Plasma thyroid hormone concentration is associated with hepatic triglyceride content in patients with type 2 diabetes

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ABSTRACT

The underlying mechanisms responsible for the development and progression of non-alcoholic fatty liver disease (NAFLD) in patients with type 2 diabetes mellitus (T2DM) are unclear. Since the thyroid hormone regulates mitochondrial function in the liver, we designed this study in order to establish the association between plasma free T4 levels and hepatic triglyceride accumulation and histological severity of liver disease in patients with T2DM and NAFLD. This is a cross-sectional study including a total of 232 patients with T2DM. All patients underwent a liver MR spectroscopy (^{1}H-MRS) to quantify hepatic triglyceride content, and an oral glucose tolerance test to estimate insulin resistance. A liver biopsy was performed in patients with a diagnosis of NAFLD. Patients were divided into 5 groups according to plasma free T4 quintiles. We observed that decreasing free T4 levels were associated with an increasing prevalence of NAFLD (from 55% if free T4≥1.18 ng/dL to 80% if free T4<0.80 ng/dL, p=0.016), and higher hepatic triglyceride accumulation by ^{1}H-MRS (p<0.001). However, lower plasma free T4 levels were not significantly associated with more insulin resistance or more severe liver histology (ie, inflammation, ballooning, or fibrosis). Decreasing levels of plasma free T4 are associated with a higher prevalence of NAFLD and increasing levels of hepatic triglyceride content in patients with T2DM. These results suggest that thyroid hormone may play a role in the regulation of hepatic steatosis and support the notion that hypothyroidism may be associated with NAFLD. No NCT number required.

Significance of this study

What is already known about this subject?

- Hypothyroidism has been associated with the presence of non-alcoholic fatty liver disease (NAFLD) diagnosed by ultrasonography.
- Liver-targeted thyroid hormone receptor agonists have been shown to reduce steatosis in animal models.

What are the new findings?

- Decreasing plasma free T4 levels are associated with an increasing prevalence of NAFLD by MR spectroscopy (^{1}H-MRS).
- Moreover, decreasing plasma free T4 levels are related to increasing levels of hepatic triglyceride accumulation by ^{1}H-MRS.
- However, these associations did not translate into any significant change in liver histological parameters (ie, inflammation, ballooning, or fibrosis).

How might it impact on clinical practice in the foreseeable future?

- Results from this article are a call for physicians to consider the association between hypothyroidism and NAFLD in their clinical practice.

Non-alcoholic fatty liver disease (NAFLD) has become the most common chronic liver disease in developed countries,[1] with an estimated prevalence of 34% in the general population of the USA.[2] It can range from benign hepatic triglyceride accumulation, known as simple steatosis, to a more severe form with inflammation and fibrosis known as non-alcoholic steatohepatitis (NASH). Unfortunately, the underlying mechanisms that contribute to the progression from simple steatosis to NASH are not well understood. It is clear, however, that some clinical conditions, such as type 2 diabetes mellitus (T2DM), favor the progression to the more severe forms of the disease.[3 4]

Defects in the ability of hepatic mitochondria to properly increase β-oxidation rate in the setting of free fatty acids oversupply (secondary to obesity and T2DM) are believed to play a key role in the development and progression of NAFLD.[5] Increased β-oxidation is believed to prevent excessive hepatic triglyceride accumulation in insulin-resistant states, while, on the other hand, metabolic byproducts of incomplete fatty acid oxidation (ie, reactive oxygen

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species) could potentially trigger and worsen hepatocyte inflammation, necrosis, and fibrosis.[6] Thyroid hormones modulate mitochondrial function by a number of mechanisms, including the uncoupling of oxidative phosphorylation, among other mechanisms.[7] Therefore, it is reasonable to think that thyroid hormone levels may have a significant impact in the regulation of hepatic fatty acid metabolism in NAFLD.

