FT3 to FT4 Ratio is Associated with Non-Alcoholic Steatohepatitis and Significant Fibrosis in Euthyroid Subjects with NAFLD

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Abstract

Background & Aims: Studies on the relationship between thyroid function and non-alcoholic fatty liver disease (NAFLD) among euthyroid subjects had shown inconsistent results. Objective of the present study was to exploring the independent relationship between thyroid function parameters and nonalcoholic steatohepatitis (NASH), significant fibrosis (SF) respectively after adjusting other well-identified risk factors.

Method: This study enrolled 307 patients with biopsy-proven NAFLD. Thyroid dysfunction defined as serum thyroid-stimulating hormone > 4.5 mIU/l or < 0.5 mIU/l and/ or free thyroxine > 14.41 pmol/l or < 7.86 pmol/l.

Results: Stepwise regression analysis showed that the fT3/fT4 ratio, an optimal thyroid function parameter, was associated with NASH and SF in euthyroid subjects with NAFLD. After multivariable analysis, the fT3/fT4 ratio (per 0.1 change) showed significant correlation with NASH (OR 2.03(1.33, 3.11), P = 0.001) and SF (OR 2.11(1.28, 3.46), P = 0.003). When ratio was stratified by quartiles (Q1-Q4) as a categorical variable, the results still significant (Q4 versus Q1 (OR for NASH 4.29(1.68, 10.91), P = 0.002; for SF 4.63(1.49, 14.43), P = 0.008, all P for linear trend < 0.05). The prevalence of NASH and SF rose significantly with increasing in quartiles. Furthermore, ratio was positively correlated with the grade of steatosis, lobular inflammation, hepatocellular ballooning and liver fibrosis stage (all P < 0.05). Subgroup analysis showed that hypertension was a possible effect modification.

Conclusion: The findings of the present study confirmed an association between fT3/fT4 ratio and NAFLD in euthyroid subjects in a dose-dependent manner, particularly in non-hypertension adults.

There is no trial registration number.

Background

Nonalcoholic Fatty Liver Disease (NAFLD) is the most common liver disease worldwide and closely associated with insulin resistance and metabolic syndrome[1]. The prevalence of NAFLD in the global population is about 25% and the incidence is increasing rapidly in parallel with the westernized diet, lack of exercise, lifestyle changes, and obesity[2]. It encompasses a spectrum of liver disease that ranges from simple accumulation of fat in liver cells to necroinflammation, liver fibrosis and cirrhosis, with the risk of hepatocellular carcinoma[3]. Nonalcoholic steatohepatitis (NASH) is considered to be a more severe part in the NAFLD spectrum, which can promote the progression of liver fibrosis faster (7 years per fibrosis stage) than without it (14 years)[4]; and liver fibrosis[5, 6] is considered to be independently associated with long-term outcomes with a high risk of death from liver-related events and all-cause mortality (e.g. cardiovascular disease), and increased with the increased stage of fibrosis[7].

In the United States, NAFLD has become the second major indication for liver transplantation and the third major cause of hepatocellular carcinoma, and is still growing [8, 9] . As the clinical consequences of

NAFLD increase, there can be severe economic burdens. The annual burden is estimated to be $103 billion ($1,613 per patient) in the United States and €35 billion (€354 to €1,163 per patient) in European countries [10] . Therefore, it is extremely important to fully understand the risk factors and pathogenesis of NAFLD and disease progression. The "multiple strike" hypothesis which include insulin resistance, hormones secreted from the adipose tissue, nutritional factors, gut microbiota and genetic and epigenetic factors largely explains the pathogenesis and progression of NAFLD, but knowledge on the mechanisms of NAFLD still remains incomplete [11] .

Given thyroid hormones play an important role in regulating body metabolism, there has been a heated discussion about the relationship between thyroid dysfunction and NAFLD. Several studies had demonstrated that hyperthyroidism (both subclinical and clinical) is a risk factor contributing to the development of steatosis, liver biopsy-proven NASH and advanced fibrosis[12–14]. A reasonable explanation is that the thyroid hormones is associated with body fat distribution, metabolic syndrome, and insulin resistance[15, 16]. Recently, some studies have shown that variation in thyroid hormones in the reference range may also have negative effects on health, same to subclinical and clinical hypothyroidism[17]. Meanwhile, the relationship between normal thyroid function and NAFLD has also gained attention. However, there is no consensus. In the previous findings, based on different sample sizes and diagnosis methods of NAFLD, they showed that low-normal thyroid function (higher plasma TSH level [2.5 to 4.5 mIU/L] with a normal thyroid hormone level)[18], low serum free thyroxine level(fT4)[19], high serum free triiodothyronine level(fT3)[20, 21], and high fT3/fT4 ratio[22] were risk factors for NAFLD respectively.

