Association Between Primary Hypothyroidism and Metabolic Dysfunction-Associated Steatotic Liver Disease: An Updated Meta-Analysis

Alessandro Mantovani[1], Alessandro Csermely[1], Josh Bilson[2], Nicolò Borella[1], Enrico Scoccia[1], Barbara Pecoraro[1], Emigela Shtembari[1], Riccardo Morandin[1], Stergios A. Polyzos[3], Luca Valenti[4,5], Herbert Tilg[6], Christopher D. Byrne[2], Giovanni Targher[7,8]

[1]Section of Endocrinology, Diabetes and Metabolism, Department of Medicine, University and Azienda Ospedaliera Universitaria Integrata of Verona, Verona, Italy

[2]National Institute for Health and Care Research, Southampton Biomedical Research Centre, University Hospital Southampton and University of Southampton, Southampton, UK

[3]First Laboratory of Pharmacology, School of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece

[4]Precision Medicine-Biological Resource Center, Transfusion Medicine, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico Milano, Milan, Italy

[5]Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy

[6]Department of Internal Medicine I, Gastroenterology, Hepatology, Endocrinology and Metabolism, Medical University Innsbruck, Innsbruck, Austria

[7]Department of Medicine, University of Verona, Italy

[8]Metabolic Diseases Research Unit, IRCCS Sacro Cuore - Don Calabria Hospital, Negrar di Valpolicella, Italy

Word count: Abstract 249; Text 4600 (*excluding* title page, summary box, references, and figure legends); n. 3 **Figures** + **Online-only Supplementary material** (n. 3 **Supplementary Tables**; n. 14 **Supplementary Figures**).

Address for correspondence:
Prof. Giovanni Targher, MD
Metabolic Diseases Research Unit
IRCCS Sacro Cuore - Don Calabria Hospital
Viale Luigi Rizzardi, 4
37024 Negrar di Valpolicella (VR), Italy
E-mail: giovanni.targher@univr.it

ABSTRACT

Objective: Epidemiological studies have reported an association between primary hypothyroidism and metabolic dysfunction-associated steatotic liver disease (MASLD). However, the magnitude of the risk and whether this risk changes with the severity of MASLD remains uncertain. We performed a meta-analysis of observational studies to quantify the magnitude of the association between primary hypothyroidism and the risk of MASLD.

Design: We systematically searched PubMed, Scopus, and Web of Science from database inception to January 31, 2024, using predefined keywords to identify observational studies in which MASLD was diagnosed by liver biopsy, imaging, or International Classification of Diseases codes. Meta-analysis was performed using random-effects modelling.

Results: We identified 24 cross-sectional and four longitudinal studies with aggregate data on ~76.5 million individuals. Primary hypothyroidism (defined as levothyroxine replacement treatment, subclinical hypothyroidism, or overt hypothyroidism) was associated with an increased risk of prevalent MASLD (n=24 studies; random-effects odds ratio 1.43, 95%CI 1.23-1.66; I^2=89%). Hypothyroidism was also associated with a substantially higher risk of metabolic dysfunction-associated steatohepatitis or advanced fibrosis (n=5 studies; random-effects odds ratio 2.84, 95%CI 2.07-3.90; I^2=0%). Meta-analysis of data from 4 longitudinal studies showed that there was a marginally non-significant association between hypothyroidism and risk of developing MASLD over a median 4.5-year follow-up (random-effects hazard ratio 1.39, 95%CI 0.98-1.97; I^2=85%). Sensitivity analyses did not modify these findings. The funnel plot did not reveal any significant publication bias.

Conclusion: This large and updated meta-analysis provides evidence that primary hypothyroidism is significantly associated with both an increased presence of, and histological severity of MASLD.

Keywords: Non-alcoholic fatty liver disease; NAFLD; metabolic dysfunction-associated steatotic liver disease; MASLD; hypothyroidism; meta-analysis

SUMMARY BOX

What is already known about this subject: Observational studies reported an association between primary hypothyroidism and metabolic dysfunction-associated steatotic liver disease (MASLD). However, the magnitude of the risk and whether this risk changes with the severity of MASLD remains uncertain.

What are the new findings: This comprehensive meta-analysis (~76.5 million participants) provides substantive evidence that primary hypothyroidism is associated with a higher risk of prevalent MASLD. Hypothyroidism is also associated with a ~2.8-fold increased risk of metabolic dysfunction-associated steatohepatitis or advanced liver fibrosis. Finally, there is a marginally non-significant association between hypothyroidism and the risk of developing MASLD.

How might it impact on clinical practice in the foreseeable future: Health care professionals should be aware that the risk of MASLD is increased in patients with primary hypothyroidism. Health care professionals should have a high index of clinical suspicion that their patients with hypothyroidism may have MASLD and these patients are at higher risk of MASH or advanced liver fibrosis.

INTRODUCTION

Metabolic dysfunction-associated steatotic liver disease (MASLD), previously known as non-alcoholic fatty liver disease (NAFLD), has become the leading cause of chronic liver diseases globally [1, 2]. MASLD affects ~30% of adults in the general population [3], ~65% of patients with type 2 diabetes [4] and more than 80% of patients with severe obesity [5]. MASLD pathophysiology involves many diverse pathways, such as altered glucose and lipid metabolism, insulin resistance, dietary factors, lipotoxicity, and energy balance dysregulation [2, 6]. Thyroid hormones, namely free thyroxine (FT4) and free triiodothyronine (FT3), play a critical role in hepatic glucose and lipid metabolism. The actions of thyroid hormones in the liver are mediated through binding to their specific nuclear receptor, i.e., THR-β1, and modulated by deiodinases, mainly deiodinase 1 [7-9]. Hepatic deiodinase 1 converts FT4 to the biologically active FT3, which is released into the bloodstream [9]. Evidence shows that patients with MASLD or metabolic dysfunction-associated steatohepatitis (MASH) have impaired intrahepatic THR-β (thyroid hormone receptor-β) signaling [9]. In particular, the MASLD/MASH-related lipotoxicity may induce a state of intrahepatic hypothyroidism, resulting in reduced conversion of FT4 to FT3 in favor of increased conversion of FT4 to the inactive metabolite reverse T3 [9].

