effects on cardiovascular system in SCH patients. L-T4 therapy had beneficial effects an observational analysis with long-term follow-up for 8 years from the English general medical practitioner research database found that L-T4 therapy was linked with lower CHD morbidity in moderately old (<70 years) and middle-aged SCH patients [34]. Several interventional RCT indicated that L-T4 therapy to restore euthyroidism improved most functional surrogate markers and coronary artery (CV) structure [9, 16, 35, 36]. Other trials revealed favourable changes in cardiac contractility and systolic time interval using L-T4 replacement [37]. Various reports also showed that L-T4 treatment of participants with mild-SCH had beneficial effects on diastolic function during rest or at exercise, cardiac systolic function, endothelial function, systemic vessel resistance,

cardiac preload, and arterial stiffness [18, 35, 38].Two meta-analyses demonstrated that L-T4 therapy had beneficial effects on serum lipid concentration in SCH patients [6, 39].

The mechanism underlying the L-T4-mediated decrease in C-IMT has not been fully elucidated. In SCH, the increased TSH is the only prominent change, and increased TSH can bind with the TSH receptor (TSHR) to perform its function. Balzan et al. [40] revealed that micro-vascular endothelial cells produce TSHR, which may help to elucidate this relationship. Recently, Tian et al. [41] published a study to identify the potential function of the TSHR and showed that increased TSH aggravated endothelial dysfunction. However, L-T4 can control the elevated TSH by reversing above-mentioned changes to reduce the mean C-IMT.

Consistent with these publications, we concluded that the mechanism underlying the L-T4 therapy-mediated decrease in mean C-IMT of SCH patients is complex. In addition to the mechanism of TSHR, hyperlipidaemia and blood pressure may also contribute to the reduction.

In a large cross-sectional study, an increase of 1.0 mU/l in serum TSH was associated with an average increase in TC values of 0.09 mm in women [37]. There was a decrease in both TC and LDL in specific groups of patients (baseline serum TSH >12 mU/l) during one

year of L-T4 substitution therapy reported by Meier et al. [42]. A decrease in LDL of 0.41 mmol/l and TC of 0.47 mmol/l was observed by Caraccio et al. [43], and the effects were noted in patients (mostly premenopausal women) with mildly elevated TSH (<10 mU/l) and Hashimoto's thyroiditis. However, there is no agreement on the effect of L–T4 substitution treatment on lipid changes [37, 42]. Six out of 13 papers in a systemic review reported that L-T4 treatment improved TC and LDL [6]. A RCT trial with the same conclusion further supported this hypothesis [19].

Among four markers of hyperlipidaemia (TC, TG, LDL, HDL), we observed non-significant differences only in HDL between pre-treatment and post-replacement conditions, which is supported by previous study with similar findings [44]. Reports by Kannel et al. [45] and Lewington et al. [46] showed that the TC/HDL ratio is more important in the progress of coronary heart disease than either TC or HDL alone. Although HDL concentration was not changed after restoration of euthyroidism by L-T4 replacement in SCH patients in our study, L-T4 treatment altered the TC/HDL ratio.

A previous study found that higher TSH, TC, and LDL are associated with increased endothelial dysfunction in SCH patients [47]. The Helsinki Heart Study reported that a 7%

reduction in LDL levels was related to a 15% decrease in the coronary heart disease incidence [48], indicating a significant benefit of L-T4 supplementation. A possible explanation is that SCH can cause endothelial dysfunction because of a decrease in NO availability, which is partly independent of dyslipidaemia, and L-T4 supplementation can reverse endothelial dysfunction [9]. Hyperlipidaemia promotes endothelial dysfunction because it can inhibit the NO synthesis pathway in endothelial cells [49]. Thus, we found that SCH not only results in endothelial dysfunction but is also an independent risk factor of dyslipidaemia; then, dyslipidaemia further amplifies endothelial dysfunction. L-T4 treatment of SCH patients can reduce these effects.

A meta-analysis showed that blood pressure was an independent risk factor for C-IMT [48]. Recently, a meta-analysis demonstrated that SCH is linked to higher SBP and DBP levels [50]. Faber showed that a drop in BP (-6%) appeared followed increased cardiac output (+14%) after 4 months of L-T4 supplementation [51]. Restoration to euthyroidism by 6 months of L-T4 therapy decreased SBP in SCH subjects as shown by Anagnostis et al. [52]. However, results showing that L-T4 treatment reduced BP have not been confirmed. Another study found that L-T4 supplementation non-significantly improved BP [30].

We know little about the mechanism of SCH-associated hypertension; nevertheless, hypothyroidism increases vascular resistance, enhances blood pressure, salt sensitivity and abnormal sodium metabolism, which may promote the development of hypertension.

For the subgroup of short duration (≤6 months), an insignificant difference of C-IMT was observed after treatment. Meanwhile, mean C-IMT in SCH subjects undergoing long-term treatment significantly increased compared to that in the pre-treatment condition. This phenomenon was confirmed in our clinical observation. The major reason is that the duration of treatment is too short to improve C-IMT. The rate of progression of IMT in the common carotid artery was approximately 0.01 mm/year.

For stratification by gender, we did not find a significant difference in female patients between pre-replacement and post-replacement. Meanwhile, for the mixed subgroup, mean C-IMT was significantly reduced after treatment. Only 3 out of 9 studies evaluated female SCH patients. Due to the lack of data, both small sample size and potential confounding factors can prevent the linkage between C-IMT degree and L-T4 treatment in SCH female patients.

In conclusion, our study suggests that C-IMT in SCH patients can be reversed by L-T4

replacement. Hyperlipidaemia and hypertension may improve after L-T4 therapy. Therefore, L-T4 supplementation can prevent or inhibit the progression of atherosclerosis, at least in SCH patients. RCTs with longer treatment durations are needed to confirm these findings.

contributorship statement

Zhao Tong and Chen Baomin made search strategies, searched papers from databases, assessed of study quality ,independently extracted data from the papers and wrote the paper Wang Haoyu as a third reviewer was asked for advice,If there was disagreement.Zhou Yingying, Wang Xinyi and Zhang Yuanyuan Participated in the revision of the paper.

competing interests statement

The author denies that he has any intention to obtain any financial interests.

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Fig.1. Process of study selection.

Abbreviations: IMT: intima-media thickness; SCH: subclinical hypothyroidism

Fig.2. Quality assessment of the included studies. Risk of bias graph (A); risk of bias summary (B).

Fig.3. Forest plots showing WMD (weighted mean differences) with 95% confidence intervals for improvement in carotid intima–media thickness comparing L-T4 treatment to the control in a fixed effects model.

Fig.4. Forest plots showing WMD (weighted mean differences) with 95% confidence intervals compared the value of C-IMT before replacement and after treatment in a fixed effects model.

Fig.5.Subgroup analyses of carotid intima-media thickness changes based on gender in a fixed effects model.

Fig.6.Subgroup analyses of carotid intima-media thickness changes based on duration of L-T4 replacement in a fixed effects model.

Long= duration of L-T4 replacement>6 months

Short= duration of L-T4 replacement≤6 months

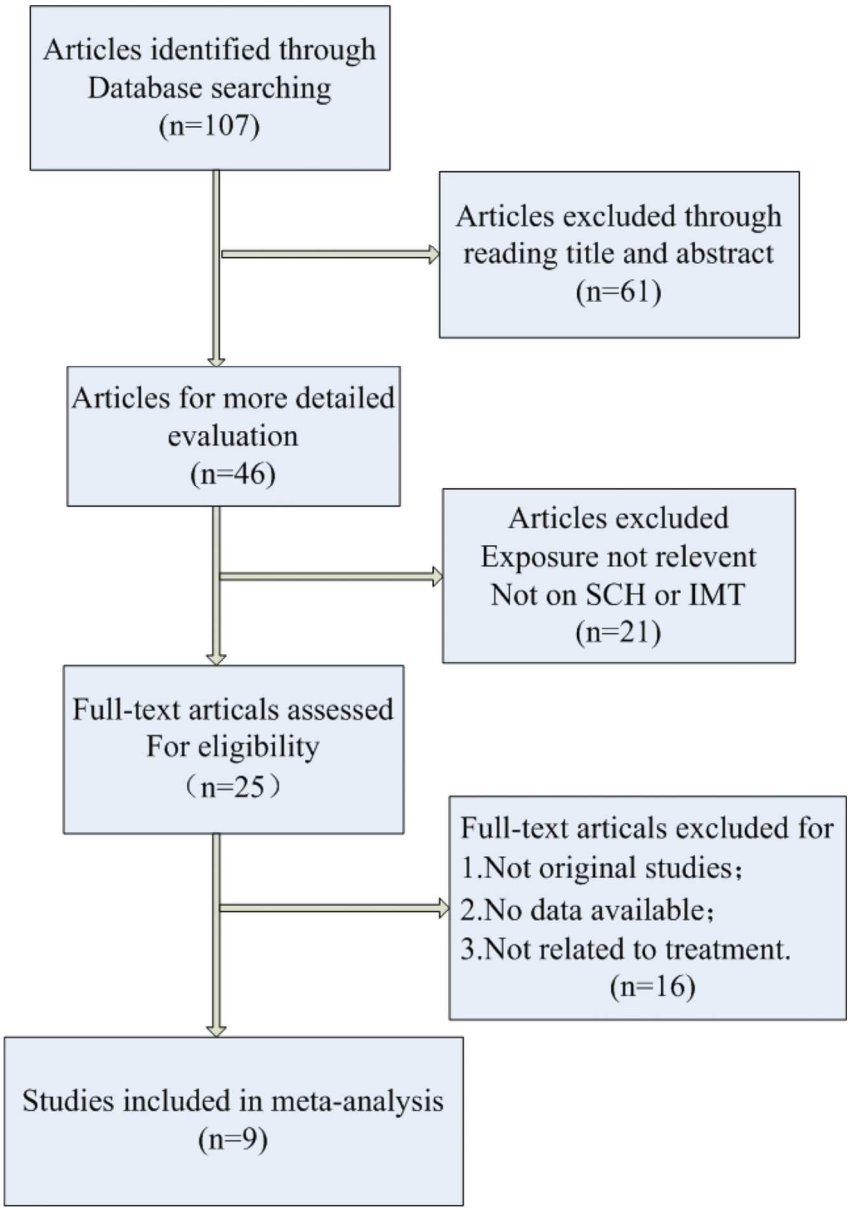

Fig.1. Process of study selection.

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Fig.2. Quality assessment of the included studies. Risk of bias graph (A).

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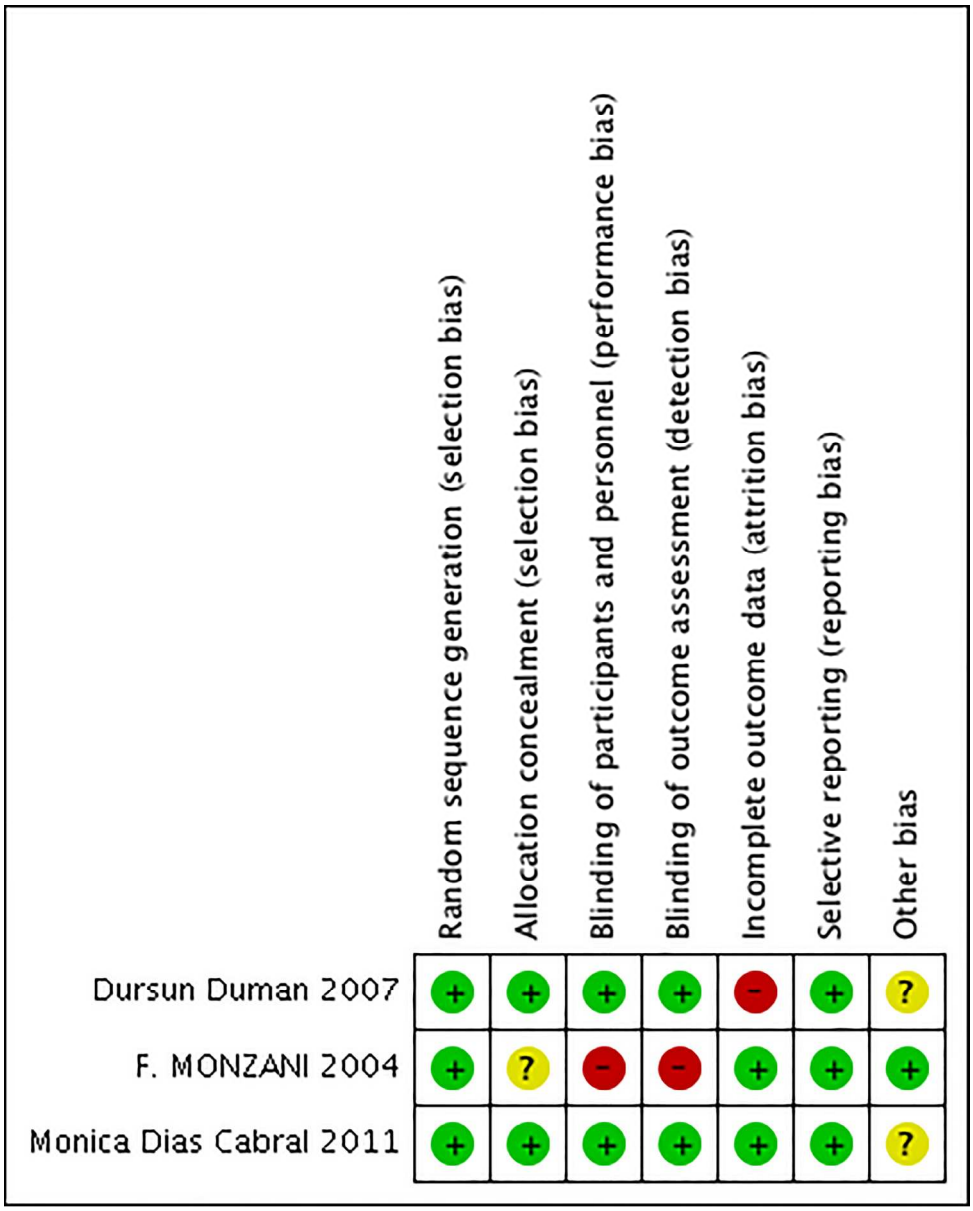

Fig.2. Quality assessment of the included studies. risk of bias summary (B)

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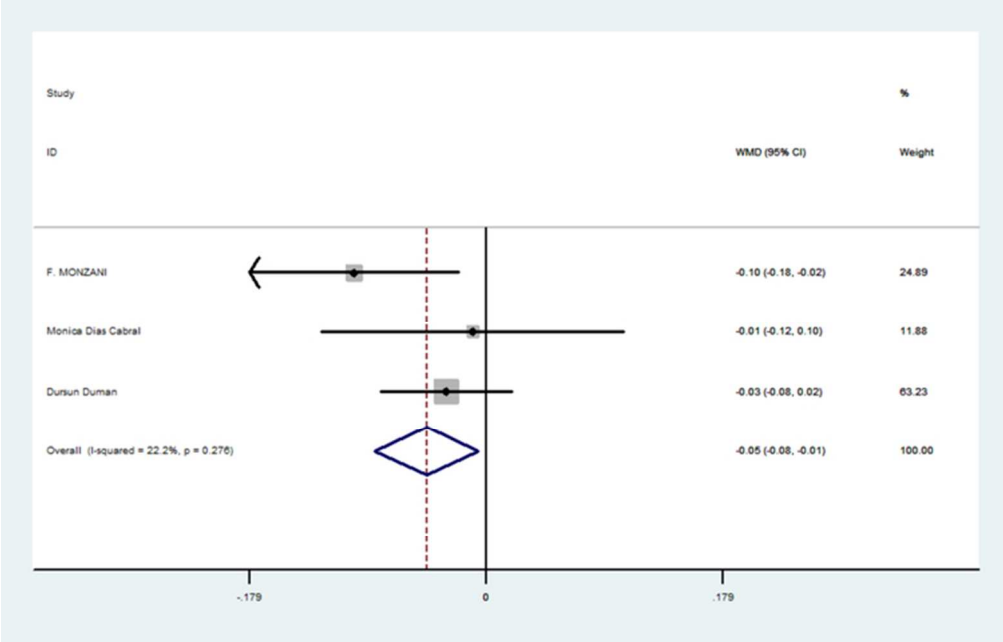

Fig.3. Forest plots showing WMD (weighted mean differences) with 95% confidence intervals for improvement in carotid intima–media thickness comparing L-T4 treatment to the control in a fixed effects model.

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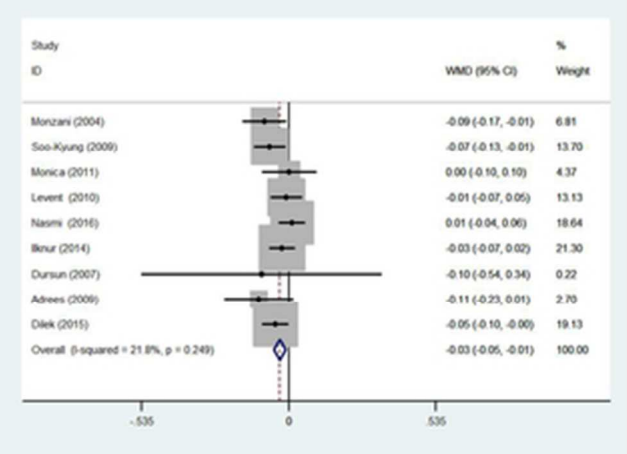

Fig.4. Forest plots showing WMD (weighted mean differences) with 95% confidence intervals compared the value of C-IMT before replacement and after treatment in a fixed effects model.

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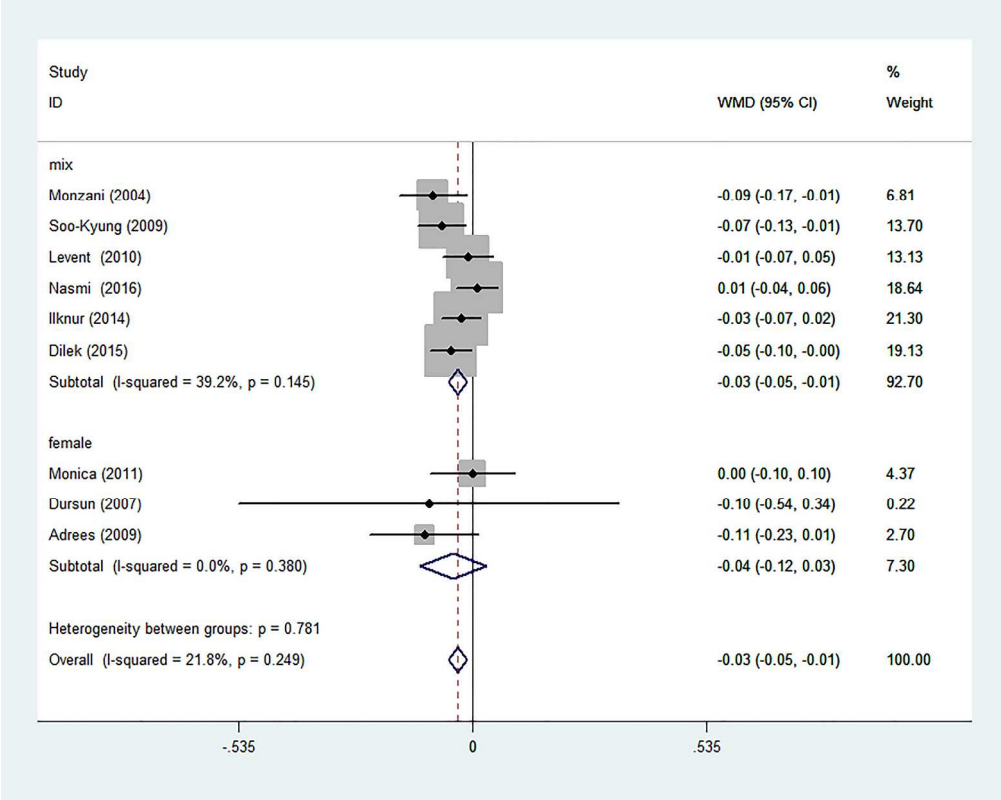

Fig.5.Subgroup analyses of carotid intima-media thickness changes based on gender in a fixed effects model.