For this reason, we performed a study in euthyroid subjects with NAFLD aimed at exploring the independent relationship between thyroid function parameters (i.e., triiodothyronine (T3), thyroxine (T4), fT3, fT4, fT3/fT4 ratio and thyroid-stimulating hormone (TSH) levels) and biopsy-proven NASH, significant fibrosis respectively after adjusting other well-identified risk factors (e.g. metabolic risk factors and insulin resistance).

Patients and study design

No informed consent was required because all the data were anonymized. The protocol was in accordance with the Helsinki Declaration and was approved by the ethics committee of the First Affiliated Hospital of Wenzhou Medical University. From our liver biopsy database at The First Affiliated Hospital of Wenzhou Medical University, the clinical records of 1371 subjects during January 2016 and July 2019 were reviewed. Strict exclusion criteria are designed and implemented: (1) ≤ 18-year-old, (2) excessive alcohol consumption (> 140 g/week for men and 70 g/week for women) evaluated by a questionnaire, (3) the steatosis (≤ 5% of liver cells) at histology and history of viral hepatitis, autoimmune hepatitis, or other forms of chronic liver disease, (4) history of malignancy, (5) history of thyroid disease, including clinical hyperthyroidism and hypothyroidism, thyroidectomy, radiofrequency ablation of thyroid gland, (6) with thyroid dysfunction defined as serum TSH > 4.5 mIU/l or < 0.5 mIU/l and/ or fT4 > 14.41 pmol/l or <

7.86 pmol/l, (7) insufficient clinical date. In the end, 307 strictly screened subjects were enrolled in this study. Data was collected from the time of liver biopsy. All variables in this study were objective results stored in the hospital computer system. The presence of diabetes mellitus (fasting blood glucose > = 7.0 mmol/L or treatment with antidiabetic drugs) was recorded. Hypertension was defined as systolic blood pressure ≥ 140 mmHg/diastolic blood pressure ≥ 90 mmHg and/or the current use of anti-hypertensive medication. Smokers were defined as those who had smoked at least one cigarette per day during the previous year.

Anthropometric and laboratory measurements

The body height and weight of the subjects were measured, while they were barefoot and wearing light clothing. The height was recorded to the nearest 1 cm, and body weight was measured to the nearest 0.1 kg. Body mass index (BMI) was calculated by dividing the weight in kilograms with the square of height in meters. Venous blood sampling was collected after overnight fasting for at least 8–12 hours and measured at the hospital Clinical Sample Test Room. Laboratory assays included albumin (g/L), total cholesterol (TC)(mmol/L), triglyceride (TG)(mmol/L), low-density lipoprotein cholesterol (LDL-c) (mmol/L), high-density lipoprotein cholesterol (HDL-c)(mmol/L), aspartate aminotransferase (ALT)(U/L), alanine aminotransferase (AST)(U/L), gamma-glutamyl transferase (γGT)(U/L), alkaline phosphatase (ALP)(U/L), type IV collagen(ng/ml), type III procollagen(ng/ml), hyaluronic acid(ng/ml), total bilirubin (TB)(μmol/L), thyroid stimulating hormone (TSH)(mIU/L), thyroxine (T4)(nmol/L), triiodothyronine (T3) (nmol/L), free thyroxine (fT4)(nmol/L), free triiodothyronine (fT3)(pmol/L), glucose(mmol/L), insulin(pmol/L), glycosylated hemoglobin (GHb)(%), platelet(*10^9), uric acid(μmol/L), creatinine(μmol/L). The fT3/fT4 ratio is the value of fT3 divided by fT4. Insulin resistance was evaluated using the homeostasis model assessment of insulin resistance (HOMA-IR): fasting blood glucose (mmol/l) * insulin (mU/l) / 22.5[23].

Liver biopsy

Liver biopsy was performed by senior operators using 16-gauge Hepafix needles under ultrasonography positioning. The liver specimens were fixed in 10% formalin, and was scored by experienced hepatologists who were blinded to the clinical data, treatment allocation, and imaging findings. A scoring system published by Kleiner et al[24] was used. The histological NAFLD Active Score (NAS) is defined as the unweighted sum of the scores for steatosis (0–3), lobular inflammation (0–3), and hepatocellular ballooning (0–2); scores therefore ranged from 0 to 8. Subjects with scores of 5 or greater were diagnosed as NASH. Fibrosis was staged as follows: stage 0 = no fibrosis; stage 1 = perisinusoidal or periportal fibrosis with 3 different patterns: 1a = mild, zone 3, perisinusoidal; 1b = moderate, zone 3, perisinusoidal fibrosis, and 1c = portal/periportal fibrosis; stage 2 = perisinusoidal and portal/periportal fibrosis; stage 3 = bridging fibrosis; stage 4 = cirrhosis. In this study, we pooled the subtype 1a, 1b, 1c of fibrosis into a single F1 score. Significant fibrosis (SF) was defined as stage 2 or greater (≥ 2).

