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Higher free triiodothyronine is associated with non-alcoholic fatty liver disease in euthyroid subjects: the Lifelines Cohort Study

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ABSTRACT

Objective. Overt hypothyroidism confers an increased risk of non-alcoholic fatty liver disease (NAFLD). The liver plays a crucial role in the metabolism of cholesterol and triglycerides; thyroid hormones interact on hepatic lipid homeostasis. Thyroid function within the euthyroid range affects a number of health issues, including atherosclerosis development and biochemical markers of increased cardiovascular risk. However, the association of thyroid hormones with NAFLD in euthyroid subjects has not been unequivocally established. We therefore determined associations of thyroid hormone parameters with NAFLD among euthyroid subjects.

Methods. The study was conducted in the Lifelines Cohort Study, a population-based cohort study of participants living in the North of the Netherlands. Only euthyroid subjects (thyroid-stimulating hormone (TSH) 0.5–4.0 mU/L, free thyroxine (FT4) 11–19.5 pmol/L and free triiodothyronine (FT3) 4.4–6.7 pmol/L) older than 18 years were included. Exclusion criteria were participants with excessive alcohol use, known hepatitis or cirrhosis, liver functions ≥ three times the upper limit, current cancer, non-white ancestry, previous or current use of thyroid medication and current use of lipid or glucose lowering medication. A priori defined liver biochemistry, thyroid function parameters and metabolic syndrome (MetS) were studied. NAFLD was defined by using the validated Fatty Liver Index (FLI); FLI ≥ 60 was categorized as NAFLD. A $P < 0.01$ was considered significant.

Results. FLI ≥ 60 was found in 4274 (21.1%) of 20,289 individuals (62.1% male, median age 46 years) with increased prevalence of MetS ($P < 0.0001$). In age- and sex-adjusted analysis FLI ≥ 60 was independently associated with a higher FT3 (OR 1.34, 95% CI 1.29–1.39, per SD increment, $P < 0.0001$) and a lower FT4 (OR 0.73, 95% CI 0.70–0.75, $P < 0.0001$) but not by TSH.

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; DIO, iodothyronine deiodinase; FLI, Fatty Liver Index; FT3, free triiodothyronine; FT4, free thyroxine; GGT, gamma-glutamyl transferase; HDL, high-density lipoprotein; IQR, interquartile range; MetS, metabolic syndrome; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NCEP ATP III, National Cholesterol Education Program Adult Treatment Panel III; NFS, NAFLD fibrosis score; OR, odds ratio; SD, standard deviation; TG, triglycerides; TSH, thyroid-stimulating hormone; VLDL, very low density lipoproteins.

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The strongest association was found for the FT3/FT4 ratio (OR 1.44, 95% CI 1.39–1.49, $P < 0.0001$). These associations remained similar after additional adjustment for the presence of MetS. In subjects with enlarged waist circumference, TSH and FT4 were lower while FT3 was higher, resulting in an increased FT3/FT4 ratio ($P < 0.0001$).

Conclusions. Euthyroid subjects with suspected NAFLD are characterized by higher FT3, lower FT4 and higher FT3/FT4 ratio, probably consequent to central obesity.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is defined as the presence of hepatic steatosis in the absence of excessive alcohol consumption [1]. NAFLD includes a broad spectrum of pathology ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), fibrosis and cirrhosis, while it also predisposes to hepatocellular carcinoma. NAFLD is considered to reflect the hepatic component of the metabolic syndrome (MetS), since there is a strong association with insulin resistance, (central) obesity, dyslipidemia and hypertension [2]. As a consequence of the obesity epidemic, NAFLD is the leading cause of chronic liver disease in the Western world. It is estimated that NAFLD occurs in 20%–30% of European adults [3]. Therefore, it is expected that subjects with NAFLD will require further identification of concurrent NASH and/or fibrosis to be a prominent target for lifestyle modification and pharmacological intervention in the near future [4].

The liver plays a crucial role in the metabolism of cholesterol and triglycerides [5], with hepatic fat accumulation being regarded as the driving force of elevated plasma triglycerides [6]. Importantly, thyroid hormones interact on hepatic lipid homeostasis through multiple pathways, including stimulation of free fatty acid delivery to the liver for re-esterification to triglycerides, and increasing fatty acid β-oxidation, thereby affecting hepatic fat accumulation [5,7–10].

Several studies have demonstrated an association between overt thyroid dysfunction and NAFLD. Subjects with hypothyroidism are about 1.5 to 2 times more likely to have biopsy-proven or ultrasonography-confirmed NAFLD [11,12]. Accordingly, a recent longitudinal study demonstrated that (subclinical) hypothyroidism is associated with NAFLD risk [13]. A systematic review indeed suggested a relation between NAFLD and hypothyroidism, although such an effect has not consistently been reported [14]. There are, however, only a few small studies, which aimed to assess the association of NAFLD with variations in thyroid function within the euthyroid range [15–19]. Higher thyroid-stimulating hormone (TSH) levels within the euthyroid range were found in subjects with NAFLD but may also relate to attenuated serum alanine aminotransferase (ALT) elevations in the context of MetS and insulin resistance [15,16]. In euthyroid subjects with NAFLD, lower free thyroxine (FT4) levels were found in some studies [15,17,18], whereas a higher free triiodothyronine (FT3) level was found in middle-aged Chinese subjects [19].

Given the importance of variations in thyroid function within the euthyroid range for a considerable number of health issues, including (subclinical) atherosclerosis and altered levels of pro-atherogenic biochemical markers [5,20], it is relevant to examine the relationship of NAFLD with thyroid function parameters in an euthyroid population. In the present cross-sectional study, we aimed to determine the relationship of NAFLD with TSH, FT4 and FT3 among participants of the Lifelines Cohort Study, representative of the general population from the North-Eastern region of the Netherlands.

2. Material and Methods

2.1. *Study Design*

The present cross-sectional study is conducted in the framework of the Lifelines Cohort Study. The Lifelines Cohort Study is a multi-disciplinary prospective population-based cohort study examining in a unique three-generation design the health and health-related behaviors of 167,729 persons living in the North-Eastern region of the Netherlands. It employs a broad range of investigative procedures in assessing the biomedical, socio-demographic, behavioral, physical and psychological factors which contribute to the health and disease of the general population, with a special focus on multi-morbidity and complex genetics [21]. All participants provided written informed consent. The medical ethics committee of the University of Groningen, the Netherlands, approved the study conforming to the Declaration of Helsinki.