The new liver-directed, selective THR-β agonists, such as resmetirom, help regulate intrahepatic thyroid hormone concentrations in patients with MASLD/MASH, potentially addressing the underlying pathophysiology related to impaired intrahepatic THR-β signaling in these patients [9]. In this regard, resmetirom has been shown to be effective for treating MASH and liver fibrosis in preclinical and randomized clinical studies, thus further supporting the role of thyroid hormones in MASLD pathophysiology [7, 9, 10].

After the publication of our previous 2018 meta-analysis of 15 observational studies (44,140 participants) that reported an association between primary hypothyroidism and the risk of NAFLD [11], it is important to note that new large observational studies have been published on this hot topic. However, the findings of these observational studies remain conflicting, and it remains uncertain whether subclinical hypothyroidism could be associated with a higher risk of MASLD.

Based on this background of evidence, we have therefore carried out a comprehensive systematic review and meta-analysis of observational studies to quantify the risk of MASLD in patients with

primary hypothyroidism. Moreover, we also aimed to assess whether there was an association between hypothyroidism and risk of having more severe histologic forms of MASLD. Clarification of the magnitude of the risk of MASLD associated with primary hypothyroidism may directly impact the development of prevention strategies for MASLD.

METHODS

Registration of review protocol

The protocol of this systematic review was registered on the Open Science Framework (OSF) database (registration DOI: https://doi.org/10.17605/OSF.IO/Z2JQ4).

Data sources and searches

This systematic review has been performed following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) and the Meta-analysis Of Observational Studies in Epidemiology (MOSE) guidelines [12, 13]. We systematically searched PubMed, Scopus, and Web of Science from database inception to January 31, 2024, to identify relevant observational studies examining the association between primary hypothyroidism and the risk of MASLD. Search free text terms were "hypothyroidism" OR "subclinical hypothyroidism" OR "thyroid dysfunction" AND “non-alcoholic fatty liver disease” OR “NAFLD” OR “non-alcoholic steatohepatitis” OR “metabolic dysfunction-associated fatty liver disease” OR “MAFLD” OR “metabolic dysfunction-associated steatotic liver disease” OR “MASLD”. Searches were restricted to human studies. No language restrictions were imposed. Furthermore, we reviewed references from relevant review articles to identify additional eligible studies not covered by the original database searches.

Study selection

Eligible studies were included in the meta-analysis if they met the following inclusion criteria: 1) observational (cross-sectional, case-control or longitudinal) studies that examined the association between primary hypothyroidism and risk of MASLD (or NAFLD); 2) studies that reported odds ratios (ORs) or hazard ratios (HRs) with 95% confidence intervals (95% CIs) values for the outcome of interest; 3) the diagnosis of MASLD (or NAFLD) was based on liver biopsy, imaging or International Classification of Diseases (ICD) codes, in the absence of significant alcohol consumption (defined as <20 g/day for women and <30 g/day for men) or other competing causes of chronic liver disease

(e.g., viral hepatitis, iron overload and use of potentially hepatotoxic drugs); and 4) the diagnosis of primary hypothyroidism was based on a self-reported history of hypothyroidism with use of levothyroxine replacement treatment or based on measurements of serum thyroid stimulating hormone (TSH), FT4 or FT3 concentrations for identifying patients with subclinical or overt hypothyroidism. Subjects included in the meta-analysis were of either sex without any age, race, or ethnicity restrictions.

The exclusion criteria of the meta-analysis were as follows: 1) congress abstracts, case reports, reviews, practice guidelines, commentaries or editorials; 2) studies in which the diagnosis of MASLD (or NAFLD) was based exclusively on serum aminotransferase levels or other blood-based biomarkers of MASLD or NAFLD (e.g., the fatty liver index); 3) studies which did not exclude individuals with significant alcohol consumption or other known causes of chronic liver disease; 4) studies that only examined the associations between continuous levels of serum TSH or thyroid hormones and the risk of MASLD; and 5) studies that included exclusively euthyroid individuals.

Data extraction and quality assessment

Data from studies eligible for the aggregate data meta-analysis were independently extracted by two investigators (AM and GT). Any disagreements between investigators about the inclusion of eligible studies were resolved by consensus and a third investigator if needed (AC).

For each eligible study, we extracted data on publication year, study design, sample size, country, population characteristics, methods used for the diagnosis of hypothyroidism and MASLD, severity of MASLD on liver histology (i.e., defined as the presence of either MASH or fibrosis stage F≥2), outcomes of interest, matching and confounding factors included in multivariable regression analyses, and length of follow-up (only for longitudinal studies). In the case of multiple publications of the same database, we included the most up-to-date or comprehensive information.

The overall quality of the studies included in the aggregate data meta-analysis was assessed using the Newcastle-Ottawa scale (NOS) by two independent authors (AM and GT). Any disparities in scoring were reviewed, and consensus obtained following discussion. The NOS scale is a validated scale for non-randomized studies in meta-analyses, which uses a star system to assess the quality of a study in three domains: selection, comparability, and outcome/exposure. The NOS assigns a

maximum of four stars for selection (or five stars in the case of cross-sectional studies), two for comparability, and three for outcome/exposure. We judged studies that received a score of at least 8 stars to be at low risk of bias, thus reflecting the highest quality.