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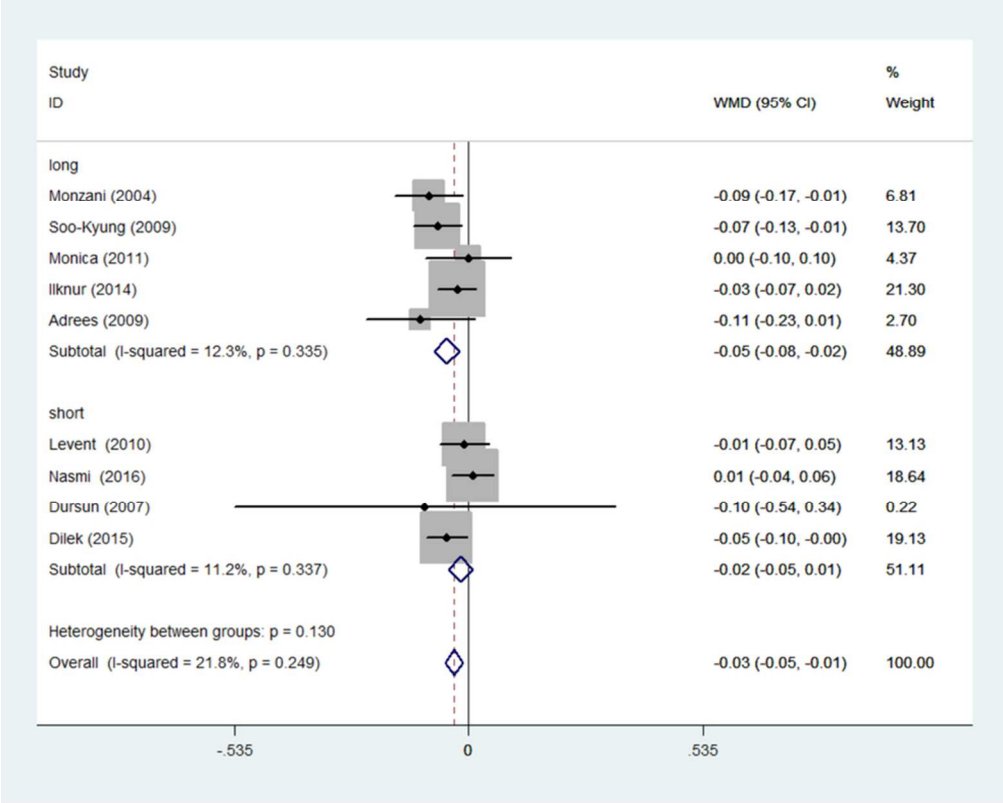

Fig.6.Subgroup analyses of carotid intima-media thickness changes based on duration of L-T4 replacement in a fixed effects model.
Long= duration of L-T4 replacement>6 months
Short= duration of L-T4 replacement≤6 months

39x31mm (600 x 600 DPI)

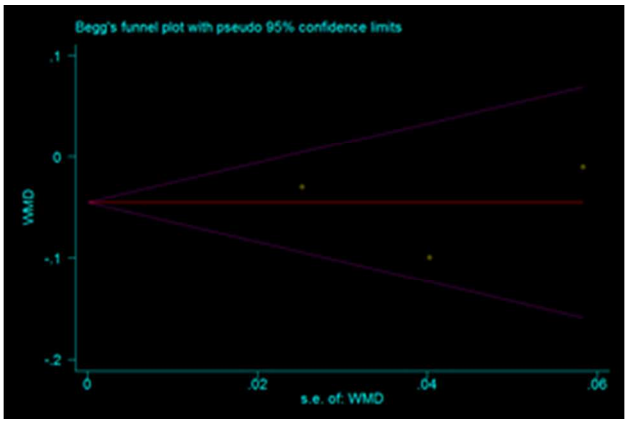

Fig.S1 Begg's funnel plot with pseudo 95% confidence limits of publication bias test for L-T4 on C-IMT in RCT studies

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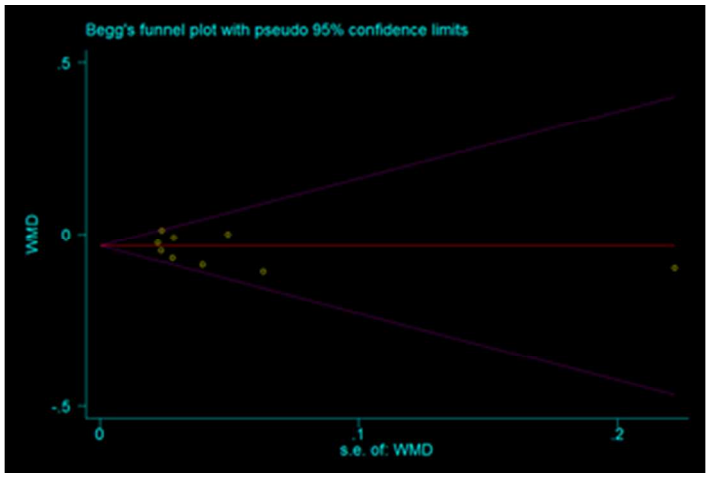

Fig.S2 Begg's funnel plot with pseudo 95% confidence limits of publication bias test for L-T4 on C-IMT in self-controlled studies

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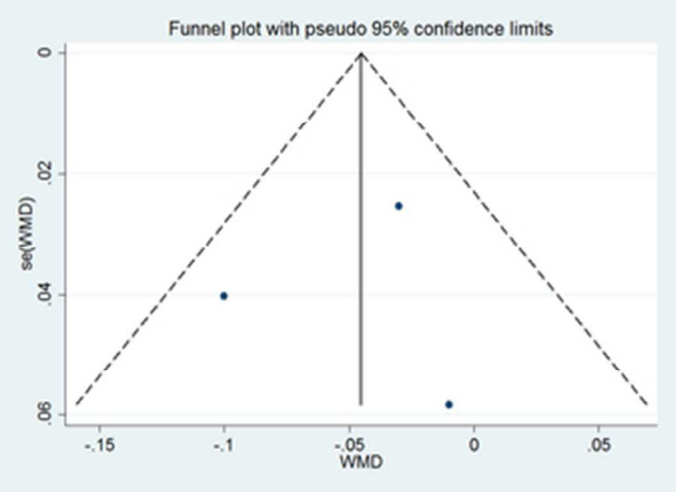

Fig.S3 Funnel plot with pseudo 95% confidence limits of publication bias test for L-T4 on C-IMT in RCT studies

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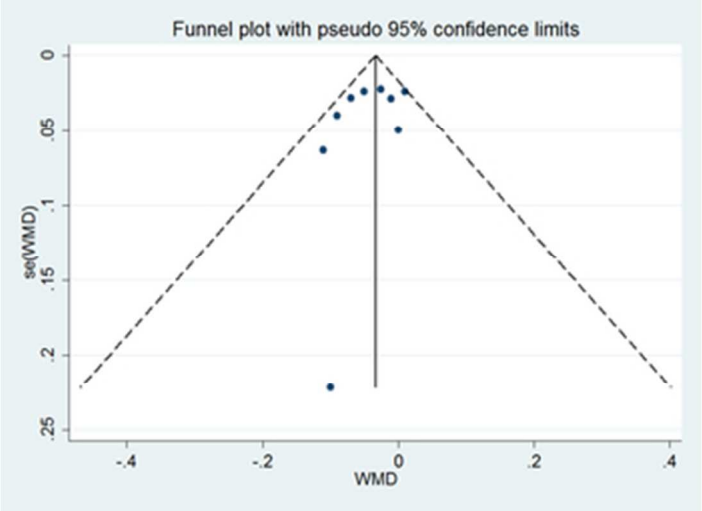

Fig.S4 Funnel plot with pseudo 95% confidence limits of publication bias test for L-T4 on C-IMT in self-controlled studies

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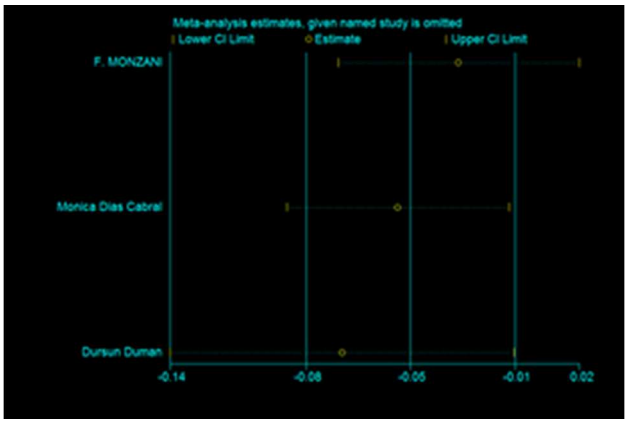

Fig.S5 Sensitivity analysis of the RCT studies included in the meta-analysis. The figure shows the WMD obtained by combined analysis of the remaining studies after the successive exclusion of each study individually. The excluded study is listed on the left, and the corresponding horizontal lines indicate the WMD and CI obtained by re-calculation after its exclusion. The CI for the overall meta-analysis of the studies is indicated by two vertical lines.

13x8mm (600 x 600 DPI)

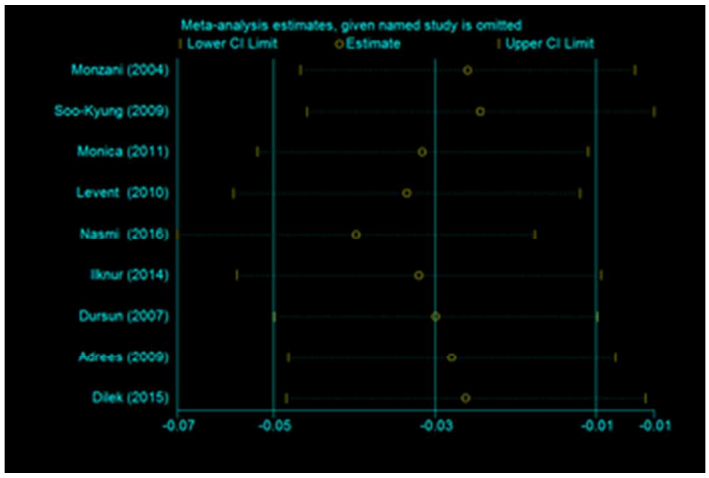

Fig.S6 Sensitivity analysis of the self-controlled studies included in the meta-analysis. The figure shows the WMD obtained by combined analysis of the remaining studies after the successive exclusion of each study individually. The excluded study is listed on the left, and the corresponding horizontal lines indicate the WMD and CI obtained by re-calculation after its exclusion. The CI for the overall meta-analysis of the studies is indicated by two vertical lines.

14x9mm (600 x 600 DPI)

Fig.S1 Begg's funnel plot with pseudo 95% confidence limits of publication bias test for L-T4 on C-IMT in RCT studies

Fig.S2 Begg's funnel plot with pseudo 95% confidence limits of publication bias test for L-T4 on C-IMT in self-controlled studies

Fig.S3 Funnel plot with pseudo 95% confidence limits of publication bias test for L-T4 on C-IMT in RCT studies

Fig.S4 Funnel plot with pseudo 95% confidence limits of publication bias test for L-T4 on C-IMT in self-controlled studies

Fig.S5 Sensitivity analysis of the RCT studies included in the meta-analysis. The figure shows the WMD obtained by combined analysis of the remaining studies after the successive exclusion of each study individually. The excluded study is listed on the left, and the corresponding horizontal lines indicate the WMD and CI obtained by re-calculation after its exclusion. The CI for the overall meta-analysis of the studies is indicated by two vertical lines.

Fig.S6 Sensitivity analysis of the self-controlled studies included in the meta-analysis. The figure shows the WMD obtained by combined analysis of the remaining studies after the successive exclusion of each study individually. The excluded study is listed on the left, and the corresponding horizontal lines indicate the WMD and CI obtained by re-calculation after its exclusion. The CI for the overall meta-analysis of the studies is indicated by two vertical lines.

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2-3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4-5
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5-6
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	6
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	7
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	6
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	8
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	8
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	8
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	8-9
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	9
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	9

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	8
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	8-9
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	10
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	11
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	17
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	15
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	18
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	18
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	18
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	19-20
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	3
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	25
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	25

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Effect of levothyroxine on the progression of carotid intima-media thickness in subclinical hypothyroidism patients: A meta-analysis

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Effect of levothyroxine on the progression of carotid intima-media thickness in subclinical hypothyroidism patients: A meta-analysis

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Abstract

Background and aims: Subclinical hypothyroidism (SCH) has been associated with increased carotid intima-media thickness (C-IMT) in recent studies, but the effects of levothyroxine (L-T4) on C-IMT in SCH patients are still controversial. The aim of the current study was to evaluate the effect of L-T4 therapy on endothelial function as determined by C-IMT in patients with SCH.

Methods: Prior to July 2016, we searched the PUBMED, EMBASE, COCHRANE LIBRARY and GOOGLE SCHOLAR databases we selected published randomized controlled trials (RCTs) and self-controlled trials.

Results: Three RCTs with 117 patients were considered suitable for the meta-analysis. The results of the meta-analysis indicated that L-T4 significantly decreased the development of C-IMT [weighted mean difference (WMD), −0.05 mm, 95% CI −0.08, −0.01; p = 0.025]. We also analysed 9 studies (self-controlled trials) with 247 patients and extracted the IMT of SCH patients before and after L-T4 treatment. After L-T4 therapy, the pooled estimate of the WMD of decreased C-IMT was −0.04 mm (95% CI −0.07, −0.02; p = 0.05). Subgroup analysis showed that L-T4 therapy was associated with a decrease in C-IMT among patients of mixed genders (WMD, -0.03 mm, 95% CI -0.06, -0.01; p = 0.145). L-T4 therapy was

associated with a decrease in C-IMT among female patients (WMD, -0.07 mm, 95% CI -0.14, -0.01; p = 0.186). Longer treatment (>6 months) also resulted in a significant decrease in C-IMT (WMD, -0.05 mm, 95% CI -0.08, -0.02; p=0.335).

Conclusion: This meta-analysis indicates that L-T4 treatment of SCH patients can reduce C-IMT, possibly as a result of the reduction of TC, TG, LDL-C, SBP, DBP, LP(a), and FMD. Decreased C-IMT was observed in SCH patients after long-term (>6 months) L-T4 treatment. RCTs with large samples are needed to verify these observations.

Strengths and limitations of this study:

Strengths

A strong quality evidence on the effect of L-T4 therapy in SCH patients is preformed.

Methodology, according to the different types of original literature, we used different method of statistical analysis and study quality evaluation. According to different heterogeneity，we adopt different effect model. This embodies the rigor of methodology

Furthermore, we analysis other metabolic parameters to better explain to effect of L-T4 therapy in SCH.

Limitations

The number of including studies was small, and the overall population of these trials was not large. Meta analysis has moderate heterogeneity. Difficult assessment of the potential publication bias mainly due to the previous two factors. Additionally, we did not perform subgroup analysis according to the level of TSH (the cutoff value is 10 mU/ml to distinguish mild SCH and severe SCH) because the original studies did not perform analyses according to the level of TSH. Finally, we did not perform subgroup analysis by mean age (>65 or <65 years) because all participants' mean age was below 65 years.

Keywords: subclinical hypothyroidism; carotid intima-media thickness; thyroxin; cardiovascular risk

Introduction

Subclinical hypothyroidism (SCH) is characterized by a normal range of free thyroxin concentrations together with increased serum thyroxin (TSH) levels. Recently, SCH was found in 5% to 10% of the general population and 6% to 10% of women (approximately 15% was found in women more than 60 years old), while the incidence in males is 2.4 to 3% [1]. Although SCH is frequently asymptomatic, approximately 30% of patients have symptoms

indicative of thyroid hormone deficiency [2, 3]. However, the clinical significance and therapeutic strategies of SCH are controversial .

C-IMT is a generally acknowledged measure of subclinical atherosclerotic alterations. It is increasingly used to evaluate vascular function in clinical analyses assessing the efficacy of interventions that reduce atherosclerosis and related diseases [4]. This parameter is listed in the European guidelines for prophylaxis of cardiovascular disease, and 0.9 mm is the threshold value for C-IMT. Progression of atherosclerosis is indicated when the value of C-IMT is over the threshold.

A meta-analysis involving 8 studies with 3602 participants found a relationship between SCH and C-IMT, especially when TSH > 10.0 mIU/l [5].L-T4 replacement should be used to treat patients with clinical hypothyroidism; however, the treatment of SCH is complex. Several placebo-controlled studies have shown the beneficial effects of L-T4 therapy on early atherosclerotic alterations and cardiovascular hazard in patients with SCH [6, 7]. Recently, in a large-scale retrospective cohort study examining the efficacy of L-T4 replacement on cardiovascular mortality, myocardial infarction and all-cause death, no beneficial effects in patients with SCH were found, except for patients under 65 years old [8]. The purpose of the

current study was to evaluate the effect of L-T4 therapy on endothelial function as evaluated by C-IMT in patients with SCH.

Methods

Search strategy

Studies published in English were found in the EMBASE, PUBMED, GOOGLE SCHOLAR and COCHRANE LIBRARY electronic databanks prior to June 2016. We identified studies that measured IMT in patients with SCH before and after treatment with L-T4. Studies were obtained using three series of key items as subject headings: 1) hypothyroidism, thyroid disease, subclinical hypothyroid, subclinical thyroid dysfunction and thyroid-stimulating hormone; 2) intima media thickness, IMT, carotid wall thickness, C-IMT and carotid atherosclerosis; 3) levothyroxine, thyroxin, L-T4, replacement, therapy and treatment. For a thorough review of the literature, we searched more studies by browsing the references of the identified reports and review articles.

Criteria for study selection

Subjects with SCH have standard free thyroxin levels and increased TSH levels. There is still debate about the TSH reference interval; several reviews have proposed a TSH upper cut off between 4.5 and 5.0 mIU/l [9, 10], but some experts suggest that the peak TSH should be decreased to 2.5-3.0 mIU/l. Because there are no consistent conclusions, no specific TSH cut off was used to define SCH, but all articles had a cut off point from 3.6 to 5.5 mIU/l. To be included, a study had to satisfy the following conditions: 1) reported SCH defined based on a thyroid function test (normal free T4 and increased TSH); 2) defined as a RCT, which compared C-IMT in SCH patients in a L-T4 treatment and control group, or a self-controlled trial, which compared C-IMT value of a patient with SCH before and after L-T4 replacement.

Study selection

Two investigators independently screened both abstracts and titles of the studies, and the studies were excluded when they did not conform to the inclusion criteria or they accorded with the exclusion criteria. Two investigators independently extracted data from the papers. If there was disagreement, a third reviewer was asked for advice. Discrepancies were solved by unanimous agreement.

Data extraction

The following information is contained in the data extraction table: 1) author 2) year of publication 3) region 4) sample size 5) sample gender 6) mean age of the cohort 7) definition of SCH 8) duration of SCH 9) SCH detection method 10) mean L-T4 dosage 11) duration of treatment 12) thyroid function after treatment 13) IMT assessment 14) IMT values (mean with SD) before and after treatment with the corresponding treatment method.

Assessment of study quality

The quality of non-randomized studies was evaluated using the MINORS method [11], which is based on the following items: a stated aim of the study, inclusion of consecutive patients, prospective collection of data, endpoint appropriate to the study aim, unbiased evaluation of endpoints, follow-up period appropriate to the major endpoint, and loss to follow-up not exceeding 5%.