2.2. *Participants*

We included subjects of Western-European origin and all participants were aged between 18 and 85 years at time of enrollment. Only euthyroid subjects participated in the present study. Euthyroidism was defined as a TSH level between 0.5 and 4.0 mU/L, FT4 level between 11 and 19.5 pmol/L and FT3 level between 4.4 and 6.7 pmol/L, i.e. within their respective institutional reference ranges. Eligible subjects had liver enzyme values <3 times the upper reference limit, i.e. for aspartate aminotransferase (AST) < 120 U/L, alanine aminotransferase (ALT) < 135 U/L, gamma-glutamyl transferase (GGT) < 165 U/L and alkaline phosphatase (ALP) < 360 U/L. Additional exclusion criteria were: missing data required to calculate the Fatty Liver Index (FLI, as outlined below) and to determine the presence of metabolic syndrome (MetS) and its components and non-white ancestry (participants were assumed to be immigrant if his/her birth country or that of one or both parents was outside the Netherlands). The representativeness of the Lifelines cohort for the North Netherlands population has been previously validated [22]. Further exclusion criteria were: excessive alcohol use (>1 alcoholic drink per day for women and >2 alcoholic drinks per day for men [23]), previously diagnosed hepatitis and/or cirrhosis, current cancer, previous or current use of thyroid medication and the use of lipid lowering or glucose lowering medication (including oral glucose lowering medication and insulin). Information about nationality, alcohol consumption, hepatitis B virus infection, liver cirrhosis, current cancer and medication use was extracted from the self-administered questionnaires.

2.3. *Data Collection*

Data collection of the Lifelines Cohort Study started in 2006 and is ongoing. Questionnaires were collected, anthropometry and blood pressure were measured and biomaterial (blood) was collected at the Lifelines research sites. A standardized protocol was used to obtain blood pressure and anthropometric measurements (height, weight and waist circumference). Systolic and diastolic blood pressures were measured 10 times during a period of 10 min, using an automated Dinamap Monitor (GE Healthcare, Freiburg, Germany). The size of the cuff was chosen according to the arm circumference. The average of the final three readings was used for each blood pressure parameter. Anthropometric measurements were measured without shoes. Body weight was measured to the nearest 0.5 kg. Height and waist circumference were measured to the nearest 0.5 cm. Height was measured with a stadiometer placing their heels against the rod and the head in Frankfort Plane position. Waist circumference was measured in standing position with a tape measure all around the body at the level midway between the lower rib margin and the iliac crest [21]. Venous blood samples were collected in the fasting state between 8.00 and 10.00 a.m. and collected into heparin-containing tubes, and centrifuged at 1885 ×*g* and the plasma aliquots were processed for laboratory measurements at the same day and stored at −80 °C. TSH, FT4 and FT3 were measured by electrochemiluminescent immunoassays on a Roche Modular E170 analyzer, using kits provided by the manufacturer (Roche, Mannheim, Germany). High-density lipoprotein (HDL) cholesterol and triglycerides (TG) were measured using routine procedures on a Roche Modular P chemistry analyzer. Glucose was assayed by the hexokinase method. GGT, ALP, ALT and AST were routinely measured according to the recommendations of the International Federation of Clinical Chemistry on a Roche Modular platform. ALT and AST were measured with pyridoxal phosphate activation. All laboratory measurements were performed at the Department of Laboratory Medicine of the University Medical Center Groningen, the Netherlands [21].

2.4. *Definitions and Calculations*

In order to categorize subjects with a high probability for the diagnosis of NAFLD the Fatty Liver Index (FLI) was used. FLI was calculated according to the formula published by Bedogni [24]. $FLI = (e^{0.953*loge\ (triglycerides)\ +\ 0.139*BMI\ +\ 0.718*loge\ (GGT)\ +\ 0.053*waist\ circumference\ -\ 15.745})/(1 + e^{0.953*loge\ (triglycerides)\ +\ 0.139*BMI\ +\ 0.718*loge\ (GGT)\ +\ 0.053*waist\ circumference\ -\ 15.745})*100$. The optimal cut-off value for the FLI has been documented to be 60 with an accuracy of 0.84, a sensitivity of 61% and a specificity of 86% for detecting NAFLD as determined by ultrasonography [24]. An FLI ≥ 60 was categorized as NAFLD. The 2016 EASL-EASD-EASO NAFLD guideline recommends that for larger scale screening studies, serum biomarkers are the preferred diagnostic with the FLI currently being considered as one of the best-validated steatosis scores [23]. To identify NAFLD patients with advanced fibrosis, the NAFLD fibrosis score (NFS) was used. To calculate the NAFLD fibrosis score the formula published by Angulo et al. was used [25]. *NAFLD Fibrosis Score (NFS) = (−1.675 + 0.037 × age (year) + 0.094 × BMI (kg/m^2) + 1.13 × fasting glucose/presence of diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio − 0.013 × platelet count (×10^9/L) − 6.6 × albumin (g/L)*. By applying a cutoff score > 0.676, the presence of advanced fibrosis could be diagnosed with a sensitivity of 43%, specificity of 96% and positive predictive value of 82% [25]. The NFS is currently considered to be one of the best validated biomarkers to diagnose fibrosis among NAFLD subjects [23,26,27].

Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). MetS was defined by the revised diagnostic criteria from the American Heart Association by the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) [28]. The NCEP ATP III criteria consist of five criteria for the MetS: *(1)* an enlarged waist circumference (≥102 cm for males and ≥88 cm for females); *(2)* elevated triglycerides (TG) (≥1.7 mmol/L); *(3)* low HDL cholesterol (<1.0 mmol/L in males and <1.3 mmol/L in females); *(4)* elevated blood pressure (systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg) and/or medication use for hypertension; *(5)* elevated fasting plasma glucose (≥5.6 mmol/L). Participants were diagnosed with the MetS when at least three out of five criteria were present. Diabetes was defined as a fasting plasma glucose ≥7.0 mmol/L.

2.5. *Statistical Analysis*

SPSS 22 (version 22.0, SPSS, Chicago, IL, USA) was used for data analysis. Normality of distribution was assessed and checked for skewness. Descriptive data are expressed in medians with interquartile ranges (IQRs) for continuous variables or in numbers with percentages. Between group differences in variables were tested with Mann–Whitney U test and categorical variables were analyzed with Chi-square test. Multivariable logistic regression analysis was performed to disclose the independent association of FLI with thyroid function parameters, MetS and individual MetS components. Separate models were made to determine associations of FT4 and FT3 and the FT3/FT4 ratio with FLI ≥ 60. All models were adjusted for age and sex. For continues variables a Z-score transformation was performed and these standardized variables were used in multivariable analysis. Odds ratios (ORs) are given per 1 standard deviation (SD) change in TSH, FT4, FT3 and the FT3/FT4 ratio with 95% confidence intervals (CIs). An FLI ≥ 60 and NFS > 0.676 were taken as the optimal cut-off for NAFLD [24] and fibrosis categorization, respectively [25]. In addition, a sensitivity analysis was performed with an FLI ≥ 90 as cut-off, corresponding to a specificity of NAFLD of 99% [24]. Given multiple comparisons, two-sided *P*-values <0.01 were considered statistically significant.

