REVIEW ARTICLE

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Are probiotics, prebiotics, and synbiotics beneficial in primary thyroid diseases? A systematic review with meta-analysis

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Abstract

Introduction and Objective. A number of studies indicate the presence of a thyroid-gut axis and the important influence of the gut microbiota on thyroid function. As prebiotics, probiotics and synbiotics show therapeutic potential in the treatment of intestinal dysbiosis, the aim of this review is to evaluate the efficacy of their supplementation in primary thyroid diseases.
Review Methods. Electronic databases (Ovid MEDLINE, Embase, CENTRAL), registers of clinical trials, and grey literature up to 6 October 2022 were searched for randomised controlled trials (RCTs) meeting pre-specified inclusion criteria. The protocol was registered in PROSPERO (CRD42021235054).
Brief description of the state of knowledge. After screening 1,721 references, two RCTs were identified, which included 136 hypothyroid participants in total. Meta-analysis of the results after eight weeks of supplementation with predominantly Lactobacillus and Bifidobacterium strains indicated a clinically and statistically nonsignificant decrease in TSH (MD –0.19 mIU/L; 95% CI –0.43 to 0.06; $I^2 = 0\%$), and no effect on fT_3 levels (MD 0.01 pg/mL; 95% CI–0.16 to 0.18; $I^2 = 0\%$). Data from single studies indicated no significant change in the levels of fT_4, thyroid auto-antibodies, BMI, levothyroxine doses, and severity of symptoms measured with validated scales. Only constipation scores showed significant improvement (MD –8.71 points in the Faecal Incontinence Questionnaire; 95% CI –15.85 to –1.57; $I^2 = 0\%$).
Summary. Low-certainty evidence from two randomised trials, suggests that routine administration of probiotics, prebiotics or synbiotics may result in little to no benefit in patients with primary hypothyroidism.

Key words

thyroid, probiotics, hypothyroidism, prebiotics, synbiotics

INTRODUCTION AND OBJECTIVE

Hypothyroidism and hyperthyroidism are commonly diagnosed conditions that have a considerable impact on metabolic rate, growth, fertility, and cognitive functions. The main clinical manifestations of thyroid disorders in iodine-sufficient areas are Graves' disease (GD) and Hashimoto's thyroiditis (HT), both caused by dysregulation of the immune system. Patients with untreated thyroid disease suffer from a wide range of symptoms, resulting in a marked impairment of quality of life [1, 2]. Furthermore, some of the symptoms may persist despite treatment and reach a state of euthyroidism [3]. A growing body of research shows a correlation between thyroid dysfunction and intestinal dysbiosis, and therefore it is hypothesised that the treatment of thyroid disorders might be facilitated by modulation of the microbiota [4–6].

It has been demonstrated that hyperthyroidism is associated with reduced concentrations of the *Lactobacillaceae* and *Bifidobacteriaceae* species, while hypothyroidism might lead to small intestine bacterial overgrowth (SIBO) [4]. Moreover, the microbiome modulates the immune response, mainly by influencing the functioning of Gut-Associated Lymphoid Tissue (GALT). GALT accounts for more than 70% of the entire immune system and plays a fundamental role in controlling the equilibrium between tolerance and immunity to nonself antigens, a process reported to be impaired in autoimmune thyroid diseases [5, 6]. In addition, the microbiome serves as a reservoir of thyroid hormones by binding triiodothyronine (T_3) and thyroxine (T_4) to bacterial thyroid-binding protein (bTBP). In turn, this mechanism may prevent fluctuations in thyroid hormone levels and necessitate modifications of levothyroxine (LT_4) supplementation. On the other hand,

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dysbiosis not only increases intestinal permeability and impairs the absorption of thyroid-important microelements (iodine, selenium, zinc and iron), but also affects thyroid hormone levels through its own deiodinase activity [4].

As the microbiome plays an indispensable role in host immunomodulation and maintenance of intestinal integrity, dysbiosis results in impaired epithelial barrier function and increased systemic inflammation. This appears to be a likely factor promoting autoimmune diseases, including autoimmune thyroid diseases. To date, a link has been shown between dysbiosis and the occurrence of many autoimmune diseases, e.g. asthma, allergies, type 1 diabetes [7]. Therefore, it was hypothesised that restoring an adequate microbiome could have a beneficial effect on the course of autoimmune thyroid diseases. Substances that have a proven ability to modulate the gut microbiome are prebiotics, probiotics and synbiotics [8–10]. These are preparations that contain beneficial microorganisms, substrates to facilitate their growth, or both [11, 12].

So far, endocrinological societies have not issued official recommendations on the role of probiotics, prebiotics, or synbiotics in the treatment of thyroid disorders [13, 14]. The current evidence is also limited by the fact that no systematic reviews have been published regarding this topic. Therefore, in order to summarise the available evidence on the efficacy of probiotics, prebiotics or synbiotics in primary thyroid diseases, a systematic review of randomised controlled trials (RCTs) was conducted.

REVIEW METHODS

On 6 March 2021, a protocol of the study was registered in PROSPERO with the Identification No. CRD42021235054. The study was performed in line with the methodology endorsed by the Cochrane Collaboration [15] and the study results reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [16]. The PRISMA flowchart is shown in Figure 1.