Methodological quality of the included RCTs was assessed using the Cochrane Handbook for Systematic Review of Interventions by several domains: generation of random sequence; allocation concealment; blinding; incomplete outcome date addressed; free of selective reporting; free of other bias.

Statistical analysis

We represented the results as weighted mean differences (WMDs) with 95% confidence intervals (CIs) for continuous variables. For continuous outcomes, if all reports had uniform units of measurement (expressed as mm), we showed the WMD and 95% CIs as the results. We evaluated statistical heterogeneity across the studies by Cochrane's Q test ($p < 0.1$ was statistically significant) and the I^2 test ($I^2 > 50\%$: high heterogeneity; I^2 = 25%-50%: moderate heterogeneity; $I^2 < 25\%$: low heterogeneity) [12]. The pooled effect size was assessed with a random-effect model when significant statistically heterogeneity was present; in contrast, we also selected a fixed-effect model. $p < 0.05$ was considered significant. The weighting of WMD in a fixed-effect model is inverse variance. However, the weighting of WMD in a random-effect model is I-V heterogeneity. We assessed publication bias with Begg's correlation test and Egger's regression method [13]. Sensitivity analysis was carried out for all papers in addition to the studies with boundary qualification. Pre-specified subgroup meta-analyses were performed to assess the between-study heterogeneity on the basis of the gender of participants (female or genders) and duration of treatment (>6 months or ≤6 months). Among 9 including studies, 3 out of them are RCTs and the other of them are self-controlled trials. We

performed Statistical analysis of three RCTs. SCH treatment group of three RCTs also have the information on C-IMT values between pre-treatment and after-treatment in SCH patients. We combined the SCH treatment group of RCTs with the other self-controlled trials to performed Statistical analysis for fully revealing the effect of L-T4 treatment in SCH patients. Statistical analyses were performed with STATA 11 for Windows.

Results

Study selection

Three RCTs [7, 14, 15] (57 patients treated with L-T4 and 60 included in the control group) were selected for the meta-analysis, with a total of 117 patients. A total of 9 publications [7, 14-21] with 247 SCH patients measured the C-IMT before and after L-T4. We selected these studies among 107 potentially related articles (Fig. 1).

Baseline characteristics and study quality

The baseline characteristics of the included trials are shown in Table 1(A)(B). The duration of therapy with L–T4 ranged from 2 to 18 months, the patient count varied from 14 to 56 subjects, and the year of the publication was from 2004 to 2016. Six trials included female

and male patients, and the other three only examined female patients. Based on the type of original research, the methodological evaluation of study quality was different. We used MINORS to assess the quality of the self-controlled trials. In general, the included self-controlled trials were of moderate quality (Table1) .The results of RCT quality are shown in Fig. 2.

Progression of C-IMT in the follow-up and subgroup analysis

A total of 3 RCTs involving 117 patients were analysed. L-T4 treatment significantly decreased C-IMT progression in SCH patients. (WMD, −0.05 mm, 95% CI −0.08, −0.01; p = 0.025). The heterogeneity was very low (I^2=22.2%) (Fig. 3)

The nine self-controlled experiments compared the value of C-IMT before replacement and after treatment. After L-T4 therapy, the thyroid function of all patients was normalized from SCH, and then, patient IMT was measured again. Because clinical heterogeneity is relatively high. We selected random-effect model. L-T4 therapy was linked to a mild but significant decrease in the development of C-IMT (WMD, −0.04 mm, 95% CI −0.07, −0.02; p = 0.050). The heterogeneity was moderate. (I2=48.3%) (Fig. 4)

Subgroup analyses stratified by gender revealed that L–T4 therapy caused a significant decrease in C-IMT among patients of mixed genders (WMD, -0.03 mm, 95% CI -0.06, -0.01; p = 0.145). The outcomes showed moderate heterogeneity (I2= 39.2%). After L-T4 treatment,

TABLE 1(A). Summary of the Characteristics of the Included Studies

Author/ Year	Sample Size	Region	Gender	Mean Age (year)	Definition of SCH	SCH Duration (month)	L-T4 Dosage (initial value)	Treatment Duration (month)	Thyroid function after treatment	CIMT(mm) (Before/After)	Quality Score
Monzani 2004	23	Italy	Mixed genders	37±11	TSH >3.6 mIU/L	at least 6 months	25 -75 ug/d	10.5[a]	normal	0.76±0.14 0.67±0.13	12
Soo-Kyung 2009	28	Korea	Mixed genders	36.0±6.2	TSH >5.5 mIU/L	newly diagnosed	67μg/d[b]	18[b]	normal	0.67±0.11 0.60±0.10	9
Monica 2011	14	Brazil	female	43.4±9.8	TSH >4.0 mIU/L	_	44.23±18.13ug/d[d]	12	normal	0.66±0.11 0.66±0.15	10
Levent 2010	38	Turkey	Mixed genders	49.8±10.0	TSH >5.0 mIU/L	at least 2 months	101±27.46μg/d[c]	6[b]	normal	0.64±0.13 0.63±0.12	11
Nasmi 2016	25	Iran	Mixed genders	35.9±7.6	TSH >4.0 mIU/L	_	50ug/d	2	normal	0.56±0.09 0.57±0.08	11
Ilknur 2014	56	Turkey	Mixed genders	41.3±14.5	TSH>4.2 mIU/L	_	25-50 u/d	_	normal	0.533±0.112 0.507±0.126	12
Dursun 2007	20	Turkey	female	36±11	TSH>4.2 mIU/L	newly diagnosed	25-100ug/d	5[b]	normal	0.65±0.09 0.55±0.08	12
Adrees 2009	20	Ireland	female	50±9	_	at least 6 months	100 ± 30 ug/d[c]	18	normal	0.82±0.2 0.71±0.2	10
Dilek 2015	23	Turkey	Mixed genders	35.2±10.7	TSH >4.0 mIU/L	at least 6 months	_	6	normal	0.51±0.09 0.46±0.07	11

Abbreviations: TSH, thyroid-stimulating hormone; CIMT, Carotid intima-media thickness; L-T4, levothyroxine.

Mixed genders=including male and female. Age and IMT are expressed as mean± s.d.

[a] median ； [b] mean； [c] mean± s.d； [d] median± interquartile range

TABLE 1(B). Summary of the Characteristics of the Included Studies

Author	TSH assay	Carotid IMT assessment
Monzani	ultrasensitive immunoradiometric assay	both the near and far wall of the right and left common carotid artery and carotid bifurcation(high-resolution ultrasonography)
Soo-Kyung	chemiluminescent immunometric assay	20 mm proximal to the origin of the carotid bulb (high-resolution ultrasonographic system)
Monica	immunochemiluminescence	both the near and far wall of the right and left common artery and the carotid bifurcation.(high-resolution ultrasound imaging)
Levent	Chemiluminescence immunometric assay	approximately 1-cm segment from both the left and right CCA proximal and distal portions (Ultrasonographic images)
Nasmi	_	sonogram B-mode images
Ilknur	electrochemiluminescence immunoassays	Three arterial wall segments of the common carotid artery were measured bilaterally after imaging from a fixed lateral transducer angle and designated（high-resolution B-mode ultrasonography）
Dursun	immunoassay	right and left extracranial carotid arteries: the CCA (1 cm proximal to the dilation of the carotid bulb), the bifurcation (the 1-cm segment proximal to the flow divider), and the internal carotid artery (the 1-cm segment in the internal branch distal to the flow divider).(high-resolution ultrasonography)
Adrees	chemiluminescent immunometric assay	1 cm proximal to the carotid bulb when a satisfactory position was found.(calibrated ultrasound machine)
Dilek	_	ultrasound system

Abbreviations: CCA, common carotid artery.

female participants with SCH displayed a significant reduction in C-IMT (WMD, -0.07 mm, 95% CI -0.14, -0.01; p = 0.186). The heterogeneity was moderate (I2= 40.5%) (Fig.5).

Subgroup analyses were performed using the duration of L-T4 replacement. Except for one reports, all trials had information on the length of the therapy. There was a significant decrease in C-IMT in the patients with long-term (>6 months) treatment (WMD, -0.05 mm, 95% CI -0.08, -0.02; p=0.335). The outcome showed low heterogeneity (I^2= 12.3%). A non-significant reduction was found in C-IMT in the participants with short-term (≤6 months) replacement therapy (WMD, -0.04 mm, 95% CI -0.08, 0.01; p=0.015). The heterogeneity was high (I^2= 71.3%). (Fig. 6).

Changes in metabolic parameters

Table 2 indicates the changes in metabolic parameters from pre-replacement to post-treatment. L-T4 therapy was linked to significant reductions in TC, TG, LDL, SBP, DBP, and LP(a) and decreased FMD. There were no significant changes in BMI, waist circumference, HDL, ApoA, ApoB and glucose between the pre-treatment and post-therapy conditions.

Sensitivity and publication bias evaluation

We carried out sensitivity analyses by sequentially eliminating one study to probe the change in the total WMD and 95% CI of C-IMT. The results of three RCT sensitivity

Table 2

The difference of the changes in metabolic parameters between pre-treatment and post-treatment.

Parameters	No. of patients	WMD (95% CI)	P-value
Waist circumference (cm)	66	-0.544(-4.041,2.954)	0.761
BMI (kg/m2)	152	-0.231(-1.044,0.582)	0.577
Glucose (mmol/L)	48	0.090(-0.109,0.289)	0.375
SBP (mmHg)	109	-9.391(-14.405,-4.377)	<0.001
DBP (mmHg)	109	-5.693(-8.471,-2.916)	<0.001
TC (mg/dl)	166	-9.037(-16.635,-1.439)	0.02
TG (mg/dl)	166	-9.842(-18.763,-0.921)	0.031
HDL (mg/dl)	166	-1.032(-4.059,1.995)	0.504
LDL (mg/dl)	166	-14.303(-19.133,-9.472)	<0.001
FMD (%)	39	3.962(2.636,5.289)	<0.001
ApoA (mg/dl)	37	-10.092(-23.173,2.989)	0.131
ApoB (mg/dl)	37	-7.718(-21.723,6.287)	0.28
LP(a) (mg/l)	34	-9.974(-12.844,-7.103)	<0.001

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; TC, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein; ApoA, Apolipoprotein A; ApoB, Apolipoprotein B; Lp(a), lipoprotein(a); WMD, weighted mean difference; CI: confidence interval;FMD, flow-mediated dilatation.

indicated no variation in the direction of WMD when each publication was removed.

(Fig.s1) . Neither Egger's test (p=0.881) nor Begg's test (p=0.602) of C-IMT in SCH

manifested publication biases. (Fig.s2) (Fig.s3). We also evaluate the sensitivity and publication bias of the nine self-controlled trial. The results showed that there was only a slight change in the WMD or 95% CI, and no change in the direction of WMD when any one study was omitted. (Fig.s4) Egger's test (p=0.458) and Begg's test (p=0.602) of C-IMT in SCH manifested publication biases. (Fig.s5) (Fig.s6) .

Discussion

This meta-analysis indicated that L-T4 has beneficial effects on the development of C-IMT. Decreased C-IMT was also observed with longer treatment (>6 months). L–T4 therapy of SCH participants also resulted in a significant reduction in TC, TG, LDL, SBP, DBP, LP(a), and FMD.

The American Heart Association/American College of Cardiology guidelines designated C-IMT along with coronary artery calcium (CAC) score as a class IIa recommendation for cardiovascular risk assessment in asymptomatic adults at intermediate risk of cardiovascular disease. For a 0.1 mm difference in C-IMT, the prospective risk of myocardial infarction increased from 10% to 15%, and the stroke risk was increased from 13% to 18% [5]. The result showed that C-IMT in SCH patients can be reversed by L-T4 replacement. The

mechanism underlying the L-T4-mediated decrease in C-IMT has not been fully elucidated. TSHR, hyperlipidaemia and blood pressure may contribute to this result.

In SCH, the increased TSH is the only prominent change, and increased TSH can bind with the TSH receptor (TSHR) to perform its function. Balzan et al. [22] revealed that micro-vascular endothelial cells produce TSHR, which may help to elucidate this relationship. Recently, Tian et al. [23] published a study to identify the potential function of the TSHR and showed that increased TSH aggravated endothelial dysfunction. However, L-T4 can control the elevated TSH by reversing above-mentioned changes to reduce the mean C-IMT.

Six out of 13 papers in a systemic review reported that L-T4 treatment improved TC and LDL [24]. A RCT trial with the same conclusion further supported this hypothesis [8].Among four markers of hyperlipidaemia (TC, TG, LDL, HDL), we observed non-significant differences only in HDL between pre-treatment and post-replacement conditions, which is supported by previous study with similar findings [25]. Reports by Kannel et al. [26] and Lewington et al. [27] showed that the TC/HDL ratio is more important in the progress of coronary heart disease than either TC or HDL alone. Although HDL concentration was not changed after restoration of euthyroidism by L-T4 replacement in SCH

patients in our study, L-T4 treatment altered the TC/HDL ratio. . Hyperlipidaemia promotes endothelial dysfunction because it can inhibit the NO synthesis pathway in endothelial cells [28]. Meanwhile, a recent paper found that SCH can cause endothelial dysfunction because of a decrease in NO availability, which is partly independent of dyslipidaemia, and L-T4 supplementation can reverse endothelial dysfunction [29]. Thus, we found that SCH not only results in endothelial dysfunction but is also an independent risk factor of dyslipidaemia; then, dyslipidaemia further amplifies endothelial dysfunction. L-T4 treatment of SCH patients can reduce these effects.

A meta-analysis showed that blood pressure was an independent risk factor for C-IMT [30]. Recently, an another meta-analysis demonstrated that SCH is linked to higher SBP and DBP levels [31].We know little about the mechanism of SCH-associated hypertension; nevertheless, hypothyroidism increases vascular resistance, enhances blood pressure, salt sensitivity and abnormal sodium metabolism, which may promote the development of hypertension.

For the subgroup of short duration (≤6 months), an insignificant difference of C-IMT was observed after treatment. Meanwhile, mean C-IMT in SCH subjects undergoing long-term

treatment significantly increased compared to that in the pre-treatment condition. This phenomenon was confirmed in our clinical observation. The major reason is that the duration of treatment is too short to improve C-IMT. The rate of progression of IMT in the common carotid artery was approximately 0.01 mm/year.

For stratification by gender, we find a significant difference in female patients between pre-replacement and post-replacement. Meanwhile, for the mixed genders subgroup, mean C-IMT was significantly reduced after treatment. Although the prevalence of SCH is a little difference between women and general population, the effects of levothyroxine (L-T4) on C-IMT are similar. This suggests that the changes of C-IMT are similar when we control thyroid function in the normal range regardless of gender.

Limitations

The number of including studies was small, and the overall population of these trials was not large. Meta analysis has moderate heterogeneity. Difficult assessment of the potential publication bias mainly due to the previous two factors. Additionally,we did not perform subgroup analysis according to the level of TSH (the cutoff value is 10 mU/ml to distinguish mild SCH and severe SCH) because the original studies did not perform analyses according to the level of TSH. Finally, we did not perform subgroup

analysis by mean age (>65 or <65 years) because all participants' mean age was below 65 years.

Conclusion

Our study suggests that C-IMT in SCH patients can be reversed by L-T4 replacement. Hyperlipidaemia and hypertension may improve after L-T4 therapy. Long L-T4 replacement duration (>6 months) is more useful compared to short-term duration for improving C-IMT in SCH patients. Therefore, L-T4 supplementation can prevent or inhibit the progression of atherosclerosis, at least in SCH patients. RCTs with more sample size are needed to confirm these findings.

contributorship statement

Zhao Tong and Chen Baomin made search strategies, searched papers from databases, assessed of study quality ,independently extracted data from the papers and wrote the paper Wang Haoyu as a third reviewer was asked for advice,If there was disagreement.Zhou Yingying, Wang Xinyi and Zhang Yuanyuan Participated in the revision of the paper. Shan Zhongyan provided some advices on the discussion section of this article.

competing interests statement

The author denies that he has any intention to obtain any financial interests.

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Fig.1. Process of study selection.

Abbreviations: IMT: intima-media thickness; SCH: subclinical hypothyroidism

Fig.2. Quality assessment of the included studies.

Fig.3. Forest plots showing WMD (weighted mean differences) with 95% confidence intervals for improvement in carotid intima–media thickness comparing L-T4 treatment to the control in a fixed effects model.

Legend for x-axis: Negative values equals improvement in IMT.

Fig.4. Forest plots showing WMD (weighted mean differences) with 95% confidence intervals compared the value of C-IMT before replacement and after treatment in a random-effect model.

Legend for x-axis: Negative values equals improvement in IMT.

Fig.5.Subgroup analyses of carotid intima-media thickness changes based on gender in a random effects model.

Legend for x-axis: Negative values equals improvement in IMT.

Fig.6.Subgroup analyses of carotid intima-media thickness changes based on duration of L-T4 replacement in a random effects model.

Legend for x-axis: Negative values equals improvement in IMT.

Long study duration= duration of L-T4 replacement>6 months

Short study duration= duration of L-T4 replacement≤6 months

Fig.S1 Begg's funnel plot with pseudo 95% confidence limits of publication bias test for L-T4 on C-IMT in RCT studies.

Fig.S2 Begg's funnel plot with pseudo 95% confidence limits of publication bias test for L-T4 on C-IMT in self-controlled studies.

Fig.S3 Funnel plot with pseudo 95% confidence limits of publication bias test for L-T4 on C-IMT in RCT studies .

Fig.S4 Funnel plot with pseudo 95% confidence limits of publication bias test for L-T4 on C-IMT in self-controlled studies.

Fig.S5 Sensitivity analysis of the RCT studies included in the meta-analysis. The figure shows the WMD obtained by combined analysis of the remaining studies after the successive exclusion of each study individually. The excluded study is listed on the left, and the corresponding horizontal lines indicate the WMD and CI

obtained by re-calculation after its exclusion. The CI for the overall meta-analysis of the studies is indicated by two vertical lines.

Fig.S6 Sensitivity analysis of the self-controlled studies included in the meta-analysis. The figure shows the WMD obtained by combined analysis of the remaining studies after the successive exclusion of each study individually. The excluded study is listed on the left, and the corresponding horizontal lines indicate the WMD and CI obtained by re-calculation after its exclusion. The CI for the overall meta-analysis of the studies is indicated by two vertical lines.