3. Results

Of the whole Lifelines Cohort Study, 40,856 participants were initially considered eligible with the required biomedical data concerning FLI, TSH, FT3 and FT4, liver enzymes and MetS classification. After applying the exclusion criteria (Fig. 1) the final study group consisted of 20,289 euthyroid subjects. Fig. 1 shows the flow chart for selection of participants.

An FLI ≥ 60 was observed in 4274 participants (21.1% of the study group). Subjects with an FLI ≥ 60 were older, were more likely to be male (62.1%) and to fulfill the criteria for MetS (46.7% vs. 4.2%) (Table 1). ALT, AST, ALP and GGT values were all higher in subjects with an FLI ≥ 60 (all $P < 0.0001$). FT4 levels were lower, whereas FT3 and the FT4/FT3 ratio were higher in subjects with an FLI ≥ 60 ($P < 0.0001$ for each). All these differences between subjects with and without an FLI ≥ 60 remained present after adjustment for age and sex. TSH levels did not differ according to FLI categorization.

In age- and sex- adjusted analysis an FLI ≥ 60 was independently associated with a higher FT3 (OR 1.34, 95% CI 1.29–1.39, $P < 0.0001$) and a lower FT4 (OR 0.73, 95% CI 0.70–0.75, $P < 0.0001$), but not with the TSH level (Table 2; model 1). In alternative analysis, an FLI ≥ 60 was associated with a higher FT3/FT4 ratio (OR 1.44, 95% CI 1.39–1.49, $P < 0.0001$) (Table 2; model 2). In subsequent analyses now also including the presence of the MetS (Table 3) or the individual MetS components (Table 4), similar independent associations of an FLI ≥ 60 with higher FT3, lower FT4 (model 1) or a higher FT3/FT4 ratio (model 2) were found. In the analyses including MetS (components) the associations of an FLI ≥ 60 with FT4, FT3 (model 1) and the FT3/FT4 ratio (model 2) were modestly attenuated. Besides a strong association of an FLI ≥ 60 with the presence of MetS (OR 17.91, 95% CI 16.17–19.82, $P < 0.0001$) (Table 3; model 1 and 2), an FLI ≥ 60 was also independently associated with each of the individual MetS components (Table 4; model 1 and 2).

To put the association of an FLI ≥ 60 in the context of central obesity, thyroid function parameters were compared between subjects with and without enlarged waist circumference (Table 5). TSH and FT4 levels were lower, but FT3 was higher in subjects with an enlarged waist circumference ($P < 0.0001$ for each). As a result, the FT3/FT4 ratio was highest in subjects with an enlarged waist circumference.

In a sensitivity analyses, age- and sex-adjusted associations of an FLI ≥ 90 with FT4, FT3 and the FT3/FT4 ratio were also found; these associations remained present after additional adjustment for the presence of MetS (Table 6).

An NFS > 0.676 was found in 49 participants (0.2%) of the whole study population (Table 7). In subjects with NFS > 0.676, TSH, FT4, FT3 and the FT3/FT4 ratio were not different in subjects with an elevated FLI vs. subjects without elevated FLI (Table 7). Among subjects with NFS < 0.676, an elevated FLI was associated with a lower FT4 and higher FT3 and FT3/FT4 ratio (Table 7).

4. Discussion

This large population-based study among strictly euthyroid individuals has demonstrated that NAFLD (defined by FLI ≥ 60) is associated with a higher FT3 and a lower FT4 level. The strongest association with an elevated FLI score was observed for the FT3/FT4 ratio. The relationships of an elevated FLI score with FT3, FT4 and the FT3/FT4 ratio were independent of age, sex, the presence of MetS and its individual components. Furthermore, the FT3/FT4 ratio was higher in subjects with an enlarged waist circumference. Taken together, the present results are in agreement with the possibility that higher FT3 levels within the euthyroid range may contribute to hepatic fat accumulation probably in the context of central obesity.

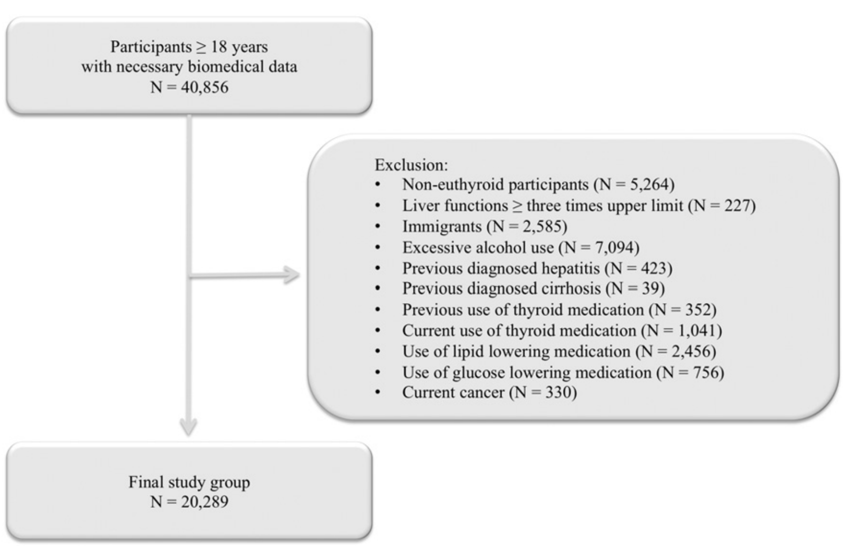

Fig. 1 – Flow chart of the study population.

Table 1 – Clinical and laboratory characteristics in subjects with and without non-alcoholic fatty liver disease estimated by the Fatty Liver Index (FLI) ≥ 60.

	FLI < 60 (N = 16,015)	FLI ≥ 60 (N = 4274)	P-value	P-value (adjusted for age and sex)
Age (years)	42 (33–49)	46 (39–51)	<0.0001	
Sex (male/female)	6188 (38.6%)/9827 (61.4%)	2655 (62.1%)/1619 (37.9%)	<0.0001	
Metabolic syndrome	668 (4.2%)	1994 (46.7%)	<0.0001	<0.0001
Enlarged waist circumference (yes)	3457 (21.6%)	3379 (79.1%)	<0.0001	<0.0001
Hyperglycemia				
≥5.6 mmol/L	998 (6.2%)	1073 (25.1%)	<0.0001	<0.0001
≥7.0 mmol/L	45 (0.3%)	88 (2.1%)	<0.0001	<0.0001
Hypertension (yes)	4677 (29.2%)	2475 (57.9%)	<0.0001	<0.0001
Elevated triglycerides (yes)	899 (5.6%)	1861 (43.5%)	<0.0001	<0.0001
Low HDL cholesterol (yes)	2436 (15.2%)	1864 (43.6%)	<0.0001	<0.0001
AST (U/L)	22 (19–26)	25 (21–29)	<0.0001	<0.0001
ALT (U/L)	18 (14–24)	28 (20–39)	<0.0001	<0.0001
GGT (U/L)	18 (14–24)	33 (24–48)	<0.0001	<0.0001
ALP (U/L)	58 (49–69)	67 (57–79)	<<0.0001	<0.0001
TSH (mU/L)	2.01 (1.49–2.65)	2.04 (1.52–2.66)	0.15	0.05
FT4 (pmol/L)	15.70 (14.60–17.00)	15.40 (14.20–16.70)	<0.0001	<0.0001
FT3 (pmol/L)	5.20 (4.90–5.60)	5.30 (5.00–5.70)	<0.0001	<0.0001
FT3/FT4 ratio	0.33 (0.31–0.36)	0.35 (0.32–0.38)	<0.0001	<0.0001