Statistical Analysis

Continuous data were presented as median (1st quartile, 3rd quartile), and categorical variables were expressed in frequency or as a percentage. First, the univariate analysis (student t-test, Mann-Whitney U test, chi-square test) were used to infer the difference between the 2 groups. It was noted that the fT3/fT4 ratio was too small; therefore, we expanded it 10 times and labelled per 0.1 change (henceforth fT3/fT4 ratio (per 0.1 change)). Second, significant variables from the univariate analysis ($P < 0.05$) were then subjected to stepwise logistic regression analysis (Probability for Stepwise enter: 0.05, remove: 0.1) to evaluate the risk factors for NASH, significant fibrosis respectively. Third, according to the recommendation of the STROBE statement[25], we simultaneously showed the results from unadjusted, minimally adjusted analyses and those from fully adjusted analyses. Fourth, Spearman's correlation analysis was performed to assess the relationship between thyroid function parameter and histological features. Fifth, subgroup analyses were performed using stratified linear regression models. The modifications and interactions of subgroups were inspected by likelihood ration tests. All of the analyses were performed with the statistical software packages R (http://www.R-project.org, The R Foundation) and EmpowerStats (http://www.empowerstats.com, X&Y Solutions, Inc., Boston, MA). P values less than 0.05 (two-sided) were considered statistically significant.

Results

Subject characteristics

The current study collective included 307 subjects (24.76%, females; 75.24%, males), 80 (men, 75.47%) and 42 subjects (men, 72.41%) were classified as biopsy-proven NASH and SF, respectively. The baseline characteristics of this cohort were shown in Tables 1 and 2. In both NASH and SF groups, there were higher level of BMI, TC, LDL-c, ALT, AST, type IV collagen, type III procollagen and HOMA-IR with the direction indicating that patients with histological severity reflected a poorer health status with more severe liver damage and active extracellular matrix synthesis and degradation. Contrary to the previously reported studies, there was no difference in prevalence of diabetes mellitus in univariate analysis, but the level of HOMA-IR was significantly greater in subjects with NASH and SF than in subjects without it (4.57 vs.3.12 in NASH; 5.09vs.3.48 in SF, all $P < 0.001$). One possible explanation was this study's cohorts were younger than in other studies, and insulin resistance predates beta-cell dysfunction[26]. With respect to thyroid function parameters, the NASH group was characterized by a higher level of TSH, T3, fT3 and fT3/fT4 ratio; and the SF group had higher level of T3, fT3 and fT3/fT4 ratio and lower fT4.

Table 1
Baseline Characteristics of NAFLD Subjects and Risk factors for NASH

	non-NASH(n = 201)	NASH(n = 106)	P' value	OR(95%CI)	P" value
Age(year)	44(35–52)	36(29–45)	0.000		
SEX(male)	151(75.12%)	80(75.47%)	0.947		
Diabetes(yes)	58(28.86%)	27(25.47%)	0.529		
Hypertension(yes)	44(21.89%)	17(16.04%)	0.222		
Smoke(yes)	37(18.41%)	20(18.87%)	0.921		
BMI(kg/m²)	26.08(24.16–28.31)	27.30(25.26–30.09)	0.000		
Albumin(g/L)	46.2(43.6–48.7)	47.2(45.23–49.08)	0.083		
TC(mmol/L)	4.87(4.06–5.61)	5.43(4.63–6.05)	0.002		
TG(mmol/L)	1.82(1.33–2.72)	2.10(1.50–2.82)	0.415		
LDL-C(mmol/L)	2.87(2.32–3.48)	3.28(2.55–3.82)	0.005		
HDL-C(mmol/L)	0.97(0.87–1.11)	0.98(0.86–1.12)	0.616		
ALT(U/L)	41(26–62)	90.5(54-132.25)	0.000	1.02(1.01,1.02)	0.000
AST(U/L)	30(23–38)	54(34-74.75)	0.000		
γGT(U/L)	46(28–76)	63(38.25-99)	0.005		
ALP(U/L)	79(66–93)	83(72–101)	0.086		
type IV collagen(ng/ml)	17.9(14.8–20.4)	20.5(17.15–25.3)	0.000		
type III procollagen(ng/ml)	17.8(15.1–20.8)	20.6(17.4-25.65)	0.000		
Hyaluronic acid(ng/ml)	44(38.4–53.4)	48.15(39.15–58.4)	0.301		
TB(μmol/L)	12(10–15)	14(10–18)	0.006		
TSH(mIU/L)	1.5(1.1–2.13)	1.59(1.18–2.22)	0.040		
T4(nmol/L)	106.87(96.14-118.52)	103.94(95.1-116.44)	0.210		
T3(nmol/L)	1.59(1.44–1.73)	1.73(1.52–1.94)	0.000		
FT4(pmol/L)	11.22(10.33–12.27)	11(9.86–12.11)	0.384		