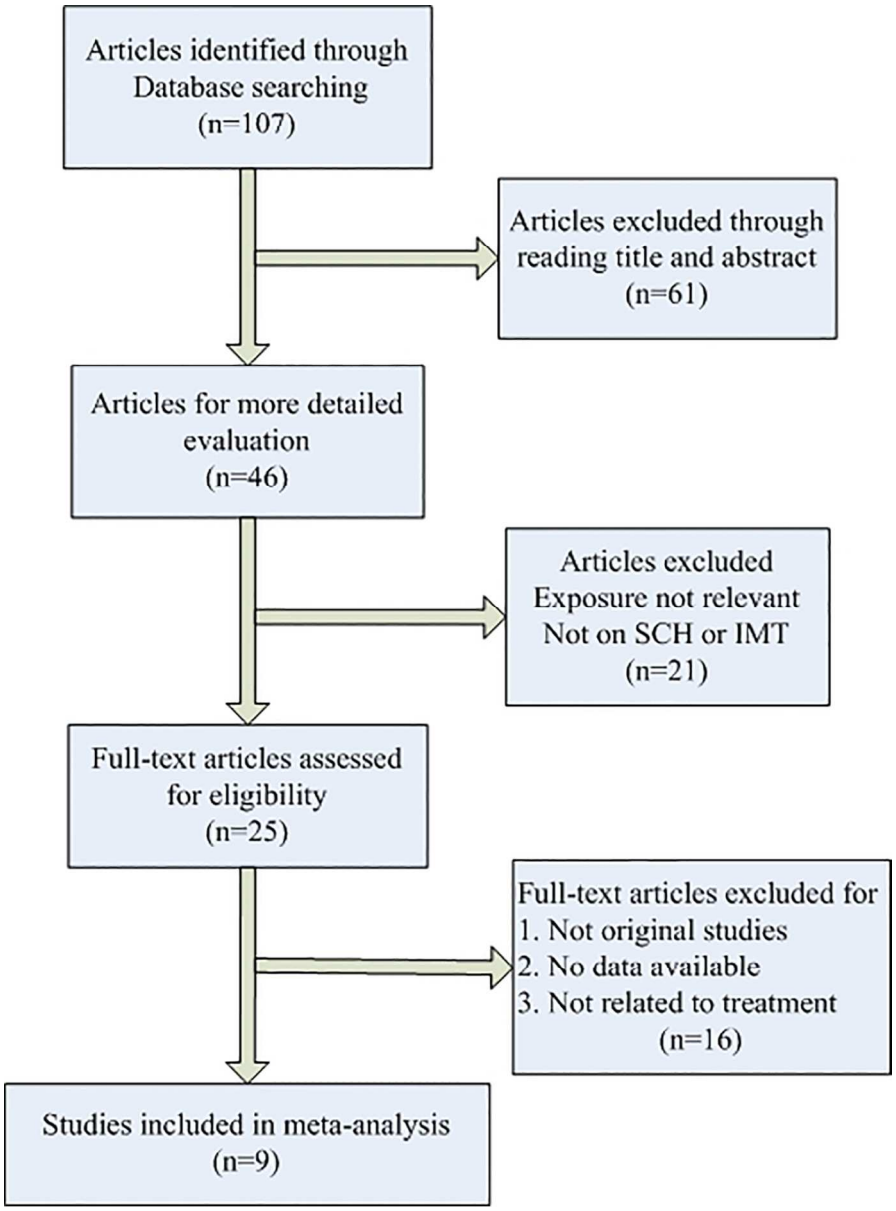

Fig.1. Process of study selection.
Abbreviations: IMT: intima-media thickness; SCH: subclinical hypothyroidism

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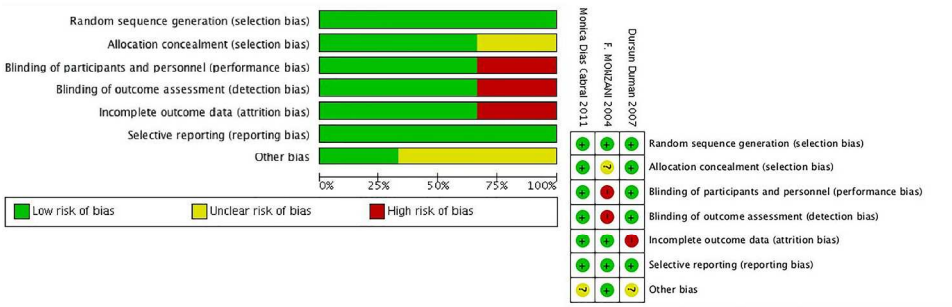

Fig.2. Quality assessment of the included studies.

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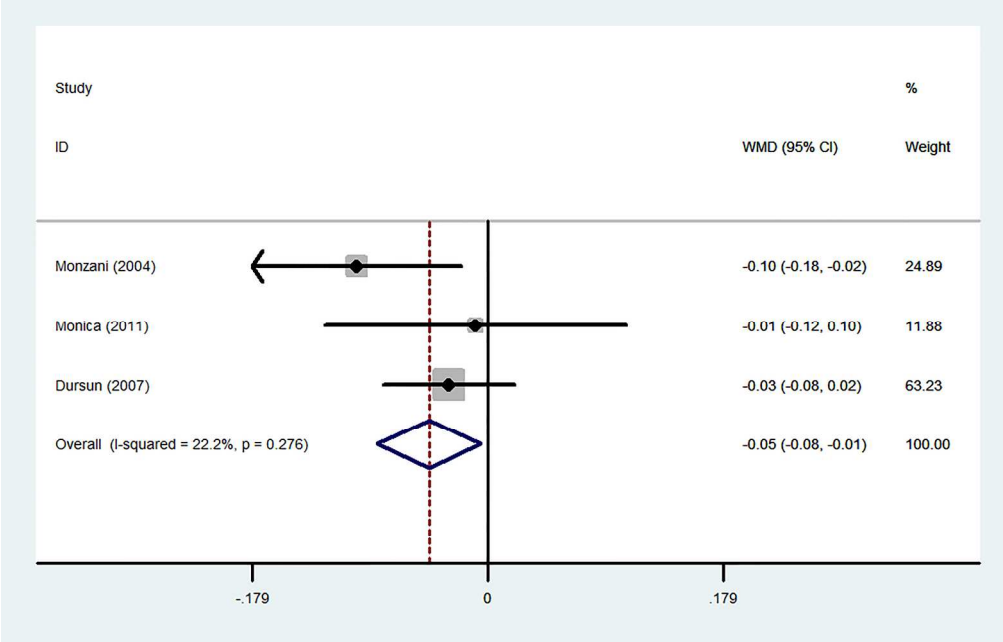

Fig.3. Forest plots showing WMD (weighted mean differences) with 95% confidence intervals for improvement in carotid intima–media thickness comparing L-T4 treatment to the control in a fixed effects model.
Legend for x-axis: Negative values equals improvement in IMT

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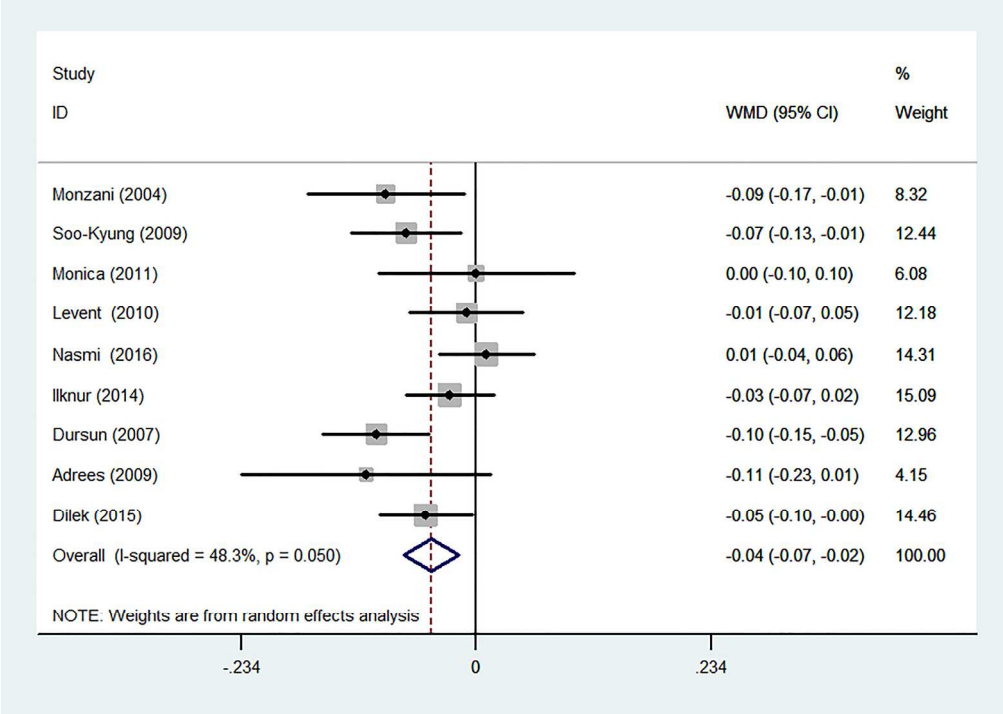

Fig.4. Forest plots showing WMD (weighted mean differences) with 95% confidence intervals compared the value of C-IMT before replacement and after treatment in a random-effect model.
Legend for x-axis: Negative values equals improvement in IMT.

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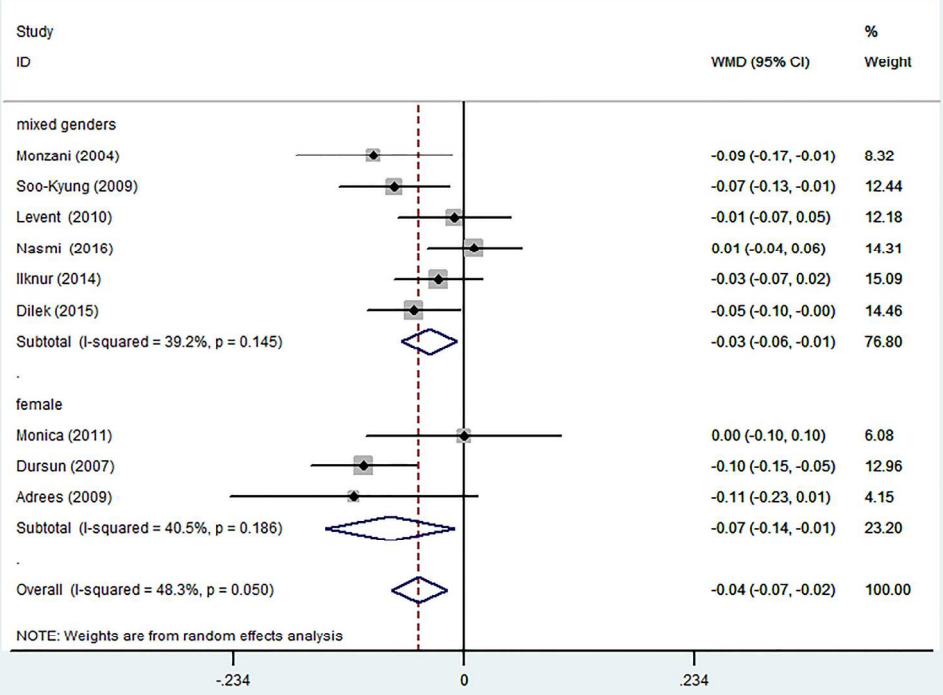

Fig.5.Subgroup analyses of carotid intima-media thickness changes based on gender in a random effects model.
Legend for x-axis: Negative values equals improvement in IMT.

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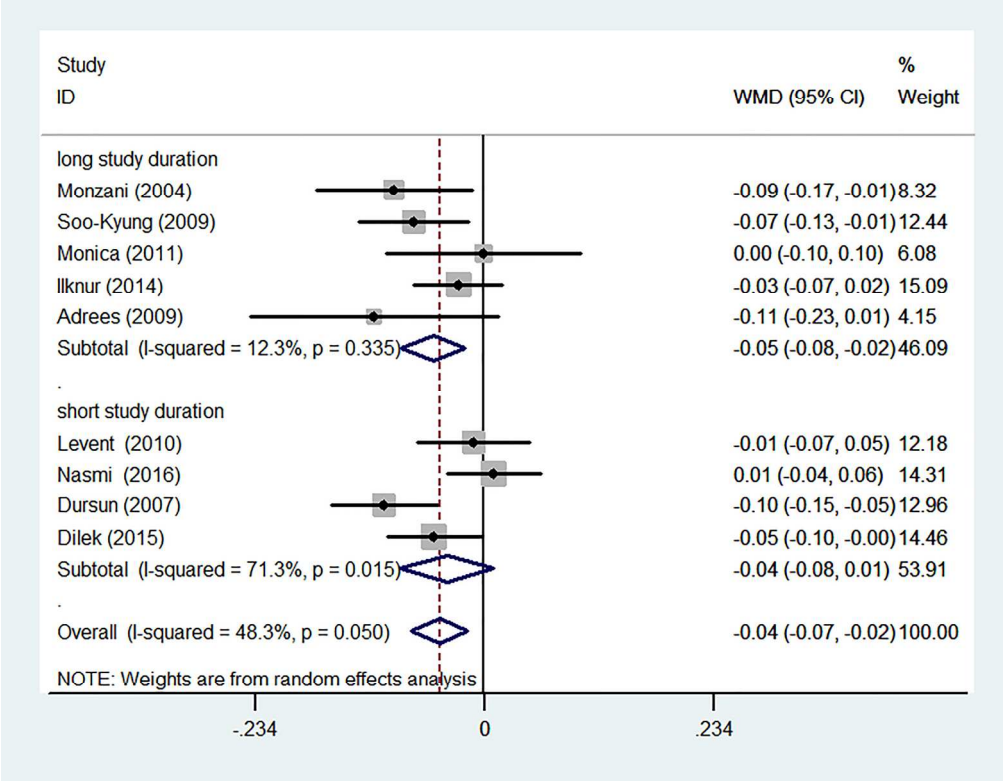

Fig.6.Subgroup analyses of carotid intima-media thickness changes based on duration of L-T4 replacement in a random effects model.
Legend for x-axis: Negative values equals improvement in IMT.
Long study duration= duration of L-T4 replacement>6 months
Short study duration= duration of L-T4 replacement≤6 months

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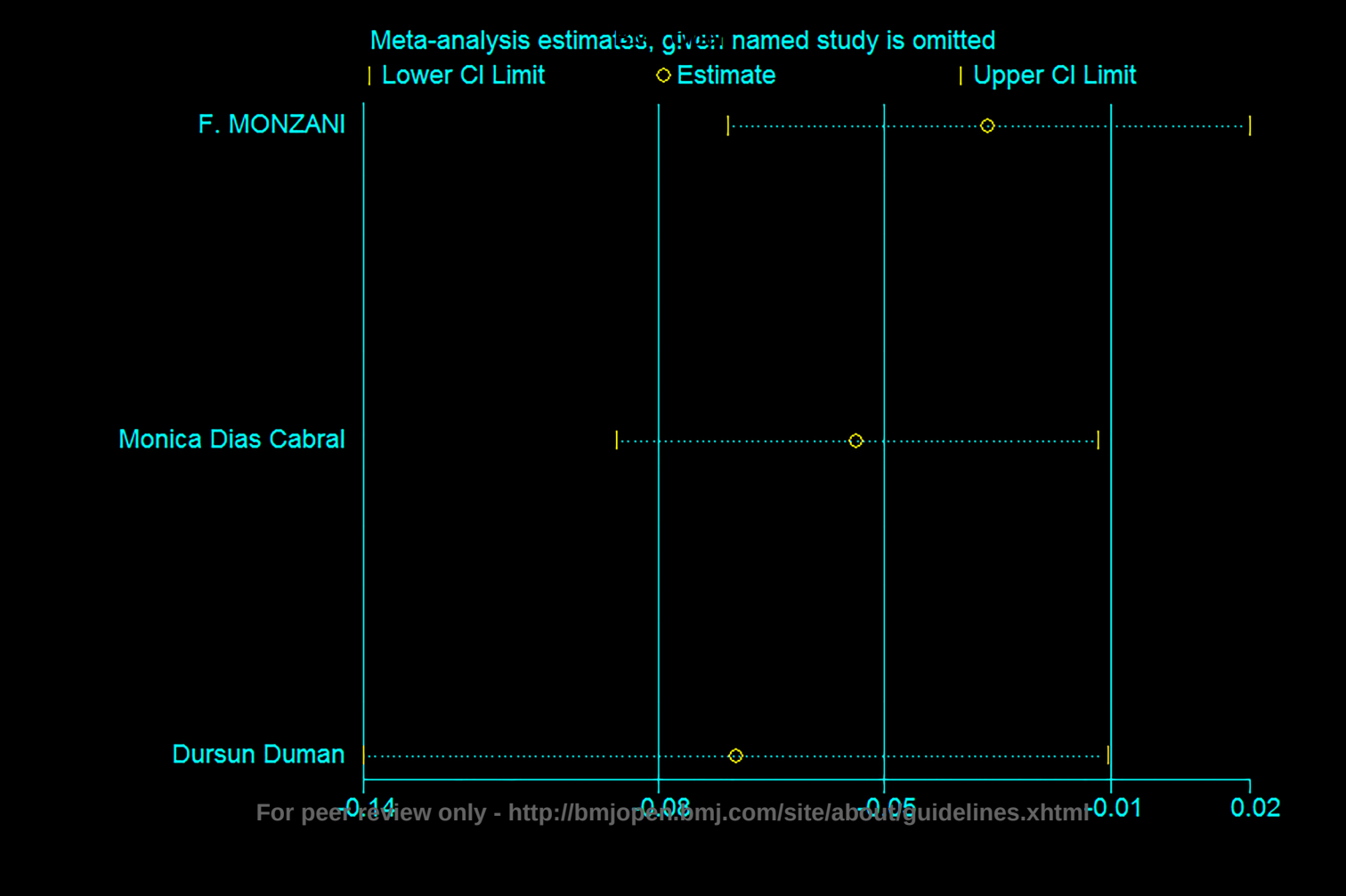