Data are given in median with interquartile ranges (IQRs) or in numbers with percentages. For continuous variables Mann–Whitney U tests and for binary variables Chi square tests were used. Age- and sex-adjusted P-values were obtained by multivariable linear regression analysis. Fatty Liver Index wax calculated by; FLI = (e $^{0.953*\text{loge (triglycerides)} + 0.139*\text{BMI} + 0.718*\text{loge (GGT)} + 0.053*\text{waist circumference} - 15.745}$)/(1 + e $^{0.953*\text{loge (triglycerides)} + 0.139*\text{BMI} + 0.718*\text{loge (GGT)} + 0.053*\text{waist circumference} - 15.745}$)*100. Metabolic syndrome was defined according to NCEP ATPIII criteria. Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; FT4, free thyroxine; FT3, free triiodothyronine; GGT, gamma-glutamyl transferase; HDL cholesterol, High Density Lipoprotein cholesterol; TSH, thyroid-stimulating hormone.

In the interpretation of the current and previous studies on the relation of thyroid function and NAFLD it should be emphasized that each individual probably has a rather narrow set-point of thyroid function status [5]. This concept underscores the clinical utility of a single dataset of thyroid hormone levels in the evaluation of cardiometabolic disorders. The relationship of FT3 with NAFLD has been studied in a few studies that all used ultrasound for establishing NAFLD [15,17–19]. Except for a study in Chinese subjects, which were also selected to have TSH, FT4 and FT3 levels each within their respective reference range, an association between NAFLD and a higher FT3 has not been previously reported. In this report, TSH and FT4 were not different between subjects with and without NAFLD [19]. In other studies, variable associations of FT4 and TSH within the euthyroid range and NAFLD have been documented with some reports showing an association with lower FT4 [17,18], which is in keeping with the present results, or higher TSH levels [15]. In one of these reports subjects with (mild) thyroid function abnormalities were not excluded [18], whereas in other studies euthyroidism was defined as a TSH and an FT4 level within the reference range without taking the FT3 level into account [13,17]. Thus, it seems plausible that patient selection could at least in part explain the discrepancies between the present and these earlier reports [15,17,18].

Triiodothyronine is commonly believed to be more biologically active as a regulator of metabolic processes than is prohormone, thyroxine [29,30]. Thus from a pathophysiological

Table 2 – Multivariable logistic regression analyses demonstrating independent associations of non-alcoholic fatty liver disease estimated by the Fatty Liver Index (FLI) ≥ 60 with thyroid function parameters.

	Model 1			Model 2		
	OR	95% CI	P-value	OR	95% CI	P-value
Age (years)	1.03	1.03–1.04	<0.0001	1.03	1.03–1.04	<0.0001
Sex (male vs. female)	2.33	2.17–2.51	<0.0001	2.35	2.19–2.52	<0.0001
TSH (per SD)	1.02	0.98–1.06	0.31	1.02	0.98–1.06	0.32
FT4 (per SD)	0.73	0.70–0.75	<0.0001			
FT3 (per SD)	1.34	1.29–1.39	<0.0001			
Ratio FT3/FT4 (per SD)				1.44	1.39–1.49	<0.0001

OR: odds ratio. 95% CI: 95% confidence interval. ORs are given per year increase in age, for men vs. women and per 1 SD increase in TSH, FT4, FT3 and the FT3/FT4 ratio and with 95% CIs. Binary logistic regression analysis was used. All models are mutually adjusted for the variables included in the analyses. Fatty Liver Index wax calculated by; FLI = (e $^{0.953*\text{loge (triglycerides)} + 0.139*\text{BMI} + 0.718*\text{loge (GGT)} + 0.053*\text{waist circumference} - 15.745}$)/(1 + e $^{0.953*\text{loge (triglycerides)} + 0.139*\text{BMI} + 0.718*\text{loge (GGT)} + 0.053*\text{waist circumference} - 15.745}$)*100. Abbreviations: FT4, free thyroxine; FT3, free triiodothyronine; TSH, thyroid-stimulating hormone.

Table 3 – Multivariable logistic regression analyses demonstrating independent associations of non-alcoholic fatty liver disease estimated by the Fatty Liver Index (FLI) ≥ 60 with thyroid function parameters and presence of the metabolic syndrome.

	Model 1			Model 2		
	OR	95% CI	P-value	OR	95% CI	P-value
Age (years)	1.02	1.02–1.02	<0.0001	1.02	1.02–1.03	<0.0001
Sex (male vs. female)	2.43	2.23–2.64	<0.0001	2.39	2.20–2.60	<0.0001
TSH (per SD)	1.01	0.97–1.05	0.59	1.01	0.97–1.06	0.52
Free T4 (per SD)	0.76	0.73–0.80	<0.0001			
Free T3 (per SD)	1.23	1.18–1.29	<0.0001			
Ratio T3/T4 (per SD)			<0.0001	1.34	1.29–1.40	<0.0001
MetS (yes/no)	17.91	16.17–19.82	<0.0001	17.86	16.13–19.77	<0.0001

OR: odds ratio. 95% CI: 95% confidence interval. ORs are given per year increase in age, for men vs. women, and per 1 SD in TSH, FT4, FT3 and the FT3/FT4 ratio and with 95% CIs. All models are mutually adjusted for the variables included in the analyses. Fatty Liver Index wax calculated by; FLI = $(e^{0.953*\log_e(\text{triglycerides}) + 0.139*\text{BMI} + 0.718*\log_e(\text{GGT}) + 0.053*\text{waist circumference} - 15.745})/(1 + e^{0.953*\log_e(\text{triglycerides}) + 0.139*\text{BMI} + 0.718*\log_e(\text{GGT}) + 0.053*\text{waist circumference} - 15.745})*100$. Metabolic Syndrome was defined according to NCEP ATPIII criteria. Abbreviations: FT4, free thyroxine; FT3, free triiodothyronine; MetS, metabolic syndrome; TSH, thyroid-stimulating hormone.