	non-NASH(n = 201)	NASH(n = 106)	P' value	OR(95%CI)	P" value
FT3(pmol/L)	5.4(5-5.8)	5.83(5.24–6.12)	0.000		
FT3/FT4(per 0.1 change)	4.9(4.3–5.3)	5.05(4.7–5.67)	0.000	1.73(1.20,2.49)	0.003
Glucose(mmol/L)	5.3(4.8–6.3)	5.4(4.9–6.18)	0.966		
GHb(%)	5.8(5.4–6.7)	5.7(5.3–6.7)	0.753		
HOMA-IR	3.12(2.11–4.77)	4.57(3.54–7.15)	0.000	1.09(1.03,1.15)	0.004
Platelet(*10^9)	242(204–284)	238.5(208–274)	0.808		
Uric acid(μmol/L)	377(317–438)	399.5(349.25-497.75)	0.000		
Creatinine(μmol/L)	68(59–76)	68(57–77)	0.617		
P' value for the univariate analysis. P" value and OR for the stepwise regression.					
Note:Above model adjusted for age, BMI, TC, LDL-C, ALT, AST, γGT, type IV collagen, type III procollagen, TB, TSH, T3, FT3, FT3/FT4(per 0.1 change), HOMA-IR, uric acid.					

Table 2
Baseline Characteristics of NAFLD Subjects and Risk factors for SF

	Non-SF(n = 249)	SF(n = 58)	P' value	OR(95%CI)	P" value
Age(year)	42(32–50)	40(30.25-50)	0.666		
SEX(male)	189(75.90%)	42(72.41%)	0.579		
Diabetes(yes)	70(28.11%)	15(25.86%)	0.730		
Hypertension(yes)	51(20.48%)	10(17.24%)	0.578		
Smoke(yes)	43(17.27%)	14(24.14%)	0.226		
BMI(kg/m²)	26.26(24.24–28.48)	27.27(25.14–30.53)	0.000		
Albumin(g/L)	46.6(44.4–49)	46.5(43.6–48)	0.520		
TC(mmol/L)	4.97(4.31–5.68)	5.57(4.63–6.28)	0.005		
TG(mmol/L)	1.90(1.33–2.77)	2.08(1.52–3.04)	0.409		
LDL-C(mmol/L)	2.94(2.36–3.55)	3.5(2.57–4.01)	0.021		
HDL-C(mmol/L)	0.97(0.87–1.11)	1(0.87–1.14)	0.347		
ALT(U/L)	47(28–83)	89(47.25–150.5)	0.000	1.01(1.00,1.02)	0.000
AST(U/L)	31(24–47)	56.5(32.25–82.5)	0.000		
γGT(U/L)	49(29–80)	66(40.25–96.75)	0.205		
ALP(U/L)	79(67–93)	83.5(71.25–101)	0.055		
type IV collagen(ng/ml)	18.1(14.9–20.8)	22.4(19.33–27.45)	0.000		
type III procollagen(ng/ml)	18.3(15.4–21.4)	22.6(18.5-26.98)	0.000		
Hyaluronic acid(ng/ml)	44(38.4–54.6)	48.55(39.12–59.35)	0.768		
TB(μmol/L)	12(10–16)	13.5(10–17)	0.959		
TSH(mIU/L)	1.53(1.11–2.15)	1.59(1.17–2.18)	0.382		
T4(nmol/L)	106.39(94.89-116.91)	106.42(96.88-123.72)	0.241		
T3(nmol/L)	1.63(1.44–1.77)	1.77(1.52–1.99)	0.000		
FT4(pmol/L)	11.21(10.31–12.39)	10.82(9.57–12.07)	0.025		

	Non-SF(n = 249)	SF(n = 58)	P' value	OR(95%CI)	P" value
FT3(pmol/L)	5.5(5.06–5.9)	5.61(5.17–6.19)	0.034		
FT3/FT4(per 0.1 change)	4.9(4.4–5.4)	5.1(4.8–5.8)	0.000	1.87(1.23,2.84)	0.003
Glucose(mmol/L)	5.2(4.8–6.1)	5.6(4.82–7.18)	0.083		
GHb(%)	5.7(5.4–6.6)	5.9(5.4–7.47)	0.538		
HOMA-IR	3.48(2.3–4.97)	5.09(3.09–9.5)	0.000	1.12(1.06,1.19)	0.000
Platelet(*10^9)	238(204–278)	244(212.75-299.75)	0.205		
Uric acid(μmol/L)	380(320–445)	397(350-490.5)	0.066		
Creatinine(μmol/L)	68(59–77)	65.5(55.25-72)	0.228		
P' value for the univariate analysis. P" value and OR for the stepwise regression.					
Note:Above model adjusted for BMI, TC, LDL-C, ALT, AST, type IV collagen, type III procollagen, T3, FT4, FT3, FT3/FT4(per 0.1 change), HOMA-IR.					

