Low-Dose Levothyroxine Reduces Intrahepatic Lipid Content in Patients With Type 2 Diabetes Mellitus and NAFLD

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Context: Nonalcoholic fatty liver disease (NAFLD) is highly prevalent in patients with type 2 diabetes mellitus (T2DM) and associated with significant morbidity and mortality. Thyroid hormone (TH) increases β-oxidation of fatty acids and decreases intrahepatic lipid content (IHLC) in rodents with NAFLD.

Objective: We investigated the possibility of low intrahepatic TH concentration in NAFLD and studied the effect of TH treatment in humans.

Design/Setting: This was a phase 2b single-arm study in six hospitals in Singapore. Intrahepatic thyroid hormone concentrations were measured in rats with induced NAFLD.

Patients: Euthyroid patients with T2DM and steatosis measured by ultrasonography.

Intervention: Levothyroxine was titrated to reach a thyroid-stimulating hormone level of 0.34 to 1.70 mIU/L before a 16-week maintenance phase.

Main Outcome Measures: The primary outcome measure was change in IHLC measured by proton magnetic resonance spectroscopy after treatment.

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Abbreviations: ^{1}H-MRS, proton magnetic resonance spectroscopy; ALT, alanine aminotransferase; fT_4, free thyroxine; HOMA-IR, homeostasis model assessment-estimated insulin resistance; IHLC, intrahepatic lipid content; LDL-C, low-density lipoprotein cholesterol; LT4, levothyroxine; MCD, methionine choline deficient; NAFLD, nonalcoholic fatty liver disease; SAT, subcutaneous adipose tissue; T2DM, type 2 diabetes mellitus; T_3, triiodothyronine; T_4, thyroxine; TH, thyroid hormone; TRβ, TH receptor-β; VAT, visceral adipose tissue.

Results: Twenty male patients were included in the per-protocol analysis [mean ± SD: age, 47.8 ± 7.8 years; body mass index (BMI), 30.9 ± 4.4 kg/m^2; baseline IHLC, 13% ± 4%]. After treatment, IHLC was decreased 12% (±SEM, 26%) relative to baseline (absolute change, −2%; 95% CI, −3 to 0; P = 0.046). Small decreases in BMI (P = 0.044), visceral adipose tissue volume (P = 0.047), and subcutaneous adipose tissue volume (P = 0.045) were observed. No significant changes in glucose regulation or lipid profile occurred.

Conclusion: This study demonstrated the efficacy and safety of low-dose TH therapy for NAFLD in men. TH or TH analogs may be beneficial for this condition. (*J Clin Endocrinol Metab* 103: 2698–2706, 2018)

Nonalcoholic fatty liver disease (NAFLD) encompasses a wide spectrum of pathologic liver conditions ranging from simple hepatosteatosis to steatohepatitis and cirrhosis. It is commonly associated with obesity, dyslipidemia, and insulin resistance (1), and now occurs in approximately 30% of the adult population in Western and Asian countries (2, 3). Among patients with type 2 diabetes mellitus (T2DM), the prevalence of NAFLD is as high as 50% (4). Its co-occurrence with T2DM significantly increases the risks for liver-related mortality, all-cause mortality, chronic kidney disease, and CVD (5–8). Impaired hepatic insulin sensitivity has been implicated in the cause and consequence of hepatosteatosis (9). Reduction of hepatic steatosis was associated with improved insulin sensitivity in subjects with T2DM (10, 11). Thus, there is a clear need for effective pharmacologic treatments in these patients.