perspective, the association of NAFLD with a higher FT3 rather than with a lower FT4 should be regarded as the most relevant. Multiple interdependent pathways involved in lipid metabolism are affected by thyroid hormone status. T3 conceivably modifies the accumulation of lipids in liver tissue, and hence is probably involved in the pathogenesis of NAFLD [9]. T3 stimulates lipolysis in adipose tissue, thereby enhancing the availability of free fatty acids, which are subsequently used as substrates for triglyceride synthesis in the liver [8,31,32]. Furthermore, T3 is able to stimulate de novo lipogenesis in the liver [33]. In agreement, hepatic lipogenesis is increased in subjects with hyperthyroidism [32]. On the other hand, a liver-targeted agonist of thyroid hormone receptor β, the subunit that is naturally expressed in hepatocytes, has been shown to diminish hepatic fat accumulation in animal studies [34]. In addition, T3 stimulates beta-oxidation of fatty acids which is anticipated to oppose hepatic lipid accumulation [9,35]. Of note, this process involves hepatic autophagy, which in the long run promotes hepatocyte damage [35], along with excessive production of reactive oxygen species by mitochondria [7,34]. Finally, thyroid hormone action may also directly affect the hepatic secretion of triglyceride-rich lipoproteins. Hyperthyroidism impairs the release of very low density lipoproteins (VLDLs) from perfused rat liver [36], whereas subclinical hypothyroidism results in increased hepatic VLDL triglyceride secretion [37]. Taken together, it is plausible that partly opposing effects of T3 on various metabolic pathways involved in hepatic lipid metabolism affect the overall effect of thyroid hormone status on hepatic fat accumulation apparently resulting in an increased prevalence of NAFLD in the context of higher FT3 levels within the euthyroid range. Remarkably among subjects with fibrosis, an elevated FLI was not

Table 4 – Multivariable logistic regression analyses demonstrating independent associations of non-alcoholic fatty liver disease estimated by the Fatty Liver Index (FLI) ≥ 60 with thyroid function parameters and the presence of individual metabolic syndrome components.

	Model 1			Model 2		
	OR	95% CI	P-value	OR	95% CI	P-value
Age (years)	1.01	1.00–1.01	0.02	1.01	1.00–1.01	0.01
Sex (male vs. female)	10.52	9.13–12.13	<0.0001	10.38	9.03–11.92	<0.0001
TSH (per SD)	1.05	1.00–1.11	0.04	1.05	1.00–1.11	0.04
Free T4 (per SD)	0.81	0.77–0.86	<0.0001			
Free T3 (per SD)	1.17	1.11–1.23	<0.0001			
Ratio T3/T4 (per SD)				1.25	1.19–1.32	<0.0001
Enlarged waist circumference (yes/no)	55.55	47.84–64.51	<0.0001	55.48	47.77–64.42	<0.0001
Hyperglycemia (yes/no)	2.42	2.11–2.78	<0.0001	2.42	2.11–2.78	<0.0001
Hypertension (yes/no)	1.71	1.54–1.89	<0.0001	1.70	1.54–1.89	<0.0001
Elevated triglycerides (yes/no)	10.46	9.18–11.91	<0.0001	10.47	9.19–11.92	<0.0001
Low HDL cholesterol (yes/no)	2.34	2.10–2.61	<0.0001	2.34	2.10–2.61	<0.0001

OR: odds ratio. 95% CI: 95% confidence interval. ORs are given per year increase in age, for men vs. women, and per 1 SD in TSH, FT4, FT3 and the FT3/FT4 ratio with 95% CIs. All models are mutually adjusted for the variables included in the analyses. Fatty Liver Index wax calculated by; FLI = $(e^{0.953*\log_e(\text{triglycerides}) + 0.139*\text{BMI} + 0.718*\log_e(\text{GGT}) + 0.053*\text{waist circumference} - 15.745})/(1 + e^{0.953*\log_e(\text{triglycerides}) + 0.139*\text{BMI} + 0.718*\log_e(\text{GGT}) + 0.053*\text{waist circumference} - 15.745})*100$. Components of the metabolic syndrome (including hyperglycemia) were defined according to NCEP ATPIII criteria. Abbreviations: FT4, free thyroxine; FT3, free triiodothyronine; HDL cholesterol, High Density Lipoprotein cholesterol; TSH, thyroid-stimulating hormone.

Table 5 – Thyroid function parameters according to enlarged waist circumference.

	Sex-stratified waist circumference		P-value
	Not enlarged N = 9555 (26.1% male, 73.9% female)	Enlarged N = 10,874 (59.1% male, 40.9% female)	
TSH (mU/L)	2.04 (1.51–2.70)	1.99 (1.47–2.62)	<0.0001
FT4 (pmol/L)	15.70 (14.60–17.00)	15.60 (14.40–16.90)	<0.0001
FT3 (pmol/L)	5.20 (4.80–5.60)	5.30 (4.90–5.70)	<0.0001
Ratio FT3/FT4	0.33 (0.31–0.36)	0.34 (0.31–0.37)	<0.0001

Data are given in median with interquartile ranges (IQR). Waist circumference was sex stratified. Enlarged waist circumference was defined as ≥102 cm in males and as ≥88 cm in females. Mann–Whitney U test was used. Abbreviations: FT4, free thyroxine; FT3, free triiodothyronine; TSH, thyroid-stimulating hormone.

associated with a higher FT3 or a higher FT3/FT4 ratio, suggesting that thyroid hormone status may predominantly impact on fatty liver as an early stage of NAFLD.

In view of the key role of thyroid hormone status on hepatic fat accumulation and lipoprotein metabolism [5,9,38], much effort has been recently paid to the development of thyromimetics, i.e. thyroid hormone receptor agonists that act by binding to specific thyroid hormone receptor hormone subunits [39,40]. Several of these agents including a liver-targeted agonist of thyroid hormone receptor β, the subunit that is naturally expressed in hepatocytes, has been shown to diminish hepatic fat accumulation in animal studies [34]. However, due to deleterious side effects, none of these drugs has been introduced into clinical practice so far, which underscores the complexity of thyroid hormone physiology [40].

It is increasingly recognized that thyroid hormone levels are associated with effects on body fat, in such a way that (centrally) euthyroid obese individuals have higher circulating FT3 [41–43]. Our current findings with respect to higher FT3 together with lower FT4 and higher TSH levels in centrally obese individuals are consistent with earlier reports [41–43]. Using a Mendelian randomization approach it was documented recently that genetic predisposition for a higher BMI is a determinant of higher FT3 levels. This suggests that adiposity may be causally implicated in the regulation of circulating thyroid hormones [43]. The mechanisms responsible for this effect of adiposity are not yet precisely known, but may involve tissue-specific alterations in iodothyronine deiodinase (DIO) expression in relation to obesity. It has been proposed that type 2 DIO is the major source of circulating T3 in humans [44], while on the other hand common genetic variation in type 1 DIO may predict the plasma FT3/FT4 ratio [45]. Among other tissues, type 1 DIO has been isolated from white adipose tissue, whereas type 2 DIO is expressed in brown adipose tissue [46,47]. Type 1 DIO is also expressed in the liver and is stimulated by leptin [46,48], which in turn may promote fatty acid oxidation, possibly contributing to hepatocyte damage [49]. The importance of (central) obesity in the pathogenesis of NAFLD is well established, and we expectedly observed a strong association of an FLI ≥ 60 with an enlarged waist circumference. We hypothesize that a higher FT3/FT4 ratio in centrally obese subjects, possibly consequent to altered DIO expression, could to some extent reflect an effect of altered thyroid hormone status on NAFLD under euthyroid conditions. Clearly, the cross-sectional design of the present study hampers to establish a sequence of metabolic changes, and the possible interrelationship of central obesity with DIO activity and the development of NAFLD needs to be prospectively delineated in future.

