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Associations among FT$_4$ level, FT$_3$/FT$_4$ ratio, and non-alcoholic fatty liver disease in Chinese patients with hypopituitarism

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Abstract. Non-alcoholic fatty liver disease (NAFLD) is the most common hepatic metabolic disorder. Thyroid function is associated with NAFLD in different populations; however, little attention has been paid in patients with hypopituitarism. To analyze the association between thyroid function and NAFLD, we included 134 patients with hypopituitarism admitted to the Tianjin Medical University General Hospital between June 2013 and May 2019. Participants were divided into the NAFLD(–) and NAFLD(+) groups based on abdominal ultrasonography findings. We evaluated 68 male and 66 female patients with hypopituitarism. The prevalence of NAFLD was 52.24%. The NAFLD(+) group had a significantly higher free triiodothyronine/free thyroxine (FT$_3$/FT$_4$) ratio than the NAFLD(–) group (p = 0.003). The NAFLD(+) group showed significantly lower levels of FT$_4$ and the growth hormone (GH) than the NAFLD(–) group (p = 0.003 and 0.016, respectively). We observed an association of the FT$_4$ level and FT$_3$/FT$_4$ ratio with NAFLD in the univariate model, which was non-significant after adjustment for metabolic parameters (BMI, HDL-C, triglycerides, serum uric acid, blood pressure, fasting glucose). To better understand the role of each metabolic parameters, we performed additional models for each of those predictors individually after adjustment for age and gender, the association between FT$_4$ level and FT$_3$/FT$_4$ ratio lost significance after adjustment for HDL-C and TG, but not for other predictors. Our findings suggest that thyroid dysfunction may be crucially involved in NAFLD by regulating whole-body metabolism, especially lipid utilization. Therefore, sufficient thyroid hormone replacement therapy for patients with hypopituitarism is recommended from the early stage.

Key words: Hypopituitarism, Non-alcoholic fatty liver disease, Free thyroxine, Free triiodothyronine/free thyroxine ratio

NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD) is the most common hepatic metabolic disorder. The increase in its prevalence is consistent with the increase in the worldwide prevalence of obesity and metabolic syndrome. The NAFLD prevalence in the general Asian population ranges from 15% to 40% [1]. NAFLD is a condition in which the fat deposited in the liver, due to causes other than excessive alcohol intake or other secondary causes, exceeds 5% of the liver's or hepatic weight [2]. NAFLD can progress to nonalcoholic steatohepatitis (NASH), with some cases progressing to liver cirrhosis and even hepatocellular carcinoma [3].

Thyroid hormones (THs) are involved in regulating body composition, lipid metabolism, and insulin resistance [4]. Hypothyroidism is associated with increased levels of low-density lipoprotein (LDL) cholesterol and triglycerides secondary to decreased activity of LDL receptors and clearance of triglyceride-rich lipoproteins [5, 6]. Excessive triglyceride accumulation in the liver can lead to steatosis, which is a component of NAFLD or NASH [7]. Specifically, NAFLD is considered a hepatic characteristic of metabolic syndrome [8]. Therefore, it is speculated that THs may be involved in the pathogenesis of NAFLD.

Hypopituitarism is the deficiency of one or more hormones produced in the anterior pituitary gland, with the most common sequential pattern being the loss of growth hormone (GH), follicle-stimulating hormone/luteinizing hormone, thyroid-stimulating hormone (TSH), and adrenocorticotropic hormone (ACTH), which may be accompanied by diabetes insipidus [9]. Patients with hypopituitarism, especially those with GH deficiency,

have an increased prevalence of metabolic syndrome characterized by central obesity, dyslipidemia, and NAFLD, compared with that of the general population [10].

Hypopituitarism is one of the rare causes of NAFLD [11], which is often accompanied by secondary hypothyroidism due to insufficient TSH secretion. Previous studies have shown that THs may play a key role in the pathogenesis of NAFLD. However, the role of thyroid dysfunction in NAFLD progress in patients with hypopituitarism remains unclear. This study aimed to analyze the clinical characteristics and association of TH levels and the presence of NAFLD in Chinese patients with hypopituitarism.

