Thyroid Function and the Risk of Nonalcoholic Fatty Liver Disease

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ABSTRACT

Background Although thyroid function is associated with several risk factors of nonalcoholic fatty liver disease (NAFLD), its role in NAFLD development remains unclear. We therefore aimed to prospectively investigate the association between variations in thyroid function and NAFLD, in a large population-based, prospective cohort study.

Methods Participants from the Rotterdam Study with thyroid function measurements at baseline and NAFLD data (ie, at baseline fatty liver index, at follow-up ultrasound) were eligible. Transient elastography was performed to assess the presence of fibrosis in patients with NAFLD, using the liver stiffness measurements ≥8 kilopascals as cutoff for clinically relevant fibrosis. The association between thyroid parameters and incident NAFLD was explored by using logistic regression models.

Results A total of 9419 participants (mean age, 64.75 years) were included. The median follow-up time was 10.04 years (interquartile range, 5.70 to 10.88 years). After adjusting for age, sex, cohort, follow-up time, use of lipid-lowering medications, and cardiovascular risk factors, higher free thyroxine levels were associated with a decreased risk of NAFLD (odds ratio [OR], 0.42; 95% confidence interval [95% CI], 0.28 to 0.63). In line, higher thyroid-stimulating hormone levels were associated with an increased risk of having clinically relevant fibrosis in NAFLD (OR, 1.49; 95% CI, 1.04 to 2.15). Compared to euthyroidism, hypothyroidism was associated with a 1.24 times higher NAFLD risk (95% CI, 1.01 to 1.53). Moreover, NAFLD risk decreased gradually from hypothyroidism to hyperthyroidism (*P* for trend, 0.003).

Conclusions Lower thyroid function is associated with an increased NAFLD risk. These findings may lead to new avenues regarding NAFLD prevention and treatment.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver condition worldwide.[1] It comprises a broad spectrum ranging from simple steatosis to nonalcoholic steatohepatitis with fibrosis, which can eventually progress to cirrhosis and hepatocellular carcinoma.[2,3] Nonalcoholic steatohepatitis-related cirrhosis is anticipated to become the leading indication for liver transplantation by 2030.[4] Moreover, accumulating evidence has shown that NAFLD, either independently or in combination with other metabolic risk factors, is associated with extrahepatic complications such as cardiovascular disease, type 2 diabetes, chronic kidney disease, malignancy, and all-cause mortality.[5] Despite improved understanding and treatment of its risk factors (eg, diabetes mellitus and dyslipidemia), prevalence of NAFLD has rapidly increased.[6] Hence, investigation of additional modifiable risk factors is urgently needed.

Thyroid hormone is the major regulator of metabolic rate. Although hypothyroidism has been implicated in the etiology of NAFLD,[7] prior studies regarding the association between thyroid function and NAFLD risk have yielded controversial results, varying from a strong [8,9] to no association.[10,11] Studies confined to euthyroid subjects have been inconsistent as well, reporting that free thyroxine (FT_4) alone,[12] thyroid-stimulating hormone (TSH) alone,[13] both,[8] or neither of them[14] are linked with NAFLD. These discrepancies are mainly due to small sample sizes and cross-sectional design of previous studies.

The only prospective study to date focused exclusively on the risk of NAFLD in subclinical hypothyroidism.[15] As a consequence, the risk of NAFLD has not been explored prospectively in the remaining categories of thyroid function, other than subclinical hypothyroidism. A recent review has also highlighted the need for prospective research on the association between normal thyroid function and NAFLD risk.[16] Moreover, it remains unclear whether and to what extent thyroid function affects fibrosis risk in NAFLD patients. Therefore, we prospectively investigated the association between variations in thyroid function and NAFLD spectrum, in a large population-based cohort study.

METHODS

Study population

The Rotterdam Study (RS) is a large, prospective, population-based cohort study, conducted among middle-aged and elderly inhabitants of the Ommoord district in Rotterdam, the Netherlands. The complete rationale and study design have been described in detail previously.[17] In brief, all residents of Ommoord aged 55 years or older were invited to participate. Firstly, 7983 participants were enrolled between 1990 and 1993 (RS I). In 2000, the study was extended with a second cohort of 3011 subjects (RS II). In 2006, a third cohort of 3932 subjects aged 45 years and over was added (RS III), and thereafter the study population comprised a total of 14926 subjects.

Participants from study cohorts RS I visit 3 (RS I.3), RS II visit 1 (RS II.1) and RS III visit 1 (RS III.1) were eligible for the study if they had thyroid function measurements and data available on ultrasound-diagnosed NAFLD at follow-up or fatty liver index (FLI) at baseline. We considered the date of baseline laboratory testing, which comprised the assessment of thyroid function and FLI components, the start date of follow-up. The end date of follow-up was considered the date of the ultrasound measurement (Supplemental Figure 1).

The Medical Ethics Committee of the Erasmus University and the Ministry of Health, Welfare and Sport of the Netherlands approved the study protocols, implementing the "Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study)". All included participants provided a written informed consent in accordance with the Declaration of Helsinki to participate in the study and to obtain information from their family physicians.