As recommended by the EASL-EASD-EASO NAFLD guidelines, the FLI algorithm, developed using data of the Dionysos Nutrition & Liver Study in Northern Italy [24], was used for detecting the presence of NAFLD. This score is described in

Table 6 – Sensitivity analyses demonstrating independent associations of non-alcoholic fatty liver disease estimated by the Fatty Liver Index (FLI) ≥ 90 with thyroid function parameters and presence of the metabolic syndrome.

	Model 1			Model 2			Model 3			Model 4		
	OR	95% CI	P-value	OR	95% CI	P-value	OR	95% CI	P-value	OR	95% CI	P-value
Age (years)	1.02	1.02–1.03	<0.0001	1.02	1.02–1.03	<0.0001	1.00	0.99–1.01	0.91	1.00	0.99–1.01	0.97
Sex (male vs. female)	1.65	1.42–1.92	<0.0001	1.69	1.46–1.95	<0.0001	1.34	1.14–1.57	<0.0001	1.35	1.16–1.57	<0.0001
TSH (per SD)	1.05	0.98–1.13	0.15	1.05	0.98–1.13	0.16	1.05	0.96–1.12	0.33	1.04	0.96–1.12	0.33
Free T4 (per SD)	0.72	0.67–0.78	<0.0001				0.83	0.77–0.90	<0.0001			
Free T3 (per SD)	1.38	1.28–1.49	<0.0001				1.18	1.09–1.29	<0.0001			
Ratio T3/T4 (per SD)				1.44	1.35–1.54	<0.0001				1.22	1.14–1.31	<0.0001
MetS (yes/no)							20.57	17.57–24.38	<0.0001	20.72	17.58–24.40	<0.0001

OR: odds ratio. 95% CI: 95% confidence interval. ORs are given per year increase in age, for men vs. women, and per 1 SD in TSH, FT4, FT3 and the FT3/FT4 ratio and with 95% CIs. All models are mutually adjusted for the variables included in the analyses. Fatty Liver Index wax calculated by; FLI = $(e^{0.953*\log_e(\text{triglycerides}) + 0.139*\text{BMI} + 0.718*\log_e(\text{GGT}) + 0.053*\text{waist circumference} - 15.745})/(1 + e^{0.953*\log_e(\text{triglycerides}) + 0.139*\text{BMI} + 0.718*\log_e(\text{GGT}) + 0.053*\text{waist circumference} - 15.745})*100$. Metabolic Syndrome was defined according to NCEP ATPIII criteria. Abbreviations: FT4, free thyroxine; FT3, free triiodothyronine; MetS, metabolic syndrome; TSH, thyroid-stimulating hormone.

Table 7 – Thyroid function parameters, according to fibrosis, estimated by NAFLD fibrosis score (NFS) and non-alcoholic fatty liver disease, estimated by the Fatty Liver Index (FLI).

	No fibrosis NFS < 0.676 N = 20,171		*P*-value	Fibrosis NFS > 0.676 N = 49		*P*-value
	No NAFLD FLI < 60 N = 15,931	NAFLD FLI ≥ 60 N = 4240		No NAFLD FLI < 60 N = 29	NAFLD FLI ≥ 60 N = 20	
TSH (mU/L)	2.01 (1.49–2.65)	2.04 (1.52–2.66)	0.14	2.17 (1.58–2.88)	2.05 (1.69–2.70)	0.95
FT4 (pmol/L)	15.70 (14.60–17.00)	15.40 (14.20–16.70)	<0.0001	16.10 (14.90–17.30)	14.70 (14.33–16.43)	0.05
FT3 (pmol/L)	5.20 (4.90–5.60)	5.30 (5.00–5.70)	<0.0001	5.30 (4.95–5.60)	4.95 (4.60–5.45)	0.08
Ratio FT3/FT4	0.33 (0.31–0.36)	0.35 (0.32–0.38)	<0.0001	0.33 (0.29–0.36)	0.33 (0.31–0.36)	0.78

Data are given in median with interquartile ranges (IQR). NAFLD fibrosis score was calculated by; NFS = (−1.675 + 0.037 × age (year) + 0.094 × BMI (kg/m^2) + 1.13 × fasting glucose/diabetes (yes =1, no =0) + 0.99 × AST/ALT ratio − 0.013 × platelet count (×10^9/L) − 6.6 × albumin (g/L)). Fatty Liver Index wax calculated by; FLI = (e $^{0.953*loge\ (triglycerides)\ +\ 0.139*BMI\ +\ 0.718*loge\ (GGT)\ +\ 0.053*waist\ circumference\ -\ 15.745}$)/(1 + e $^{0.953*loge\ (triglycerides)\ +\ 0.139*BMI\ +\ 0.718*loge\ (GGT)\ +\ 0.053*waist\ circumference\ -\ 15.745}$)*100. Mann–Whitney U test was used. Abbreviations: FT4, free thyroxine; FT3, free triiodothyronine; TSH, thyroid-stimulating hormone.

the NAFLD guideline as one of the 3 best-validated steatosis scores so far [23], and has been proven highly accurate in detecting fatty liver (accuracy of 0.84 and specificity of 86%) for an FLI ≥ 60; area under the receiver operating characteristic curve 0.83 [24,50]. The NFS has been developed in a predominantly Caucasian (90%) cohort [25] and is described in the NAFLD guideline as one of the best biomarkers for the detection of fibrosis [23]. This score has been externally validated in ethnically different NAFLD populations, with consistent results [23,27]. Both the FLI and NFS seem to perform best in Caucasians, which is probably related to the ethnical difference in fat distribution [23–25,27]. However, the FLI and NFS scores are not an absolute measure of hepatic fat accumulation and fibrosis. While histological examination of a liver biopsy is still the golden standard for diagnosing NAFLD and fibrosis, liver biopsy also has well-known limitations with respect to invasiveness and sampling variability. As an alternative, imaging techniques are time consuming, expensive and also not feasible in large observational studies. Given these considerations, the recent EASL-EASD-EASO NAFLD guidelines have adopted that serum biomarkers are the preferred diagnostic tool for large scale screening studies [23].