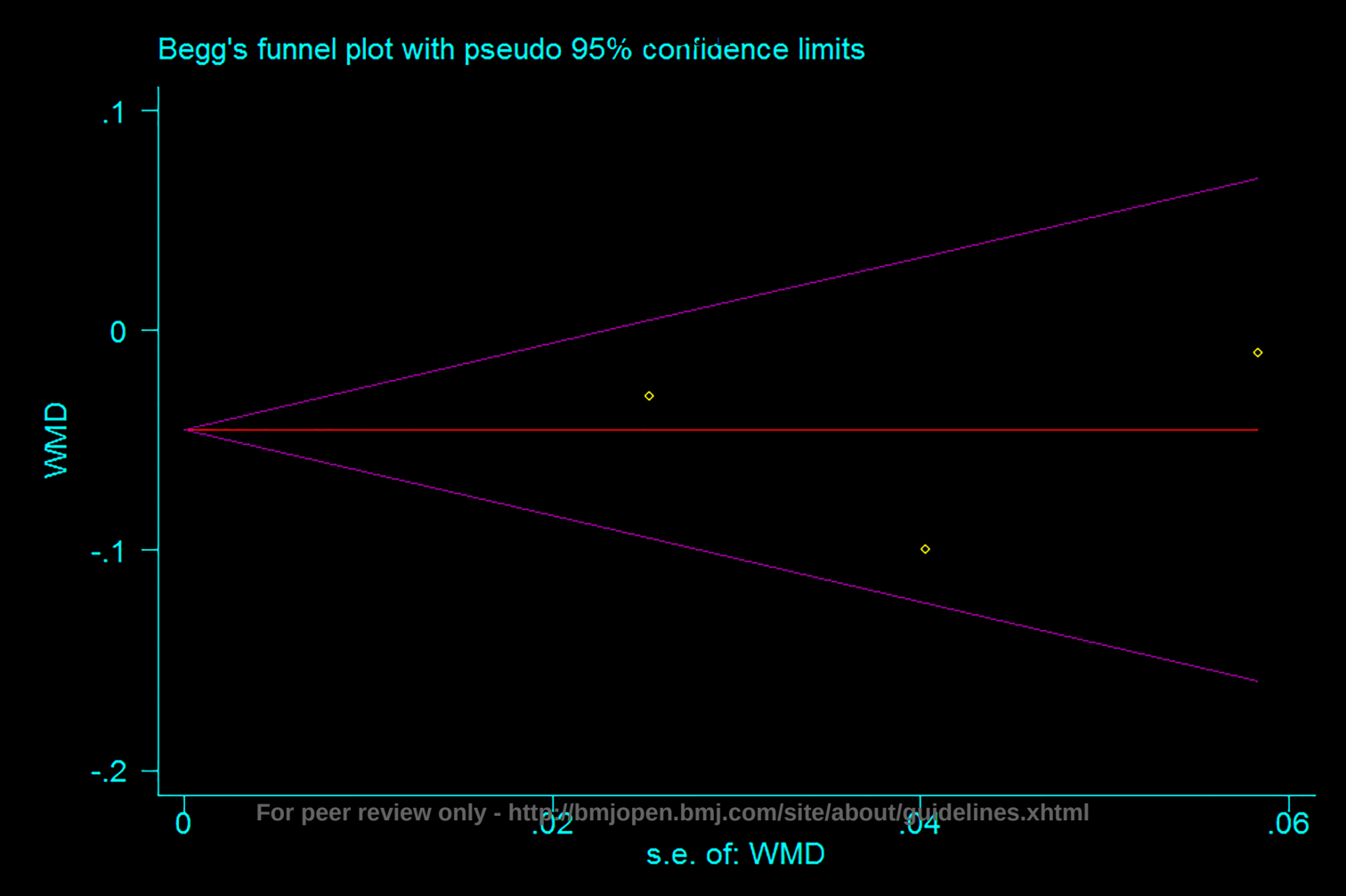

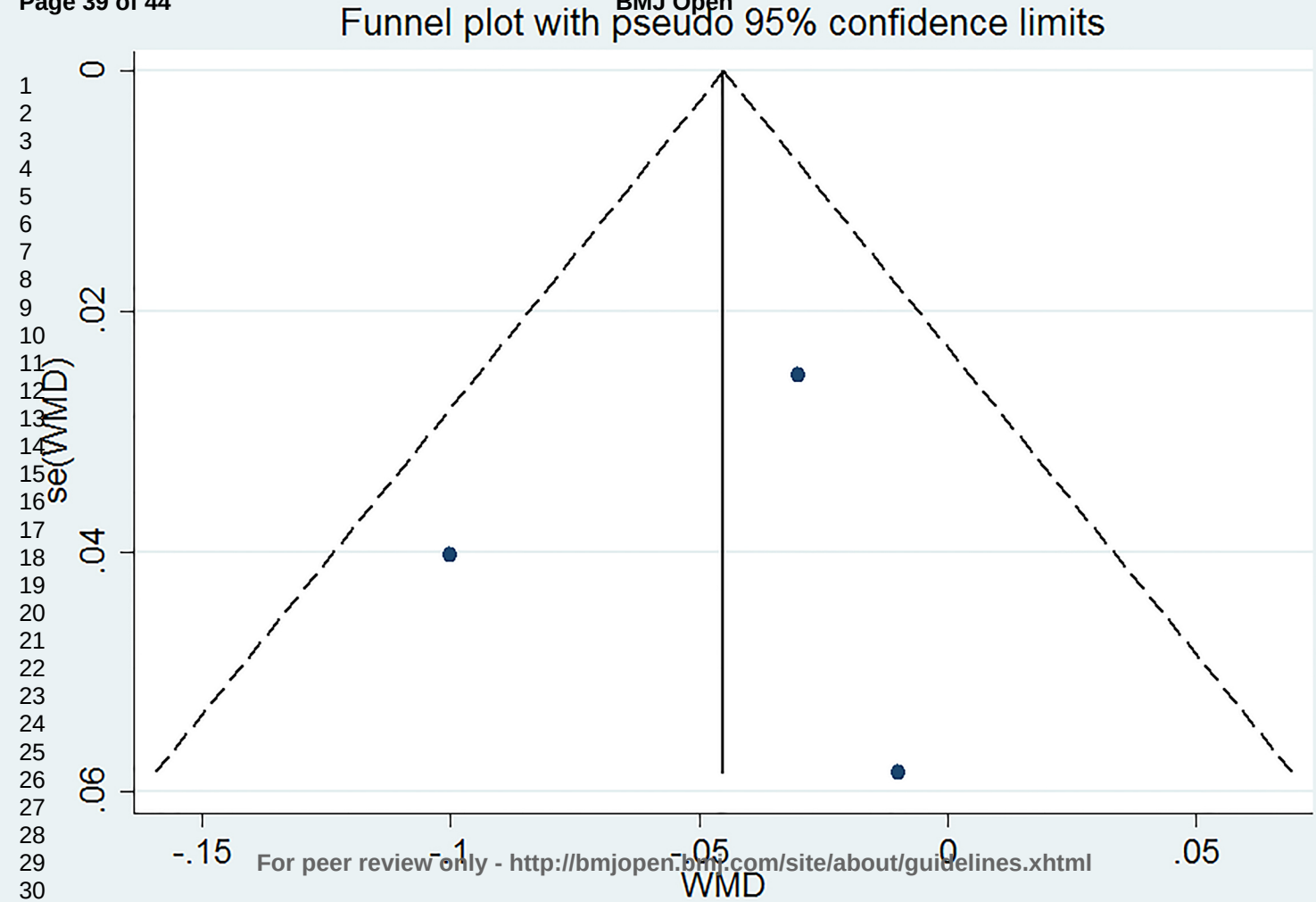

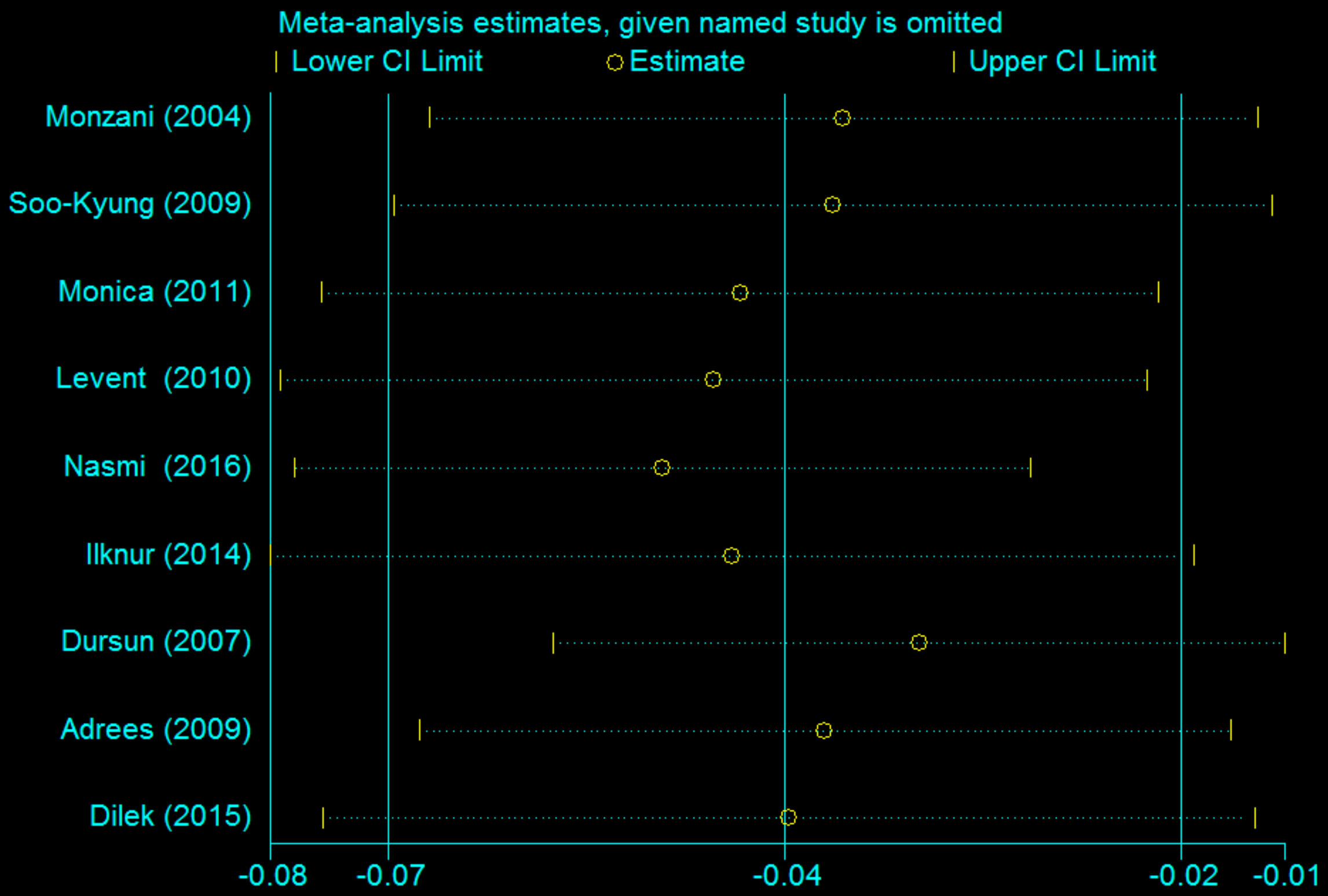

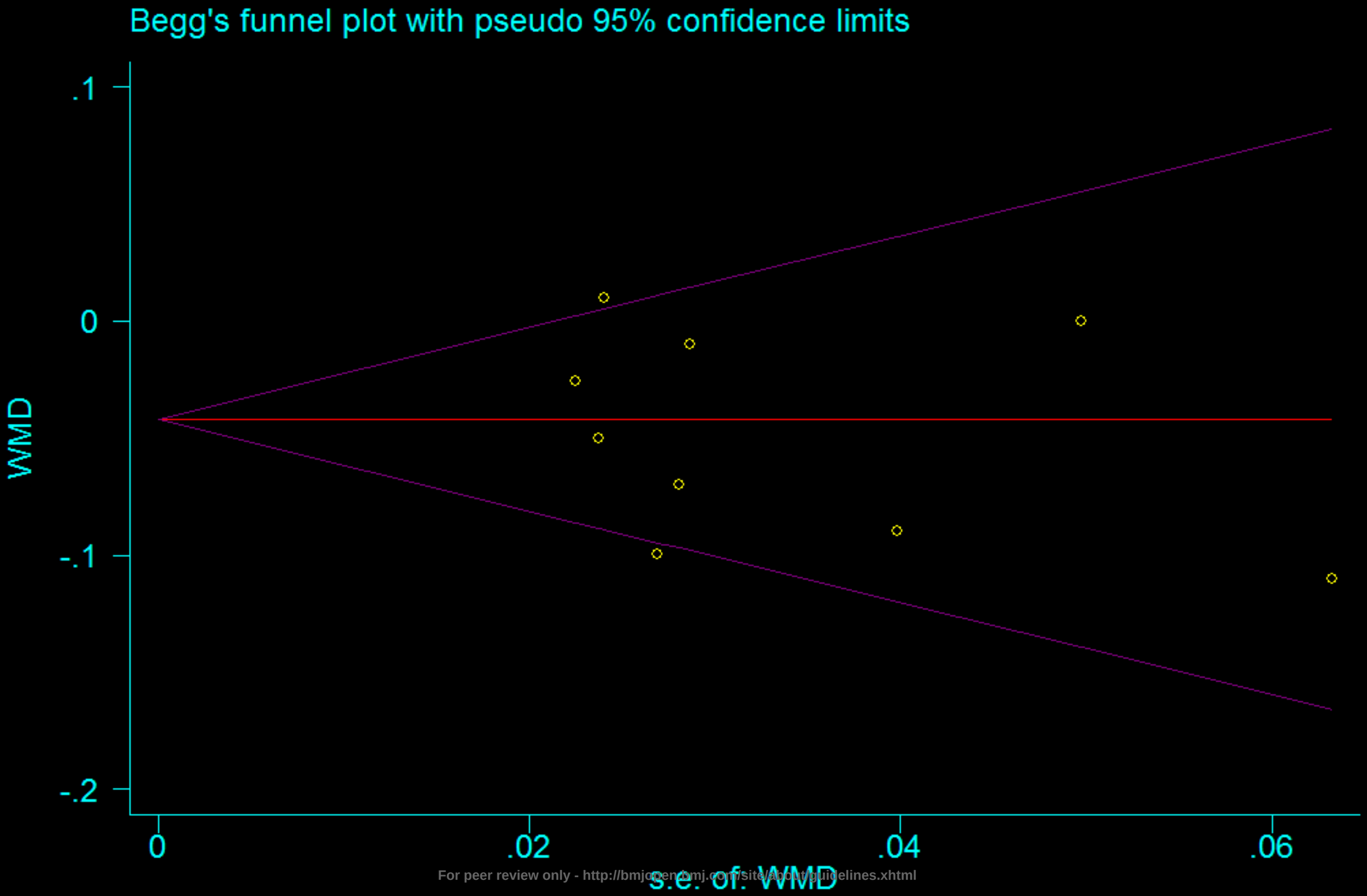

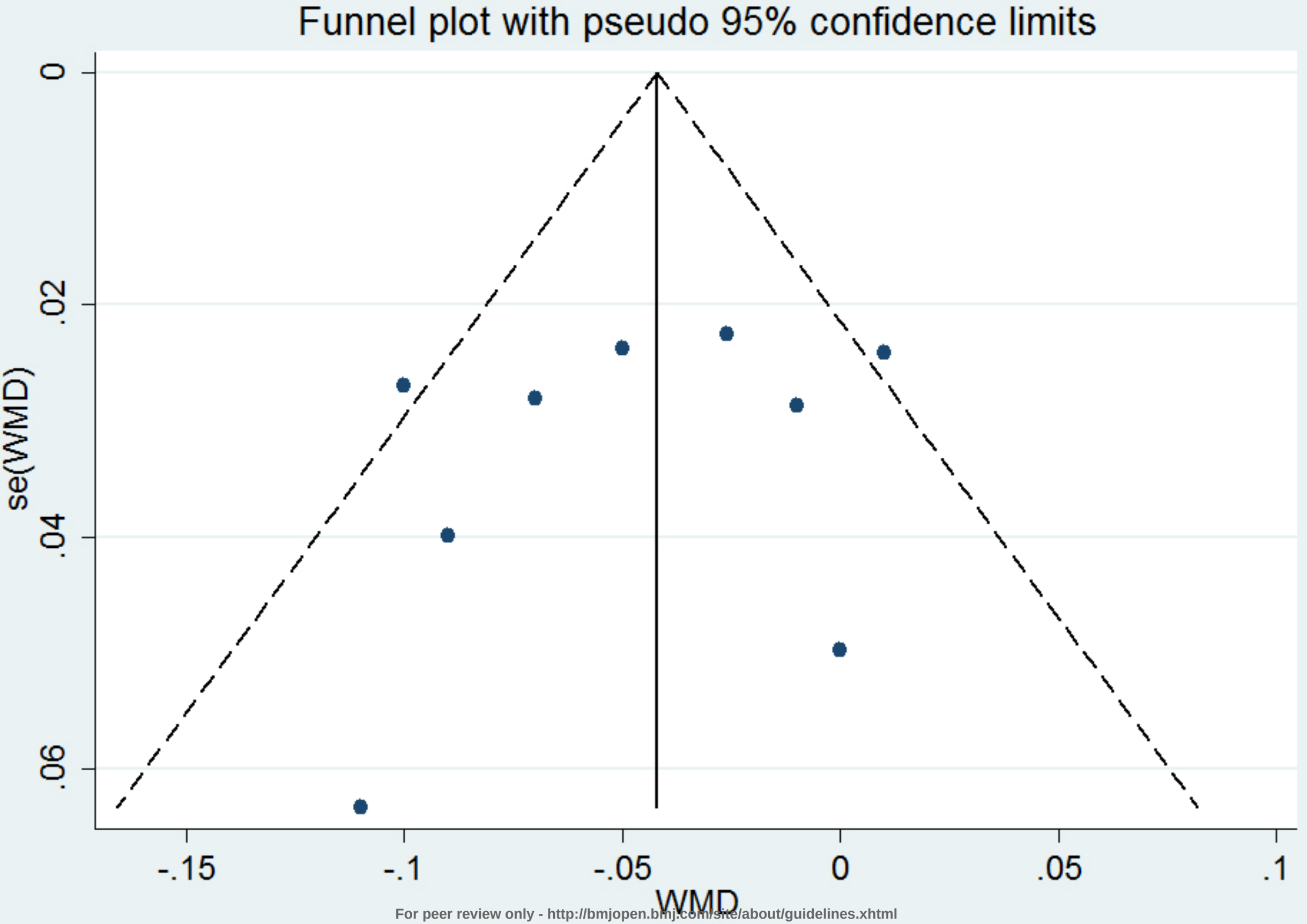

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2-3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4-5
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5-6
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	6
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	7
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	6
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	8
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	8
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	8
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	8-9
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	9
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	9

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	8
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	8-9
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	10
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	11
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	17
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	15
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	18
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	18
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	18
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	19-20
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	3
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	25
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	25

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

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BMJ Open

Effect of levothyroxine on the progression of carotid intima-media thickness in subclinical hypothyroidism patients: A meta-analysis

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Primary Subject Heading:	Diabetes and endocrinology
Secondary Subject Heading:	Diabetes and endocrinology, Nutrition and metabolism, Cardiovascular medicine
Keywords:	subclinical hypothyroidism, carotid intima-media thickness, thyroxin, cardiovascular risk

Effect of levothyroxine on the progression of carotid intima-media thickness in subclinical hypothyroidism patients: A meta-analysis

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Abstract

Background and aims: Subclinical hypothyroidism (SCH) has been associated with increased carotid intima-media thickness (C-IMT) in recent studies, but the effects of levothyroxine (L-T4) on C-IMT in SCH patients are still controversial. The aim of the current study was to evaluate the effect of L-T4 therapy on endothelial function as determined by C-IMT in patients with SCH.

Methods: Prior to July 2016, we searched the PUBMED, EMBASE, COCHRANE LIBRARY and GOOGLE SCHOLAR databases we selected published randomized controlled trials (RCTs) and self-controlled trials.

Results: Three RCTs with 117 patients were considered appropriate for the meta-analysis. The results of the meta-analysis indicated that L-T4 significantly decreased the development of C-IMT [weighted mean difference (WMD), −0.05 mm, 95% CI −0.08, −0.01; p = 0.025]. We also analysed 9 studies (self-controlled trials) with 247 patients and extracted the IMT of SCH patients before and after L-T4 treatment. After L-T4 therapy, the pooled estimate of the WMD of decreased C-IMT was −0.04 mm (95% CI −0.07, −0.02; p = 0.05). Subgroup analysis showed that L-T4 therapy was associated with a decrease in C-IMT among patients of mixed genders (WMD, -0.03 mm, 95% CI -0.06, -0.01; p = 0.145). L-T4 therapy was

associated with a decrease in C-IMT among female patients (WMD, -0.07 mm, 95% CI -0.14, -0.01; p = 0.186). Longer treatment (>6 months) also resulted in a significant decrease in C-IMT (WMD, -0.05 mm, 95% CI -0.08, -0.02; p=0.335).

Conclusion: This meta-analysis indicates that L-T4 treatment of SCH patients can reduce C-IMT, possibly as a result of the reduction of TC, TG, LDL-C, SBP, DBP, LP(a), and FMD. Decreased C-IMT was observed in SCH patients after long-term (>6 months) L-T4 treatment. RCTs with large samples are needed to verify these observations.

Strengths and limitations of this study:

Strengths

1.A strong quality evidence on the effect of L-T4 therapy in SCH patients is preformed. Methodology, according to the different types of original literature, we used different method of statistical analysis and study quality evaluation. According to different heterogeneity，we adopt different effect model. This embodies the rigor of methodology

2.We analysis other metabolic parameters to better explain to effect of L-T4 therapy in SCH.

Limitations

1.The number of including studies was small, and the overall population of these trials was not large. Meta analysis has moderate heterogeneity. Difficult assessment of the potential publication bias mainly due to the previous two factors.

2.We did not perform subgroup analysis according to the level of TSH (the cutoff value is 10 mU/ml to distinguish mild SCH and severe SCH) because the original studies did not perform analyses according to the level of TSH.

3.We did not perform subgroup analysis by mean age (>65 or <65 years) because all participants' mean age was below 65 years.