Risk factors of NASH, SF respectively

Stepwise logistic regression analysis was performed to evaluate the risk factors for NASH, SF respectively. Variables that were significant in univariate analysis would enter the regression analysis. The results showed that ALT (OR 1.02(1.01, 1.02)), fT3/fT4 ratio(per 0.1 change) (OR 1.73(1.20,2.49)) and HOMA-IR (OR 1.09(1.03,1.15)) were remained in the final equation for NASH (Table 1) and similarly showed that ALT (OR 1.01(1.00,1.02)), fT3/fT4 ratio(per 0.1 change) (OR 1.87(1.23,2.84)) and HOMA-IR (OR 1.12(1.06,1.19)) as risk factors for subjects with SF (Table 2). A notable finding was that fT3/fT4 ratio, an optimal thyroid function parameter, was found to be significantly associated with risk factor for NASH and SF.

Correlation between fT3/fT4 ratio and histological features

Univariate linear regression models were used to evaluate the associations between fT3/fT4 ratio and NASH, SF respectively. Meanwhile, we showed the non-adjusted and adjusted models in Table 3. For NASH, in the crude model, fT3/fT4 ratio (per 0.1 change) showed significant correlation with NASH (OR 1.89(1.37, 2.60), P = 0.000). In the minimally adjusted model □ (adjusted age, sex), the effect size showed no obvious change (OR 1.88(1.35, 2.63), P = 0.000). After adjusting for other covariates (model □), these associations were consistently maintained (OR 2.03(1.33, 3.11), P = 0.001). As for SF, fT3/fT4 ratio (per 0.1 change) showed significant correlation with SF in crude model (OR 1.91(1.32, 2.76)), model □ (OR

1.92(1.33, 2.79)) and model □ (OR 2.11(1.28, 3.46)) (all P value < 0.05). For the purpose of sensitivity analysis, we also handled fT3/fT4 ratio (per 0.1 change) as a categorical variable (Quartiles, Q1(3.1–4.4), Q2(4.5–4.8), Q3(4.9–5.4), Q4(5.5–8.1)). The correlation between fT3/fT4 ratio (per 0.1 change) and NASH, SF respectively was robust in a dose-dependent manner. After adjusting for covariates (model □), these associations had been maintained (OR for NASH 4.29(1.68, 10.91), P = 0.002; OR for SF 4.63(1.49, 14.43) P = 0.008), significant linear trend was observed (all P for trend < 0.05). In addition, the prevalence rate of NASH and SF showed an increasing trend as fT3/fT4 ratio increased (Fig. 1). Spearman's correlation analysis showed that the fT3/fT4 ratio was positively related to more severe histological features of NAFLD presenting the higher grade of steatosis, lobular inflammation, hepatocellular ballooning, and liver fibrosis stage (all P < 0.05) (Fig. 2).

Table 3
Relationship between fT3/fT4 ratio and NASH, SF respectively in different models

Variable	Crude Model		Adjust I		Adjust II	
	OR (95%CI)	P value	OR (95%CI)	P value	OR (95%CI)	P value
FT3/FT4(per 0.1 change) as a continuous variable						
NASH	1.89 (1.37, 2.60)	0.000	1.88 (1.35, 2.63)	0.000	2.03 (1.33, 3.11)	0.001
SF	1.91 (1.32, 2.76)	0.000	1.92 (1.33, 2.79)	0.001	2.11 (1.28, 3.46)	0.003
FT3/FT4(per 0.1 change) as a categorical variable (quartile)						
NASH						
Q1(3.1–4.4)	Refrence		Refrence		Refrence	
Q2(4.5–4.8)	2.36 (1.07, 5.19)	0.032	2.20 (0.99, 4.93)	0.054	2.72 (1.01, 7.35)	0.048
Q3(4.9–5.4)	2.17 (1.07, 4.40)	0.032	1.88 (0.91, 3.89)	0.088	2.29 (0.96, 5.48)	0.062
Q4(5.5–8.1)	3.75 (1.84, 7.63)	0.000	3.58 (1.73, 7.43)	0.001	4.29 (1.68, 10.91)	0.002
P for trend	1.47 (1.18, 1.83)	0.001	1.44 (1.15, 1.81)	0.001	1.51 (1.13, 2.01)	0.005
SF						
Q1(3.1–4.4)	Refrence		Refrence		Refrence	
Q2(4.5–4.8)	1.92 (0.70, 5.22)	0.204	1.91 (0.70, 5.22)	0.208	2.05 (0.60, 6.97)	0.251
Q3(4.9–5.4)	1.79 (0.72, 4.45)	0.208	1.79 (0.71, 4.47)	0.216	2.19 (0.72, 6.63)	0.165
Q4(5.5–8.1)	3.57 (1.49, 8.54)	0.004	3.58 (1.49, 8.61)	0.004	4.63 (1.49, 14.43)	0.008
P for trend	1.47 (1.12, 1.93)	0.005	1.48 (1.12, 1.94)	0.005	1.60 (1.13, 2.27)	0.009
Abbreviations: Non-alcoholic steatohepatitis(NASH), significant fibrosis(SF), odds ratio(OR), confidence interval(CI).						
Crude model did not adjust for other covariants.						
Model □adjusted for age and sex.						