Materials and Methods

Participants and data collection

We evaluated 178 patients with hypopituitarism who were admitted to the Department of Endocrinology and Metabolism of Tianjin Medical University General Hospital between June 2013 and May 2019. Hypopituitarism was diagnosed based on clinical manifestations, examination of pituitary-related hormones, and provocative tests. Diagnosis of hypopituitarism [12]: (1) Growth hormone deficiency (GHD) was diagnosed by a GH peak below 3 μg/L in the insulin tolerance test (ITT) in adults or below 5 μg/L in the ITT (or clonidine stimulation test in children); (2) Hypogonadotropic hypogonadism (HH) was defined by a reduction in the levels of total testosterone lower than the minimum limit of the reference values for the method used and for women was based on the presence of amenorrhea, reduced estradiol and inadequately normal or low FSH/LH concentrations; (3) Secondary hypothyroidism was diagnosed from serum free thyroxine (FT_4) level below the laboratory reference range in conjunction with a low, normal, or mildly elevated TSH; (4) Secondary adrenal insufficiency was considered in patients with serum cortisol levels lower than 5 μg/dL in an appropriate clinical context or cortisol peak lower than 18 μg/dL in the ITT; (5) Central diabetes insipidus was defined by the presence of high serum osmolarity (>295 mOsm/kgH$_2$O), while the corresponding urine osmolality should lower than 300 mOsm/kgH$_2$O in fluid deprivation tests. The exclusion criteria was as follows: (1) hypopituitarism secondary to functional pituitary tumors, including acromegaly, prolactinoma, TSH adenoma, and ACTH-dependent Cushing syndrome; (2) autoimmune diseases, including glucocorticoid-treated hypophysitis; (3) primary thyroid disease; (4) viral hepatitis and alcoholic fatty liver (weekly alcohol consumption >21 and 14 units for men and women, respectively); and (5) malignancy. Finally, 134 patients (66 females and 68 males; Fig. 1) were

included in this study. There were no patients with liver cirrhosis. We retrospectively collected anthropometric indicators, laboratory tests, and abdominal ultrasonography findings. Based on the abdominal ultrasonography findings, the participants were divided into the NAFLD(−) and NAFLD(+) groups.

Data on patients' age, sex, race, smoking status, current alcohol consumption, cause and duration of hypopituitarism, laboratory findings, abdominal ultrasonography results, complications, comorbidies, and hormone replacement were collected from the standardized electronic inpatient record. Trained doctors measured height, weight, and blood pressure using a standard criterion. Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters squared). The duration of hypopituitarism was calculated starting from the date of clinical diagnosis. The glucocorticoid replacement dose was expressed as the daily dose of hydrocortisone and the equivalent dose of 20 mg hydrocortisone was 5 mg prednisolone. The replacement dose of thyroid hormone was expressed as the daily dose of levothyroxine. Following an overnight fasting period of ≥10 h, blood samples were obtained between 08:00 and 10:00 a.m. for biochemical analysis and examination of pituitary-related hormone levels and other parameters.

Further, based on the standardized criteria, NAFLD was diagnosed as hepatic steatosis according to the abdominal ultrasonography findings [13]. Abdominal ultrasonography, for assessment of hepatic steatosis, was performed for all participants by a trained operator who was blinded to the participants' clinical and laboratory characteristics, using a SIEMENS S3000 machine. NAFLD was diagnosed based on the presence of hepatic brightness, hepatorenal echo contrast, deep attenuation, and vascular blurring on ultrasonography [14].

Statistical analysis

For the descriptive analysis, categorical variables are expressed as the percentage and frequency. Numerical variables are expressed as the mean ± standard deviation or medians and interquartile range, based on the sample distribution. Between group differences were evaluated using Student's *t*-tests and Mann-Whitney *U* tests for non-categorical variables and the chi-square and Fischer's exact tests for categorical variables.