Data synthesis and analysis

The primary outcome measure of the meta-analysis was the presence of MASLD (or NAFLD) for cross-sectional studies or the risk of developing incident MASLD (or NAFLD) over the follow-up for longitudinal studies. The ORs (for cross-sectional studies) or HRs (for longitudinal studies) and their 95% CIs were considered as the effect size (ES) for all the eligible studies. When studies reported ORs/HRs with varying degrees of covariate adjustment, we extracted those that reflected the maximum extent of adjustment for potentially confounding variables. When studies used multiple definitions of hypothyroidism, we chose the ORs (for cross-sectional studies) or HRs (for longitudinal studies) and their 95% CIs for defining hypothyroidism that identified the most severe endocrine disorder. The adjusted OR/HRs of all eligible studies were pooled, and an overall ES estimate was calculated using a random-effects model since high heterogeneity was expected for a meta-analysis of observational studies.

The statistical heterogeneity among studies was evaluated by the chi-square test and the I^2-statistic, which estimates the percentage of variability across studies due to heterogeneity rather than chance alone. The proportion of heterogeneity accounted for by between-study variability was assessed using the I^2-statistic and adjudicated to be significant if the I^2-index was >50% [14]. The possibility of publication bias was examined using the visual inspection of funnel plots and the Egger's regression asymmetry test [15].

To explore the possible sources of (expected) heterogeneity among the studies and to test the robustness of the observed associations, we also performed stratification-sensitivity analyses by study population (adults vs. children/adolescents), study country, methodologies used for diagnosing hypothyroidism and MASLD, severity of MASLD histology (presence of either MASH or fibrosis stage F≥2) and quality of studies (≥8 stars on the NOS scale, i.e., studies with low risk of bias). We also tested for possible excessive influence of individual studies using a meta-analysis influence test that eliminated each of the included studies at a time. Finally, we performed univariable meta-regression analyses to test the impact of specific moderator variables (i.e., age,

sex, body mass index, percentage of pre-existing type 2 diabetes, hypertension, and serum TSH concentrations) on the effect size for the association between hypothyroidism and MASLD.

All statistical tests were two-sided and used a significance level of $p<0.05$. We used R version 4.3.2 (R Core Team 2023, R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>) for all statistical analyses with the following packages: *meta* (version 7.0-0) and *metafor* (version 4.4-0).

RESULTS

Literature search and characteristics of included studies

Supplementary Figure 1 shows the PRISMA flow diagram of the meta-analysis. After examining the titles and abstracts of these publications and excluding duplicates, we identified 60 potentially eligible studies from three large electronic databases (PubMed, Web of Science and Scopus) from the inception to January 31, 2024. We further excluded 32 studies because of unsatisfactory inclusion criteria or unsatisfactory outcome measures (as specified in **Supplementary Table 1**). Consequently, we identified 28 unique observational studies (24 cross-sectional and four longitudinal) for inclusion in the meta-analysis.

The main characteristics of these studies are summarized in **Supplementary Table 2** and **Table 3**, respectively. Twenty-four studies had a cross-sectional design [16-38], 21 of which recruited adult individuals [16-35] and three studies enrolled overweight or obese children and adolescents [36-38]. Four studies had a longitudinal design [39-42]. Regarding the cross-sectional studies (**Supplementary Table 2**), the diagnosis of MASLD was based on liver biopsy (n=5 studies), ultrasonography or other imaging methods (n=16 studies), and ICD-10/SNOMED-CT codes (n=3 studies). The diagnosis of primary hypothyroidism was either based on a self-reported history of hypothyroidism with the use of levothyroxine replacement therapy or based on serum TSH and thyroid hormone measurements identifying subclinical hypothyroidism (i.e., elevated TSH with normal thyroid hormones) or overt hypothyroidism (elevated TSH with low thyroid hormones). Eight studies were carried out in Asia (China, South Korea, Iran, India and Philippines), seven in Europe

(Germany, Spain and Italy), six in the USA and two in Mexico, while a study included an international cohort of individuals. Eight of these 24 studies obtained at least 8 stars on the NOS scale, thus reflecting a low risk of bias; three studies obtained 7 stars, and the remaining 13 studies obtained 6 stars or less. Overall, these 24 cross-sectional studies included a total of 76,424,439 adult individuals (22.3% men; age 45±14 years; BMI 28.4±5.5 kg/m^2; 17% had a diagnosis of MASLD; 7.6% had a diagnosis of primary hypothyroidism [as described above]; 9% had established type 2 diabetes) and 3,009 overweight or obese children and adolescents (age 12±2 years; 45.9% had a diagnosis of MASLD; 12.8% had hypothyroidism).

Cross-sectional studies on the association between hypothyroidism and MASLD

The distribution of cross-sectional studies (involving 76,427,448 individuals) by estimate of the association between primary hypothyroidism and the risk of MASLD is plotted in **Figure 1.** We found that hypothyroidism was significantly associated with a higher risk of prevalent MASLD (pooled random-effects OR 1.43, 95% CI 1.23-1.66; I^2=89%). Specifically, this pooled risk was 1.35 (95% CI 1.16-1.56; I^2=90%, n=21 studies) in studies enrolling adults and 2.30 (95% CI 1.63-3.24, I^2=0%, n=3 studies) in those involving overweight or obese children and adolescents. Since we have always used the fully adjusted OR estimates for each study, this pooled random-effects OR was independent of age, sex, ethnicity, adiposity measures, diabetes, dyslipidemia, or other common metabolic risk factors (as specified in Table 1).