Assessment of thyroid function

We performed thyroid function tests in the 3 independent Rotterdam Study cohorts using the same method and assay. Thyroid function assessment was performed for TSH, FT_4, and thyroid peroxidase antibodies (TPOAbs) in baseline serum samples stored at -80°C (The electrochemiluminescence immunoassay ECLIA, Roche). We determined the reference range of TSH (0.4 to 4.0 mIU/L) and FT_4 (0.85 to 1.95 ng/dL [to convert to picomoles per liter, multiply by 12.871]), according to national guidelines and previous reports from the Rotterdam Study.[18] Thyroid function was defined as euthyroid if serum TSH was within the reference range. Subclinical hypothyroidism was defined as serum TSH >4.0 mIU/L and FT_4 levels within the reference range.

Overt hypothyroidism was defined as serum TSH >4.0 mIU/L and FT_4 levels <0.85 ng/dL. Subclinical hyperthyroidism was defined as serum TSH <0.4 mIU/L and FT_4 levels within the reference range. Overt hyperthyroidism was defined as serum TSH <0.4 mIU/L and FT_4 levels >1.95 ng/dL. Levels of TPOAb >35 kU/ml were regarded as positive, as recommended by the assay manufacturer.

Assessment of NAFLD

Assessment of NAFLD comprised abdominal ultrasonographies at follow-up and FLI measurements at baseline. To assess incident NAFLD during follow-up, abdominal ultrasonography was performed by a single trained technician and subsequently images were reevaluated by an experienced hepatologist.[17] NAFLD was defined by the presence of liver steatosis on abdominal ultrasound, in the absence of secondary causes as excessive alcohol consumption (>14 alcoholic beverages weekly), hepatitis B surface antigen, and/or hepatitis C virus positivity, and use of fatty liver inducing pharmacological agents (ie, amiodarone, tamoxifen, corticosteroids, and methotrexate).

At baseline, ultrasound measurements were not available and instead, we utilized FLI measurements. FLI, an algorithm based on levels of triglycerides, gamma-glutamyl transferase, body mass index (BMI) and waist circumference, was calculated by the formula previously described by Bedogni et al.[19] The accuracy of FLI in the detection of NAFLD has been demonstrated in various studies, including the Rotterdam Study.[20-22] FLI ≥60 has a probability of 82.3% to identify the presence of NAFLD.[22] Therefore, we used a cutoff of 60 to classify participants into low and high probability of NAFLD, after primarily excluding subjects with a secondary cause of hepatic steatosis.

Liver stiffness (LS) was examined using transient elastography (Fibroscan; Echosens). LS measurements were performed by a single operator, on the right lobe of the liver, through the intercostal spaces, with the participant lying flat on his back with the right arm laying in maximal abduction. Either M- or XL-probe was applied, based on the manufacturer's instructions. Reliability of LS measurements was defined according to the criteria by Boursier et al.[23] LS measurements were considered poorly reliable if interquartile range /median LS >0.30 with median LS ≥7.1 kilopascals (kPa). A total of 48 participants with NAFLD diagnosis had unreliable LS measurements and were therefore excluded from the analyses involving LS. LS

≥8.0 kPa was used as a cutoff suggesting clinically relevant fibrosis. A high positive predictive value of this cutoff has been previously reported.[24,25]

Additional measurements

Information was obtained from each participant through a home questionnaire concerning demographics, medical history, alcohol intake, tobacco smoking, and medication use. Blood lipids, glucose, gamma-glutamyl transferase, were measured using automatic enzymatic procedures (Roche Diagnostics GmbH). BMI was calculated as weight in kilograms divided by height in meters squared. Waist circumference was measured in centimeters, at the level midway between the lower rib margin and the iliac crest with participants in standing position without heavy outer garments and with emptied pockets, breathing out gently. Blood pressure was calculated as the average of two consecutive measurements, realized in the sitting position at the right upper arm with a random-zero-sphygmomanometer. Hypertension was defined as a systolic blood pressure ≥140 mmHg or a diastolic blood pressure ≥90 mmHg or the use of blood pressure-lowering drugs prescribed for hypertension. Diabetes mellitus was defined as fasting plasma glucose level ≥7 mmol/L, non-fasting plasma glucose level ≥11.1 mmol/L (when fasting samples were absent) or the use of antidiabetic medications.

Statistical analysis

We prospectively assessed the association between thyroid parameters (TSH, FT_4, and TPOAb) and incident NAFLD, by using logistic regression models. Subsequently, we restricted the analyses to those with baseline FLI values <60, to minimize the possibility of misclassification of cases with incident NAFLD.

We explored differences in the risk of NAFLD throughout tertiles of FT_4, taking the highest tertile as reference. After our primary analyses, we performed sensitivity analyses, restricting to subjects with TSH and FT_4 within the reference ranges, excluding thyroid medication users and participants with previous thyroid surgery.

Next, we evaluated the risk of NAFLD throughout thyroid status categories of participants, taking euthyroid subjects as reference group. After excluding thyroid medication users and participants with previous thyroid surgery, we investigated the association between thyroid function/status and the risk of having a combination of NAFLD and LS ≥8.0 kPa.

After excluding thyroid medication users and participants with previous thyroid surgery, we cross-sectionally assessed the association between thyroid function and NAFLD, performing logistic regression analysis. Here, NAFLD was defined on basis of categorized FLI, in the absence of secondary causes of hepatic steatosis.