It has long been known that thyroid hormone (TH) can induce favorable metabolic effects (12). TH-derived therapies have been extensively studied for dyslipidemia, due to the ability of TH to reduce low-density lipoprotein cholesterol (LDL-C) dramatically (13, 14). Several TH receptor-β (TRβ) agonists that target the main TH receptor isoform in the liver have been investigated but have not been used clinically, because of adverse effects. However, new compounds such as MGL-3196 are being investigated in phase 2 trials. Apart from dyslipidemia, there is also mounting evidence that TH is beneficial in NAFLD. There were clear associations between NAFLD and overt hypothyroidism [*i.e.*, high TSH and low free thyroxine (fT_4) levels], and subclinical hypothyroidism (high TSH and normal fT_4 levels) (15–19). In addition, patients with higher baseline TSH levels and no evidence of liver disease were more likely to develop NAFLD (20). Liver biopsy specimens from patients with hepatosteatosis to cirrhosis showed decreased hepatic deiodinase 1, an enzyme that converts thyroxine (T_4) to the biologically active triiodothyronine (T_3), suggesting increased intrahepatic hypothyroidism (21). In liver samples of patients who underwent bariatric surgery, the major genes that had altered expression in NAFLD were regulated by TH (22). In support of this intrahepatic hypothyroidism, TH and TH analogs decreased hepatosteatosis in various rodent models of NAFLD (23–26). Taken together, these data suggest that treatment with TH may be beneficial in patients with NAFLD, because TH concentration and/or action might be impaired.

To address this issue, we first measured TH concentrations in a rat model of NAFLD. Our findings then provided further rationale for performing a single-arm intervention study to investigate whether short-term, low-dose levothyroxine (LT4) therapy decreased intrahepatic lipid content (IHLC) as measured by proton magnetic resonance spectroscopy (^{1}H-MRS) in Asian male patients with T2DM and NAFLD.

Materials and Methods

Animal model of NAFLD

Animal experiments were approved by the Austin Health Animal Ethics Committee and performed according to the National Health and Medical Research Council of Australia Guidelines for animal experimentation. Male Sprague Dawley rats (10 weeks old) were fed a control diet (methionine choline replete diet; MP Biomedicals, Solon, OH) or methionine choline deficient (MCD) diet (MP Biomedicals) for 12 weeks. Rats were allowed unlimited access to their specific food and water and kept in cages of up to two animals. Rats were euthanized with sodium pentobarbital and liver tissue was collected. Liver T_3 [Chemical Abstracts Service (CAS) no. 6893-02-3], T_4 (CAS no. 51-48-9) and 3,3′,5′-triiodo-L-thyronine, or reverse T_3 (CAS no. 5817-39-0) were measured using liquid chromatography–tandem mass spectrometry, as previously described (27). Serum free T_3 level was measured using an immunoassay (Cobas 06437206 190) measured by a Roche Cobas 602 immunoassay automated analyzer. Total RNA was isolated using TRIzol (Sigma-Aldrich, St. Louis, MO). Total RNA (1 μg) was reverse transcribed by the iScript Select cDNA synthesis kit (catalog no. 170–8896; Bio-Rad, Hercules, CA) in accordance with the manufacturer's instructions. Quantitative polymerase chain reaction was performed using the QuantiTect SYBR Green PCR Kit (QIAGEN, Venlo, Netherlands) in accordance to the manufacturer's instructions (forward primer sequence for iodothyronine deiodinase 1: TGGCCATGGGCCAAAAGACCG; reverse: CCAGGGGTCTGCTGCCTTGAAT). Actin levels (forward primer sequence: TCCACCCGCGAGTACAACCTTC; reverse: ATCGTCATCCATGGCGAACTGG) were measured for normalization and fold change was calculated using the comparative threshold cycle method.