Subgroup analyses

We undertook subgroup analyses to explore the possible sources of heterogeneity across the cross-sectional studies. As shown in **Supplementary Figure 2**, the association between hypothyroidism and the risk of MASLD was consistent even when the comparison was stratified by study country, with the higher random-effects ORs observed in studies conducted in the USA (n=6 studies; random-effects OR 1.59, 95% CI 1.55-1.64; I^2=0%) and Europe (n=7 studies; random-effects OR 1.52, 95% CI 1.13-2.06; I^2=62%), as well as in the single international study (n=1 study; random-effects OR 2.79, 95% CI 1.10-7.06; I^2=not applicable). This association was marginally non-significant in Asian studies (n=7 studies; random-effects OR 1.26, 95% CI 0.99-1.61; I^2=60%).

Stratifying the studies by different methods used for diagnosing MASLD (**Supplementary Figure 3**), the association between hypothyroidism and the risk of prevalent MASLD was significant in studies

in which MASLD was evaluated by liver histology (n=5 studies; random-effects OR 2.05, 95% CI 1.55-2.71; I^2=0%), ultrasonography (n=15 studies; random-effects OR 1.35, 95% CI 1.09-1.66; I^2=78%) or ICD−10/SNOMED−CT codes (n=3 studies; random-effects OR 1.43, 95% CI 1.17-1.75; I^2=98%).

Stratifying the studies by different methodologies used for identifying primary hypothyroidism (**Supplementary Figure 4**), the association between hypothyroidism and the risk of MASLD was significant both in studies in which the diagnosis was based on a self-reported history of hypothyroidism with the use of levothyroxine replacement and in those in which the diagnosis was based on abnormal serum thyroid function tests (subclinical or over hypothyroidism).

Stratifying the studies by risk of bias evaluated using NOS scale (**Supplementary Figure 5**), the association between hypothyroidism and risk of MASLD was significant in studies with a low risk of bias (NOS scale ≥8) (n=7 study; random-effects OR 1.63, 95% CI 1.39-1.93; I^2=20%) and in those with a medium-high risk of bias (NOS scale <8) (n=17 study; random-effects OR 1.31, 95% CI 1.08-1.59; I^2=92%).

Sensitivity analyses and meta-regressions

A sensitivity analysis using the one-study remove (leave-one-out) approach to test the influence of each study on the overall effect size showed that eliminating each of the studies from the pooled primary analysis did not have any significant effect on the overall risk of prevalent MASLD (**Supplementary Figure 6**). The results of univariable meta-regression analyses to examine the impact of potential moderator variables showed a significant association between the proportion of patients with pre-existing diabetes and risk of MASLD (**Supplementary Figure 7**). Conversely, meta-regression analyses did not show any significant effects of age (**Supplementary Figure 8**), sex (**Supplementary Figure 9**), BMI (**Supplementary Figure 10**), hypertension (**Supplementary Figure 11**), or serum TSH levels (**Supplementary Figure 12**) on the association between hypothyroidism and MASLD (in cross-sectional studies of adult patients).

Publication bias

As shown in **Supplementary Figure 13**, the Egger's test did not show any statistically significant asymmetry of the funnel plot for cross-sectional studies (p=0.526), thus suggesting that the

publication bias was low. Since there were only four longitudinal cohort studies, we did not perform a formal publication bias analysis for these studies.

Cross-sectional studies on the association between hypothyroidism and more severe MASLD

The distribution of eligible studies by estimate of the association between primary hypothyroidism and the risk of more severe MASLD is plotted in **Figure 2.** Five liver biopsy-based studies [16, 18, 25, 26, 43], involving 9,640 adult individuals from different countries, provided data for this pooled secondary analysis. We found that hypothyroidism was associated with a ~2.8-fold increased risk of having MASH or fibrosis stage F≥2 (n=5 studies; random-effects OR 2.84, 95% CI 2.07-3.90; I^2=0%). This risk was independent of age, sex, ethnicity, adiposity measures, diabetes, and other common metabolic risk factors. As shown in **Figure 2**, the association between hypothyroidism and MASLD severity was consistent even when the comparison was stratified by MASH or fibrosis stage F≥2, separately.

Longitudinal studies on the association between hypothyroidism and incident MASLD

Four retrospective cohort studies [39-42] examined the association between primary subclinical/overt hypothyroidism and the risk of developing MASLD (**Supplementary Table 3**). Overall, these studies included a total of 31,518 middle-aged individuals from China, South Korea, or the Netherlands (48.4% men; age 52±15 years; BMI 24.4±2.5 kg/m^2; 10.8% of whom had a diagnosis of primary hypothyroidism at baseline, most frequently subclinical hypothyroidism). During a median follow-up of 4.5 years (interquartile range: 3.3-6.2 years), there were 3,969 new cases of incident MASLD. In all studies, the diagnosis of MASLD was based on ultrasonography. Three of these studies obtained at least 8 stars on the NOS scale, thus reflecting a low risk of bias.

The distribution of cohort studies by estimate of the association between primary hypothyroidism and the risk of developing MASLD is plotted in **Figure 3**. We found that hypothyroidism was marginally associated with an increased risk of developing MASLD over a median of 4.5 years (random-effects HR 1.39, 95% CI 0.98-1.97; I^2=85%). Notably, as shown in **Supplementary Figure 14**, the one-study remove (leave-one-out) approach to test the influence of each study on the overall effect size showed that the association between hypothyroidism and the risk of developing MASLD became statistically significant (random-effects HR 1.59, 95%CI 1.12-2.24; I^2=74%) after excluding the study by Lee *et al*. [40] from the pooled analysis.