Eligibility criteria – Study design. Only RCTs were included since studies of this design are characterised by the lowest risk of bias, and therefore provide the most reliable evidence of the effectiveness of investigated interventions.

Population. The analysed population included adult patients (aged 18 years or older) diagnosed with a primary thyroid disease manifested by abnormal levels of thyroid-stimulating hormone (TSH), free thyroxine (fT_4), free triiodothyronine (fT_3), or the presence of thyroid autoantibodies. The condition could be associated with either hyper-, hypo-, or euthyroidism (according to any valid criteria specified by the authors). Studies on participants diagnosed with thyroid cancer were excluded.

Intervention. Participants were allocated to one of two or more different treatment regimens, with at least one group receiving oral probiotics, prebiotics, or synbiotics supplementation. These substances could be applied as a single or add-on therapy. No restrictions were imposed related to the composition, dose, or duration of the treatment. Additional co-interventions were allowed if deemed sufficiently similar across all trial groups.

The World Health Organisation's (WHO) definition of probiotics was used: 'live microorganisms that, when administered in adequate amounts, confer a health benefit on the host', as well as the International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus to describe prebiotics: 'a substrate that is selectively utilised by host microorganisms conferring a health benefit', and synbiotics – 'a mixture comprising live microorganisms and substrate(s) selectively utilised by host microorganisms that confers a health benefit on the host' [11, 12, 17].

Comparator. Acceptable forms of comparators were placebo, no intervention, care as usual, or application of other probiotic, prebiotic, or synbiotic preparations.

Outcomes. The primary outcomes were as follows:
1) change in the severity of symptoms related to hypo- or hyperthyroidism (any type of symptoms reported individually or diagnosed with a validated scale);
2) change in hormone levels (TSH, fT_3, fT_4);
3) progression of euthyroid autoimmune disease towards hypo- or hyperthyroidism (number of patients in the euthyroid phase of autoimmune thyroid disease at baseline who exceeded the reference values for TSH and fT_4 for hypo- or hyperthyroidism during follow-up);
4) changes in dose of anti-thyroid drugs or levothyroxine (LT_4);
5) occurence of any adverse events.

Secondary outcomes included:
1) change in thyroid autoantibodies levels, such as anti-thyroid peroxidase (TPOAb), antithyroglobulin (TgAb), and TSH receptor antibodies (TRAb);
2) change in the quality of life (QoL) measured with validated scales;
3) change in weight and Body Mass Index (BMI).

Search methods for identification of studies. Electronic databases (Ovid MEDLINE, Embase, CENTRAL), registers of clinical trials (ClinicalTrials.gov, European Trials Register), as well as grey literature (Networked Digital Library of Theses and Dissertations (NDLTD), Digital Access to Research Theses (DART) Europe E-theses Portal, Open Grey) were searched from inception to 13 June 2021. The entire search process was updated on 6 October 2022. No language or date restrictions were applied. Search strategies are provided in Supplementary File 1. Reference lists of all included studies, systematic reviews, and relevant guidelines were checked for additional studies.

Selection of studies. Identified references were checked for duplicates using EndNote (Clarivate Analytics, Philadelphia, PA). Rayyan QCRI was used to screen titles and abstracts. At least two reviewers (KZ, KK, MJS, ZS, EA, or ML) independently screened titles, abstracts, and full texts. To ensure a common understanding of the inclusion criteria, both phases were preceded by pilot screenings. Any conflicts were resolved through discussion or consulting the third reviewer (MMB). Studies that were not completed until the end of the review process were classified as 'ongoing'. Studies that could not be included or excluded from the analysis due to insufficient data or unavailability of full texts, were labelled as 'awaiting classification'. Authors of ongoing and unpublished trials were contacted via e-mail at least twice.

Data extraction and management. At least two reviewers (KZ, KK, MJS, ZS, EA, ML, or AK) independently extracted data from the included studies using a Microsoft Excel 2010 spreadsheet. Any discrepancies were resolved through discussion or by contacting a third reviewer (MMB). If a study was reported in more than one publication, data from all reports were extracted directly into a single data collection form and treated as one unit of interest [18–20]. An attempt was made to retrieve the missing relevant data by contacting the authors.

Assessment of risk of bias (RoB). RoB for each included study was independently evaluated by at least two reviewers (KZ, KK, MJS, EA, ZS, ML, or AK) using the Cochrane Risk of Bias Tool [21]. Any disagreements were resolved by discussion or involving a third reviewer (MMB).

Assessment of reporting bias. Since at least ten included studies are needed for certain outcomes to produce viable funnel plots or statistical tests, it was not possible to formally assess publication bias (small study effects) [22].

Analysis and synthesis of data. Statistical analyses were performed using Review Manager 5 (RevMan) software. Continuous data were presented as mean differences (MDs) with 95% confidence intervals (CIs). If the clinical questions of the studies were deemed similar enough, the data was combined in a meta-analysis. Since the presence of heterogeneity was expected between trials, a random-effects model (Der Simonian and Laird) was used [23]. Because the study by Talebi et al. [18–20] reported data as mean ± standard error (SE), standard deviation (SD) was calculated using RevMan.