Intervention of LT4 in patients with T2DM

Study design and patients

The multicenter, single-arm study was conducted at six centers in Singapore between March 2014 and July 2016. Eligible patients were between 21 and 60 years old with stable T2DM (HbA1c, ≤10%). Patients also had to have a liver ultrasound showing presence of steatosis and have alanine aminotransferase (ALT) levels less than three times the upper limit of normal, per the institution's specified reference range, with a baseline TSH level between 1 and 10 mIU/L and fT_4 level in the normal range. In addition, patients could not have changes in statins or oral antidiabetic medication for the last 2 months or a >10-unit change in insulin dose, if they were receiving insulin, as documented in the medical records. Exclusion criteria included a history of viral hepatitis (except for hepatitis A or hepatitis E diagnosed at least 1 year before), consumption of ethanol >30 g/d (*i.e.*, three drinks per day or 21 drinks per week, with about 10 g of alcohol per drink), and advanced liver disease with a baseline NAFLD fibrosis score >0.675 (*i.e.*, stage 3 or 4 fibrosis). Additional inclusion and exclusion criteria are given in the Supplemental Materials and Methods.

Procedures

A common clinical protocol was approved by all institutional review boards and was conducted in accordance with the principles described in the Declaration of Helsinki. All patients provided written informed consent before study participation.

Screening fasting blood samples were obtained together with ECG, review of medical history, medications for the past 6 months, physical examination, and liver ultrasound, if not performed in the last 6 months, to determine eligibility. All laboratory assessments were done where the patient was screened. Patients who met the eligibility criteria underwent imaging to assess baseline IHLC, abdominal subcutaneous adipose tissue (SAT) volume, and visceral adipose tissue (VAT) volume. Low-dose oral LT4 was then titrated to attain a target TSH level between 0.34 and 1.70 mIU/L. Upon reaching the target range, the last titrated LT4 dose was used to commence a 16-week maintenance phase. The full protocol for commencement and titration of thyroid hormone is shown in the Supplemental Materials and Methods. Reduction of IHLC measured by ^{1}H-MRS can be achieved during this timeframe (28).

Patients were then called back at weeks 8 and 16 (*i.e.*, middle and end of the maintenance phase) for a physical examination, and measurements of TSH, fT_4, HbA1c, and random glucose levels. At the end of the study, the key assessments of efficacy were measured if the LT4 therapy had been maintained for at least 2 months with good compliance (*i.e.*, the amount of LT4 ingested divided by the amount the patient should have ingested was ≥70%, based on prescriptions). All patients continued to receive standard care. Telephone follow-up to encourage drug compliance was provided by the study coordinator at weeks 4 and 12 of the maintenance phase.

Imaging protocol

IHLC was assessed as a percentage using ^{1}H-MRS, a sensitive method for detecting steatosis (29, 30). SAT and VAT volumes were assessed using MRI. All the study MRI scans were performed at a single site (Clinical Imaging Research Center, Singapore) using the Tim Trio 3T scanner (Siemens, Erlangen, Germany). All readings and postprocessing were done by a single imaging expert to minimize interreader variability. A detailed description of the imaging protocol is shown in the Supplemental Materials and Methods.

Outcomes

The primary outcome measure was the absolute change in percentage IHLC between baseline and the end of the maintenance phase (week 16) after titration to serum TSH of 0.34 to 1.70 mIU/L with LT4 treatment. Secondary outcomes were changes in VAT and SAT volumes, and HbA1c, total serum cholesterol, high-density lipoprotein, LDL, and total triglyceride levels from baseline to 16 weeks after titration of TH. Adverse events were assessed at every visit.

Statistical analysis

We performed the primary per-protocol analysis in the population of patients treated with LT4 for 16 weeks after successful TSH titration (0.34 to 1.70 mIU /L) with ≥70% compliance. The safety analysis was done in all patients who received at least one dose of LT4. The primary and secondary end points were compared at baseline and after LT4 treatment with a paired sample *t* test, with significance set at two-sided $P < 0.05$). *Post hoc* subgroup analysis was performed for age >50 years. Correlations were measured with the Pearson correlation coefficient (*R*). *Post hoc* stepwise multiple linear regression was performed for change in the primary outcome. All correlations described in this article are not dependent on outliers.