Keywords: subclinical hypothyroidism; carotid intima-media thickness; thyroxin; cardiovascular risk

Introduction

Subclinical hypothyroidism (SCH) is characterized by a normal range of free thyroxin concentrations together with increased serum thyroxin (TSH) levels. Recently, SCH was found in 5% to 10% of the general population and 6% to 10% of women (approximately 15% was found in women more than 60 years old), while the incidence in males is 2.4 to 3% [1].

Although SCH is frequently asymptomatic, approximately 30% of patients have symptoms indicative of thyroid hormone deficiency [2, 3]. However, the clinical significance and therapeutic strategies of SCH are controversial .

C-IMT is a generally acknowledged measure of subclinical atherosclerotic alterations. It is increasingly used to evaluate vascular function in clinical analyses to assess the efficacy of interventions that reduce atherosclerosis and related diseases [4]. This parameter is listed in the European guidelines for prophylaxis of cardiovascular disease, and 0.9 mm is the threshold value for C-IMT. Progression of atherosclerosis is indicated when the value of C-IMT is over the threshold.

A meta-analysis involving 8 studies with 3602 participants found a relationship between SCH and C-IMT, especially when TSH > 10.0 mIU/l [5].L-T4 replacement should be used to treat patients with clinical hypothyroidism; however, the treatment of SCH is complex. Several placebo-controlled studies have shown the beneficial effects of L-T4 therapy on early atherosclerotic alterations and cardiovascular hazard in patients with SCH [6, 7]. Recently, in a large-scale retrospective cohort study examining the efficacy of L-T4 replacement on cardiovascular mortality, myocardial infarction and all-cause death, no beneficial effects in

patients with SCH were found, except for patients under 65 years old [8]. The purpose of the current study was to evaluate the effect of L-T4 therapy on endothelial function as evaluated by C-IMT in patients with SCH.

Methods

Search strategy

Studies published in English were found in the EMBASE, PUBMED, GOOGLE SCHOLAR and COCHRANE LIBRARY electronic databanks prior to June 2016. We identified studies that measured IMT in patients with SCH before and after treatment with L-T4. Studies were identified and evaluated by two authors (Zhao T, Chen BM) using following words (the following is an example for Pubmed): ((("hypothyroidism"[MeSH Terms] OR "hypothyroidism"[All Fields]) OR (subclinical[All Fields] AND ("hypothyroidism"[MeSH Terms] OR "hypothyroidism"[All Fields]))) AND ("thyroxine"[MeSH Terms] OR "thyroxine"[All Fields])) AND ("carotid intima-media thickness"[MeSH Terms] OR ("carotid"[All Fields] AND "intima-media"[All Fields] AND "thickness"[All Fields]) OR "carotid intima-media thickness"[All Fields] OR ("carotid"[All Fields] AND "intima"[All Fields] AND "media"[All Fields] AND "thickness"[All Fields]) OR "carotid intima media

thickness"[All Fields]). For a thorough review of the literature, we searched more studies by browsing the references of the identified reports and review articles.

Criteria for study selection

Subjects with SCH have standard free thyroxin levels and increased TSH levels. There is still controversial about the TSH reference interval; several reviews have proposed a TSH upper cut off between 4.5 and 5.0 mIU/l [9, 10], but some experts suggest that the peak TSH should be decreased to 2.5-3.0 mIU/l. Because there are no consistent conclusions, no specific TSH cut off was used to define SCH, but all articles had a cut off point from 3.6 to 5.5 mIU/l. To be included, a study had to satisfy the following conditions: 1) reported SCH defined based on a thyroid function test (normal free T4 and increased TSH); 2) defined as a RCT, which compared C-IMT in SCH patients in a L-T4 treatment and control group, or a self-controlled trial, which compared C-IMT value of a patient with SCH before and after L-T4 replacement.

Study selection

Two investigators independently screened both abstracts and titles of the studies, and the studies were excluded when they did not conform to the inclusion criteria or they accorded with the exclusion criteria. Two investigators independently extracted data from the papers. If

there was disagreement, a third reviewer was asked for advice. Discrepancies were solved by unanimous agreement.

Data extraction

The following information is contained in the data extraction table: 1) author 2) year of publication 3) region 4) sample size 5) sample gender 6) mean age of the cohort 7) definition of SCH 8) duration of SCH 9) SCH detection method 10) mean L-T4 dosage 11) duration of treatment 12) thyroid function after treatment 13) IMT assessment 14) IMT values (mean with SD) before and after treatment with the corresponding treatment method.

Assessment of study quality

The quality of non-randomized studies was evaluated using the MINORS method [11], which is based on the following items: a stated aim of the study, inclusion of consecutive patients, prospective collection of data, endpoint appropriate to the study aim, unbiased evaluation of endpoints, follow-up period appropriate to the major endpoint, and loss to follow-up not exceeding 5%.

Methodological quality of the included RCTs was assessed using the Cochrane Handbook for Systematic Review of Interventions by several domains: generation of random sequence; allocation concealment; blinding; incomplete outcome date addressed; free of selective reporting; free of other bias.

Statistical analysis

We represented the results as weighted mean differences (WMDs) with 95% confidence intervals (CIs) for continuous variables. For continuous outcomes, if all reports had uniform units of measurement (expressed as mm), we showed the WMD and 95% CIs as the results. We evaluated statistical heterogeneity across the studies by Cochrane's Q test ($p < 0.1$ was statistically significant) and the I^2 test ($I^2 > 50\%$: high heterogeneity; $I^2 = 25\%$-50%: moderate heterogeneity; $I^2 < 25\%$: low heterogeneity) [12]. In 2011, Kulinskaya et al suggested a method based on fractional degrees of freedom (df) which substantially improve the original chi-square distribution with s-1 df, where is the number of studies included in the meta analysis [13]. The pooled effect size was assessed with a random-effect model when significant statistically heterogeneity was present; in contrast, we also selected a fixed-effect model. $p < 0.05$ was considered significant. The weighting of WMD in a fixed-effect model

is inverse variance. However, the weighting of WMD in a random-effect model is D-L method (DerSimonian and Laired method) [14] .We assessed publication bias with Begg's correlation test and Egger's regression method [15]. Sensitivity analysis was carried out for all papers in addition to the studies with boundary qualification. Pre-specified subgroup meta-analyses were performed to assess the between-study heterogeneity on the basis of the gender of participants (female or genders) and duration of treatment (>6 months or ≤6 months). Among 9 including studies, 3 out of them are RCTs and the other of them are self-controlled trials. We performed Statistical analysis of three RCTs. SCH treatment group of three RCTs also have the information on C-IMT values between pre-treatment and after-treatment in SCH patients. We combined the SCH treatment group of RCTs with the other self-controlled trials to performed Statistical analysis for fully revealing the effect of L-T4 treatment in SCH patients. Statistical analyses were performed with STATA 11 for Windows.

Results

Study selection

Three RCTs [7, 16, 17] (57 patients treated with L-T4 and 60 included in the control group) were selected for the meta-analysis, with a total of 117 patients. A total of 9 publications [7, 16-23] with 247 SCH patients measured the C-IMT before and after L-T4. We selected these studies among 107 potentially related articles (Fig. 1).

Baseline characteristics and study quality

The baseline characteristics of the included trials are shown in Table 1(A)(B). The duration of therapy with L–T4 ranged from 2 to 18 months, the patient count varied from 14 to 56 subjects, and the year of the publication was from 2004 to 2016. Six trials included female and male patients, and the other three only examined female patients. Based on the type of original research, the methodological evaluation of study quality was different. We used MINORS to assess the quality of the self-controlled trials. In general, the included self-controlled trials were of moderate quality (Table1) .The results of RCT quality are shown in Fig. 2.

Progression of C-IMT in the follow-up and subgroup analysis

A total of 3 RCTs involving 117 patients were analysed. L-T4 treatment significantly decreased C-IMT progression in SCH patients. (WMD, −0.05 mm, 95% CI −0.08, −0.01; p = 0.025). The heterogeneity was very low (I^2=22.2%) (Fig. 3)

The nine self-controlled experiments compared the value of C-IMT before replacement and after treatment. After L-T4 therapy, the thyroid function of all patients was normalized from SCH, and then, patient IMT was measured again. Because clinical heterogeneity was relatively high. We selected random-effect model. L-T4 therapy was associated with a mild but significant decrease in the development of C-IMT (WMD, −0.04 mm, 95% CI −0.07, −0.02; p = 0.050). The heterogeneity was moderate. (I2=48.3%) (Fig. 4)

Subgroup analyses stratified by gender revealed that L–T4 therapy caused a significant decrease in C-IMT among patients of mixed genders (WMD, -0.03 mm, 95% CI -0.06, -0.01; p = 0.145). The outcomes showed moderate heterogeneity (I2= 39.2%). After L-T4 treatment,

TABLE 1(A). Summary of the Characteristics of the Included Studies

Author/ Year	Sample Size	Region	Gender	Mean Age (year)	Definition of SCH	SCH Duration (month)	L-T4 Dosage (initial value)	Treatment Duration (month)	Thyroid function after treatment	CIMT(mm) (Before/After)	Quality Score
Monzani 2004	23	Italy	Mixed genders	37±11	TSH >3.6 mIU/L	at least 6 months	25 -75 ug/d	10.5[a]	normal	0.76±0.14 0.67±0.13	12
Soo-Kyung 2009	28	Korea	Mixed genders	36.0±6.2	TSH >5.5 mIU/L	newly diagnosed	67μg/d[b]	18[b]	normal	0.67±0.11 0.60±0.10	9
Monica 2011	14	Brazil	female	43.4±9.8	TSH >4.0 mIU/L	_	44.23±18.13ug/d[d]	12	normal	0.66±0.11 0.66±0.15	10
Levent 2010	38	Turkey	Mixed genders	49.8±10.0	TSH >5.0 mIU/L	at least 2 months	101±27.46μg/d[c]	6[b]	normal	0.64±0.13 0.63±0.12	11
Nasmi 2016	25	Iran	Mixed genders	35.9±7.6	TSH >4.0 mIU/L	_	50ug/d	2	normal	0.56±0.09 0.57±0.08	11
Ilknur 2014	56	Turkey	Mixed genders	41.3±14.5	TSH>4.2 mIU/L	_	25-50 u/d	_	normal	0.533±0.112 0.507±0.126	12
Dursun 2007	20	Turkey	female	36±11	TSH>4.2 mIU/L	newly diagnosed	25-100ug/d	5[b]	normal	0.65±0.09 0.55±0.08	12
Adrees 2009	20	Ireland	female	50±9	_	at least 6 months	100 ± 30 ug/d[c]	18	normal	0.82±0.2 0.71±0.2	10
Dilek 2015	23	Turkey	Mixed genders	35.2±10.7	TSH >4.0 mIU/L	at least 6 months	_	6	normal	0.51±0.09 0.46±0.07	11

Abbreviations: TSH, thyroid-stimulating hormone; CIMT, Carotid intima-media thickness; L-T4, levothyroxine.

Mixed genders=including male and female. Age and IMT are expressed as mean± s.d.

[a] median ； [b] mean； [c] mean± s.d； [d] median± interquartile range

TABLE 1(B). Summary of the Characteristics of the Included Studies

Author	TSH assay	Carotid IMT assessment
Monzani	ultrasensitive immunoradiometric assay	both the near and far wall of the right and left common carotid artery and carotid bifurcation(high-resolution ultrasonography)
Soo-Kyung	chemiluminescent immunometric assay	20 mm proximal to the origin of the carotid bulb (high-resolution ultrasonographic system)
Monica	immunochemiluminescence	both the near and far wall of the right and left common artery and the carotid bifurcation.(high-resolution ultrasound imaging)
Levent	Chemiluminescence immunometric assay	approximately 1-cm segment from both the left and right CCA proximal and distal portions (Ultrasonographic images)
Nasmi	_	sonogram B-mode images
Ilknur	electrochemiluminescence immunoassays	Three arterial wall segments of the common carotid artery were measured bilaterally after imaging from a fixed lateral transducer angle and designated（high-resolution B-mode ultrasonography）
Dursun	immunoassay	right and left extracranial carotid arteries: the CCA (1 cm proximal to the dilation of the carotid bulb), the bifurcation (the 1-cm segment proximal to the flow divider), and the internal carotid artery (the 1-cm segment in the internal branch distal to the flow divider).(high-resolution ultrasonography)
Adrees	chemiluminescent immunometric assay	1 cm proximal to the carotid bulb when a satisfactory position was found.(calibrated ultrasound machine)
Dilek	_	ultrasound system

Abbreviations: CCA, common carotid artery.

female participants with SCH displayed a significant reduction in C-IMT (WMD, -0.07 mm, 95% CI -0.14, -0.01; p = 0.186). The heterogeneity was moderate (I2= 40.5%) (Fig.5).

Subgroup analyses were performed using the duration of L-T4 replacement. Except for one reports, all trials had information on the length of the therapy. There was a significant decrease in C-IMT in the patients with long-term (>6 months) treatment (WMD, -0.05 mm, 95% CI -0.08, -0.02; p=0.335). The outcome showed low heterogeneity (I^2= 12.3%). A non-significant reduction was found in C-IMT in the participants with short-term (≤6 months) replacement therapy (WMD, -0.04 mm, 95% CI -0.08, 0.01; p=0.015). The heterogeneity was high (I^2= 71.3%). (Fig. 6).

Changes in metabolic parameters

Table 2 indicates the changes in metabolic parameters from pre-replacement to post-treatment. L-T4 therapy was linked to significant reductions in TC, TG, LDL, SBP, DBP, and LP(a) and decreased FMD. There were no significant changes in BMI, waist circumference, HDL, ApoA, ApoB and glucose between the pre-treatment and post-therapy conditions.

Sensitivity and publication bias evaluation

We carried out sensitivity analyses by sequentially eliminating one study to probe the change in the total WMD and 95% CI of C-IMT. The results of three RCT indicated no variation

Table 2

The difference of the changes in metabolic parameters between pre-treatment and post-treatment.

Parameters	No. of patients	WMD (95% CI)	P-value
Waist circumference (cm)	66	-0.544(-4.041,2.954)	0.761
BMI (kg/m2)	152	-0.231(-1.044,0.582)	0.577
Glucose (mmol/L)	48	0.090(-0.109,0.289)	0.375
SBP (mmHg)	109	-9.391(-14.405,-4.377)	<0.001
DBP (mmHg)	109	-5.693(-8.471,-2.916)	<0.001
TC (mg/dl)	166	-9.037(-16.635,-1.439)	0.02
TG (mg/dl)	166	-9.842(-18.763,-0.921)	0.031
HDL (mg/dl)	166	-1.032(-4.059,1.995)	0.504
LDL (mg/dl)	166	-14.303(-19.133,-9.472)	<0.001
FMD (%)	39	3.962(2.636,5.289)	<0.001
ApoA (mg/dl)	37	-10.092(-23.173,2.989)	0.131
ApoB (mg/dl)	37	-7.718(-21.723,6.287)	0.28
LP(a) (mg/l)	34	-9.974(-12.844,-7.103)	<0.001

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; TC, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein; ApoA, Apolipoprotein A; ApoB, Apolipoprotein B; Lp(a), lipoprotein(a); WMD, weighted mean difference; CI: confidence interval;FMD, flow-mediated dilatation.

in the direction of WMD when each publication was removed. (Fig.s1) . Neither Egger's test (p=0.881) nor Begg's test (p=0.602) of C-IMT in SCH manifested publication biases. (Fig.s2) (Fig.s3). We also evaluate the sensitivity and publication bias of the nine self-controlled trial. The results showed that there was only a slight change in the WMD or 95% CI, and no change in the direction of WMD when any one study was omitted. (Fig.s4) Egger's test

(p=0.458) and Begg's test (p=0.602) of C-IMT in SCH manifested publication biases. (Fig.s5) (Fig.s6) .

Discussion

This meta-analysis indicated that L-T4 has beneficial effects on the development of C-IMT. Decreased C-IMT was also observed with longer treatment (>6 months). L–T4 therapy of SCH participants also resulted in a significant reduction in TC, TG, LDL, SBP, DBP, LP(a), and FMD.

The American Heart Association/American College of Cardiology guidelines designated C-IMT along with coronary artery calcium (CAC) score as a class IIa recommendation for cardiovascular risk assessment in asymptomatic adults at intermediate risk of cardiovascular disease. For a 0.1 mm difference in C-IMT, the prospective risk of myocardial infarction increased from 10% to 15%, and the stroke risk was increased from 13% to 18% [5]. The result showed that C-IMT in SCH patients can be reversed by L-T4 replacement. The mechanism underlying the L-T4-mediated decrease in C-IMT has not been fully elucidated. TSHR, hyperlipidaemia and blood pressure may contribute to this result.

In SCH, the increased TSH is the only prominent change, and increased TSH can bind with the TSH receptor (TSHR) to perform its function. Balzan et al. [24] revealed that micro-vascular endothelial cells produce TSHR, which may help to elucidate this relationship. Recently, Tian et al. [25] published a study to identify the potential function of the TSHR and showed that increased TSH aggravated endothelial dysfunction. However, L-T4 can control the elevated TSH by reversing above-mentioned changes to reduce the mean C-IMT.

Six out of 13 papers in a systemic review reported that L-T4 treatment improved TC and LDL [26]. A RCT trial with the same conclusion further supported this hypothesis [8]. Among four markers of hyperlipidaemia (TC, TG, LDL, HDL), except for HDL we observed non-significant differences between pre-treatment and post-replacement conditions, which is supported by previous study with similar findings [27]. Reports by Kannel et al. [28] and Lewington et al. [29] showed that the TC/HDL ratio is more important in the progress of coronary heart disease than either TC or HDL alone. Although HDL concentration was not changed after restoration of euthyroidism by L-T4 replacement in SCH patients in our study, L-T4 treatment altered the TC/HDL ratio. . Hyperlipidaemia promotes endothelial dysfunction because it can inhibit the NO synthesis pathway in endothelial cells [30].

Meanwhile, a recent paper found that SCH can cause endothelial dysfunction because of a decrease in NO availability, which is partly independent of dyslipidaemia, and L-T4 supplementation can reverse endothelial dysfunction [31]. Thus, we found that SCH not only results in endothelial dysfunction but is also an independent risk factor of dyslipidaemia; then, dyslipidaemia further amplifies endothelial dysfunction. L-T4 treatment of SCH patients can reduce these effects.

A meta-analysis showed that blood pressure was an independent risk factor for C-IMT [32]. Recently, an another meta-analysis demonstrated that SCH is linked to higher SBP and DBP levels [33].We know little about the mechanism of SCH-associated hypertension; nevertheless, hypothyroidism increases vascular resistance, enhances blood pressure, salt sensitivity and abnormal sodium metabolism, which may promote the development of hypertension.

For the subgroup of short duration (≤6 months), an insignificant difference of C-IMT was observed after treatment. Meanwhile, mean C-IMT in SCH subjects undergoing long-term treatment significantly increased compared to that in the pre-treatment condition. This phenomenon was confirmed in our clinical observation. The major reason is that the duration

of treatment is too short to improve C-IMT. The rate of progression of IMT in the common carotid artery was approximately 0.01 mm/year.

For stratification by gender, we find a significant difference in female patients between pre-replacement and post-replacement. Meanwhile, for the mixed genders subgroup, mean C-IMT was significantly reduced after treatment. Although the prevalence of SCH is a little difference between women and general population, the effects of levothyroxine (L-T4) on C-IMT are similar. This suggests that the changes of C-IMT are similar when we control thyroid function in the normal range regardless of gender.

Limitations

The number of including studies was small, and the overall population of these trials was not large. Meta analysis has moderate heterogeneity. Difficult assessment of the potential publication bias mainly due to the previous two factors. Additionally,we did not perform subgroup analysis according to the level of TSH (the cutoff value is 10 mU/ml to distinguish mild SCH and severe SCH) because the original studies did not perform analyses according to the level of TSH. Finally, we did not perform subgroup analysis by mean age (>65 or <65 years) because all participants' mean age was below 65 years.