Supporting this hypothesis, low thyroid hormone levels have been associated with a higher prevalence of NAFLD by ultrasonography,[8–10] and hypothyroidism has been found to be more prevalent in patients with NASH.[11] Moreover, liver-targeted thyroid hormone receptor agonists have been shown to reduce steatosis in animal models.[12]

A major shortcoming of previous clinical studies has been their reliance on liver ultrasound, a methodology that has been recently shown to have important limitations for the diagnosis of NAFLD and does not allow for quantification of liver fat.[13] The only study on patients with biopsy-proven NASH did not report on thyroid hormone levels or severity of liver disease.[11]

The aim of this study was to carefully assess the association between thyroid hormone levels and the amount of hepatic triglyceride accumulation measured by MR spectroscopy (^{1}H-MRS) and severity of liver disease by histology.

METHODS

Subjects

Patients with T2DM were recruited from San Antonio, Texas and Gainesville, Florida from the general population and from referrals from internal medicine and hepatology clinics. Patients of any gender, between 18 and 70 years of age, were included in the study, as long as they had a previous diagnosis of T2DM, were not receiving levothyroxine replacement, and their plasma thyroid-stimulating hormone (TSH) levels were within the normal range (0.27–4.2 mIU/L). Volunteers were also excluded if they had a history of alcohol abuse (≥30 g/day for males and ≥20 g/day for females), type 1 diabetes, any evidence of clinically significant renal, pulmonary, or heart disease, or any liver disease other than NASH (ie, chronic viral hepatitis, autoimmune hepatitis, hemochromatosis, Wilson's disease, drug-induced hepatitis). Patients receiving pioglitazone or vitamin E were also excluded. The study was approved by both Institutional Review Boards and a written informed consent was obtained from each patient prior to their participation.

Study design

A complete medical history, physical examination, ECG, and blood and urine chemistries were performed in all patients at screening, including plasma TSH and free thyroxine (T4). Other metabolic measurements included plasma glucose, and insulin measured at six different time points during a 2 h oral glucose tolerance test (OGTT). All patients underwent a two-step screening for NAFLD with liver ^{1}H-MRS, followed by a liver biopsy if the hepatic triglyceride content was higher than 5.5%.

Liver fat content

For the measurement of hepatic fat content, localized proton nuclear MR spectra of the liver were acquired on three different areas of the liver (30×30×30 mm each) using the methodology previously described.[13–15] Percentage of hepatic triglyceride content was calculated as the area under the curve (AUC) of fat peak divided by (fat +water peak AUC)×100. Measurements were corrected for T1 and T2 relaxation. A liver fat content of >5.5% was considered diagnostic of NAFLD as previously defined.[16] Total body fat content was measured by dual energy X-ray absorptiometry (DXA; Hologic Inc, Waltham, Massachusetts, USA).

Analytical measurements

Plasma TSH (normal range 0.27–4.2 mIU/L) and FT4 (normal range: 0.93–1.70 ng/dL) were measured with an electrochemiluminescence sandwich immunoassay on a COBAS 8000 (Roche Diagnostics). Plasma acylcarnitines were measured by liquid chromatography (LC)-based mass spectrometry (MS) after an overnight fast in a subgroup of 63 patients with a diagnosis of NASH. Free carnitine and acylcarnitines were extracted and quantified as previously reported by our group.[17] Detection was performed on a Thermo TSQ Access triple quadrupole MS with an Accela 1200 LC pump and HESI source. Acylcarnitines were separated on an ACE PFP-C18 column (100×2.1 mm, 2 µm pore size) at 40°C. Mobile phases consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The gradient began at 0.5% B, rose to 90% B over 10 min, remained isocratic for 4 min, went back to starting in 0.1 min, and equilibrated for 7 min. Data were collected using the selected reaction monitoring (SRM) mode by monitoring reactions fragmenting to m/z 85.3. MS was operated in a positive mode with a spray voltage of 3 kV, capillary temperature of 300°C, and vaporizer temperature at 300°C. Quantification of tissue acylcarnitines was done by comparison of individual ion peak areas to their respective internal ^{13}C standard (Cambridge Isotope Laboratories, Inc).