Based on a report of a randomized controlled trial of 8-week resistance exercise in patients with NAFLD, we estimated 6% as the SD of change in IHLC from baseline to posttreatment in our sample size calculation. Thirty-four patients were required to detect a 3% change in IHLC with 80% power, based on paired-sample *t* test at a 5% significance level (two sided). The sample size was increased to 43 to allow for a 20% dropout rate. Patient enrollment was stopped early due to slow recruitment and end of funding. SPSS (version 24) was used for statistical analysis. Mean data are reported ± SD.

Results

Reduced TH in animal model of NAFLD

We measured TH concentration in an animal model of NAFLD to investigate whether intrahepatic TH concentrations were reduced. Rats fed an MCD diet to induce NAFLD had lower intrahepatic T_3 concentrations than rats receiving a control diet (control, 3.96 ± 0.92 ng/g vs MCD 2.42 ± 0.38 ng/g; Supplemental Fig. 1). However, no differences in serum T_3 concentrations were observed (Supplemental Fig. 1). Hepatic mRNA levels of deiodinase 1, an enzyme that converts T_4 to the biologically active T_3, were reduced by 55% in MCD rats ($P < 0.0001$; Supplemental Fig. 1). Taken together, these results showed decreased intrahepatic T_3 concentration in this rodent model of NAFLD, despite normal serum T_3 concentration. This animal study thus

provided further rationale for TH supplementation in patients with NAFLD.

LT4 treatment in patients with type 2 diabetes and NAFLD

Patient characteristics

We screened 30 patients for this study (Fig. 1). One patient had a device that precluded MRI. A total of 29 patients started titration of LT4. Three patients discontinued study medication during the titration phase. During the maintenance phase, six patients did not adhere to the protocol. Accordingly, a total of 20 male Asian patients with T2DM and hepatosteatosis who received the maintenance dose for 16 weeks after proper titration in the target range with >70% compliance were included in the per-protocol analysis (Fig. 1). Their mean age was 47.8 ± 7.8 years and mean BMI was 30.9 ± 4.4 kg/m^2. Baseline characteristics are shown in Table 1. Patients' medications included metformin (n = 19), statins (n = 16), vitamin E (n = 2), sulfonylureas (n = 11), angiotensin-converting enzyme inhibitors/angiotensin II receptor blockers (n = 9), dipeptidyl peptidase-4 inhibitors (n = 6), calcium antagonists (n = 5), insulin (n = 4), β-blockers (n = 4), fibrates (n = 3), and ezetimibe (n = 1).

TSH and fT$_4$

In all patients, TH treatment was titrated to a low-normal TSH level between 0.34 and 1.70 mIU/L, because the lowest prevalence of NAFLD had been found previously below this upper limit (18, 19). The lower limit for TSH was based on the lowest and most common cutoff for normal range among the six hospitals from which patients were recruited. At baseline, we measured serum TSH level of 1.86 ± 0.76 mIU/L and serum fT$_4$ level of 14 ± 3 pmol/L [Fig. 2(a)]. During the titration phase (mean, 54.5 days) TSH significantly decreased to 1.41± 0.25 mIU/L whereas fT$_4$ remained unchanged during the titration and maintenance phases [Fig. 2(b)]. The median maintenance dose was 18.75 μg of LT4 and ranged from 12.5 μg every 2 days to 87.5 μg daily. This indicates that low-dose LT4 reduced TSH levels within the normal range without causing any change in fT$_4$ levels.