DISCUSSION

This updated meta-analysis that included 28 observational studies (24 cross-sectional and 4 longitudinal) with aggregate data on approximately 76.5 million individuals from different countries provides substantive evidence that primary hypothyroidism was significantly associated with a higher risk of prevalent MASLD as detected by liver biopsy, imaging methods or ICD codes (pooled random-effects OR 1.43, 95% CI 1.23-1.66; I^2=89%). This risk remained essentially unchanged when the comparison was stratified by study country, different modalities for diagnosing hypothyroidism or MASLD, NOS quality scale, or when we analyzed separately the published studies that included adult individuals and pediatric population. Notably, this risk remained significant in those studies where statistical analysis was adjusted for age, sex, ethnicity, adiposity measures, pre-existing diabetes, dyslipidemia, and other common metabolic risk factors (as specified in Supplementary Table 2). Furthermore, in studies with liver biopsy data, hypothyroidism was associated with a ~2.8-fold increased risk of either MASH or fibrosis stage F≥2 (n=5 studies; random-effects OR 2.84, 95% CI 2.07-3.90; I^2=0%). Our meta-regression analyses also showed a significant association between the proportion of patients with pre-existing diabetes and increased risk of hypothyroidism-related MASLD, suggesting that the association between hypothyroidism and MASLD may be partly mediated by the rates of diabetes in different studies (thus explaining a part of the high heterogeneity among studies). This also represents further evidence of a close link existing between primary hypothyroidism, type 2 diabetes, and MASLD [44]. Finally, meta-analysis of data from 4 retrospective longitudinal studies showed that there was a borderline association between subclinical hypothyroidism and increased risk of developing MASLD over a median follow-up of 4.5 years (random-effects HR 1.39, 95% CI 0.98-1.97; I^2=85%).

This is the largest and most comprehensive meta-analysis assessing the relationship between primary hypothyroidism and the risk of MASLD. Our findings corroborate and further expand the findings of our previous meta-analysis of 15 observational studies (involving 44,140 participants) [11] and two other smaller meta-analyses published in 2018 and 2021 [45, 46]. Unlike these three previous meta-analyses, we included much more observational studies (increasing the total number of individuals from almost 44,000 in our earlier meta-analysis to about 76.5 million individuals from different countries). In addition, we have included a larger number of liver biopsy-based studies, thereby proving a more reliable estimate of the association between hypothyroidism and the risk of having MASH or fibrosis stage F≥2. Although we observed that subclinical hypothyroidism was

marginally associated with a higher risk of incident MASLD over a median follow-up of 4.5 years, we believe that the most likely explanations for this partly unexpected result are the relatively small sample size of the eligible cohort studies, the lack of information on the duration of hypothyroidism, the short duration of the follow-up, and the use of ultrasonography for detecting MASLD (that is not sensitive enough to detect mild steatosis). That said, it should also be noted that the one-study remove (leave-one-out) approach showed that the association between hypothyroidism and incident MASLD became statistically significant (random-effects HR 1.59, 95% CI 1.12-2.24) after excluding the study by Lee et al. [40] from the pooled analysis.

Taken together, the findings of our meta-analysis may have clinical implications; more specifically, individuals with primary hypothyroidism should be screened for MASLD since these patients are at higher risk of having MASH or advanced fibrosis. It should be noted that none of the studies included in the meta-analysis had data available to investigate the association between hypothyroidism and the risk of major adverse liver outcomes. Furthermore, no published studies have examined the effect of thyroid hormone replacement therapy when examining the risk of MASLD in patients with subclinical hypothyroidism. However, a post-hoc analysis of a small randomized controlled trial of patients with subclinical hypothyroidism showed that levothyroxine replacement therapy for 15 months resulted in significant reductions of serum liver enzymes and hepatic steatosis on ultrasound in these patients [47]. In addition, a small phase 2b single-arm study of 20 euthyroid patients with type 2 diabetes and MASLD showed that low-dose levothyroxine treatment for 16 weeks was able to reduce hepatic fat content measured by proton magnetic resonance spectroscopy [48].

A detailed discussion of the putative biological mechanisms linking primary hypothyroidism and MASLD is beyond the scope of this meta-analysis. Briefly, thyroid hormone levels are key modulators of energy homeostasis with established effects on hepatic glucose and lipid metabolism [7-9, 49]. It is well known that overt hypothyroidism promotes MASLD development and progression, possibly by inducing overweight/obesity, dyslipidemia, dysglycemia/insulin resistance, low-grade inflammation, and increased oxidative stress [7-9]. In the Rotterdam study, a population-based cohort study, the authors reported a negative linear association between serum FT4 levels and the risk of incident MASLD and a positive linear association for serum TSH levels. Moreover, compared to euthyroidism, the risk of MASLD appeared to decrease in hyperthyroidism [41]. Experimentally, it has also been reported that elevated serum TSH levels itself may induce hepatic fat accumulation

through upregulation of sterol regulatory element-binding protein-1c (SREBP-1c) activity [50]. Furthermore, evidence shows that intrahepatic THR-β signaling is impaired in the liver of patients with MASLD or MASH [9]. Preclinical studies supported a role of THR-β in MASLD development, as the THR-β pathway signaling modulates hepatic *de novo* lipogenesis, cholesterol synthesis, fatty acid β-oxidation, and the circulating levels of various lipids, such as low-density lipoprotein and lipoprotein(a) [9]. In recent years, there has been considerable scientific interest in developing liver-directed, selective THR-β agonists for treating MASLD [7-9]. In this regard, resmetirom has provided promising results for treating MASH and liver fibrosis in preclinical and clinical studies, thus further implying a role of thyroid hormones in the development and progression of MASLD [7, 9]. Recently, Harrison *et al.* reported the results of the ongoing phase-3 MAESTRO-NASH trial, in which 966 adults with MASH and liver fibrosis were randomly assigned to receive for 52 weeks once-daily resmetirom at a dose of 80 mg or 100 mg or placebo [10]. These investigators showed that both resmetirom doses were superior to placebo for the primary endpoints of MASH resolution without fibrosis worsening and fibrosis improvement by at least one stage with no worsening of MASH [10]. Therefore, FDA conditionally approved on 14 March, 2024 resmetirom as the first drug for the treatment of adults with non-cirrhotic MASH and significant liver fibrosis [51]