In longitudinal analyses, we first adjusted for age, sex, cohort, alcohol intake, smoking, and follow-up time (Model 1). Further adjustments were made for the use of lipid-lowering medications, total cholesterol, triglycerides, BMI, hypertension, and diabetes mellitus (Model 2). Lipids, BMI, hypertension, and diabetes mellitus could act as confounders as well as possible mediators depending on the presumed pathway through which thyroid function is related to NAFLD and therefore included in the multivariable model (Model 2). In mediation analyses, we calculated the percentage of excess risk mediated $((\text{odds ratio } [\text{OR}]_{\text{con adj}} - \text{OR}_{\text{con+med adj}})/(\text{OR}_{\text{con adj}} - 1)) \times 100\%$, where $\text{OR}_{\text{con adj}}$ is the confounder-adjusted OR and $\text{OR}_{\text{con+med adj}}$ is the confounder and mediator–adjusted OR.

In cross-sectional analyses, we adjusted for the aforementioned covariates, excluding lipids and BMI, as these variables are used to calculate FLI. High-density lipoprotein cholesterol and waist circumference were not included as covariates in the multivariable model to avoid multicollinearity. TSH was naturally log transformed in the continuous analyses in order to approximate a normal distribution. We checked for risk modification by adding an interaction term of the exposure (TSH or FT_4) with covariates of the multivariable model, but none of the interaction terms were significant. There was no departure from linearity for the TSH and FT_4 analyses, assessed by adding quadratic terms of covariates in the multivariable model. Multiple imputations were performed in case of missing covariates (<2% for all covariates). Statistical analyses were conducted using IBM SPSS version 21 (IBM Corp) and R statistical software (R-project, Institute for Statistics and Mathematics, R Core Team [2013], version 3.0.2). Reporting is done according to the Strengthening of the Reporting of Observational Studies in Epidemiology Statement.

RESULTS

We included a total of 9419 eligible participants with thyroid function measurements at baseline and data available on ultrasound-diagnosed NAFLD at follow-up or FLI at baseline.

Table 1 and Supplemental Table 1 summarize the baseline characteristics of included participants. The mean age was 64.7 years and 56.5% were females. Amongst 5324 participants in whom follow-up data were available, we documented 1763 cases of incident hepatic steatosis, of which 1217 cases of incident NAFLD (median follow-up time, 10.0 years; interquartile range, 5.7 to 10.9 years). A total of 546 subjects with hepatic steatosis had secondary causes, comprising 460 subjects with excessive alcohol consumption, 54 subjects with known steatosis-inducing drugs, 15 subjects with viral hepatitis, and 17 with combinations of the above. After excluding thyroid medication users and participants with previous thyroid surgery, reliable LS measurements were available in 805 participants with ultrasound-diagnosed NAFLD, of which 69 (8.6%) had LS ≥8.0 kPa.

Table 1. Baseline characteristics of 9419 participants*

Age, years	64.7 (9.7)
Women, n (%)	5321 (56.5)
Smoking, n (%)	
Current	1989 (21.1)
Past	4490 (47.7)
Never	2940 (31.2)
Use of lipid-lowering medications, n (%)	1508 (16.0)
Use of thyroid medication, n (%)	296 (3.1)
Total cholesterol, mmol/l	5.7 (1.0)
High-density lipoprotein cholesterol, mmol/l	1.4 (0.4)
Triglycerides, mmol/l	1.5 (0.8)
Body mass index, kg/m^2	27.2 (4.2)
Waist circumference, cm	93.7 (12.1)
Hypertension, n (%)	5881 (62.4)
Diabetes mellitus, n (%)	1073 (11.4)
TSH, mIU/L, median (IQR)	1.9 (1.3-2.8)
FT_4, ng/dL	1.2 (0.1)
TPOAb positive, n (%)	1240 (13.2)

*Data are mean (standard deviation), unless otherwise specified. Abbreviations: TSH, thyroid-stimulating hormone; IQR, interquartile range; FT_4, free thyroxine; TPOAb, thyroid peroxidase antibodies.

Thyroid function and the risk of NAFLD

The risk of NAFLD decreased gradually with higher FT_4 levels (OR, 0.33; 95% confidence interval [95% CI], 0.22 to 0.48 per 1ng/dL) (Table 2). These results remained similar after further adjustments for cardiovascular risk factors (OR, 0.42; 95% CI, 0.28 to 0.63), and also after restricting the analyses to participants with baseline FLI <60 (OR, 0.42; 95% CI, 0.24 to 0.74). In the multivariable-adjusted model, participants in the lowest FT_4 tertile had a 1.31 times higher risk of NAFLD, compared with those in the highest tertile (95% CI, 1.11 to 1.56; Supplemental Table 2). There was a positive linear association between TSH levels and NAFLD risk (OR, 1.09; 95% CI, 1.01 to 1.19 per 1 logTSH), which was attenuated after additional adjustment for cardiovascular risk factors (OR, 1.07; 95% CI, 0.98 to 1.17; Table 2). After separate and simultaneous additions of cardiovascular risk factors to Model 1, BMI and triglycerides were held accountable for the attenuation (Supplemental Table 3). The percentage of excess risk mediated by BMI and triglycerides was 22.2% in the association of TSH with