Multivariable logistic regression models were applied to assess the associations among metabolic parameters (including BMI, triglycerides, high-density lipoprotein cholesterol (HDL-C), and serum uric acid), GH levels, FT_4 levels, FT_3/FT_4 ratio, and NAFLD presence. Subsequently, additional multivariable models adjusted as follows to estimate the effects of FT_4 levels and the FT_3/FT_4 ratio on the odds of NAFLD presence were performed:
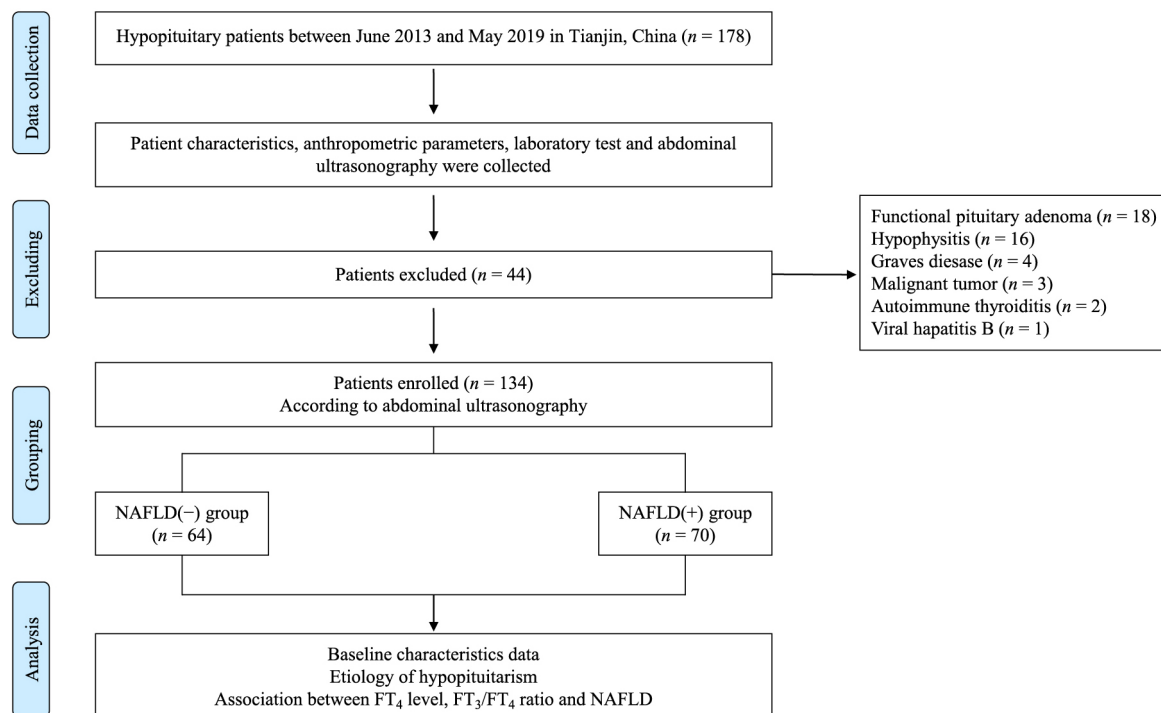
Fig. 1 Schematic overview of study data, calculations and analyses

NAFLD, non-alcoholic fatty liver disease; FT$_3$, free triiodothyronine; FT$_4$, free thyroxine

Model 1: adjusted for age and sex

Model 2: adjusted for variables in model 1, duration, and smoking

Model 3: adjusted for variables in model 2 and daily dose of levothyroxine

Model 4: adjusted for variables in model 2 and metabolic parameters (BMI, HDL-C, triglycerides, serum uric acid, blood pressure, and fasting glucose)

To adjust for each metabolic parameter (BMI, HDL-C, triglycerides, serum uric acid, blood pressure, and fasting glucose), we used additional models for each of the aforementioned predictors after adjustment for age and sex.

Statistical analyses were performed using SPSS Statistics version 25.0 (IBM Corp., Armonk, New York). A p value <0.05 was considered to indicate statistical significant.

Study approval

In this study, informed consent were not obtained because we used an electronic data set with consistent medical records from the Department of Endocrinology and Metabolism, which did not include personally identifiable information. The study protocol was approved by the Tianjin Medical University General Hospital Institutional Review Board with waiver of individual informed consent (approved number IRB2020-YX-029-01).