Conclusion

Our study suggests that C-IMT in SCH patients can be reversed by L-T4 replacement. Hyperlipidaemia and hypertension may improve after L-T4 therapy. Long L-T4 replacement duration (>6 months) is more useful compared to short-term duration for improving C-IMT in SCH patients. Therefore, L-T4 supplementation can prevent or inhibit the progression of atherosclerosis, at least in SCH patients. RCTs with more sample size are needed to confirm these findings.

contributorship statement

Zhao Tong and Chen Baomin made search strategies, searched papers from databases, assessed of study quality ,independently extracted data from the papers and wrote the paper Wang Haoyu as a third reviewer was asked for advice,If there was disagreement.Zhou Yingying, Wang Xinyi and Zhang Yuanyuan Participated in the revision of the paper. Shan Zhongyan provided some advices on the discussion section of this article.

competing interests statement

The author denies that he has any intention to obtain any financial interests.

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Fig.1. Process of study selection.

Abbreviations: IMT: intima-media thickness; SCH: subclinical hypothyroidism

Fig.2. Quality assessment of the included studies.

Fig.3. Forest plots showing WMD (weighted mean differences) with 95% confidence intervals for improvement in carotid intima–media thickness comparing L-T4 treatment to the control in a fixed effects model.

Legend for x-axis: Negative values equals improvement in IMT.

Fig.4. Forest plots showing WMD (weighted mean differences) with 95% confidence intervals compared the value of C-IMT before replacement and after treatment in a random-effect model.

Legend for x-axis: Negative values equals improvement in IMT.

Fig.5.Subgroup analyses of carotid intima-media thickness changes based on gender in a random effects model.

Legend for x-axis: Negative values equals improvement in IMT.

Fig.6.Subgroup analyses of carotid intima-media thickness changes based on duration of L-T4 replacement in a random effects model.

Legend for x-axis: Negative values equals improvement in IMT.

Long study duration= duration of L-T4 replacement>6 months

Short study duration= duration of L-T4 replacement≤6 months

Fig.S1 Begg's funnel plot with pseudo 95% confidence limits of publication bias test for L-T4 on C-IMT in RCT studies.

Fig.S2 Begg's funnel plot with pseudo 95% confidence limits of publication bias test for L-T4 on C-IMT in self-controlled studies.

Fig.S3 Funnel plot with pseudo 95% confidence limits of publication bias test for L-T4 on C-IMT in RCT studies .

Fig.S4 Funnel plot with pseudo 95% confidence limits of publication bias test for L-T4 on C-IMT in self-controlled studies.

Fig.S5 Sensitivity analysis of the RCT studies included in the meta-analysis. The figure shows the WMD obtained by combined analysis of the remaining studies after the successive exclusion of each study individually. The excluded study is listed on the left, and the corresponding horizontal lines indicate the WMD and CI

obtained by re-calculation after its exclusion. The CI for the overall meta-analysis of the studies is indicated by two vertical lines.

Fig.S6 Sensitivity analysis of the self-controlled studies included in the meta-analysis. The figure shows the WMD obtained by combined analysis of the remaining studies after the successive exclusion of each study individually. The excluded study is listed on the left, and the corresponding horizontal lines indicate the WMD and CI obtained by re-calculation after its exclusion. The CI for the overall meta-analysis of the studies is indicated by two vertical lines.

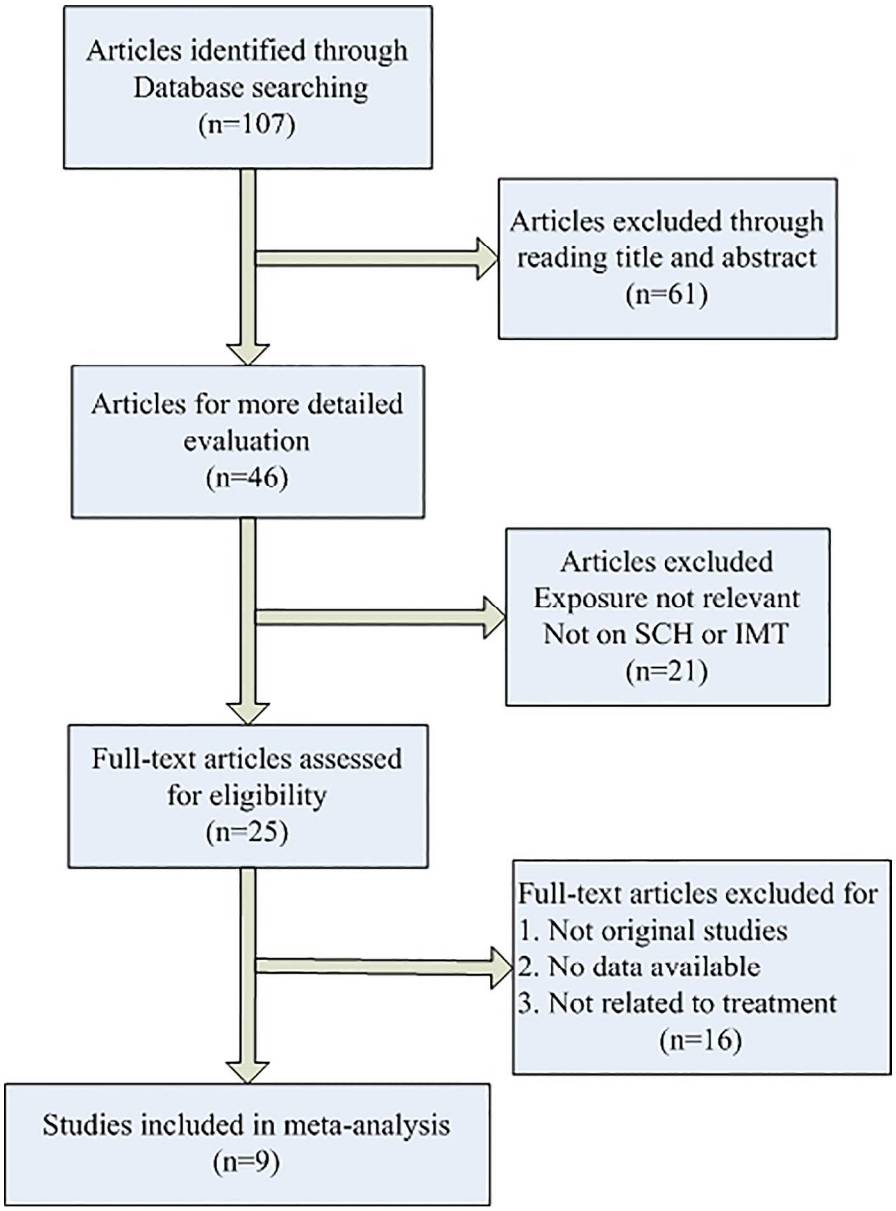

Fig.1. Process of study selection. ‼ ┼ Abbreviations: IMT: intima-media thickness; SCH: subclinical hypothyroidism.

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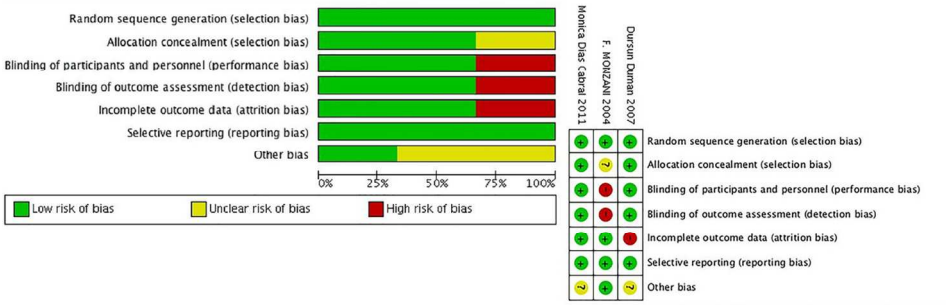

Fig.2. Quality assessment of the included studies.

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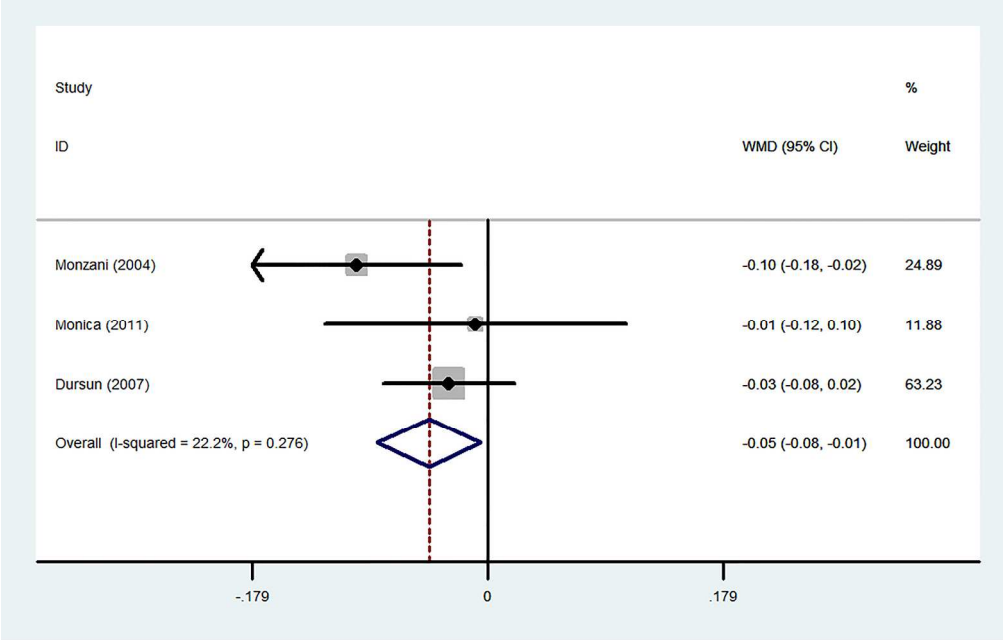

Fig.3. Forest plots showing WMD (weighted mean differences) with 95% confidence intervals for improvement in carotid intima–media thickness comparing L-T4 treatment to the control in a fixed effects model.
Legend for x-axis: Negative values equals improvement in IMT.

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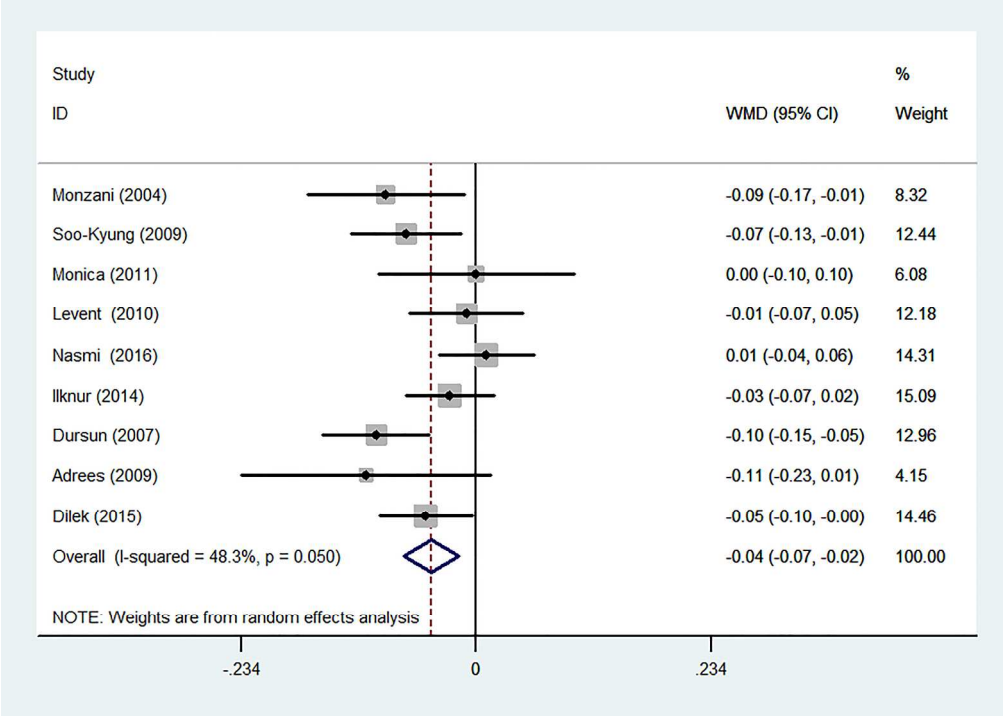

Fig.4. Forest plots showing WMD (weighted mean differences) with 95% confidence intervals compared the value of C-IMT before replacement and after treatment in a random-effect model.
Legend for x-axis: Negative values equals improvement in IMT.

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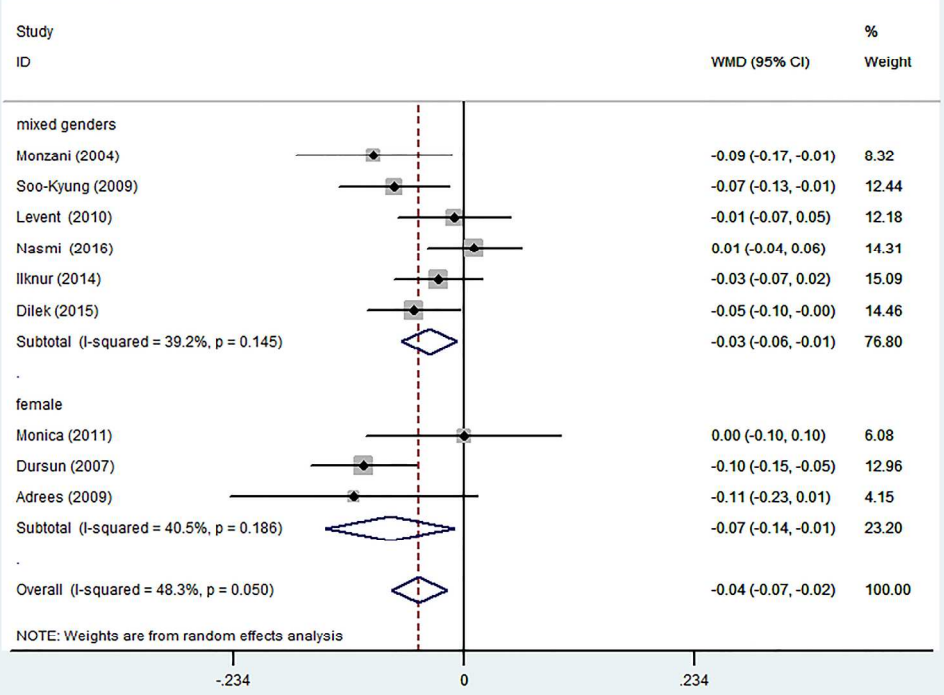


Fig.5.Subgroup analyses of carotid intima-media thickness changes based on gender in a random effects model.
Legend for x-axis: Negative values equals improvement in IMT.

138x106mm (600 x 600 DPI)