Heterogeneity was assessed by visually inspecting forest plots and considering Chi^2 and I^2 statistics [21]. Whenever heterogeneity was observed, its potential causes were investigated by examining individual studies. Due to the small number of included studies it was not possible to perform planned subgroup or sensitivity analyses.

Certainty of evidence. The certainty of the evidence for the primary outcomes was evaluated independently by two authors (KK, ZS, MJS, or MMB) according to the Grading of Recommendations, Assessment, Development and Evaluations (GRADE) methodology [24].

DESCRIPTION OF THE STATE OF KNOWLEDGE

Study selection. Initial and updated searches yielded 2,174 records; following deduplication, 1,721 records were screened based on titles and abstracts. Finally, 15 records were identified: 3 ongoing trials (4 records), 4 studies awaiting classification (5 records), and 2 full-text publications (6 records). Figure 1 presents the PRISMA flowchart of the study selection process. Lists of studies classified as included, excluded, ongoing, and awaiting classification are provided in Supplementary File 2.

Characteristics of included studies. Table 1 summarises the characteristics of the included trials; full details are available in Supplementary File 3. Both included studies were RCTs with parallel design and comprised altogether 136 participants, of which 68 were randomised to the intervention and 68 to the control group. All participants were diagnosed with primary hypothyroidism, received LT_4 replacement therapy, and had TSH in the normal range at baseline. The vast majority of patients were female: 92.6% and 97.1% for the intervention and control groups, respectively.

All participants continued their LT_4 regimen (concentrations were individually adjusted according to clinical guidelines). Spaggiari et al. [25] randomised patients to an 8-week supplementation with a probiotic (VSL#3® including various strains of mostly *Lactobacillus* and *Bifidobacterium*) or to a control group that did not receive any intervention. All participants underwent monthly assessment that was continued for another 8 weeks after the intervention phase. In the study by Talebi et al. [18–20], patients received an 8-week treatment with either synbiotic (containing predominantly *Lactobacillus* and *Bifidobacterium* strains) or placebo capsules, with assessment performed immediately after the end of the intervention.

In both studies, patients were given instructions regarding the storage of supplements and their appropriate method of application. Calculated compliance for the Spaggiari et al. trial was 79.2%, while Talebi et al. planned to exclude participants if they consumed less than 90% of the supplements, and reported no exclusions.

Risk of bias. Figure 2 shows the RoB assessment (details available in Supplementary File 3). Both included trials reported random sequence generation and allocation concealment (Spaggiari et al. [25], however, did not clarify how the process was performed). Adequate blinding of participants, personnel, and outcome assessors for both subjective and objective outcomes was ensured by Talebi et al. [18–20]. Due to the single-blind design, the Spaggiari et al. [25] research was judged to be at high risk of bias regarding blinding of participants, as well as blinding of outcome assessors for subjective outcomes. Neither study was free from attrition bias. Study protocols were available for both studies, but Talebi et al. [18–20], although they planned to include the effect of synbiotics on QoL in the protocol, did not report these results in the publication, resulting in a selective outcome reporting bias. No other bias was detected.

Primary outcomes. Table 2 summarises data concerning reported primary and secondary outcomes, which are described in more detail below.

Change in severity of symptoms. Only Talebi et al. [18–20] reported data regarding this outcome, namely changes in the Multidimensional Fatigue Inventory (MFI), Fatigue Severity Scale (FSS), Depression, Anxiety and Stress Scale (DASS-21); the severity of constipation was measured with the Faecal Incontinence Questionnaire, and appetite scores (visual analogue scales) [26–28]. Of all these symptoms, only the severity of constipation was significantly diminished in the synbiotic group compared to the placebo (MD -8.71 points; 95% CI -15.85 to -1.57) (Tab. 2). The results concerning other investigated symptoms showed no improvement. The certainty of the evidence for all outcomes was low (Tab. 2 and Supplementary File 3).

Change in hormone levels. Talebi et al. reported changes in TSH and fT_3 levels after 8 weeks of supplementation, whereas Spaggiari et al. provided mean differences in levels of TSH,

Table 1. Characteristics of randomised trials analysing the effect of probiotics, prebiotics, and synbiotics in primary thyroid diseases