Table 2. Longitudinal association between thyroid function and NAFLD risk

	Events/TN	OR (95% CI) Model 1	OR (95% CI) Model 2
All participants			
TSH	1216/5321	**1.09 (1.01; 1.19)**	1.07 (0.98; 1.17)
FT_4	1217/5320	**0.33 (0.22; 0.48)**	**0.42 (0.28; 0.63)**
Baseline FLI < 60			
TSH	553/3379	**1.13 (1.00; 1.27)**	1.08 (0.95; 1.23)
FT_4	553/3376	**0.42 (0.24; 0.74)**	**0.52 (0.29; 0.92)**

Model 1: age, sex, cohort, alcohol intake, smoking, and follow-up time. Model 2: Model 1, use of lipid-lowering medications, total cholesterol, triglycerides, body mass index, hypertension, and diabetes mellitus. ORs of TSH are denoted per 1 unit increase of natural log transformed TSH (mIU/L). ORs of FT_4 are denoted per 1 unit increase in FT_4 (ng/dL). Abbreviations: NAFLD, nonalcoholic fatty liver disease; TN, total number; OR, odds ratio; CI, confidence interval; TSH, thyroid-stimulating hormone; FT_4, free thyroxine; FLI, fatty liver index.

Table 3. Longitudinal association of thyroid status with NAFLD risk

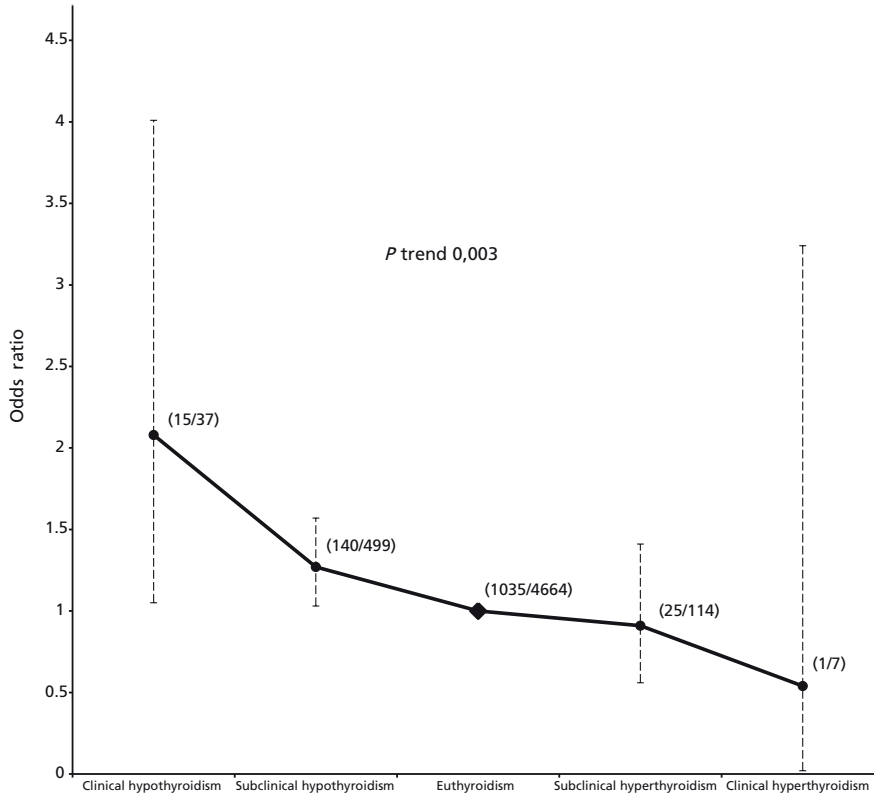
	Events/TN	OR (95% CI) Model 1	OR (95% CI) Model 2
Hypothyroidism*	155/536	**1.32 (1.08; 1.62)**	**1.24 (1.01; 1.53)**
Euthyroidism	1035/4664	1 (Reference)	1 (Reference)
Hyperthyroidism*	26/121	0.88 (0.56; 1.36)	0.88 (0.54; 1.37)

Model 1: age, sex, cohort, alcohol intake, smoking, and follow-up time. Model 2: Model 1, use of lipid-lowering medications, total cholesterol, triglycerides, body mass index, hypertension, and diabetes mellitus. * includes subclinical and clinical range. Abbreviations: NAFLD, nonalcoholic fatty liver disease; TN, total number; OR, odds ratio; CI, confidence interval.

NAFLD and 13.4% in the association of FT_4 with NAFLD; that is 22.2% and 13.4% of the respective associated effect size of TSH and FT_4 on NAFLD is explained by BMI and triglycerides. No significant association was observed for TPOAb and NAFLD risk (OR, 1.09; 95% CI, 0.89 to 1.32; Supplemental Table 2).

There was a significant trend (*P* for trend, 0.003) in the decrease of NAFLD risk, across categories of thyroid function from clinical hypothyroidism to clinical hyperthyroidism (OR from 2.08 to 0.54; Figure 1, Supplemental Table 4). Compared to euthyroidism, hypothyroidism was associated with a 1.24 times higher risk of NAFLD (95% CI, 1.01 to 1.53; Table 3).