Our study has several strengths. Considering a sample size of approximately 20,000 individuals, this is the largest study to date, which reports on the association of NAFLD with thyroid function parameters in patients with euthyroidism. Additionally, all participants included in the Lifelines Cohort Study have been well characterized, with extensive validated questionnaires and standardized (anthropometric) measurements. Laboratory measurements were performed in fasting serum samples in a single reference laboratory [21]. Furthermore, the Lifelines study population has been validated to be representative of the population of the North of the Netherlands [22]. Several other methodological aspects and limitations of our study need to be considered. First, we excluded subjects with concurrent major diseases including cancer to circumvent bias due to non-thyroidal illness. We also excluded subjects using lipid and glucose lowering medication to obviate alleged effects on hepatic lipid metabolism and to avoid confounding on the assessment of NAFLD. Consequently, this has resulted in underrepresentation of diabetic and more severe dyslipidemic subjects in our study population. Indeed although 10.2% of subjects had elevated plasma glucose, a fasting plasma glucose ≥7.0 mmol/L, as diabetes criterion, was present in only 0.6% of the selected participants compared to 2.5% in the entire Lifelines cohort [22]. Also, subjects of non-white ancestry were excluded in order to select a Western-European population. While this likely limits extrapolation of our findings to other ethnicities, this was done in view of the limited percentage of immigrants in our region (Fig. 1), and our choice to use the FLI score and NFS for NAFLD and fibrosis assessment. Second, we performed a cross-sectional study. Therefore, cause–effect relationships cannot be established with certainty. Third, since levels of anti-thyroid peroxidase and anti-thyroglobulin autoantibodies were not measured in the Lifelines Cohort Study, we cannot explore the possible influence of impending thyroid autoimmunity on the relationship of NAFLD with thyroid function. Fourth, since self-reported questionnaires were used for a number of relevant variables, we cannot exclude the possibility that misreporting could have resulted in a limited degree of misclassification. Fifth, the FLI score includes the variables waist circumference, BMI, GGT and TG. Therefore, apparent differences in these variables between subjects with and without an elevated FLI score, as well as the extent to which the corresponding MetS components were associated with an elevated FLI should be interpreted with caution. Finally, in sensitivity analyses increasing the FLI cut-off value from 60 to 90, thereby increasing its specificity at the expense of sensitivity [24], comparable associations of an FLI ≥ 90 with thyroid function parameters were observed, supporting the robustness of our findings.

In conclusion, euthyroid subjects with suspected NAFLD are characterized by a higher FT3, a lower FT4 and in particular a higher FT3/FT4 ratio, probably consequent to central obesity. From a clinical point of view, the present findings suggest potentially adverse consequences of higher T3 exposure within euthyroid range, and would underscore the necessity to evaluate the safety of T4-T3 combination treatment in subjects with overt hypothyroidism on NAFLD development.

Author Contributions

Conception and design of the study: EB, LTW, TS, KNF, HB and RD. Data collection and analysis: EB, MA and RD. Interpretation of data: EB, LTW, HB and RD. Drafting the manuscript: EB, LTW, HB and RD. All authors have revised and approved the submitted manuscript.

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Conflicts of Interests

The authors declared no conflict of interests.

REFERENCES

[1] Angulo P. Nonalcoholic fatty liver disease. N Engl J Med 2002;346:1221–31.

[2] Pagano G, Pacini G, Musso G, Gambino R, Mecca F, Depetris N, et al. Nonalcoholic steatohepatitis, insulin resistance, and metabolic syndrome: further evidence for an etiologic association. Hepatology 2002;35:367–72.

[3] Loomba R, Sanyal AJ. The global NAFLD epidemic. Nat Rev Gastroenterol Hepatol 2013;10:686–90.

[4] Alkhouri N, Feldstein AE. Noninvasive diagnosis of nonalcoholic fatty liver disease: are we there yet? Metab Clin Exp 2016;65:1087–95.

[5] van Tienhoven-Wind LJN, Dullaart RPF. Low-normal thyroid function and novel cardiometabolic biomarkers. Nutrients 2015;7:1352–77.

[6] Adiels M, Taskinen M-R, Packard C, Caslake MJ, Soro-Paavonen A, Westerbacka J, et al. Overproduction of large VLDL particles is driven by increased liver fat content in man. Diabetologia 2006;49:755–65.

[7] Cordeiro A, Souza LL, Einicker-Lamas M, Pazos-Moura CC. Non-classic thyroid hormone signalling involved in hepatic lipid metabolism. J Endocrinol 2013;216:R47–57.

[8] Pucci E, Chiovato L, Pinchera A. Thyroid and lipid metabolism. Int J Obes Relat Metab Disord 2000;24(Suppl. 2):S109–12.

[9] Heimberg M, Olubadewo JO, Wilcox HG. Plasma lipoproteins and regulation of hepatic metabolism of fatty acids in altered thyroid states. Endocr Rev 1985;6:590–607.

[10] van Tienhoven-Wind L, Dullaart RPF. Low normal thyroid function as a determinant of increased large very low density lipoprotein particles. Clin Biochem 2015;48:489–94.

[11] Pagadala MR, Zein CO, Dasarathy S, Yerian LM, Lopez R, McCullough AJ. Prevalence of hypothyroidism in nonalcoholic fatty liver disease. Dig Dis Sci 2012;57:528–34.

[12] Chung GE, Kim D, Kim W, Yim JY, Park MJ, Kim YJ, et al. Non-alcoholic fatty liver disease across the spectrum of hypothyroidism. J Hepatol 2012;57:150–6.

[13] Bano A, Chaker L, Plompen EPC, Hofman A, Dehghan A, Franco OH, et al. Thyroid function and the risk of non-alcoholic fatty liver disease: the Rotterdam study; 2016. J Clin Endocrinol Metab. http://dx.doi.org/10.1210/jc.2016-1300.

[14] Eshraghian A, Hamidian JA. Non-alcoholic fatty liver disease and thyroid dysfunction: a systematic review. World J Gastroenterol 2014;20:8102–9.

[15] Tao Y, Gu H, Wu J, Sui J. Thyroid function is associated with non-alcoholic fatty liver disease in euthyroid subjects. Endocr Res 2015;40:74–8.

[16] Dullaart RPF, van den Berg EH, van der Klauw MM, Blokzijl H. Low normal thyroid function attenuates serum alanine aminotransferase elevations in the context of metabolic syndrome and insulin resistance in white people. Clin Biochem 2014;47:1028–32.

[17] Xu C, Xu L, Yu C, Miao M, Li Y. Association between thyroid function and nonalcoholic fatty liver disease in euthyroid elderly Chinese. Clin Endocrinol (Oxf) 2011;75:240–6.

[18] Ittermann T, Haring R, Wallaschofski H, Baumeister SE, Nauck M, Dörr M, 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.

[19] Liu G, Zheng X, Guan L, Jiang Z, Lin H, Jiang Q, et al. Free triiodothyronine levels are positively associated with non-alcoholic fatty liver disease in euthyroid middle-aged subjects. Endocr Res 2015;40:188–93.