Study name, year, country	I/C	Description of intervention and control groups, follow-up	Analysed, n	Age - years, mean (SD)	Female, n (%)	Diagnosis, n (%)	Reported outcomes
Spaggiari et al. 2017 [25] Italy	I	PROBIOTIC + LT_4 1 probiotic sachet/d for 8 weeks, then an 8-week period of follow-up; throughout the study LT_4 replacement therapy Probiotic VSL#3®: 450×10⁹ CFU of live freeze-dried bacteria (i.e. Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus paracasei, Lactobacillus bulgaricus, and Streptococcus thermophilus)	39[a]	49.6 (10.39)	36 (92.3)	Autoimmune hypothyroidism: 28 (71.8) Non-autoimmune hypothyroidism: 11 (28.2)	Change in hormone levels: TSH, fT_3, fT_4 Change in LT_4 dose[b] Presence of adverse events Change in BMI and weight[b]
	C	No intervention + LT_4 replacement therapy	41[a]	49.33 (11.38)	41 (100)	Autoimmune hypothyroidism: 30 (73.2) Non-autoimmune hypothyroidism: 11 (26.8)	
Talebi et al. 2016, 2019, 2020 [18–20] Iran	I	SYNBIOTIC + LT_4 1 capsule/d of 500mg Familact for 8 weeks; LT_4 replacement therapy throughout the study period Synbiotic Familact: 7×10⁹ CFU Lactobacillus casei, 2×10⁹ CFU Lactobacillus acidophilus, 1.5×10⁹ CFU Lactobacillus rhamnosus, 2×10⁸ CFU Lactobacillus bulgaricus, 2×10¹⁰ CFU Bifidobacterium breve, 7×10⁹ CFU Bifidobacterium longum, 1.5×10¹⁰ CFU Streptococcus Thermophilus), FOS, lactose, magnesium stearate, talc	29	42.37 (11.96)	27 (93.1)	Autoimmune hypothyroidism: 20 (69) Non-autoimmune hypothyroidism: 9 (31)	Change in hormone levels: TSH, fT_3. Change in LT_4 dose. Presence of adverse events. Change in thyroid autoantibodies: TPOAb. Change in the severity of symptoms: MFI, FSS, Appetite scores, Constipation scores, DASS-21. Change in BMI
	C	PLACEBO + LT_4 1 placebo capsule/d for 8 weeks (containing 375 mg starch, 22 mg lactose, 1 mg magnesium stearate, 1 mg silicon dioxide, and 1 mg talc); LT_4 replacement therapy throughout the study	27	43.96 (9.25)	25 (92.6)	Autoimmune hypothyroidism: 11 (40.7) Non-autoimmune hypothyroidism: 16 (59.3)	

BMI - Body Mass Index; CFU - colony-forming unit; DASS-21 - Depression, Anxiety and Stress Scale (21 Items); FOS - fructooligosaccharide; FSS - Fatigue Severity Scale; fT_3 - free triiodothyronine; fT_4 - free thyroxine; I/C - intervention/control group; LT_4 - levothyroxine; MFI - Multidimensional Fatigue Inventory; RCT - randomized controlled trial; SD - standard deviation; TPOAb - thyroid peroxidase antibodies; TSH - thyroid-stimulating hormone
[a] Data regarding intention-to-treat (ITT) analysis were obtained upon mail contact
[b] Data provided as point estimates (mean ± SD) at each follow-up, no change values available

fT_3, and fT_4 between baseline and each of the 4 visits (after 4, 8, 12, and 16 weeks) [18–20, 25]. As both studies reported data after 8 weeks of supplementation, meta-analyses for TSH and fT_3 changes were performed at this time point. The pooled results indicated that supplementation with preparations including predominantly Lactobacillus and Bifidobacterium strains might be associated with a decrease in TSH levels; however, the results failed to reach statistical significance (MD –0.19 mIU/L; 95% CI –0.43 to 0.06) (Tab. 2, Fig. 3). The results demonstrated no impact on fT_3 (MD 0.01 pg/mL; 95% CI –0.16 to 0.18) (Tab. 2, Fig. 4). The study by Spaggiari et al. also reported no effect on fT_4 (MD –0.88 pg/mL; 95% CI –2.66 to 0.90) (Tab. 2). There was no important heterogeneity. The certainty of the evidence for all outcomes was low. Detailed results regarding other follow-ups are presented in Supplementary File 3.

Progression of a euthyroid autoimmune thyroid disease toward hypo- or hyperthyroidism. None of the included studies reported the progression of a euthyroid autoimmune thyroid disease toward hypo- or hyperthyroidism.

Change in anti-thyroid drugs or levothyroxine (LT4) dose. Participants in both studies received LT4 replacement therapy; dose adjustments were made based on serum

Table 2. Effect of eight-week probiotic (Spaggiari 2017) or synbiotics (Talebi 2020) supplementation on thyroid parameters, fatigue, depression, constipation and occurrence of side effects

Outcomes	Effect size MD (95% CI)	Participants, n (studies)	I2, %	Certainty of evidence (GRADE)
TSH (μIU/L)[d]	–0.19 (–0.43, 0.06)	136 (2)	0	low[a,b]
fT_3 (pg/mL)[d]	0.01 (–0.16, 0.18)	136 (2)	0	low[a,b]
fT_4 (pg/mL)[d]	–0.88 (–2.66, 0.90)	80 (1)	NA	low[a,b]
TPOAb (IU/mL)	10.84 (–8.08, 29.76)	56 (1)	NA	low[a,b]
LT_4 dose (μg/day)	–0.84 (–4.27, 2.59)	56 (1)	NA	low[a,b]
FSS[e]	–2.40 (–9.66, 4.86)	56 (1)	NA	low[a,b]
Depression (DASS-21)[f]	1.23 (–0.35, 2.81)	56 (1)	NA	low[a,b]
Constipation[g]	–8.71 (–15.85, –1.57)	56 (1)	NA	low[a,b]
Adverse events	No adverse events reported	136 (2)	NA	low[a,c]