Figure 1. Longitudinal association between thyroid status and NAFLD.



Point estimates for NAFLD (nonalcoholic fatty liver disease) were plotted against thyroid status of participants, taking euthyroid subjects as reference, after adjusting for age, sex, cohort, alcohol intake, smoking, follow-up time. Euthyroidism was defined as TSH (thyroid-stimulating hormone) within reference range (0.4 to 4.0 mIU/L); overt hypothyroidism as TSH >4.0 mU/L and FT_4 (free thyroxine) <0.85 ng/dL; subclinical hypothyroidism as TSH >4.0 mU/L and FT_4 0.85 to 1.95 ng/dL; overt hyperthyroidism as TSH <0.4 mU/L and FT_4 >1.95 ng/dL; subclinical hyperthyroidism as TSH <0.4 mU/L and FT_4 0.85 to 1.95 ng/dL. Dashed lines represent confidence intervals. Within brackets: NAFLD events/ Total number.

Cross-sectional analyses, based on categorized FLI, demonstrated a significant association of TSH (OR, 1.11; 95% CI, 1.04 to 1.18) and FT_4 (OR, 0.45; 95% CI, 0.34 to 0.60) with NAFLD (Supplemental Table 5). We found similar results in sensitivity analyses conducted only among euthyroid subjects, after excluding thyroid medication users and participants with previous thyroid surgery (Supplemental Table 2 and Supplemental Table 5).

Thyroid function and the risk of having a combination of NAFLD and LS ≥8 kPa

There was a positive association between TSH levels and the risk of having a combination of NAFLD and LS ≥8.0 kPa (OR, 1.55; 95% CI, 1.09 to 2.20). In line, higher FT_4 levels were associated with a lower risk of having a combination of NAFLD and LS ≥8.0 kPa, but not significantly (OR, 0.41; 95% CI, 0.09 to 1.73) (Table 4). The risk of having a combination of NAFLD and LS ≥8.0 kPa decreased gradually from hypothyroidism to hyperthyroidism (*P* for trend, 0.002) (Table 4).

Compared with euthyroidism, subclinical hypothyroidism was associated with a 2.30 times higher risk of having a combination of NAFLD and LS ≥8.0 kPa (95% CI, 1.12 to 4.31; Table 4). Results remained similar after further adjustments for

Table 4. Longitudinal association of thyroid function and status with the risk of having a combination of NAFLD and LS ≥8 kPa*†

	NAFLD with LS ≥8.0 kPa/ TN	OR (95% CI) Model 1	OR (95% CI) Model 2
Thyroid function and the risk of having combined NAFLD & LS ≥8.0 kPa			
TSH	69/4762	**1.55 (1.09; 2.20)**	**1.49 (1.04; 2.15)**
FT_4	69/4762	0.41 (0.09; 1.73)	0.59 (0.13; 2.59)
Thyroid status and the risk of having combined NAFLD & LS ≥8.0 kPa			
Clinical hypothyroidism	2/31	5.93 (0.93; 20.85)	**6.64 (1.04; 23.98)**
Subclinical hypothyroidism	11/408	**2.30 (1.12; 4.31)**	**2.14 (1.04; 4.07)**
Euthyroidism	55/4240	1 [Reference]	1 [Reference]
Subclinical hyperthyroidism	1/81	0.87 (0.04; 4.11)	0.80 (0.04; 3.91)
Clinical hyperthyroidism	NA	NA	NA
P value for trend		0.002	0.004

Model 1: age, sex, cohort, alcohol intake, smoking, and follow-up time. Model 2: Model 1, use of lipid-lowering medications, total cholesterol, triglycerides, body mass index, hypertension, and diabetes mellitus. *LS ≥8.0 kilopascals suggests clinically relevant fibrosis. †For this analysis, we excluded thyroid medication users and participants with previous thyroid surgery. ORs of TSH are denoted per 1 unit increase of natural log transformed TSH (mIU/L). ORs of FT_4 are denoted per 1 unit increase in FT_4 (ng/dL). Abbreviations: NAFLD, nonalcoholic fatty liver disease; LS, liver stiffness; kPa, kilopascals, TN, total number; OR, odds ratio; CI, confidence interval; TSH, thyroid-stimulating hormone; FT_4, free thyroxine; NA, not applicable.

cardiovascular risk factors (Table 4). In euthyroid subjects, higher TSH and lower FT_4 concentrations were associated with an increased risk of having a combination of NAFLD and LS ≥8.0 kPa, but not significantly (OR, 1.13; 95% CI, 0.63 to 2.03 for TSH; OR, 0.81; 95% CI, 0.11 to 5.75 for FT_4).

DISCUSSION

The current study is the first prospective population-based study to evaluate the relation between the whole spectrum of thyroid function and subsequent risk of NAFLD. We demonstrated a negative linear association between FT_4 levels and incident NAFLD, even among euthyroid subjects, as well as a positive linear association for TSH levels. Moreover, the risk of NAFLD progressively decreased from a hypothyroid to a hyperthyroid state. Hypothyroidism was associated with a higher NAFLD risk compared to euthyroidism. Lower thyroid function was also associated with an increased risk of having NAFLD with fibrosis. We demonstrate for the first time that subclinical hypothyroidism is associated with an increased risk of having NAFLD with fibrosis in the general population.