Liver biopsy

An ultrasound-guided liver biopsy was performed in patients with NAFLD by ^{1}H-MRS. Only 9% of patients (n=15) refused a liver biopsy. Biopsies were evaluated in a blinded fashion (ie, with the pathologist unaware of the participants' identity or clinical information). Histological characteristics for the diagnosis of NASH were determined using standard criteria.[18]

Statistical analysis

Data were expressed as the percentage or mean±SE for categorical and numeric variables, respectively. Numeric variables without a normal distribution were expressed as the median (IQR). Categorical variables were compared performing χ^2 or Fisher's exact test. Comparisons among groups were performed with the analysis of variance (ANOVA) (Bonferroni method for post hoc testing) or Kruskal-Wallis test. Pearson or Spearman correlations were used for numerical variables according to their characteristics. A two-tailed p value of <0.05 was considered to indicate statistical significance. Analyses were performed with Stata V.11.1 (StataCorp LP, College Station, Texas, USA).

RESULTS

Demographic and clinical characteristics

A total of 232 middle-aged patients with T2DM, mainly overweight and obese, were recruited from San Antonio, Texas and Gainesville, Florida, with a slight over-representation of the Hispanic ethnicity when compared to the general population of the USA (Caucasians 54%, Hispanics 32%, and African-Americans 13%).

Patients were divided into five groups depending on plasma free T4 quintiles: (1) <0.80; (2) 0.80–0.89; (3) 0.90–1.01; (4) 1.02–1.17; and (5) ≥1.18 ng/dL. As can be observed in table 1, there were no significant differences in gender, body mass index (BMI), glycated hemoglobin, fasting plasma insulin, or lipid panel among the five groups. Increasing levels of plasma free T4 were associated with higher diastolic blood pressure. Since only patients with normal TSH were included, we did not find any significant variation in plasma TSH among the groups (1.6 [1.3–2.1] vs 1.6 [1.3–2.1] vs 1.8 [1.2–2.6] vs 1.7 [1.3–2.4] vs 1.5 [1.0–2.0] mIU/L, respectively, p=0.77) although the group with higher plasma free T4 level had a slightly lower absolute TSH concentration.

Role of plasma free T4 in hepatic triglyceride accumulation

In figure 1A, we have summarized the prevalence of NAFLD by ^{1}H-MRS in patients with T2DM depending on their plasma free T4 levels. As can be observed, we found a significant increase in the prevalence of NAFLD by ^{1}H-MRS with decreasing plasma free T4 levels (from 55% to 63% in the groups with plasma free T4 ≥0.80 ng/dL to 80% if free T4<0.80 ng/dL, p for trend 0.016). Of note, this occurred regardless of similar BMI and diabetes control, as previously observed in table 1. The quantification of hepatic triglyceride content by ^{1}H-MRS among these groups followed a similar pattern, with decreasing values of plasma free T4 associated with higher hepatic triglyceride accumulation (figure 1B; p for trend <0.001). Even when only patients with a diagnosis of NAFLD by ^{1}H-MRS were considered, the amount of hepatic triglyceride content tended to increase with decreasing plasma free T4 levels (16±1% vs 16±2% vs 15±2% vs 14±2% vs 13 ±1%, p for trend 0.08). In accordance with these findings, plasma free T4 was significantly correlated with hepatic triglyceride accumulation (r=−0.26, p=0.001). Moreover, this remained true even after adjusting for age, glycated hemoglobin, and BMI (partial r=−0.27, p<0.001). Both plasma alanine and aspartate aminotransferases trended down with increasing plasma free T4 (table 1).

Role of plasma free T4 concentration on insulin resistance

In order to determine if the association between thyroid hormones and the amount of hepatic triglyceride accumulation was secondary to changes in insulin resistance, we then assessed the homeostatic model assessment (HOMA-IR; fasting state) and the Matsuda Index (during the OGTT) among the five groups of patients. No differences were observed in either measurement of insulin resistance among the groups (HOMA-IR: 3.3 [1.5–7.1] vs 3.8 [2.2–5.7] vs 3.3 [2.5–5.4] vs 4.0 [2.4–8.7] vs 3.3 [1.4–5.3], p=0.72; Matsuda Index: 2.6 [1.6–5.4] vs 2.5 [1.5–3.5] vs 2.8 [1.9–4.3] vs 2.5 [1.5–5.4] vs 2.8 [2.1–4.8], p=0.62). Since these indexes represent insulin resistance at the levels of the liver (HOMA-IR), or a combination of liver plus skeletal muscle (Matsuda), we used suppression