[20] Dullaart RPF, de Vries R, Roozendaal C, Kobold ACM, Sluiter WJ. Carotid artery intima media thickness is inversely related to serum free thyroxine in euthyroid subjects. Clin Endocrinol (Oxf) 2007;67:668–73.

[21] Scholtens S, Smidt N, Swertz MA, Bakker SJL, Dotinga A, Vonk JM, et al. Cohort profile: LifeLines, a three-generation cohort study and biobank. Int J Epidemiol 2015;44:1172–80.

[22] Klijs B, Scholtens S, Mandemakers JJ, Snieder H, Stolk RP, Smidt N. Representativeness of the LifeLines cohort study. PLoS One 2015;10:e0137203.

[23] European Association for the Study of the Liver (EASL), European Association for the Study of Diabetes (EASD), European Association for the Study of Obesity (EASO). EASL-EASD-EASO clinical practice guidelines for the management of non-alcoholic fatty liver disease. Diabetologia 2016;59:1121–40.

[24] Bedogni G, Bellentani S, Miglioli L, Masutti F, Passalacqua M, Castiglione A, et al. The fatty liver index: a simple and accurate predictor of hepatic steatosis in the general population. BMC Gastroenterol 2006;6:33.

[25] Angulo P, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology 2007;45:846–54.

[26] Castera L, Vilgrain V, Angulo P. Noninvasive evaluation of NAFLD. Nat Rev Gastroenterol Hepatol 2013;10:666–75.

[27] European Association for Study of Liver, Asociacion Latinoamericana para el Estudio del Higado. EASL-ALEH clinical practice guidelines: non-invasive tests for evaluation of liver disease severity and prognosis. J Hepatol 2015;63: 237–64.
[28] Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute scientific statement. Circulation 2005;112:2735–52.
[29] Abdalla SM, Bianco AC. Defending plasma T3 is a biological priority. Clin Endocrinol (Oxf) 2014;81:633–41.
[30] Cheng S-Y, Leonard JL, Davis PJ. Molecular aspects of thyroid hormone actions. Endocr Rev 2010;31:139–70.
[31] Elks ML, Manganiello VC. Effects of thyroid hormone on regulation of lipolysis and adenosine 3′,5-′monophosphate metabolism in 3T3-L1 adipocytes. Endocrinology 1985;117: 947–53.
[32] Cachefo A, Boucher P, Vidon C, Dusserre E, Diraison F, Beylot M. Hepatic lipogenesis and cholesterol synthesis in hyperthyroid patients. J Clin Endocrinol Metab 2001;86:5353–7.
[33] Muci MR, Gnoni GV. Short-term effects of triiodothyronine on exogenous and de novo synthesized fatty acids in rat hepatocytes. Biochem Int 1991;25:807–13.
[34] Cable EE, Finn PD, Stebbins JW, Hou J, Ito BR, van Poelje PD, et al. Reduction of hepatic steatosis in rats and mice after treatment with a liver-targeted thyroid hormone receptor agonist. Hepatology 2009;49:407–17.
[35] Sinha RA, You S-H, Zhou J, Siddique MM, Bay B-H, Zhu X, et al. Thyroid hormone stimulates hepatic lipid catabolism via activation of autophagy. J Clin Invest 2012;122:2428–38.
[36] Wilcox HG, Heimberg M. Effects of hyperthyroidism on synthesis, secretion and metabolism of the VLDL apoproteins by the perfused rat liver. Biochim Biophys Acta 1991;1081: 246–52.
[37] Fabbrini E, Magkos F, Patterson BW, Mittendorfer B, Klein S. Subclinical hypothyroidism and hyperthyroidism have opposite effects on hepatic very-low-density lipoprotein-triglyceride kinetics. J Clin Endocrinol Metab 2012;97:E414–8.
[38] Baxter JD, Webb P. Thyroid hormone mimetics: potential applications in atherosclerosis, obesity and type 2 diabetes. Nat Rev Drug Discov 2009;8:308–20.
[39] Coppola M, Glinni D, Moreno M, Cioffi F, Silvestri E, Goglia F. Thyroid hormone analogues and derivatives: actions in fatty liver. World J Hepatol 2014;6:114–29.
[40] Elbers LPB, Kastelein JJP, Sjouke B. Thyroid hormone mimetics: the past, current status and future challenges. Curr Atheroscler Rep 2016;18:14.
[41] De Pergola G, Ciampolillo A, Paolotti S, Trerotoli P, Giorgino R. Free triiodothyronine and thyroid stimulating hormone are directly associated with waist circumference, independently of insulin resistance, metabolic parameters and blood pressure in overweight and obese women. Clin Endocrinol (Oxf) 2007;67:265–9.
[42] Reinehr T, Isa A, de Sousa G, Dieffenbach R, Andler W. Thyroid hormones and their relation to weight status. Horm Res 2008;70:51–7.
[43] Taylor PN, Richmond R, Davies N, Sayers A, Stevenson K, Woltersdorf W, et al. Paradoxical relationship between body mass index and thyroid hormone levels: a study using Mendelian randomization. J Clin Endocrinol Metab 2016;101: 730–8.
[44] Maia AL, Kim BW, Huang SA, Harney JW, Larsen PR. Type 2 iodothyronine deiodinase is the major source of plasma T3 in euthyroid humans. J Clin Invest 2005;115:2524–33.
[45] Panicker V, Cluett C, Shields B, Murray A, Parnell KS, Perry JRB, et al. A common variation in deiodinase 1 gene DIO1 is associated with the relative levels of free thyroxine and triiodothyronine. J Clin Endocrinol Metab 2008;93: 3075–81.
[46] Ortega FJ, Jílková ZM, Moreno-Navarrete JM, Pavelka S, Rodriguez-Hermosa JI, Kopeck Ygrave J, et al. Type I iodothyronine 5'-deiodinase mRNA and activity is increased in adipose tissue of obese subjects. Int J Obes (Lond) 2012;36: 320–4.
[47] Bianco AC, Kim BW. Deiodinases: implications of the local control of thyroid hormone action. J Clin Invest 2006;116: 2571–9.
[48] Macek Jílková Z, Pavelka S, Flachs P, Hensler M, Kůs V, Kopecký J. Modulation of type I iodothyronine 5'-deiodinase activity in white adipose tissue by nutrition: possible involvement of leptin. Physiol Res 2010;59:561–9.
[49] Minokoshi Y, Kim Y-B, Peroni OD, Fryer LGD, Müller C, Carling D, et al. Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. Nature 2002;415: 339–43.
[50] Fedchuk L, Nascimbeni F, Pais R, Charlotte F, Housset C, Ratziu V, et al. Performance and limitations of steatosis biomarkers in patients with nonalcoholic fatty liver disease. Aliment Pharmacol Ther 2014;40:1209–22.