There are various pathways via which the beneficial effects of thyroid hormone on NAFLD risk can be mediated. Thyroid dysfunction is related to several cardiovascular risk factors that are in turn associated with an increased NAFLD risk (eg, higher BMI and dyslipidemia). When we add BMI and triglycerides into the model, the risk estimates of the association between thyroid function and NAFLD attenuate, indeed suggesting a mediating role of these factors.

Studies in rodents have demonstrated a regression of hepatic steatosis after treatment with liver-targeted thyroid hormone receptor agonists.[26-28] Thyroid hormone induces intrahepatic lipolysis through lipophagy, that involves the sequestration and degradation of lipid droplets within hepatic lysosomes.[29] Moreover, thyroid hormone receptor-mediated lipophagy enhances fatty acid oxidation, which may accelerate the clearance of liver lipids and reduce hepatosteatosis.[29]

Conversely, the decreased activity of hepatic lipases that occurs under hypothyroid conditions can promote NAFLD via decreased triglyceride clearance and hepatic triglyceride accumulation.[30] In addition, the insulin resistance state associated with hypothyroidism [31] can contribute to NAFLD by concomitantly inducing "de novo" lipogenesis and generating a flux of free fatty acids from adipose tissue to the liver.[32] Furthermore, decreased thyroid hormones might affect circulating levels of

adipocytokines, such as tumor necrosis factor-α, leptin and adiponectin.[32,33] Altered adipocytokines may then contribute to hepatic inflammation and fibrosis, by exerting direct hepatotoxic effects or promoting oxygen radicals.[34]

A putative role of thyroid autoimmunity has also been suggested in NAFLD pathogenesis, because various autoantibodies such as antinuclear antibodies and antismooth muscle antibodies, have been reported in patients with NAFLD.[35] However, our findings do not support this hypothesis, as there was no association between TPOAb and NAFLD.

Our findings consistently demonstrate that low thyroid function is associated with an increased risk of developing NAFLD, as well as higher risk of having NAFLD with fibrosis. Therefore, it can be hypothesized that a hypothyroid state might accelerate the progression of liver steatosis to fibrosis. Alternatively, low thyroid function might contribute on the development of liver fibrosis, independently of steatosis. Additional prospective research is needed to address these underlying mechanisms and possible mediating role of cardiovascular risk factors.

The results of the present study confirm a negative linear association between FT_4 levels and the risk of NAFLD. Based on the negative feedback regulation of hypothalamus-pituitary-thyroid axis, we would expect an analogous opposite association for TSH. Although there was a positive linear relationship between TSH levels and NAFLD risk, it attenuated among euthyroid subjects and after adjustment for cardiovascular risk factors. Several comparable studies exploring the association between thyroid function and different clinical end points have shown that FT_4, rather than TSH, is significantly related to the outcome risk,[18,36,37] particularly within the euthyroid range.[18,36] This may be ascribed to the distinct central and peripheral effects of thyroid hormone, as pituitary gland and liver differ in thyroid hormone transporters, receptors and deiodinases.[38] Also, genetic determinants and ageing can modify the TSH-FT_4 set point of the feedback mechanism, accounting for the weaker TSH-FT_4 association predominantly among euthyroid subjects.[39,40]

Our study has several important strengths. To our knowledge, it represents the first population-based prospective study to assess the effect of the whole spectrum of thyroid function on NAFLD and presence of clinically relevant fibrosis. The large sample size allowed us to conduct multiple sensitivity analyses. Other strengths include the extensive data on potential confounding factors and the laboratory assessment of thyroid parameters. In addition, we minimized the possibility of misclassification of cases with incident NAFLD, by excluding individuals with baseline

FLI ≥60 (thus highest probability of already having NAFLD), which however did not affect our results.

One limitation of our study is that we could not restrict the analysis to participants with baseline FLI values <30, due to a large sample size reduction (over 70% of the total population and over 80% of the NAFLD cases). Moreover, the diagnosis of NAFLD was based on ultrasonographic examination, whereas liver biopsy is considered the gold standard for the detection of mild steatosis or liver fibrosis. However, liver biopsies are not conducted routinely in NAFLD diagnosis and are considered unethical in population-based studies, because of invasiveness and potential complications. Also, abdominal ultrasonography has a sensitivity of 80-90% for detecting liver steatosis compared with histology, and its accuracy for diagnosing steatosis meets other imaging modalities.[41] In addition, transient elastography is considered reproducible and effective in liver fibrosis assessment.[24,25] Thyroid parameters were tested only at baseline and we lacked information regarding their variations over time. However, this would generate an underestimation of the association strength, rather than a spurious finding. Serum triiodothyronine measurements were not available in our study. Nevertheless, thyroid function is clinically defined by the combined TSH and FT_4 measurement. Furthermore, the generalizability of our findings to non-Caucasian populations remains uncertain. Finally, we cannot dismiss the possibility of residual confounding in an observational study design, even though we accounted for a large number of covariates.