Table 1 Demographic and clinical characteristics

	Quintile 1 <0.80 ng/dL (n=47)	Quintile 2 0.80–0.89 ng/dL (n=54)	Quintile 3 0.90–1.01 ng/dL (n=40)	Quintile 4 1.02–1.17 ng/dL (n=45)	Quintile 5 ≥1.18 ng/dL (n=46)	p Value
Age (years)	55±1	56±1	57±1	60±1	59±1	0.02
Gender (male, %)	72	80	88	89	87	0.18
BMI (kg/m^2)	33.4±0.7	34.3±0.7	33.7±0.8	32.6±0.7	33.9±0.7	0.51
Total body fat (%)	32±1	35±1	34±1	36±1	36±1	0.08
HbA1c (%)	7.0±0.2	7.0±0.2	7.0±0.2	7.4±0.2	7.5±0.2	0.16
Plasma insulin (µU/mL)	15±2	15±2	13±1	13±2	14±2	0.89
Use of T2DM medications						
Metformin (%)	63	68	79	78	88	0.09
Sulfonylureas (%)	41	47	32	52	38	0.55
Insulin (%)	23	19	28	30	19	0.77
AST (U/L)	30 (24–45)	32 (23–54)	27 (21–36)	24 (18–28)	28 (21–36)	0.003
ALT (U/L)	39 (25–59)	40 (27–70)	36 (23–48)	30 (18–47)	31 (21–49)	0.09
Systolic blood pressure (mm Hg)	128±2	132±2	132±3	135±2	136±3	0.22
Diastolic blood pressure (mm Hg)	75±1	76±1	78±2	80±2	82±2	0.002
Use of lipid-lowering medication (%)	84	74	90	89	79	0.17
Cholesterol (mg/dL)	165±5	164±6	158±5	174±7	160±6	0.42
Triglycerides (mg/dL)	137 (97–192)	148 (109–192)	120 (87–166)	142 (99–238)	136 (94–197)	0.63
LDL-C (mg/dL)	94±5	92±5	89±4	98±6	86±5	0.49
HDL-C (mg/dL)	38±1	39±1	40±2	39±1	42±2	0.28

ALT, alanine transaminase ;AST, aspartate transaminase; BMI, body mass index; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; T2DM, type 2 diabetes mellitus.

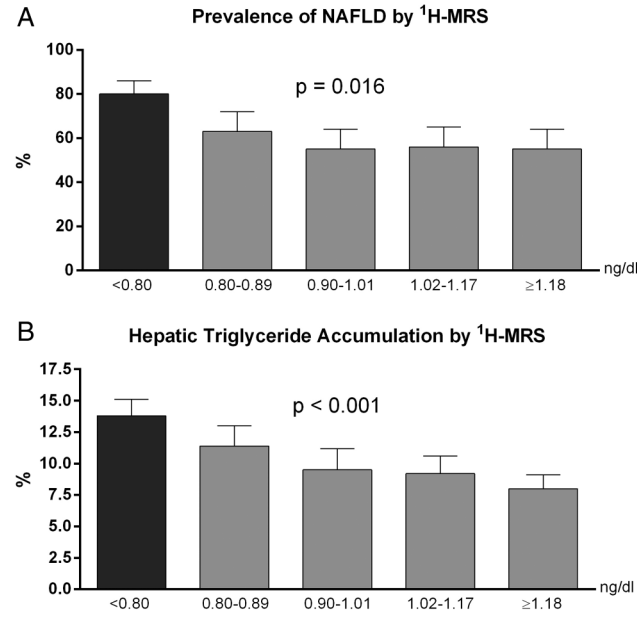

Figure 1 Prevalence of non-alcoholic fatty liver disease (NAFLD) (A) and hepatic triglyceride (TG) content measured by MR spectroscopy (^{1}H-MRS; B) in patients with type 2 diabetes mellitus over the spectrum of plasma free T4 levels.

of plasma free fatty acids (FFA) during the OGTT as a measure of insulin resistance in adipose tissue. Again, no difference was observed among the groups (66±3% vs 67 ±2% vs 72±3% vs 70±3 vs 66±4%, p=0.43).