DASS-21 – Depression, Anxiety and Stress Scale (21 Items); FSS – Fatigue Severity Scale; NA – not applicable
[a] Risk of bias: downgraded 1 level due to the high risk of bias in included studies.
[b] Imprecision: downgraded 1 level due to 95% CI including both benefit and harm and low number of participants.
[c] Imprecision: downgraded 1 level due to the low number of participants.
[d] Unpublished data from the Spaggiari study, information obtained from the authors.
[e] FSS: 9-item scale with a minimum score 9 and maximum 63 [26].
[f] DASS-21 comprises 3 subscales (depression, anxiety and stress) with 7 items in each subscale. The maximum score for the depression subscale is 21 [27].
[g] Constipation: assessed with Faecal Incontinence Questionnaire (https://colonrectaldocs.com/wp-content/uploads/2017/08/FecalIncontinenceQuestionnaire.pdf)

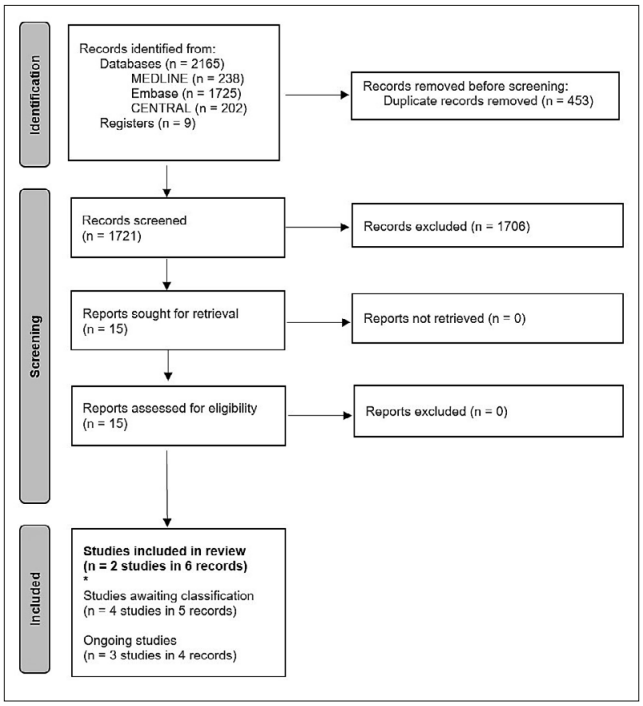

Figure 1. The process of selecting papers according to the PRISMA methodology, showing the subsequent phases of the systematic review

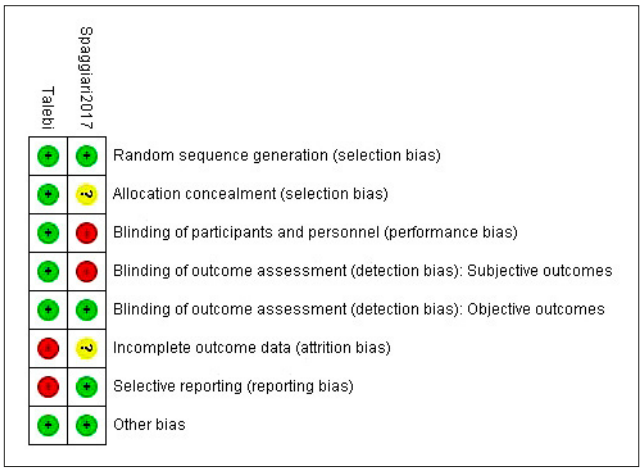

Figure 2. Risk of bias in the Spaggiari 2017 study and in the Talebi 2020 publications

TSH levels. Data from Talebi et al. showed no statistically significant changes in the LT4 dose (MD –0.84 µg/day; 95% CI –4.27 to 2.59) (Tab. 2). Spaggiari et al. also reported no significant difference in the mean daily LT4 dose (µg/day and µg/kg/day) between groups (p = 0.419); although as data were provided as point estimates at each follow-up, it was insufficient to calculate MD and SD (Supplementary File 4).

Presence of adverse events. Both studies reported no adverse events related to the supplementation.

Secondary outcomes

Change in the levels of thyroid auto-antibodies. Talebi et al. reported a non-statistically significant increase in serum TPOAb levels in the study group (Tab. 2).

Change in the Quality of Life (QoL). None of the included studies reported on QoL.

Change in weight and Body Mass Index (BMI). Talebi et al. reported no change in BMI upon supplementation (MD 0.21; 95% CI -0.04 to 0.46). Spaggiari et al. provided results regarding weight and BMI as point estimates at each follow-up (Supplementary File 4).

DISCUSSION

This systematic review comprised 2 RCTs performed in people with primary hypothyroidism: one comparing the efficacy of probiotics to no intervention, and the other comparing the efficacy of synbiotics to placebo. As preparations utilised in both studies comprised similar bacteria strains (predominantly *Lactobacilli* and *Bifidobacteria*), this justified meta-analysing available data for relevant outcomes. The pooled results indicate that after 8 weeks of supplementation with predominantly *Lactobacillus* and *Bifidobacterium* strains, the observed change in TSH was statistically and clinically insignificant [18–20, 25, 29]. There was also no effect regarding levels of fT_3 and fT_4, change in LT4 doses, thyroid autoantibodies levels, and BMI. Regarding the severity of symptoms, only constipation scores (measured with the Faecal Incontinence Questionnaire) improved after 8 weeks of synbiotic supplementation compared to placebo.