Conclusions

Individuals with hypothyroidism are at increased risk of NAFLD compared with euthyroid subjects. The current study also reveals a negative linear association between FT_4 levels and the subsequent risk of NAFLD, even within the euthyroid reference range. Lower thyroid function is associated with an increased risk of fibrosis in NAFLD patients. Our findings highlight the need for future investigations on preventive measures (eg, screening of thyroid function in NAFLD patients) and possible therapeutic interventions (eg, decision of treatment in subclinical thyroid dysfunction).

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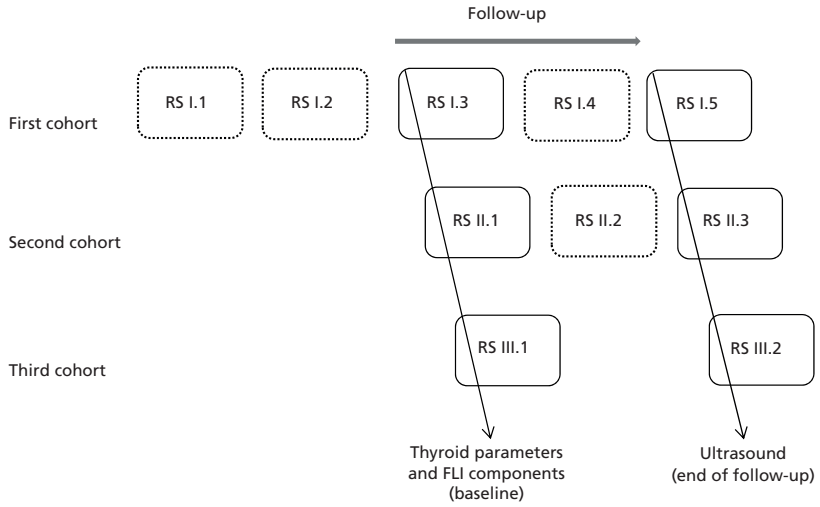
SUPPLEMENTAL MATERIAL

Supplemental Figure 1. Assessment of thyroid function and NAFLD in the Rotterdam Study.



Supplemental Table 1. Baseline characteristics of 9419 participants in three RS cohorts

	RS I	RS II	RS III
Total number	3694	2355	3370
Age, years	72.2 (6.8)	64.3 (7.7)	56.8 (6.7)
Women, n (%)	2139 (57.9)	1281 (54.4)	1901 (56.4)
Smoking, n (%)			
Current	628 (17.0)	468 (19.9)	893 (26.5)
Past	1849 (50.0)	1173 (49.8)	1468 (43.6)
Never	1217 (33.0)	714 (30.3)	1009 (29.9)
Use of lipid-lowering medications, n (%)	459 (12.4)	308 (13.1)	741 (22.0)
Use of thyroid medications, n (%)	104 (2.8)	70 (3.0)	122 (3.6)
Total cholesterol, mmol/l	5.8 (0.9)	5.7 (0.9)	5.5 (1.6)
HDL-C, mmol/l	1.4 (0.4)	1.4 (0.4)	1.4 (0.4)
Triglycerides, mmol/l	1.5 (0.7)	1.5 (0.8)	1.5 (0.9)
Body mass index, kg/m^2	26.8 (3.9)	27.1 (3.9)	27.7 (4.5)
Waist circumference, cm	93.4 (11.4)	93.7 (11.8)	93.8 (12.9)
Hypertension, n (%)	2816 (76.2)	1423 (60.4)	1642 (48.7)
Diabetes mellitus, n (%)	532 (14.4)	265 (11.3)	276 (8.2)
TSH, mIU/L, median (IQR)	1.8 (1.2-2.7)	1.8 (1.2-2.7)	2.02 (1.4-2.8)
FT_4, ng/dL	1.2 (0.1)	1.2 (0.1)	1.2 (0.1)
TPOAb positive, n (%)	473 (12.8)	326 (13.8)	441 (13.1)

*Data are mean (standard deviation), unless otherwise specified. Abbreviations: RS, Rotterdam Study; HDL-C, high density lipoprotein cholesterol; TSH, thyroid-stimulating hormone; IQR, interquartile range; FT_4, free thyroxine; TPOAb, thyroid peroxidase antibodies.

Supplemental Table 2. Longitudinal association of thyroid parameters with NAFLD risk

	Events/TN	OR (95% CI) Model 1	OR (95% CI) Model 2
FT_4 in tertiles and NAFLD risk			
All participants			
FT_4 (ng/dL) tertiles			
0.10-1.13	466/1774	**1.47 (1.25; 1.73)**	**1.31 (1.11; 1.56)**
1.14-1.27	420/1776	**1.31 (1.12; 1.55)**	**1.27 (1.07; 1.51)**
1.27-2.37	331/1770	1 [Reference]	1 [Reference]
P value for trend		<0.001	0.003
*Thyroid function within the reference ranges**			
FT_4 (ng/dL) tertiles			
0.85-1.14	385/1503	**1.54 (1.29; 1.84)**	**1.36 (1.12; 1.64)**
1.14-1.27	339/1500	**1.33 (1.11; 1.60)**	**1.25 (1.05; 1.53)**
1.28-1.89	264/1501	1 [Reference]	1 [Reference]
P value for trend		<0.001	0.003
Thyroid function within the reference ranges* and NAFLD risk			
TSH	988/4504	0.97 (0.83; 1.13)	0.92 (0.78; 1.08)
FT_4	988/4504	**0.25 (0.15; 0.41)**	**0.37 (0.22; 0.63)**
TPOAb and NAFLD risk			
TPOAb	1217/5316	1.10 (0.91; 1.31)	1.09 (0.89; 1.32)†