Role of plasma free T4 concentration on the severity of liver disease by histology

The prevalence of definite NASH in patients with NAFLD was similar among the five groups (71% vs 59% vs 61% vs

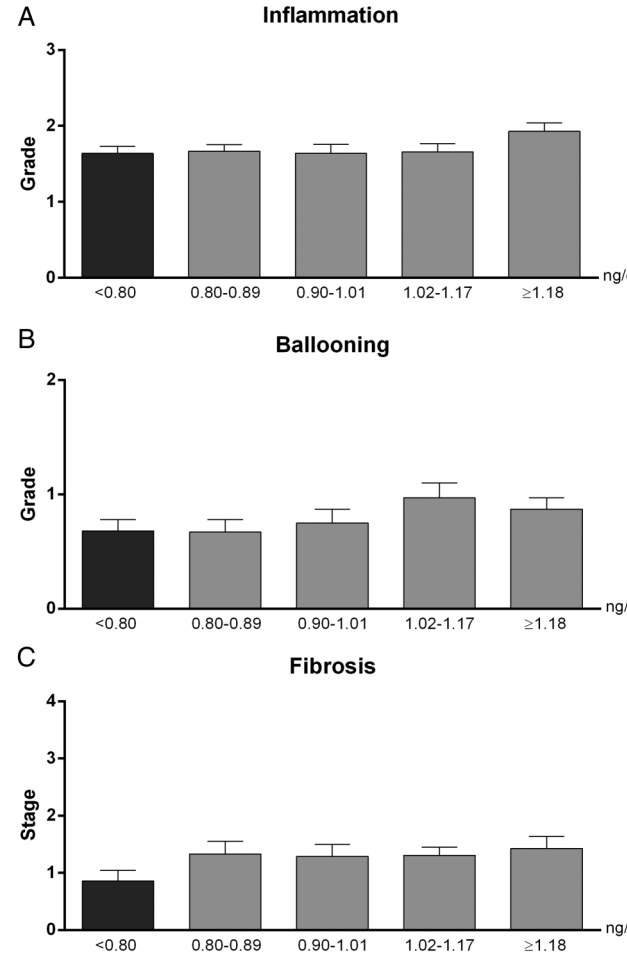

Figure 2 Severity of hepatic inflammation (A), ballooning (B), and fibrosis (C) according to plasma free T4 levels in patients with type 2 diabetes mellitus (T2DM). p Values calculated by non-parametric test for trend across ordered groups were only statistically significant for fibrosis (ie, p=0.03).

76% vs 80%, p=0.28). In figure 2, we have summarized the histological severity of liver disease according to the different plasma free T4 levels. As can be observed, increasing plasma free T4 levels were not significantly associated with worse inflammation (panel A) or ballooning (panel B). On the contrary, steatosis grade by histology decreased with increasing plasma free T4 levels (p=0.05), similar to what we observed with hepatic triglyceride content by ^{1}H-MRS. Regarding fibrosis stage (panel C), patients with low plasma free T4 showed a slightly lower fibrosis score compared to patients with plasma free T4≥0.80 ng/dL (p=0.04 comparing patients with plasma free T4<0.80 ng/dL against the other 4 groups), but no difference was observed in fibrosis stage among the other four groups with increasing plasma free T4.

Role of plasma free T4 on mitochondrial function in patients with T2DM and NASH

In order to indirectly assess the impact of plasma free T4 on mitochondrial fatty acid oxidation and the formation of relevant lipid metabolites, we measured plasma acylcarnitines by targeted metabolomics in a small group of patients (n=63) with NASH (50±1 years, 67% male, BMI 30.2 ±0.4 kg/m^2). Plasma acetyl and butyrylcarnitine were significantly associated with plasma free T4 (r=0.27, p=0.03 and r=0.27, p=0.04, respectively). Meanwhile, free carnitine was negatively associated with plasma free T4 (r=−0.30, p=0.02). Of note, all these correlations were independent of hepatic triglyceride content, suggesting a direct link between free T4 and acylcarnitines.