Results

Clinical characteristics of included participants

Table 1 presents the baseline clinical characteristics of the participants according to the presence or absence of NAFLD. We performed a comparative analysis. There were 70 (F/M, 31/39) and 64 (F/M, 35/29) patients in the NAFLD(+) and NAFLD(−) groups, respectively. The overall prevalence of NAFLD was 52.24%. The NAFLD(+) group had a significantly higher FT$_3$/FT$_4$ ratio than that of the NAFLD(−) group (0.34 [0.27–0.42] vs. 0.28 [0.22–0.36], p = 0.003). Further, the NAFLD(+) group had a significantly lower mean FT$_4$ and GH levels than that of the NAFLD(−) group (9.42 [7.27–12.70] vs. 12.34 [8.79–15.30], p = 0.003 and 0.05 [0.05–0.11] vs. 0.07 [0.05–0.23], p = 0.016; respectively). Additionally, the NAFLD(+) group had significantly higher BMI, alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transpeptidase, triglyceride (TG), uric acid (UA), daily dose of levothyroxine, and lower HDL-C level than that of the NAFLD(−) group.

Etiology of hypopituitarism and distribution of diagnosed hormone deficiency

Table 2 presents the etiology and types of tumors in the included patients with hypopituitarism. There were between-group differences in the causes of hypopituitarism and tumor types (p = 0.025 and 0.034, respectively).

Table 1 Baseline characteristics of hypopituitary patients between group NAFLD(+) and group NAFLD(−)

Parameters	NAFLD(−)	NAFLD(+)	*p* value
No. of participants	64	70	
Male *n* (%)	29 (45.31)	39 (55.71)	0.229
Age (yr)	56.50 (44.50–66.75)	56.50 (35.25–66.00)	0.599
Duration (yr)	3.00 (0.23–10.00)	2.00 (0.75–7.50)	0.926
BMI (kg/m^2)	23.34 (20.46–25.58)	26.90 (25.01–29.09)	0.000*
SBP (mmHg)	120.00 (110.00–140.00)	120.00 (110.00–135.50)	0.756
DBP (mmHg)	80.00 (70.00–85.00)	80.00 (70.00–84.25)	0.984
Smoking *n* (%)	11 (17.91)	12 (17.14)	0.993
Daily dose of hydrocortisone	20 (10–40)	20 (10–30)	0.166
Daily dose of levothyroxine	25 (0–50)	25 (12.50–62.50)	0.035*
Routine blood tests			
WBC (×10^9/L)	6.80 ± 2.29	7.33 ± 2.24	0.177
RBC (×10^{12}/L)	3.97 ± 0.54	4.12 ± 0.41	0.081
Hb (g/L)	121.03 ± 14.71	125.10 ± 11.72	0.080
PLT (×10^9/L)	247.49 ± 69.74	232.93 ± 80.16	0.269
FIB (g/L)	3.07 ± 0.64	2.86 ± 0.78	0.101
D-Dimer (ng/mL)	482.50 (285.00–812.00)	394.50 (257.00–585.25)	0.123
Liver enzymes			
ALB (g/L)	42.00 (38.00–45.00)	42.00 (40.00–45.50)	0.495
ALT (U/L)	15.00 (10.00–22.00)	26.00 (15.00–47.00)	0.000*
AST (U/L)	18.00 (15.00–25.00)	23.00 (17.00–33.00)	0.004*
ALP (U/L)	66.00 (52.00–86.50)	63.00 (51.00–90.50)	0.837
GGT (U/L)	16.00 (12.50–23.00)	30.00 (22.00–63.00)	0.000*
LDH (U/L)	198.08 ± 47.98	216.52 ± 55.86	0.047*
TBIL (μmol/L)	7.70 (5.50–10.85)	8.80 (6.30–11.85)	0.051
DBIL (μmol/L)	2.60 (1.90–3.60)	3.10 (2.30–4.58)	0.026*
Metabolic parameters			
TC (mmol/L)	4.94 (4.04–5.66)	5.03 (4.20–5.98)	0.431
TG (mmol/L)	1.32 (0.87–1.82)	2.13 (1.36–2.82)	0.000*
LDL-C (mmol/L)	2.93 ± 1.09	2.99 ± 1.08	0.752
HDL-C (mmol/L)	1.165 (0.87–1.43)	0.97 (0.79–1.16)	0.002*
GLU (mmol/L)	4.80 (4.30–5.30)	5.10 (4.40–5.88)	0.071
UA (μmol/L)	244.22 ± 86.13	316.06 ± 109.52	0.000*
Diabetes *n* (%)	15 (35.71)	27 (64.29)	0.059
Hypertension *n* (%)	22 (47.83)	24 (52.17)	0.991
Pituitary related hormone			
TSH (μIU/mL)	1.52 (0.81–3.24)	1.31 (0.43–3.36)	0.386
FT$_3$ (pmol/L)	3.34 ± 0.91	3.25 ± 0.70	0.534
FT$_4$ (pmol/L)	12.34 (8.79–15.30)	9.42 (7.27–12.70)	0.003*
FT$_3$/FT$_4$	0.28 (0.22–0.36)	0.34 (0.27–0.42)	0.003*
ACTH (pg/mL)	17.60 (10.62–28.30)	15.80 (8.90–26.23)	0.509
Cor (μg/mL)	10.72 (4.43–21.75)	6.78 (2.88–16.33)	0.076
GH (ng/mL)	0.07 (0.05–0.23)	0.05 (0.05–0.11)	0.016*
IGF-1 (ng/mL)	45.70 (26.80–120.00)	64.90 (30.60–114.00)	0.492