Model 1: age, sex, cohort, alcohol intake, smoking, and follow-up time. Model 2: Model 1, use of lipid-lowering medications, total cholesterol, triglycerides, body mass index, hypertension, and diabetes mellitus. *Normal reference ranges of thyroid function were defined as serum TSH levels of 0.4 to 4.0 mIU/L and serum FT_4 levels of 0.85 to 1.95 ng/dL. For this analysis, we excluded thyroid medication users and participants with previous thyroid surgery. †Additionally adjusted for lnTSH. ORs of TSH are denoted per 1 unit increase of natural log transformed TSH (mIU/L). ORs of FT_4 are denoted per 1 unit increase in FT_4 (ng/dL). Abbreviations: NAFLD, nonalcoholic fatty liver disease; TN, total number; OR, odds ratio; CI, confidence interval; TSH, thyroid-stimulating hormone; FT_4, free thyroxine; TPOAb, thyroid peroxidase antibodies.

Supplemental Table 3. Longitudinal association of thyroid function with NAFLD risk

	OR (95% CI) Model 1	OR (95% CI) Model 2[a]	OR (95% CI) Model 2[b]
TSH	**1.09 (1.01; 1.19)**	**1.09 (1.01; 1.19)**	1.07 (0.98; 1.17)
FT_4	**0.33 (0.22; 0.48)**	**0.32 (0.22; 0.48)**	**0.42 (0.28; 0.63)**

Model 1: age, sex, cohort, alcohol intake, smoking, and follow-up time. Model 2[a]: Model 1, use of lipid-lowering medications, total cholesterol, hypertension, and diabetes mellitus; Model 2[b]: Model 2[a], triglycerides, and body mass index. ORs of TSH are denoted per 1 unit increase of natural log transformed TSH (mIU/L). ORs of FT_4 are denoted per 1 unit increase in FT_4 (ng/dL). Abbreviations: NAFLD, nonalcoholic fatty liver disease; OR, odds ratio; CI, confidence interval; TSH, thyroid-stimulating hormone; FT_4, free thyroxine.

Supplemental Table 4. Longitudinal association of thyroid status with NAFLD risk

	NAFLD/TN	Median TSH (IQR)	Mean FT_4 (Extreme values)	OR (95% CI)
Clinical hypothyroidism	15/37	17.19 (8.54-40.75)	0.64 (0.10-0.84)	**2.08 (1.05; 4.01)**
Subclinical hypothyroidism	140/499	5.15 (4.44-6.43)	1.13 (0.86-1.67)	**1.27 (1.03; 1.57)**
Euthyroidism	1035/4664	1.86 (1.34-2.54)	1.21 (0.74-2.07)	1 [Reference]
Subclinical hyperthyroidism	25/114	0.17 (0.06-0.31)	1.36 (0.90-1.91)	0.91 (0.56; 1.41)
Clinical hyperthyroidism	1/7	0.10 (0.01-0.16)	2.09 (1.97-2.37)	0.54 (0.02; 3.24)
				P for trend 0.003

Adjusted for age, sex, cohort, alcohol intake, smoking, and follow-up time. Abbreviations: NAFLD, nonalcoholic fatty liver disease; TN, total number; TSH, thyroid-stimulating hormone; IQR, interquartile range; FT_4, free thyroxine; OR, odds ratio; CI, confidence interval.

Supplemental Table 5. Cross-sectional association between thyroid function and NAFLD defined by FLI*

	Total number	OR (95% CI) Model 1	OR (95% CI) Model 2
All participants			
TSH	8777	**1.11 (1.05; 1.19)**	**1.11 (1.04; 1.18)**
FT_4	8779	**0.45 (0.34; 0.59)**	**0.45 (0.34; 0.60)**
Thyroid function within the reference ranges†			
TSH	7668	**1.12 (1.01; 1.24)**	1.11 (0.99; 1.24)
FT_4	7668	**0.41 (0.29; 0.57)**	**0.41 (0.29; 0.58)**

Model 1: age, sex, cohort, alcohol intake, and smoking. Model 2: Model 1, hypertension, and diabetes mellitus. *For this analysis, we excluded thyroid medication users and participants with previous thyroid surgery. NAFLD was based on categorized baseline FLI, with 60 as a cutoff, in the absence of secondary causes of hepatic steatosis (>14 alcoholic beverages weekly, viral hepatitis, use of fatty liver inducing pharmacological agents). †Normal reference ranges of thyroid function were defined as serum TSH levels of 0.4 to 4.0 mIU/L and serum FT_4 levels of 0.85 to 1.95 ng/dL. ORs of TSH are denoted per 1 unit increase of natural log transformed TSH (mIU/L). ORs of FT_4 are denoted per 1 unit increase in FT_4 (ng/dL). Abbreviations: NAFLD, nonalcoholic fatty liver disease; FLI, fatty liver index; OR, odds ratio; CI, confidence interval; TSH, thyroid-stimulating hormone; FT_4, free thyroxine.