The present meta-analysis has important limitations inherent to the design of the eligible studies. First, the retrospective design of the cross-sectional studies does not allow us to define the time line between the development of primary hypothyroidism and that of MASLD. However, Mendelian randomization studies are consistent with a causal association between overt hypothyroidism and MASLD [52]. Second, although most eligible studies adjusted the results for known risk factors and potential confounders, the possibility of residual confounding by unmeasured factors cannot be entirely excluded. Third, the cross-sectional studies of the meta-analysis used different definitions of primary hypothyroidism (ranging from a self-reported history of hypothyroidism with the use of thyroid hormone therapy to abnormal thyroid function tests with variably different TSH laboratory cutoffs for defining subclinical hypothyroidism). At first glance, the association between self-reported history of hypothyroidism with the use of levothyroxine replacement and risk of MASLD seems surprising. However, as the use of thyroid replacement therapy is only a surrogate for the diagnosis of hypothyroidism, the results from eligible studies using this definition of hypothyroidism should be interpreted cautiously because data regarding the time of diagnosis of hypothyroidism, as well as accurate results of thyroid function tests were not extensively available. It is likely that most

patients included in the meta-analysis were euthyroid (due to levothyroxine replacement therapy) or only had subclinical hypothyroidism (but, as expected, few participants had overt primary hypothyroidism). This is also observable from data shown in Supplementary **Table 2** and **Table 3**, where most eligible studies reported mean serum TSH levels within the reference range. In addition, serum TSH and thyroid hormone levels were not routinely monitored, and serum thyroid antibodies were not constantly measured in all eligible studies. Furthermore, detailed information on the duration of subclinical or overt hypothyroidism was not available in most studies. This limited our ability to investigate a potential dose-response relationship between the severity of hypothyroidism and MASLD. Fourth, most studies used ultrasonography to diagnose MASLD, whereas only a minority of the studies used liver biopsy, which is the "gold standard" for diagnosing and staging MASLD. Fifth, although we used a random-effects model, the interpretation of some meta-analysis results requires some caution because of the observed medium-high heterogeneity for the pooled primary analysis of studies and the relatively low quality of the studies, suggesting a medium-high risk of bias according to the NOS. Using subgroup analyses, meta-regressions and sensitivity analyses, we explored the possible sources of statistical heterogeneity. However, we believe that more detailed analyses of the causes of heterogeneity will require collaborative pooling of individual participant data from future large studies. Finally, although all eligible studies have used the NAFLD terminology, for this meta-analysis, we have assumed that, as the two fatty liver disease definitions are mainly overlapping [53], the increase in the risk conferred by hypothyroidism was comparable for NAFLD and MASLD, which should be confirmed in future studies. Finally, although a selective reporting bias of eligible studies could not be excluded (because we did not include "grey" literature in the meta-analysis), we believe our comprehensive search has made it unlikely that any published studies were missed.

Despite these limitations, our updated meta-analysis also has important strengths. This meta-analysis is the most comprehensive assessment of the association between hypothyroidism and the risk of prevalent and incident MASLD. The large number of individuals with hypothyroidism and MASLD provided sufficient statistical power to quantify the relationship between hypothyroidism and the risk of MASLD. Moreover, we have used standardized risk estimates from all included studies to allow a consistent combination of estimates across studies. Finally, the funnel plot did not show any significant asymmetry, thus suggesting that the risk of publication bias was low.

In conclusion, this comprehensive meta-analysis of observational studies provides evidence for a significant and independent association between primary hypothyroidism and the risk of MASLD. This risk remained significant in those studies whose results were adjusted for age, sex, adiposity measures, pre-existing diabetes, dyslipidemia and/or other common metabolic risk factors. Furthermore, hypothyroidism was associated with a substantially higher risk of having MASH or fibrosis stage F≥2. It remains to be established whether patients with subclinical hypothyroidism have a higher risk of developing MASLD. Future well-designed studies (possibly using vibration-controlled transient elastography or magnetic resonance-based techniques) are needed to further validate these findings, as well as mechanistic studies to better decipher the complex mechanisms underlying the association between primary hypothyroidism and MASLD.

Data availability statement: All supporting data of the meta-analysis are available within the article (and in the online-only Supplementary Material).

Funding: There was no funding source for this study.

Acknowledgments: CDB is supported in part by the Southampton National Institute for Health and Care Research (NIHR) Biomedical Research Centre (NIHR203319). LV is supported by Italian Ministry of Health (Ministero della Salute), Ricerca Finalizzata RF-2016-02364358, Rete Cardiologica "CV-PREVITAL", RF-2021-12373889, Italian Ministry of Health, Ricerca Finalizzata PNR-MAD-2022-12375656; Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Ricerca corrente "Liver BIBLE" (PR-0391); The European Union, programme "Photonics" under grant agreement "101016726", HORIZON-MISS-2021-CANCER-02; Ministry of University and Research MUR PNRR-CN3, PRIN 2022. GT is supported in part by grants from the University School of Medicine of Verona, Verona, Italy.

Conflicts of interest statement: The Authors have no potential conflicts of interest to disclose.

Author Contributions: AM and GT were involved in the conception of the study, the analysis and interpretation of the results and wrote the first draft of the manuscript. AC, NB, JB, BP, ES and RM were involved in the conduct of the study and searched the published articles. JB, SAP, LV, HT and

CDB were involved in the interpretation of the results and contributed to the discussion. All authors edited, reviewed, and approved the final version of the manuscript.

Research Ethics Approval: This study involves human participants but was not approved by an Ethics Committee. Approval from an Ethics Committee is not necessary as this is a meta-analysis of published observational studies that had already obtained informed consent from participants and ethical approval by their local Ethics committees.