* $p < 0.05$

Abbreviations: NAFLD, non-alcoholic fatty liver disease; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; WBC, white blood cells; RBC, red blood cells; Hb, hemoglobin; PLT, blood platelet; FIB, Fibrinogen; ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, γ-glutamyltrans ferase; LDH, lacitc dehydrogenase; TBIL, total bilirubin; DBIL, direct bilirubin; TC, total cholesterol; TG, triglyceride; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; GLU, glucose; UA, uric acid; TSH, thyroid stimulating hormone; FT$_3$, free triiodothyronine; FT$_4$, free thyroxine; ACTH, adrenocorticotropic hormone; Cor, cortisol; GH, growth hormone; IGF-1, insulin like growth factor 1

Except for unclear causes, surgery was the most common cause of hypopituitarism. Based on the postoperative pathological results, non-functionging pituitary adenoma was the most common tumor type in the NAFLD(+) group. Table 2 also shows the distribution of diagnosed hormone deficiency among the study participants. There was no significant between-group difference in the distribution of diagnosed hormone deficiency (p = 0.553). To confirm the physiological replacement of deficient hormones, following standard clinical practice, patients with hypopituitarism undergoing hormone replacement underwent regular clinical and biochemical tests. The prevalence of GHD was as high as 82.86% and 78.12% in NAFLD(+) and NAFLD(−) groups, respectively. In addition, we found that the prevalence of adult-onset GHD was 61.43% and 56.25% in NAFLD(+) and NAFLD(−) groups, respectively while that of childhood-onset GHD was 21.43% and 21.88%, respectively. There was no statistical difference between the two groups in the prevalence of adult-onset and childhood-onset GHD (p = 0.803). However, no patient in the study underwent GH replacement therapy.

Risk factors for NAFLD in patients with hypopituitarism

We performed logistic regression analysis to delineate the nature of the relationships among BMI, TG, HDL-C, UA, GH, FT$_4$, FT$_3$/FT$_4$ ratio, and NAFLD as independent variables (Table 3). Univariate analysis revealed no association between GH levels and NAFLD. Compared with the NAFLD(−) group, the NAFLD(+) group had significantly lower FT$_4$ levels and a higher FT$_3$/FT$_4$ ratio (Table 1). Univariate logistic analysis revealed a significant association of the FT$_4$ level and FT$_3$/FT$_4$ ratio with NAFLD (Table 3), which remained significant after adjustment for age, sex, duration of hypopituitarism, smoking, and daily levothyroxine dose. However, the association was not significant after adjustment for metabolic parameters (Table 4).

Further analysis of each predictor revealed that the association between FT$_4$ levels and FT$_3$/FT$_4$ ratio lost significance after adjustment for HDL-C and TG, but not other predictors (Table 5).