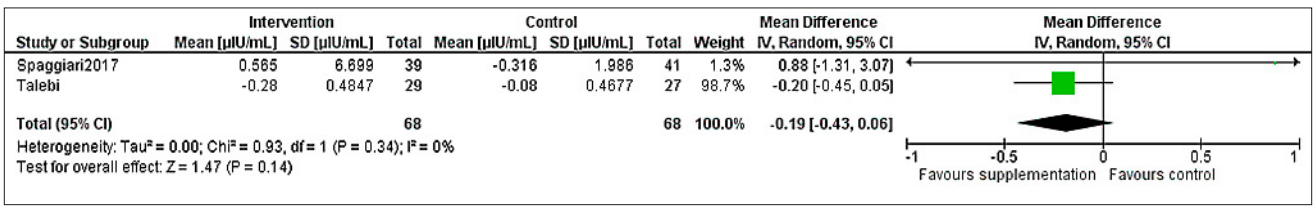

Figure 3. Change in TSH levels after 8 weeks of supplementation with probiotics (Spaggiari 2017) or synbiotics (Talebi 2020)

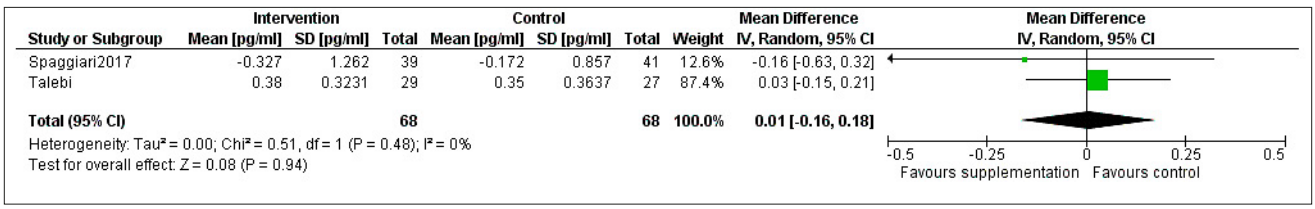

Figure 4. Change in fT_3 levels after 8 weeks of supplementation with probiotics (Spaggiari 2017) or synbiotics (Talebi 2020)

The available evidence reveals that intestinal dysbiosis favours the HT development [30, 31]. Hypothyroid patients have significantly lower concentrations of *Bifidobacterium* than healthy subjects. Alterations in *Lactobacillus* and other species vary between studies [32]. Participants within the probiotic group in the study of Spaggiari et al. and in the synbiotic group in the study of Talebi et al. received similar probiotic strains, predominantly *Lactobacillus* and *Bifidobacterium*. Nevertheless, neither Talebi et al. nor Spaggiari et al. assessed gut microbiota composition before starting the supplementation. Hence, it is unclear whether the applied concentrations were adequate to change the intestinal microbiome of the participants. Furthermore, both included RCTs provided results from a relatively short study period (2-month intervention followed directly by outcome assessment or a two-month follow-up). This prevented drawing meaningful conclusions regarding the long-term effects of the intervention, especially whether the beneficial effects remain after discontinuation of supplementation [33].

However, David et al. demonstrated that the gut microbiome responded to an altered diet within 5 days [34]. These findings also underline the importance of thorough dietary assessment, which was performed only by Talebi et al. [18–20]. The observed significant improvement in the constipation scores is in line with the results of previously published studies. Kaminski et al. conducted an umbrella review on the efficacy of probiotics in the treatment of chronic idiopathic constipation and concluded that probiotics might improve intestinal motility [35]. A meta-analysis by Zhang et al. showed that consumption of probiotics significantly improved stool frequency bowel movements [36].

To the best of the knowledge of the authors of the current study, this is the first systematic review that summarises available data on the efficacy of probiotics, prebiotics, and synbiotics in adults with hypothyroidism. Although preliminary data from the literature showed that disturbances of the gut microbiota are involved in the onset and development of primary thyroid diseases, there is still insufficient evidence that supplementation with probiotics, prebiotics, and synbiotics can improve the state of the microbiota, and thus influence the effects of the disease or alleviate its symptoms. The findings are particularly important as over the past few years there has been a tremendous increase in interest in probiotics. Furthermore, a substantial number of patients with primary thyroid disease use pro-/pre- or synbiotics as supplements. However, the results of the current meta-analysis did not confirm the efficacy of these substances in the context of thyroid hormone balance, BMI, levothyroxine replacement dose or severity of symptoms.