Variable	Crude Model	Adjust I	Adjust II
Model □ adjusted for age, sex, diabetes, hypertension, smoke, BMI, albumin, TC, TG, LDL-C, HDL-C, ALT, AST, γGT, ALP, type IV collagen, type III procollagen, hyaluronic acid, TB, HOMA-IR, platelet, uric acid, creatinine.			

The results of subgroup analyses

As shown in Table 4, the test for interactions was significant for hypertension (P for interaction = 0.011 in NASH, 0.042 in SF), while the test for interactions were not statistically significant for sex, age, smoke, diabetes, HOMA-IR and BMI both in NASH and SF groups (all P values for interactions were larger than 0.05). We observed that there was evidence for an interaction fT3/fT4 ratio and hypertension. The effect sizes of fT3/fT4 ratio on NASH and SF showed significant differences in subjects with or without hypertension. FT3/FT4 ratio (per 0.1 change) was associated with NASH and SF in subjects who were without hypertension (OR for NASH 2.77(1.66, 4.62); OR for SF 2.78(1.54, 5.04)), but had no relationship with NASH and SF in hypertension group.

Table 4
Effect size of fT3/fT4 ratio on NASH, SF respectively in subgroups

Variable	N	OR (95%CI) for NASH	P value	OR (95%CI) for SF	P value
Sex			0.330		0.743
Male	231	1.80 (1.10, 2.93)		2.21 (1.24, 3.96)	
Female	76	2.83 (1.27, 6.35)		1.84 (0.71, 4.76)	
Age(year)			0.725		0.880
< 50	228	1.86 (1.13, 3.06)		2.11 (1.14, 3.91)	
>=50	79	2.17 (1.05, 4.51)		2.29 (1.00, 5.22)	
Smoke			0.624		0.648
Yes	57	1.69 (0.72, 3.95)		2.54 (0.97, 6.62)	
No	250	2.15 (1.33, 3.49)		1.97 (1.11, 3.49)	
Hypertension			0.011		0.042
Yes	61	0.77 (0.31, 1.87)		0.83 (0.28, 2.41)	
No	246	2.77 (1.66, 4.62)		2.78 (1.54, 5.04)	
Diabetes			0.103		0.109
Yes	85	1.25 (0.61, 2.55)		1.14 (0.47, 2.76)	
No	222	2.60 (1.53, 4.40)		2.76 (1.49, 5.09)	
HOMA-IR			0.450		0.540
< 2.5	84	1.62 (0.76, 3.48)		2.73 (0.95, 7.84)	
>=2.5	223	2.29 (1.39, 3.77)		1.89 (1.08, 3.29)	
BMI			0.528		0.292
< 24	61	2.56 (1.05, 6.21)		3.69 (1.07, 12.67)	
>=24	246	1.86 (1.16, 2.99)		1.85 (1.09, 3.14)	

Abbreviations: Non-alcoholic steatohepatitis(NASH), significant fibrosis(SF), odds ratio(OR), confidence interval(CI).

Note 1:Above model adjusted for age, sex, diabetes, hypertension, smoke, BMI, albumin, TC, TG, LDL-C, HDL-C, ALT, AST, γGT, ALP, type IV collagen, type III procollagen, hyaluronic acid, TB, HOMA-IR, platelet, uric acid, creatinine.

Note 2:In each case, the model is not adjusted for the stratification variable.

Discussion

In this cross-sectional study performed among 307 euthyroid subjects, the main finding was that the fT3/fT4 ratio was associated with biopsy-proven NASH and SF in subjects with NAFLD, independently of well-known metabolic risk factors. Besides, in the subgroup analyses, an effect modification by hypertension on the association was found. Inconsistent with our conclusion, a study in euthyroid elderly Chinese, which diagnosed NAFLD based on ultrasonography and included thyroid function parameters (TSH, fT3 and fT4), found that serum fT4 level was significantly associated with the risk for NAFLD (odds ratio 0.847)[19]. Another study with a similar approach from China found high levels of serum fT3 was significantly associated with NAFLD among middle-aged euthyroid subjects independently of known metabolic risk factor (odds ratio: 1.253)[20]. Kim et al, based on noninvasive marker to diagnose liver fibrosis, found a significant correlation between low–normal thyroid function / TSH with advanced fibrosis in the US general population[18]. On the one hand different diagnostic methods of NAFLD are the most important reason for inconsistent results, on the other hand the aforementioned studies did not set the variable fT3/fT4 ratio. Liver biopsy is the gold standard for NAFLD diagnosis, the accuracy of using noninvasive markers and ultrasonography for the diagnosis of NALFD may influenced by the misclassification[27] and hyposensitivity[28]. Eline et al[21] and Fatma et al[22] previously reported that higher fT3/fT4 ratio but not TSH is a risk factor for NAFLD independent of metabolic parameters, which is consistent with ours.