Table 2 Etiology of hypopituitarism, type of tumor and distribution of diagnosed hormone deficiency in patients with hypopituitarism n (%)

	NAFLD(−) (n = 64)	NAFLD(+) (n = 70)	p value
Etiology			0.025*
Surgery	18 (28.12)	32 (45.71)	
Radiotherapy	2 (3.12)	6 (8.57)	
Surgery and radiotherapy	3 (4.69)	5 (7.14)	
Vacuolar sella syndrome	6 (9.38)	9 (12.86)	
Sheehan syndrome	3 (4.69)	4 (5.71)	
Sellar lesions	13 (20.31)	4 (5.71)	
Others	19 (29.69)	10 (14.29)	
Type of tumor			0.034*
Non-functioning pituitary adenoma	22 (34.38)	25 (35.71)	
Craniopharyngioma	5 (7.81)	14 (20.00)	
Intracranial germ cell tumor	0 (0.00)	3 (4.29)	
Hypophyseal cyst	5 (7.81)	1 (1.43)	
Distribution of diagnosed hormone deficiency			0.553
Adrenal	59 (92.19)	59 (84.29)	
Thyroid	39 (60.94)	58 (82.86)	
Growth hormone	50 (78.12)	58 (82.86)	
Gonadotrophines	64 (100)	70 (100)	
Diabetes insipidus	6 (9.38)	11 (15.71)	

* p < 0.05
Abbreviations: NAFLD, non-alcoholic fatty liver disease

Discussion

NAFLD is considered the liver disease component of metabolic syndrome. Hypopituitarism, which induces a condition similar to metabolic syndrome, is associated with NAFLD. We found that the NAFLD prevalence in patients with hypopituitarism (52.24%) was notably higher than that in the generation population (25%,

reported in 2016) [15]; however, it was similar to that reported in Chinese adult patients with hypopituitarism (54%) [16]. More attention should be paid to NAFLD caused by hypopituitarism since such cases can rapidly progress to cirrhosis even at a young age [17].

Our findings suggested that surgery, especially for non-functioning pituitary adenoma and craniopharyngioma, was the most common etiology of hypopituitarism with NAFLD. Surgical resection may damage the pituitary gland and hypothalamus. Since the hypothalamus is involved in regulating the appetite center and sympathetic nervous system, hypothalamic damage can also cause eating disorders and decreased sympathetic nerve activity, in addition to GH deficiency [18].

Disturbances in the endocrine hypothalamic-pituitary axis are associated with NAFLD, including GH deficiency, hypothyroidism, and hypogonadism [19]. The NAFLD prevalence in patients with hypopituitarism-related GH deficiency is approximately 60%. Additionally, GH supplementation therapy can reverse non-alcoholic steatohepatitis [20]. In this study, the prevalence of GHD was 82.86% in NAFLD(+) group. However, due to the difficulty in purchasing and high cost of growth hormone, most patients could not acquire it, and none of the patients with hypopituitarism, we included, received growth hormone therapy.

Table 3 Univariate logistic regression analysis of the association between NAFLD and related factors

	OR	95%CI	*p* value
BMI	1.371	1.204–1.561	0.000*
TG	1.798	1.230–2.627	0.002*
HDL-C	0.206	0.068–0.618	0.005*
UA	1.007	1.003–1.011	0.000*
GH	0.673	0.372–1.219	0.192
FT_4	0.862	0.784–0.947	0.002*
FT_3/FT_4 ratio	1.048	1.014–1.083	0.006*

FT_3/FT_4 ratio = FT_3/FT_4*100
* $p < 0.05$
Abbreviations: NAFLD, non-alcoholic fatty liver disease; BMI, body mass index; TG, triglyceride; HDL-C, high density lipoprotein cholesterol; UA, uric acid; GH, growth hormone; FT_3, free triiodothyronine; FT_4, free thyroxine

Table 4 Logistic regression for NAFLD outcome using FT_4 level and FT_3/FT_4 ratio

	Model 1		Model 2		Model 3		Model 4	
	OR	*p* value	OR	*p* value	OR	*p* value	OR	*p* value
FT_4	0.867	0.004*	0.859	0.003*	0.878	0.013*	0.881	0.074
FT_3/FT_4 ratio	1.047	0.011*	1.057	0.005*	1.051	0.012*	1.016	0.543