This study is distinguished by a number of strengths as well as a few weaknesses. The main strengths of the study include adhering to the pre-registered protocol with transparent and rigorous methods, including a systematic and comprehensive literature search of electronic databases, registers of clinical trials, and grey literature. Each step of the review was conducted independently by at least 2 authors. Furthermore, the study authors were contacted and most of the crucial missing data and necessary clarifications were received. Interpretation of the results is limited by the number of patients; the results and conclusions are based on 2 RCTs involving a total of 136 participants, which is less than optimal for adequate precision for continuous variables [37]. In addition, there was a clear female predominance in both the intervention and control groups, which could even exceed the gender disparity in the prevalence of hypothyroidism [38].

Furthermore, the small number of included trials made formal assessment of publication bias impossible. Since only results regarding hypothyroid patients were available, it was not possible to perform pre-planned analyses on patients diagnosed with hyperthyroidism or the euthyroid phase of autoimmune thyroid disorders. Three ongoing studies were found (1 on Hashimoto's thyroiditis, and 2 on Graves' disease), but to date, no results from these studies have been published.

SUMMARY

Evidence from 2 RCTs investigating the efficacy of probiotics and synbiotics in adults with hypothyroidism suggests that an 8-week supplementation with predominantly Lactobacillus and Bifidobacterium strains has no significant effect on TSH and fT_3 levels. Results from a single study indicated that probiotics also have no significant impact on the levels of fT_4 and thyroid autoantibodies, BMI, or LT4 doses. The included studies did not report adverse effects. Low certainty evidence derived from a single study suggests that synbiotics may relieve the severity of constipation in hypothyroid patients, but more robust evidence is needed to consider synbiotic supplementation in these patients. Future studies should also take into account the composition of gut microbiota, evaluate the effects of long-term supplementation, as well as examine whether beneficial effects are maintained after cessation of the intervention.

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REFERENCES

1. Chaker L, Bianco AC, Jonklaas J, et al. Hypothyroidism. Lancet. 2017;390:1550–1562. https://doi.org/10.1016/S0140-6736(17)30703-1
2. de Leo S, Lee SY, Braverman LE. Hyperthyroidism. Lancet. 2016;388:906–918. https://doi.org/10.1016/S0140-6736(16)00278-6
3. Jordan B, Uer O, Buchholz T, et al. Physical fatigability and muscle pain in patients with Hashimoto thyroiditis. J Neurol. 2021;268:2441–2449. https://doi.org/10.1007/s00415-020-10394-5
4. Knezevic J, Starchl C, Berisha AT, et al. Thyroid-Gut-Axis: How Does the Microbiota Influence Thyroid Function? Nutrients. 2020;12:1–16, https://doi.org/10.3390/nu12061769
5. Köhling HL, Plummer SF, Marchesi JR, et al. The microbiota and autoimmunity: Their role in thyroid autoimmune diseases. Clin Immunol. 2017;183:63–74, https://doi.org/10.1016/j.clim.2017.07.001
6. Virili C, Fallahi P, Antonelli A, et al. Gut microbiota and Hashimoto's thyroiditis. Rev Endocr Metab Disord. 2018;19:293–300. https://doi.org/10.1007/s11154-018-9467-y