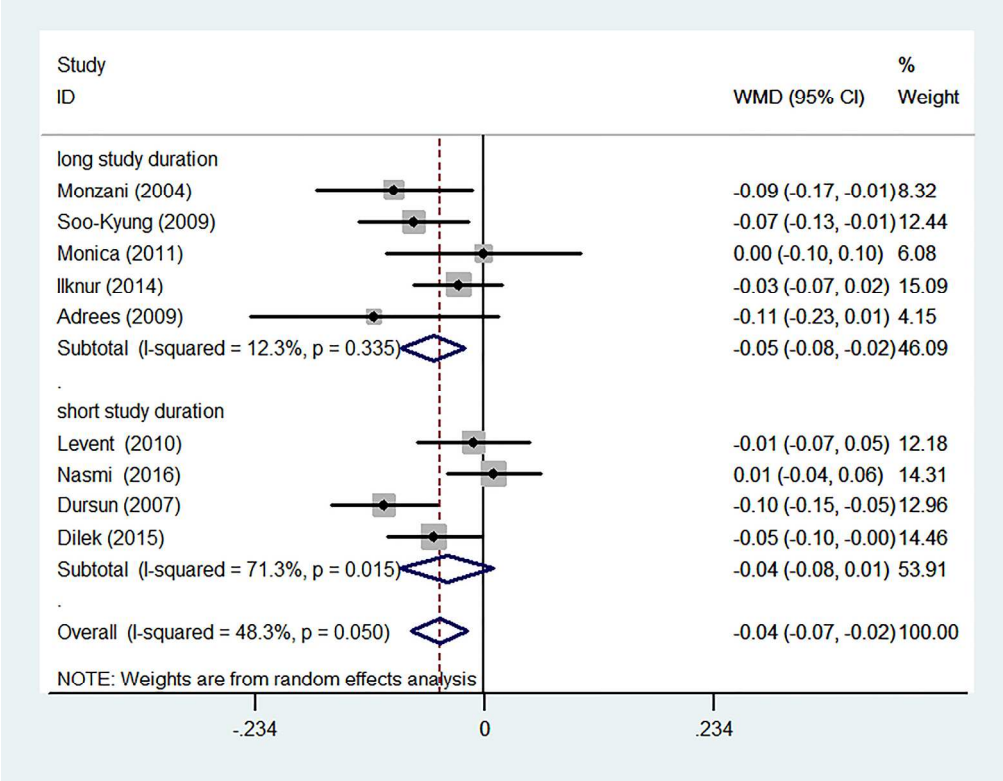
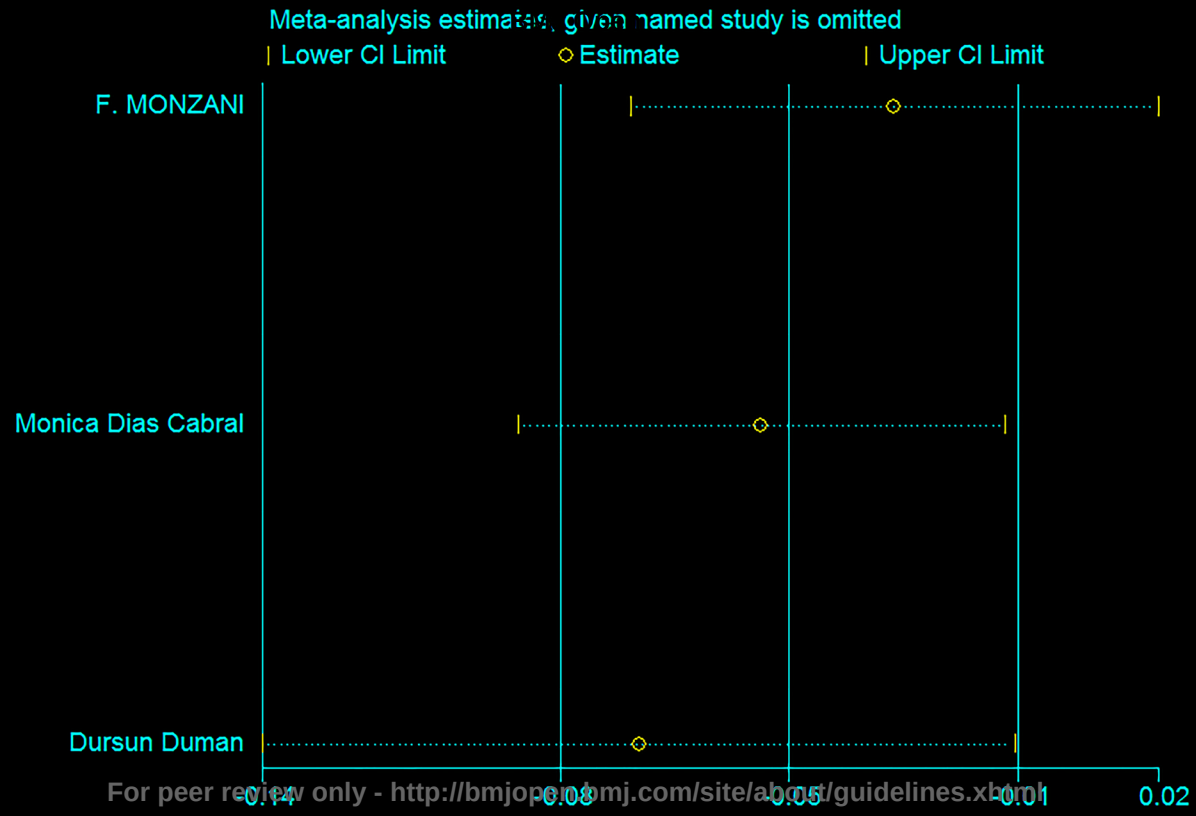
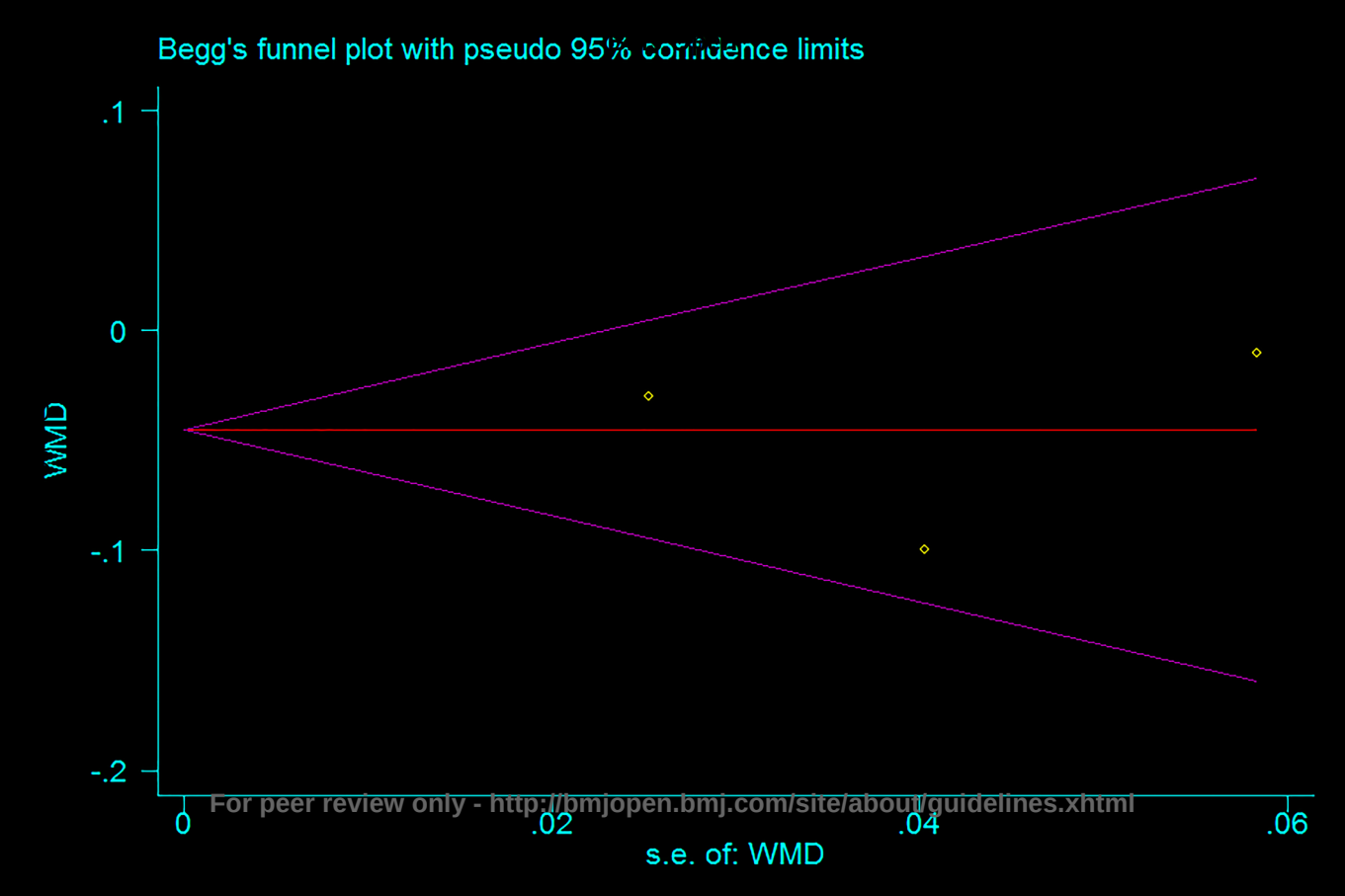
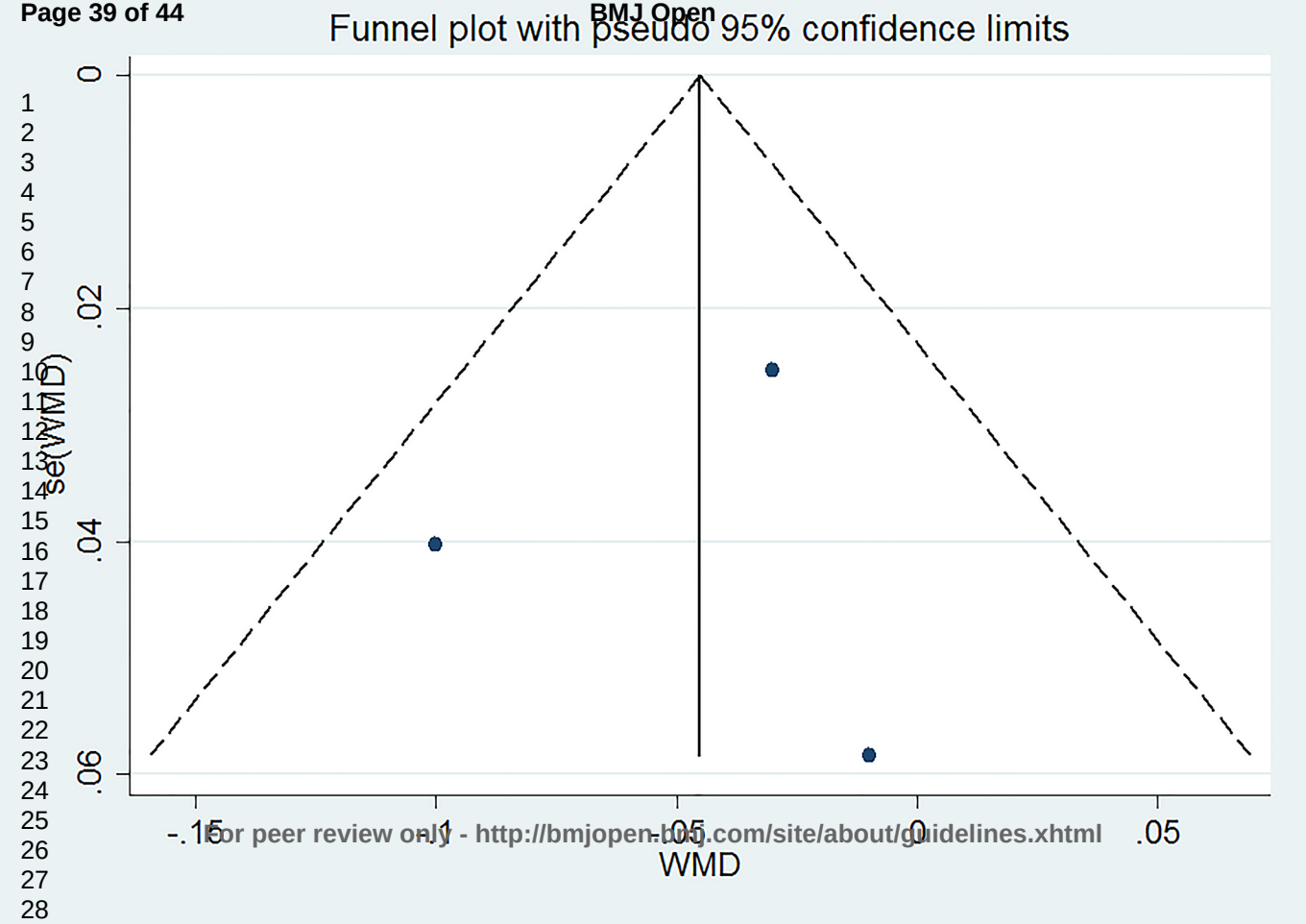


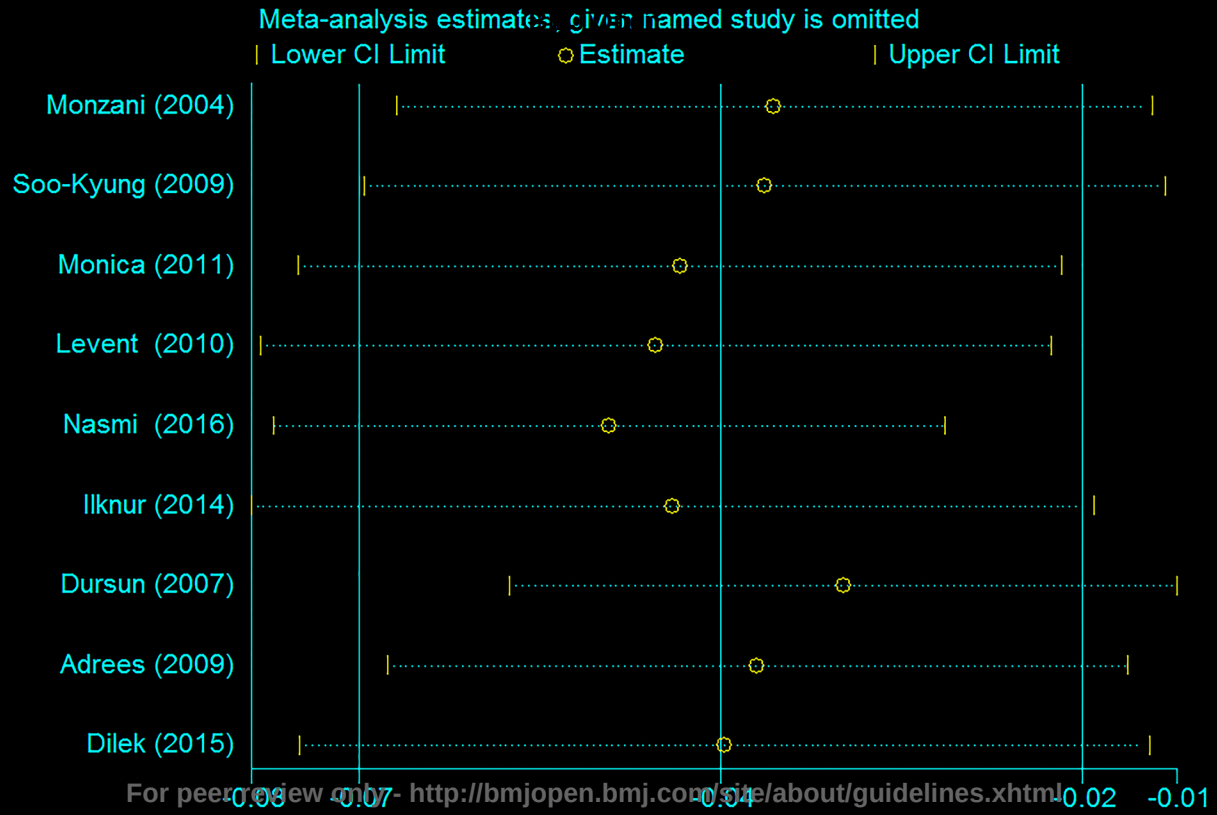
Fig.6.Subgroup analyses of carotid intima-media thickness changes based on duration of L-T4 replacement in a random effects model.
Legend for x-axis: Negative values equals improvement in IMT.
Long study duration= duration of L-T4 replacement>6 months
Short study duration= duration of L-T4 replacement≤6 months

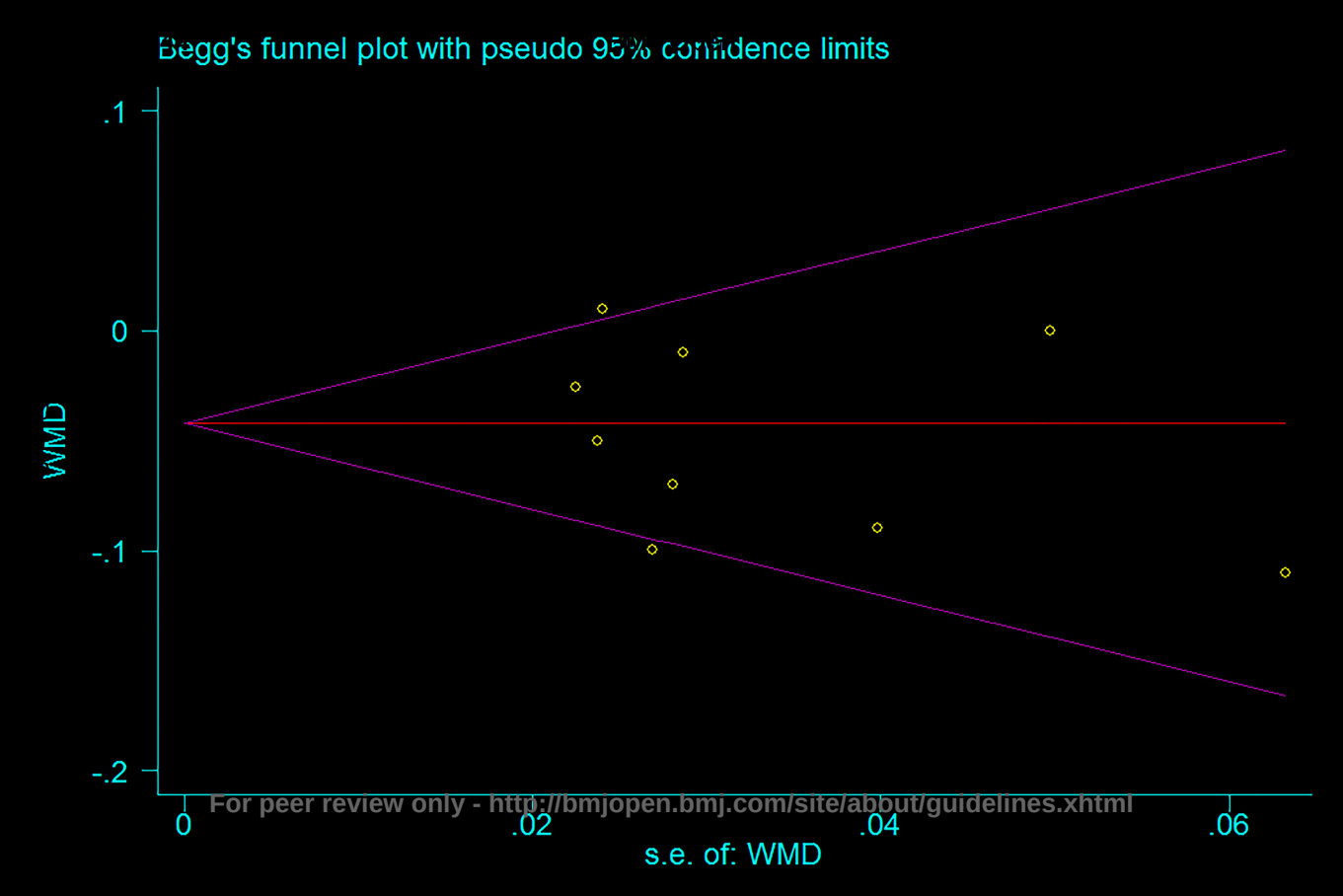
140x109mm (600 x 600 DPI)

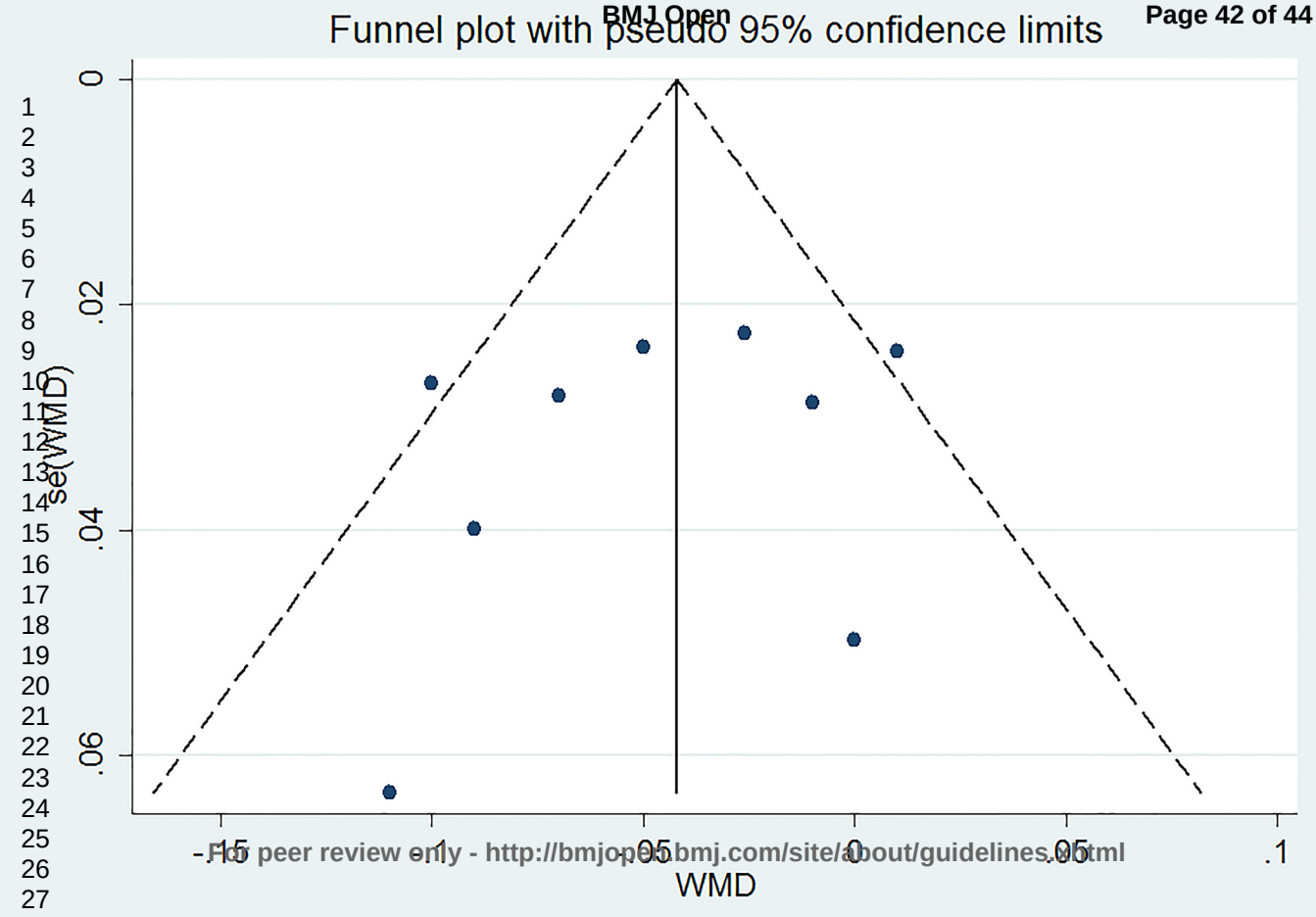












PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2-3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4-5
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5-6
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	6
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	7
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	6
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	8
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	8
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	8
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	8-9
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	9
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	9

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	8
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	8-9
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	10
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	11
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	17
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	15
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	18
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	18
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	18
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	19-20
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	3
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	25
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	25

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

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