FT_3/FT_4 ratio = FT_3/FT_4*100
Model 1: adjusted for age, gender
Model 2: adjusted for variables in model 1 and also adjusted for duration and smoking
Model 3: adjusted for variables in model 2 and also adjustesd for daily dose of levothyroxine
Model 4: adjusted for variables in model 2 and use of metabolic parameters (BMI, HDL-C, triglycerides, blood pressure, fasting glucose, uric acid)
* $p < 0.05$
Abbreviations: NAFLD, non-alcoholic fatty liver disease; BMI, body mass index; HDL-C, high density lipoprotein cholesterol; FT_3, free triiodothyronine; FT_4, free thyroxine

Table 5 Logistic regression for NAFLD outcome with individual risk factors

	BMI		HDL-C		TG		SBP		Glucose		UA	
	OR	*p* value	OR	*p* value	OR	*p* value	OR	*p* value	OR	*p* value	OR	*p* value
FT_4	0.852	0.004*	0.906	0.059	0.907	0.067	0.859	0.003*	0.854	0.003*	0.897	0.040*
FT_3/FT_4	1.048	0.025*	1.029	0.134	1.027	0.168	1.048	0.011*	1.045	0.021*	1.024	0.211

FT_3/FT_4 ratio = FT_3/FT_4*100
All data were adjusted for age and gender
* $p < 0.05$
Abbreviations: NAFLD, non-alcoholic fatty liver disease; BMI, body mass index; HDL-C, high density lipoprotein cholesterol; TG, triglyceride; SBP, systolic blood pressure; UA, uric acid; FT_3, free triiodothyronine; FT_4, free thyroxine

Kang SJ *et al.* enrolled 76 adolescents and young adult patients with childhood-onset hypopituitarism and 74 controls matched by age and BMI. Among patients with hypopituitarism, a lower insulin-like growth factor-1 standard deviation score (SDS) was associated with steatosis [21]. In our study, the NAFLD(+) group showed significantly lower GH levels than the NAFLD(–) group. However, logistic analysis revealed no significant association between GH levels and NAFLD. The reason for different results from previous studies may be due to the different ages of the included study population. In addition, Zhu H *et al.* established age- and sex-specific reference ranges for serum IGF-I on a large scale in a Chinese population. The +2SD, +SD, median, –SD, and –2SD of the serum IGF-I levels in healthy Chinese adults were obtained for both sexes [22]. Since the population in our study was patients with hypothyroidism, the IGF-1 level of the majority of patients were lower than -2 standard deviations, the exact IGF-1 SDS could not be used to analyze the GH deficiency.

Sex hormones are related to the presence and severity of NAFLD. Mueller NT *et al.* [23] found that sex hormones were associated with distinct histologic features of NAFLD in children and adolescents. The levels of testosterone in men and women are different, and the levels of LH, FSH, and progesterone in females are affected by the menstrual cycle and menopause. Since the levels of sex hormones could not be analyzed in this study, we could not clarify the associations between sex hormones and NAFLD. Therefore, we speculate that other pituitary-related hormones could be involved in the pathophysiology of NAFLD in patients with hypopituitarism.

It has been demonstrated that a relationship existed between NAFLD and thyroid dysfunction [24]. Silveira *et al.* observed thyroid dysfunction in approximately 25% of patients with NAFLD [25]. A matched case-control study reported a two-fold higher risk of hypothyroidism in patients with hepatic steatosis than that in controls [26]. Numerous studies have shown an inverse relationship of serum TH levels with NAFLD since patients with clinical and subclinical hypothyroidism have an increased risk of NAFLD and vice versa [27-29]. A study confirmed that independent of insulin resistance, the FT$_3$/FT$_4$ ratio was a predictor of NAFLD in both patients with euthyroidism and hypothyroidism [30]. Local conversion of T$_4$ to the active form T$_3$ by type 2 iodothyronine deiodinase (DIO$_2$) is a key mechanism of thyroid hormone regulation. The FT$_3$/FT$_4$ ratio was evaluated as an indirect index for increased conversion of FT$_4$ to FT$_3$, reflecting deiodinase activity [31]. A cross-sectional study has indicated that FT$_3$/FT$_4$ ratio is strongly associated with insulin resistance in patients with euthyroidism and hypothyroidism [32]. An increased FT$_3$/FT$_4$ ratio was associated with metabolic syndrome and some of its components, including obesity, high TG, fasting blood glucose levels, and reduced HDL-C levels in individuals with normal thyroid function [33, 34]. The increase in the FT$_3$/FT$_4$ ratio could be the consequence of adaptation to adverse metabolic conditions including dyslipidemia, insulin resistance, and metabolic syndrome, which enhance the activity of DIO$_2$. Thus, a higher FT$_3$/FT$_4$ ratio possibly consequent to altered DIO expression, could to some extent reflect an effect of altered thyroid hormone status on NAFLD [31].