7. de Oliveira GLV, Leite AZ, Higuchi BS, et al. Intestinal dysbiosis and probiotic applications in autoimmune diseases. Immunology. 2017;152:1–12. https://doi.org/10.1111/imm.12765
8. Hemarajata P, Versalovic J. Effects of probiotics on gut microbiota: mechanisms of intestinal immunomodulation and neuromodulation. Therap Adv Gastroenterol. 2013;6:39–51. https://doi.org/10.1177/1756283X12459294
9. Wang S, Xiao Y, Tian F, et al. Rational use of prebiotics for gut microbiota alterations: Specific bacterial phylotypes and related mechanisms. J Funct Foods. 2020;66:103838. https://doi.org/10.1016/j.jff.2020.103838
10. Sergeev IN, Aljutaily T, Walton G, et al. Effects of Synbiotic Supplement on Human Gut Microbiota, Body Composition and Weight Loss in Obesity. Nutrients. 2020;12:222. https://doi.org/10.3390/nu12010222
11. Hill C, Guarner F, Reid G, et al. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol. 2014;11:506–514. https://doi.org/10.1038/nrgastro.2014.66
12. Swanson KS, Gibson GR, Hutkins R, et al. The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of synbiotics. Nat Rev Gastroenterol Hepatol. 2020;17:687–701. https://doi.org/10.1038/s41575-020-0344-2
13. Jonklaas J, Bianco AC, Bauer AJ, et al. Guidelines for the treatment of hypothyroidism: prepared by the American Thyroid Association task force on thyroid hormone replacement. Thyroid. 2014;24:1670–1751. https://doi.org/10.1089/thy.2014.0028
14. Ross DS, Burch HB, Cooper DS, et al. 2016 American Thyroid Association Guidelines for Diagnosis and Management of Hyperthyroidism and Other Causes of Thyrotoxicosis. Thyroid. 2016;26:1343–1421, https://doi.org/10.1089/thy.2016.0229
15. Higgins JPT, Thomas J, Chandler J, et al. Cochrane Handbook for Systematic Reviews of Interventions version 6.3 (updated February 2022) [Internet]. Cochrane 2022
16. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. Syst Rev. 2021;10:89. https://doi.org/10.1186/s13643-021-01626-4
17. Gibson GR, Hutkins R, Sanders ME, et al. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nat Rev Gastroenterol Hepatol. 2017;14:491–502. https://doi.org/10.1038/nrgastro.2017.75
18. Talebi S, Karimifar M, Heidari Z, et al. The effect of synbiotic supplementation on anthropometric indices, appetite, and constipation in people with hypothyroidism: A randomized, double-blind, placebo-controlled trial. Phytother Res. 2020;34:2712–2720. https://doi.org/10.1002/ptr.6710
19. Talebi S, Karimifar M, Heidari Z, et al. The effects of synbiotic supplementation on thyroid function and inflammation in hypothyroid patients: A randomized, double-blind, placebo-controlled trial. Complement Ther Med. 2020;48:102234. https://doi.org/10.1016/j.ctim.2019.102234
20. Talebi S, Karimifar M, Heidari Z, et al. The effects of synbiotic supplementation on fatigue management and mental health status in levothyroxine-treated patients with hypothyroidism: A randomized, double-blind, placebo-controlled clinical trial study. J Isfahan Med Sch 2016;38:74–84. https://doi.org/10.22122/JIMS.V38I564.12428
21. Higgins JPT, Altman DG, Gøtzsche PC, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ. 2011;343:d5928. https://doi.org/10.1136/bmj.d5928
22. Sterne JAC, Sutton AJ, Ioannidis JPA, et al. Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomised controlled trials. BMJ. 2011;343:d4002. https://doi.org/10.1136/bmj.d4002
23. der Simonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials. 1986;7:177–188. https://doi.org/10.1016/0197-2456(86)90046-2
24. Guyatt GH, Oxman AD, Vist GE, et al. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. BMJ. 2008;336:924–926. https://doi.org/10.1136/bmj.39489.470347.AD
25. Spaggiari G, Brigante G, De Vincentis S, et al. Probiotics Ingestion Does Not Directly Affect Thyroid Hormonal Parameters in Hypothyroid Patients on Levothyroxine Treatment. Front Endocrinol (Lausanne). 2017;8:316. https://doi.org/10.3389/fendo.2017.00316
26. Krupp LB, Larocca NG, Muir Nash J, et al. The fatigue severity scale. Application to patients with multiple sclerosis and systemic lupus erythematosus. Arch Neurol. 1989;46:1121–1123. https://doi.org/10.1001/archneur.1989.00520460115022
27. Henry JD, Crawford JR. The short-form version of the Depression Anxiety Stress Scales (DASS-21): construct validity and normative data in a large non-clinical sample. Br J Clin Psychol. 2005;44:227–239. https://doi.org/10.1348/014466505X29657
28. Flint A, Raben A, Blundell JE, et al. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. Int J Obes Relat Metab Disord. 2000;24:38–48. https://doi.org/10.1038/sj.ijo.0801083
29. Andersen S, Bruun NH, Pedersen KM, et al. Biologic variation is important for interpretation of thyroid function tests. Thyroid. 2003;13:1069–1078. https://doi.org/10.1089/105072503770867237
30. Zhao F, Feng J, Li J, et al. Alterations of the Gut Microbiota in Hashimoto's Thyroiditis Patients. Thyroid. 2018;28:175–186. https://doi.org/10.1089/thy.2017.0395
31. Ishaq HM, Mohammad IS, Guo H, et al. Molecular estimation of alteration in intestinal microbial composition in Hashimoto's thyroiditis patients. Biomed Pharmacother. 2017;95:865–874. https://doi.org/10.1016/j.biopha.2017.08.101
32. Cayres LC de F, de Salis LVV, Rodrigues GSP, et al. Detection of Alterations in the Gut Microbiota and Intestinal Permeability in Patients With Hashimoto Thyroiditis. Front Immunol. 2021;12:579140. https://doi.org/10.3389/fimmu.2021.579140
33. Fröhlich E, Wahl R. Microbiota and Thyroid Interaction in Health and Disease. Trends Endocrinol Metab. 2019;30:479–490. https://doi.org/10.1016/j.tem.2019.05.008
34. David LA, Maurice CF, Carmody RN, et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature. 2014;505:559–563. https://doi.org/10.1038/nature12820
35. Kaminski M, Skonieczna-Zydecka K, Loniewski I, et al. Are probiotics useful in the treatment of chronic idiopathic constipation in adults? A review of existing systematic reviews, meta-analyses, and recommendations. Prz Gastroenterol. 2020;15:103–118. https://doi.org/10.5114/pg.2019.86747
36. Zhang C, Jiang J, Tian F, et al. Meta-analysis of randomized controlled trials of the effects of probiotics on functional constipation in adults. Clin Nutr. 2020;39:2960–2969, https://doi.org/10.1016/j.clnu.2020.01.005
37. Guyatt GH, Oxman AD, Kunz R, et al. GRADE guidelines 6. Rating the quality of evidence-imprecision. J Clin Epidemiol. 2011;64:1283–1293. https://doi.org/10.1016/j.jclinepi.2011.01.012
38. Dong YH, Fu DG. Autoimmune thyroid disease: mechanism, genetics and current knowledge. Eur Rev Med Pharmacol Sci. 2014;18:3611–3618.