DISCUSSION

The present work is the first study assessing, in a quantitative way, hepatic triglyceride content by ^{1}H-MRS in patients with T2DM grouped across the spectrum of plasma free T4 levels. More importantly, it is the first study to assess the histological severity of liver disease in NAFLD among these groups. Overall, our results suggest that decreasing plasma free T4 levels (even within the spectrum of normal plasma TSH concentration) are associated with increasing levels of hepatic triglyceride accumulation, but do not play a role in the development of NASH. Of note, worse hepatic steatosis occurred in patients well matched for all other clinical characteristics, such as BMI and glycated hemoglobin, another strength of the current study. These findings have significant clinical implications suggesting an important role for subtle decreases in plasma thyroid hormone concentration relative to hepatic steatosis (but not steatohepatitis), and provide a pathophysiological basis for earlier observations of lower plasma free T4 levels being associated with a worse cardiovascular risk profile.[19]

Since free T4 is the biologically active form of the hormone, we decided to generate groups according to its plasma levels, regardless of having a normal plasma TSH level. In order to avoid differences generated by patients who were being treated for hypothyroidism, all patients receiving T4 replacement were excluded from the study. In addition, plasma free T4 levels were not found to be associated with insulin resistance, or diabetes control, excluding these variables as potential confounding factors in the association between free T4 levels and hepatic triglyceride content.

The concept that thyroid dysfunction may be associated with NAFLD has been controversial. In a retrospective study by Liangpunsakul and Chalasani,[11] patients with NASH were found to have a higher prevalence of hypothyroidism. However, all patients with hypothyroidism in this study were receiving T4 replacement, and were therefore most likely euthyroid (thus not supporting a pathogenic role of thyroid hormones in NAFLD). This was also the case in a study by Pagadala *et al*[20] in patients with biopsy-proven NAFLD. Other studies tried to confirm the association between NAFLD and low plasma thyroid hormones, but have been small, poorly controlled for potential confounding factors, or have relied on liver ultrasound for the diagnosis of NAFLD and did not perform liver biopsies to assess histology.[8–10]

Differences in hepatic triglyceride content with different plasma free T4 levels did not result in any significant worsening of other histological parameters (inflammation, ballooning, or fibrosis). We observed a trend toward lower plasma alanine and aspartate aminotransferases with increasing plasma free T4. These results are in accordance with previous reports,[21] and emphasize results from our own laboratory suggesting that plasma alanine and aspartate aminotransferases are a better reflection of the severity of hepatic triglyceride accumulation rather than of other histological parameters such as inflammation, ballooning, or fibrosis.[22] The fact that plasma free T4 levels were significantly correlated with acetyl and butyrylcarnitine (independently of hepatic triglyceride content) supports the role of free T4 in modulating β-oxidation in patients with NAFLD.

The use of liver-targeted thyroid hormone receptor agonists has raised some enthusiasm in the NAFLD field.[12 23] Indeed, in a provocative study by Bohinc *et al*,[24] reduced hepatic exposure to thyroid hormone was associated with worse liver disease. However, an opposite effect is also potentially possible, as elevated free T4 levels (and the resulting overactivation of mitochondria) can promote reactive oxygen species production, and consequently worsen liver histology, even when reducing the total amount of hepatic triglyceride content. However, our results imply that thyroid hormones may not have any impact on liver histology. Nevertheless, in our cohort of clinically asymptomatic patients, they mostly had normal or only slightly reduced plasma free T4 levels, with no patient diagnosed with overt hypothyroidism. Therefore, future work should assess the effects of overt hypothyroidism, and its reversal, on liver disease in patients with T2DM and NAFLD.

In summary, we have provided evidence that decreasing levels of plasma free T4 are associated with a higher prevalence of NAFLD and increasing levels of hepatic triglyceride content in patients with T2DM. Moreover, we showed that this did not result in any worsening of liver histological parameters (inflammation, ballooning, or fibrosis). Whether liver-targeted thyroid hormone receptor agonists have a role to play in the treatment of patients with NASH remains unknown, and only future randomized controlled trials will provide the answer to this. Meanwhile, the association observed in this study between lower plasma free T4 levels and NAFLD is a call for physicians to consider the potential association between hypothyroidism and NAFLD in their clinical practice.

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Patient consent Obtained.