These findings suggest that thyroid dysfunction could promote NAFLD development. Moreover, most patients with hypopituitarism present with secondary hypothyroidism. In our study, TH replacement therapy was performed in 97 (72.39%) patients. We found that the NAFLD(+) group had significantly lower FT$_4$ levels and a higher FT$_3$/FT$_4$ ratio than the NAFLD(–) group. Logistic regression analysis revealed an association of the FT$_4$ level and FT$_3$/FT$_4$ ratio with the presence of NAFLD in patients with hypopituitarism. Our findings are consistent with previous reports in other populations.

Although there are various hypotheses regarding the pathogenesis of NAFLD, the relationship among NAFLD, hypothyroidism, and metabolic syndrome remains unclear. The association between thyroid dysfunction and NAFLD could be mediated by metabolic parameters, including BMI, HDL-C levels, TG levels, UA levels, blood pressure, and fasting glucose, since there was no significant association after adjustment for those variables. Further analysis of each predictor revealed that the association between FT$_4$ level and FT$_3$/FT$_4$ ratio lost significance after adjustment for HDL-C and TG, but not other predictors. This could be attributed to THs being crucially involved in regulating systemic metabolism and liver lipid utilization [35]. Hypothyroidism can promote NAFLD development by causing hyperlipidemia. TH, T$_4$, and the more biologically active form T$_3$ stimulate fatty acid (FA) β-oxidation and oxidative phosphorylation in the liver [36]. In the liver, crosstalk between TH receptors and peroxisome proliferator-activated receptor alpha activates the expression of genes involved in FA β-oxidation [37-39]. Hepatic autophagy of fat droplets and their conversion to free FA in fused autolysosomes (lipophagy) could be essential for TH-mediated FA β-oxidation [40]. Briefly, since THs are involved in regulating hepatic lipid utilization, hypothyroidism-induced NAFLD could involve an intrahepatic mechanism. Specifically, the TH signal is impaired, which reduces hepatic FA utilization, with FAs being instead esterified and accumulated as TGs [37, 39]. A clinical study on Asian male patients with diabetes and hepatic steatosis found that low-dose levothyroxine

supplementation for 4 months could significantly reduce hepatic fat content on magnetic resonance spectroscopy [41].

To the best of our knowledge, this is the first study to evaluate the relationship of the FT_4 level and FT_3/FT_4 ratio with NAFLD in patients with hypopituitarism. However, this study had some limitations. First, since hypopituitarism is a rare endocrine disease, the sample size of the study was small. Thus, our findings cannot be generalized to other populations. Second, liver biopsy is considered appropriate for accurate diagnosis of NAFLD; however, NAFLD was diagnosed using abdominal ultrasonography for the study participants. The reported sensitivity and specificity of ultrasound are 53–100% and 77–98%, respectively [42]. Third, we did not measure insulin levels; therefore, we could not evaluate the association between insulin resistance and NAFLD. Fourth, we could not collect data on the thyroid function and abdominal ultrasonography findings of the patients before hormone replacement therapy because most patients were not initially diagnosed with hypopituitarism. To exclude the effect of thyroid hormone replacement on NAFLD, we adjusted for the daily dose of levothyroxine in Model 3. Finally, since this was a retrospective cross-sectional study, we could not determine causal relationships. Therefore, prospective, multi-center, cohort studies and animal experiments are warranted to elucidate the pathophysiology of NAFLD in patients with hypopituitarism.

In summary, we observed a high prevalence of NAFLD among patients with hypopituitarism. Therefore, NAFLD should be closely monitored in patients with hypopituitarism, especially those who underwent cranial surgeries. Additionally, thyroid dysfunction may be crucially involved in NAFLD by regulating whole-body metabolism, especially lipid utilization. Therefore, sufficient TH replacement therapy for patients with hypopituitarism is recommended from the early stage.

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Disclosure

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