The pathophysiological mechanisms between fT3/fT4 ratio and NAFLD are multifactorial and not fully understood. It has been hypothesized that as an indicator of peripheral deiodinase activity, the higher fT3/fT4 ratio, higher conversion rate from fT4 to fT3, is a compensatory response to improve energy consumption[29]. An increase in expression of the genes for type I iodothyronine 5'-deiodinase and activity in adipose tissue of obese humans had been observed[30]. Moreover, a positive correlation between fT3/fT4 ratio and BMI, waist circumference, HOMA-IR in a cohort of euthyroid women have been reported[31]. All of the above could partially explain the potential relationship between fT3/fT4 ratio and NAFLD.

Our study also explored a possible effect modification by hypertension on the association between ratio and NASH, SF respectively. We concluded that the association was significant in subjects without hypertension, but not with hypertension. Gu et al has demonstrated that FT3 and FT4 are positively related to the prevalence of elevated blood pressure in euthyroid adults, which may have caused the phenomenon observed in our study[32]. However, further studies are required to validate and explicate the effect modification noted in our study.

Although our cross-sectional study did not reveal an in-depth mechanism, our finding illustrates the necessity to actively assess serum fT3/fT4 ratio in patients with NAFLD. From a clinical point of view, this present finding suggests the assessment of the fT3/fT4 ratio should be included in the multidisciplinary baseline assessment of patients with NAFLD, and that decreasing the fT3/fT4 ratio may be a promising potential therapy for curing NAFLD or preventing progression. Several studies[33, 34]had

demonstrated a beneficial effect of low dose Levothyroxine replacement therapy on NAFLD with a decrease in the prevalence of NAFLD and serum liver enzymes.

To our knowledge, our study is the first and largest analysis to date evaluating the association between thyroid function parameters and biopsy-proven NAFLD in euthyroid population. However, there are also limitations to this study. Firstly, the cross-sectional design of the study was possible to investigate associations but not causalities. Secondly, since the levels of thyroid antibody were not detected, we were unable to explore the possible effects of the impending thyroid autoimmunity on the association between NAFLD and thyroid function. Finally, this study does not reflect institutional and regional diversities because the cohorts of this study were composed of Chinese patients who were recruited at a single hospital.

Conclusion

This study shows that the fT3/fT4 ratio was independently associated with NASH and significant fibrosis in a dose-dependent manner in euthyroid subjects with NAFLD, particularly in non-hypertension adults. These associations persisted after further adjustment for obesity, metabolic risk factors, and insulin resistance. Future studies should elucidate the exact role of fT3/fT4 ratio in the development or progression of NAFLD, and explore the feasibility of adjusting ratio in the prevention and treatment of NAFLD.

Abbreviations

NAFLD: non-alcoholic fatty liver disease; NASH: nonalcoholic steatohepatitis; fT4: free thyroxine; fT3: free triiodothyronine; T3: triiodothyronine; T4: thyroxine; TSH: thyroid-stimulating hormone; BMI: body mass index; TC: total cholesterol; TG: triglyceride; LDL-c: low-density lipoprotein cholesterol; HDL-c: high-density lipoprotein cholesterol; ALT: aspartate aminotransferase; AST: alanine aminotransferase; γGT: gamma-glutamyl transferase; ALP: alkaline phosphatase; TB: total bilirubin; GHB: glycosylated hemoglobin; HOMA-IR: homeostasis model assessment of insulin resistance; NAS: NAFLD active score; SF: significant fibrosis; OR: odds ratio

Declarations

Ethics approval and consent to participate

The study was approved by the ethics committee of the First Affiliated Hospital of Wenzhou Medical University(Wenzhou, Zhengjiang, China). No informed consent was required because all the data were anonymized.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

All other authors declare that they have no competing interests.

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Authors' contributions

HBX and JMW designed this study, HBX, YQG, JZZ, ZQX and YL Collected data. HBX and JMW conducted the statistical analyse. HBX, YQG, JZZ and ZQX interpreted data. HBX and JMW reviewed the result and wrote manuscript. HBX, YQG, ZQX, YL and JZZ revised manuscript. All authors read and approved manuscript.

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Conflict of interest

The authors declare that they do not have anything to disclose regarding any funding or conflict of interest with respect to this manuscript entitled, "FT3 to FT4 ratio is associated with non-alcoholic steatohepatitis and significant fibrosis in euthyroid subjects with NAFLD".