Patient and Public Involvement: It was not appropriate or possible to involve patients or the public in the design, or conduct, or reporting, or dissemination plans of our research**.**

REFERENCES

1. Rinella ME, Lazarus JV, Ratziu V, et al. A multisociety Delphi consensus statement on new fatty liver disease nomenclature. J Hepatol 2023;79:1542-56.
2. Targher G, Byrne CD, Tilg H. MASLD: a systemic metabolic disorder with cardiovascular and malignant complications. Gut 2024;73:691-702.
3. Younossi ZM, Golabi P, Paik JM, et al. The global epidemiology of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH): a systematic review. Hepatology 2023;77:1335-47.
4. En Li Cho E, Ang CZ, Quek J, et al. Global prevalence of non-alcoholic fatty liver disease in type 2 diabetes mellitus: an updated systematic review and meta-analysis. Gut 2023;72:2138-48.
5. Quek J, Chan KE, Wong ZY, et al. Global prevalence of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in the overweight and obese population: a systematic review and meta-analysis. Lancet Gastroenterol Hepatol 2023;8:20-30.
6. Tilg H, Adolph TE, Dudek M, et al. Non-alcoholic fatty liver disease: the interplay between metabolism, microbes and immunity. Nat Metab 2021;3:1596-607.
7. Cusi K. Selective Agonists of Thyroid Hormone Receptor Beta for the Treatment of NASH. N Engl J Med 2024;390:559-61.
8. Lonardo A, Ballestri S, Mantovani A, et al. Pathogenesis of hypothyroidism-induced NAFLD: Evidence for a distinct disease entity? Dig Liver Dis 2019;51:462-70.
9. Byrne CD, Targher G, Tilg H. Thyroid hormone receptor-beta agonists: new MASLD therapies on the horizon. Gut 2024;73:573-81.
10. Harrison SA, Bedossa P, Guy CD, et al. A Phase 3, Randomized, Controlled Trial of Resmetirom in NASH with Liver Fibrosis. N Engl J Med 2024;390:497-509.
11. Mantovani A, Nascimbeni F, Lonardo A, et al. Association Between Primary Hypothyroidism and Nonalcoholic Fatty Liver Disease: A Systematic Review and Meta-Analysis. Thyroid 2018;28:1270-84.
12. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71.
13. Stroup DF, Berlin JA, Morton SC, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. JAMA 2000;283:2008-12.
14. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med 2002;21:1539-58.
15. Egger M, Davey Smith G, Schneider M, et al. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997;315:629-34.
16. Liangpunsakul S, Chalasani N. Is hypothyroidism a risk factor for non-alcoholic steatohepatitis? J Clin Gastroenterol 2003;37:340-3.
17. Ittermann T, Haring R, Wallaschofski H, et al. Inverse association between serum free thyroxine levels and hepatic steatosis: results from the Study of Health in Pomerania. Thyroid 2012;22:568-74.
18. Pagadala MR, Zein CO, Dasarathy S, et al. Prevalence of hypothyroidism in nonalcoholic fatty liver disease. Dig Dis Sci 2012;57:528-34.
19. Chung GE, Kim D, Kim W, et al. Non-alcoholic fatty liver disease across the spectrum of hypothyroidism. J Hepatol 2012;57:150-6.
20. Zhang J, Sun H, Chen L, et al. Relationship between serum TSH level with obesity and NAFLD in euthyroid subjects. J Huazhong Univ Sci Technolog Med Sci 2012;32:47-52.

21. Eshraghian A, Dabbaghmanesh MH, Eshraghian H, et al. Nonalcoholic fatty liver disease in a cluster of Iranian population: thyroid status and metabolic risk factors. Arch Iran Med 2013;16:584-9.
22. Posadas-Romero C, Jorge-Galarza E, Posadas-Sanchez R, et al. Fatty liver largely explains associations of subclinical hypothyroidism with insulin resistance, metabolic syndrome, and subclinical coronary atherosclerosis. Eur J Endocrinol 2014;171:319-25.
23. Ludwig U, Holzner D, Denzer C, et al. Subclinical and clinical hypothyroidism and non-alcoholic fatty liver disease: a cross-sectional study of a random population sample aged 18 to 65 years. BMC Endocr Disord 2015;15:41.
24. Lingad-Sayas RC, Montano CN, Isidro MJC. Prevalence of elevated TSH and its association with dyslipidemia and NAFLD among Filipino adult executive check-up patients in a tertiary hospital. Philipp J Intern Med 2017;55:1-8.
25. Kim D, Kim W, Joo SK, et al. Subclinical Hypothyroidism and Low-Normal Thyroid Function Are Associated With Nonalcoholic Steatohepatitis and Fibrosis. Clin Gastroenterol Hepatol 2018;16:123-31 e1.
26. Kim D, Yoo ER, Li AA, et al. Low-Normal Thyroid Function Is Associated With Advanced Fibrosis Among Adults in the United States. Clin Gastroenterol Hepatol 2019;17:2379-81.
27. Martinez Escude A, Pera G, Arteaga I, et al. Relationship between hypothyroidism and non-alcoholic fatty liver disease in the Spanish population. Med Clin (Barc) 2020;154:1-6.
28. Labenz C, Kostev K, Armandi A, et al. Impact of thyroid disorders on the incidence of non-alcoholic fatty liver disease in Germany. United European Gastroenterol J 2021;9:829-36.
29. Grewal H, Joshi S, Sharma R, et al. Non-alcoholic fatty liver disease in patients with hypothyroidism presenting at a rural tertiary care centre in north India. Trop Doct 2021;51:181-4.
30. Almomani A, Hitawala AA, Kumar P, et al. Prevalence of hypothyroidism and effect of thyroid hormone replacement therapy in patients with non-alcoholic fatty liver disease: A population-based study. World J Hepatol 2022;14:551-8.
31. Sheikhi V, Heidari Z. Association of Subclinical Hypothyroidism with Nonalcoholic Fatty Liver Disease in Patients with Type 2 Diabetes Mellitus: A Cross-Sectional Study. Adv Biomed Res 2022;11:124.
32. Zuarth-Vazquez J, Moreno-Castaneda L, Soriano-Marquez JP, et al. Low-Normal Thyroid Function Is Not Associated with Either Non-Alcoholic Fatty Liver Disease or with Metabolic Dysfunction-Associated Fatty Liver Disease. Life (Basel) 2023;13.
33. Boustany A, Onwuzo S, Zeid HKA, et al. Non-alcoholic steatohepatitis is independently associated with a history of gestational diabetes mellitus. J Gastroenterol Hepatol 2023;38:984-8.
34. Chen YL, Tian S, Wu J, et al. Impact of Thyroid Function on the Prevalence and Mortality of Metabolic Dysfunction-Associated Fatty Liver Disease. J Clin Endocrinol Metab 2023;108:e434-e43.
35. Fan H, Li L, Liu Z, et al. Low thyroid function is associated with an increased risk of advanced fibrosis in patients with metabolic dysfunction-associated fatty liver disease. BMC Gastroenterol 2023;23:3.
36. Pacifico L, Bonci E, Ferraro F, et al. Hepatic steatosis and thyroid function tests in overweight and obese children. Int J Endocrinol 2013;2013:381014.
37. Kaltenbach TE, Graeter T, Oeztuerk S, et al. Thyroid dysfunction and hepatic steatosis in overweight children and adolescents. Pediatr Obes 2017;12:67-74.
38. Di Sessa A, Cembalo Sambiase Sanseverino N, De Simone RF, et al. Association between non-alcoholic fatty liver disease and subclinical hypothyroidism in children with obesity. J Endocrinol Invest 2023;46:1835-42.
39. Xu L, Ma H, Miao M, et al. Impact of subclinical hypothyroidism on the development of non-alcoholic fatty liver disease: a prospective case-control study. J Hepatol 2012;57:1153-4.