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REFERENCES

1 Loomba R, Sanyal AJ. The global NAFLD epidemic. *Nat Rev Gastroenterol Hepatol* 2013;10:686–90.
2 Browning JD, Szczepaniak LS, Dobbins R, *et al*. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology* 2004;40:1387–95.
3 Adams LA, Sanderson S, Lindor KD, *et al*. The histological course of nonalcoholic fatty liver disease: a longitudinal study of 103 patients with sequential liver biopsies. *J Hepatol* 2005;42:132–8.
4 El-Serag HB, Hampel H, Javadi F. The association between diabetes and hepatocellular carcinoma: a systematic review of epidemiologic evidence. *Clin Gastroenterol Hepatol* 2006;4:369–80.
5 Cusi K. Role of obesity and lipotoxicity in the development of nonalcoholic steatohepatitis: pathophysiology and clinical implications. *Gastroenterology* 2012;142:711–725.e6.
6 Sunny NE, Parks EJ, Browning JD, *et al*. Excessive hepatic mitochondrial TCA cycle and gluconeogenesis in humans with nonalcoholic fatty liver disease. *Cell Metab* 2011;14:804–10.
7 Yehuda-Shnaidman E, Kalderon B, Bar-Tana J. Thyroid hormone, thyromimetics, and metabolic efficiency. *Endocr Rev* 2014;35:35–58.
8 Tao Y, Gu H, Wu J, *et al*. Thyroid function is associated with non-alcoholic fatty liver disease in euthyroid subjects. *Endocr Res* 2015;40:74–8.
9 Xu C, Xu L, Yu C, *et al*. Association between thyroid function and nonalcoholic fatty liver disease in euthyroid elderly Chinese. *Clin Endocrinol (Oxf)* 2011;75:240–6.
10 Chung GE, Kim D, Kim W, *et al*. Non-alcoholic fatty liver disease across the spectrum of hypothyroidism. *J Hepatol* 2012;57:150–6.
11 Liangpunsakul S, Chalasani N. Is hypothyroidism a risk factor for non-alcoholic steatohepatitis? *J Clin Gastroenterol* 2003;37:340–3.
12 Cable EE, Finn PD, Stebbins JW, *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.
13 Bril F, Ortiz-Lopez C, Lomonaco R, *et al*. Clinical value of liver ultrasound for the diagnosis of nonalcoholic fatty liver disease in overweight and obese patients. *Liver Int* 2015;35:2139–46.
14 Bril F, Maximos M, Portillo-Sanchez P, *et al*. Relationship of vitamin D with insulin resistance and disease severity in non-alcoholic steatohepatitis. *J Hepatol* 2015;62:405–11.
15 Portillo-Sanchez P, Bril F, Maximos M, *et al*. High prevalence of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus and normal plasma aminotransferase levels. *J Clin Endocrinol Metab* 2015;100:2231–8.
16 Szczepaniak LS, Nurenberg P, Leonard D, *et al*. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. *Am J Physiol Endocrinol Metab* 2005;288:E462–468.
17 Sunny NE, Kalavalapalli S, Bril F, *et al*. Cross-talk between branched-chain amino acids and hepatic mitochondria is compromised in nonalcoholic fatty liver disease. *Am J Physiol Endocrinol Metab* 2015;309:E311–19.
18 Sanyal AJ, Brunt EM, Kleiner DE, *et al*. Endpoints and clinical trial design for nonalcoholic steatohepatitis. *Hepatology* 2011;54:344–53.
19 Kim ES, Shin JA, Shin JY, *et al*. Association between low serum free thyroxine concentrations and coronary artery calcification in healthy euthyroid subjects. *Thyroid* 2012;22:870–6.
20 Pagadala MR, Zein CO, Dasarathy S, *et al*. Prevalence of hypothyroidism in nonalcoholic fatty liver disease. *Dig Dis Sci* 2012;57:528–34.
21 Dullaart RP, van den Berg EH, van der Klauw MM, *et al*. 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.
22 Maximos M, Bril F, Portillo Sanchez P, *et al*. The role of liver fat and insulin resistance as determinants of plasma aminotransferase elevation in nonalcoholic fatty liver disease. *Hepatology* 2015;61:153–60.
23 Perra A, Simbula G, Simbula M, *et al*. Thyroid hormone (T3) and TRbeta agonist GC-1 inhibit/reverse nonalcoholic fatty liver in rats. *FASEB J* 2008;22:2981–9.
24 Bohinc BN, Michelotti G, Xie G, *et al*. Repair-related activation of hedgehog signaling in stromal cells promotes intrahepatic hypothyroidism. *Endocrinology* 2014;155:4591–601.