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Figures

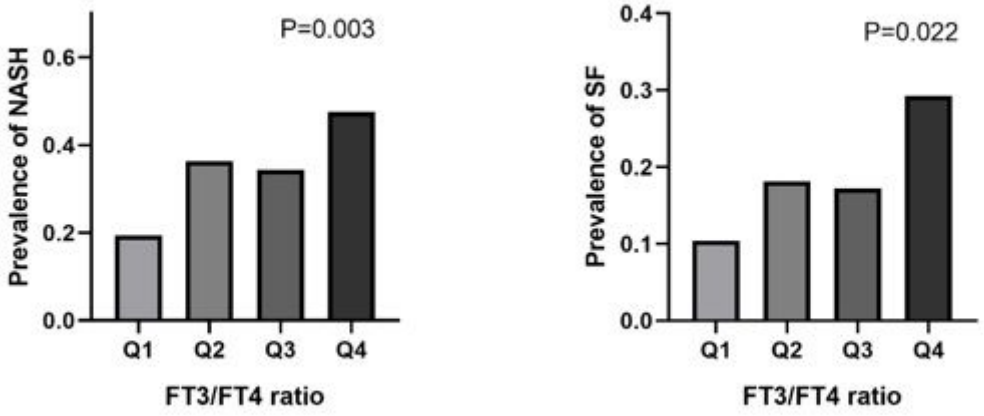

Figure 1

Prevalence of nonalcoholic steatohepatitis (NASH) and significant liver fibrosis (SF) according to fT3/fT4 ratio quartiles.

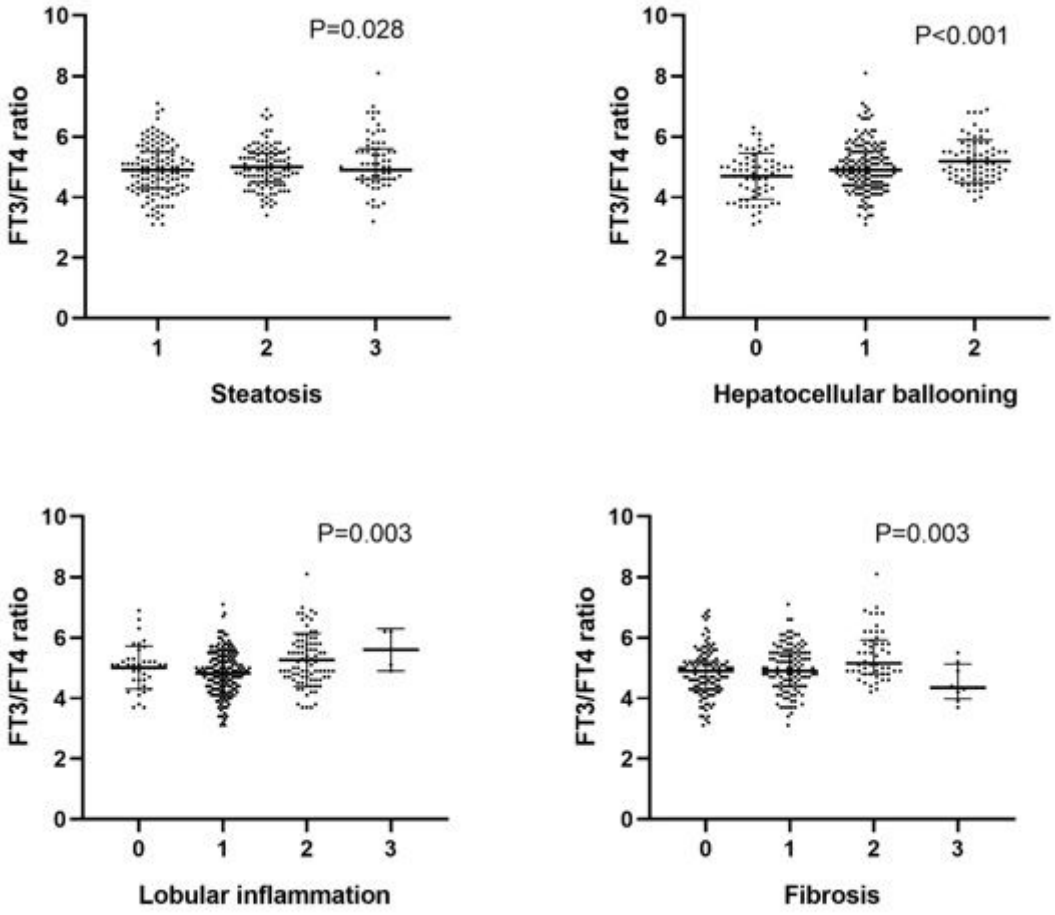

Figure 2

Correlation analysis between fT3/fT4 ratio and histological features spectrum of NAFLD.