40. Lee KW, Bang KB, Rhee EJ, et al. Impact of hypothyroidism on the development of non-alcoholic fatty liver disease: A 4-year retrospective cohort study. Clin Mol Hepatol 2015;21:372-8.
41. Bano A, Chaker L, Plompen EP, et al. Thyroid Function and the Risk of Nonalcoholic Fatty Liver Disease: The Rotterdam Study. J Clin Endocrinol Metab 2016;101:3204-11.
42. Wang S, Xia D, Fan H, et al. Low thyroid function is associated with metabolic dysfunction-associated steatotic liver disease. JGH Open 2024;8:e13038.
43. Kouvari M, Valenzuela-Vallejo L, Axarloglou E, et al. Thyroid function, adipokines and mitokines in metabolic dysfunction-associated steatohepatitis: A multi-centre biopsy-based observational study. Liver Int 2024;44:848-64.
44. Saatmann N, Schon M, Zaharia OP, et al. Association of thyroid function with non-alcoholic fatty liver disease in recent-onset diabetes. Liver Int 2024;44:27-38.
45. Guo Z, Li M, Han B, et al. Association of non-alcoholic fatty liver disease with thyroid function: A systematic review and meta-analysis. Dig Liver Dis 2018;50:1153-62.
46. Zeng X, Li B, Zou Y. The relationship between non-alcoholic fatty liver disease and hypothyroidism: A systematic review and meta-analysis. Medicine (Baltimore) 2021;100:e25738.
47. Liu L, Yu Y, Zhao M, et al. Benefits of Levothyroxine Replacement Therapy on Nonalcoholic Fatty Liver Disease in Subclinical Hypothyroidism Patients. Int J Endocrinol 2017;2017:5753039.
48. Bruinstroop E, Dalan R, Cao Y, et al. Low-Dose Levothyroxine Reduces Intrahepatic Lipid Content in Patients With Type 2 Diabetes Mellitus and NAFLD. J Clin Endocrinol Metab 2018;103:2698-706.
49. Efstathiadou ZA, Kita MD, Polyzos SA. Thyroid dysfunction and non-alcoholic fatty liver disease. Minerva Endocrinol 2018;43:367-76.
50. Yan F, Wang Q, Lu M, et al. Thyrotropin increases hepatic triglyceride content through upregulation of SREBP-1c activity. J Hepatol 2014;61:1358-64.
51. https://www.fda.gov/news-events/press-announcements/fda-approves-first-treatment-patients-liver-scarring-due-fatty-liver-disease.
52. Xie J, Huang H, Liu Z, et al. The associations between modifiable risk factors and nonalcoholic fatty liver disease: A comprehensive Mendelian randomization study. Hepatology 2023;77:949-64.
53. Hagstrom H, Vessby J, Ekstedt M, et al. 99% of patients with NAFLD meet MASLD criteria and natural history is therefore identical. J Hepatol 2024;80:e76-e7.

FIGURE LEGENDS

Figure 1. Forest plot and pooled estimates of the effect of primary hypothyroidism on the risk of prevalent MASLD in the eligible cross-sectional studies (n=24) stratified by population type (adult *vs*. pediatric population).

Figure 2. Forest plot and pooled estimates of the effect of primary hypothyroidism on the histological severity of MASLD (i.e., defined as the presence of MASH or fibrosis stage F≥2) in the eligible cross-sectional studies with liver biopsy data (n=5).

Figure 3. Forest plot and pooled estimates of the effect of primary hypothyroidism on the risk of developing incident MASLD in the eligible longitudinal cohort studies (n=4).