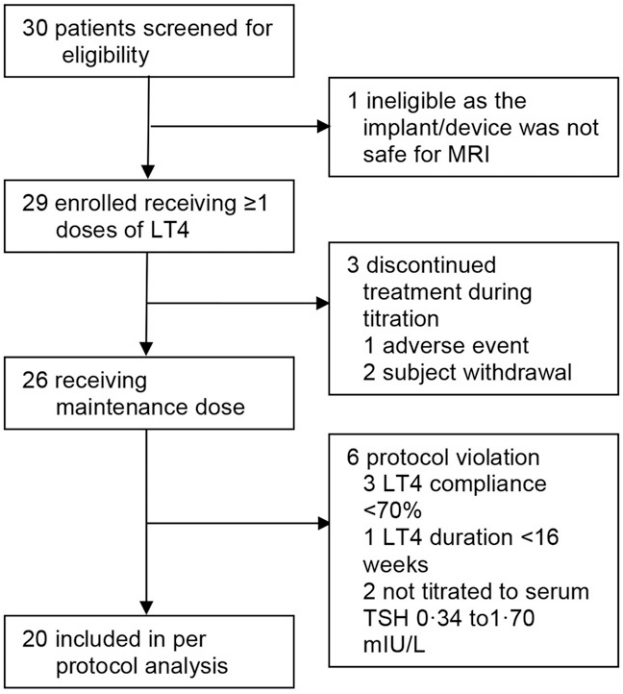

Figure 1. Study profile.

IHLC

We investigated whether low-dose LT4 decreased IHLC measured by ^{1}H-MRS after 16 weeks on maintenance dose. Average baseline IHLC in the study population was 13% (range, 8% to 23%). After treatment, there was a decrease in IHLC of 2% (absolute difference, 95% CI, −3 to 0; relative difference −12% ± 26%; P = 0.046), with 15 of 20 patients showing a decrease in IHLC [Fig. 3(a)]. Patients ≥50 years old had a significantly larger decrease in IHLC [<50 years, 2% ± 8%; ≥50 years, −23% ± 6%, P = 0.024; Fig. 3(b)].

We performed a stepwise multiple linear regression analysis to develop a model for predicting relative change in IHLC with the study parameters. A multivariate model including age (coefficient, −1.469; 95% CI, −2.566 to −0.372; P = 0.012) and change in BMI (coefficient, 19.051; 95% CI, 8.093 to 30.008; P = 0.002) accounted for 53% of the variance in IHLC (adjusted R^2 = 0.53; P = 0.001), with both variables independently correlated with IHLC (Supplemental Fig. 2). Of note, serum TSH and fT$_4$ levels measured after treatment and determination of the maintenance dose did not correlate with change in IHLC. To conclude, we found decreased IHLC after treatment with low-dose LT4 for 16 weeks.

HbA1c and homeostasis model assessment-estimated insulin resistance

Our secondary outcome measures included changes in HbA1c, lipid profile and body composition. HbA1c, a marker for glycemic control in patients with T2DM, was monitored at baseline, end of titration, 8 weeks of maintenance and 16 weeks maintenance because of known effects of TH stimulating gluconeogenesis. We found no change in HbA1c levels during LT4 treatment [repeated measurements generalized linear model P = 0.950; Fig. 4(a)]. In addition, there were no significant changes in fasting serum glucose and insulin levels, or homeostasis model

Table 1. Patient Characteristics and Clinical Parameters Before and After Levothyroxine Treatment

	Baseline	LT4 Treatment	Change	
	Mean (Range)	Mean (Range)	Mean (SD)	*P* Value
Age, y	48 (29–60)			
Body weight, kg	87.1 (68.6–116.0)	86.1 (67.9–116.4)	−1.0 (2.1)	0.042
BMI, kg/m^2	30.9 (25.0–40.6)	30.6 (25.6–40.4)	−0.4 (0.8)	0.044
Heart rate, bpm	78 (61–88)	80 (66–97)	2 (10)	0.289
Systolic blood pressure, mm Hg	133 (117–159)	133 (114–152)	−1 (12)	0.837
Diastolic blood pressure, mm Hg	81 (64–97)	80 (62–98)	0 (10)	0.857
Serum creatinine, μmol/L	76 (44–112)			
HbA1c, mmol/mol	62 (36–86)	62 (39–88)	0.0 (0.8)	0.800
Fasting glucose, mmol/L	7.8 (5.2–11.0)	8.2 (4.8–14.0)	0.4 (2.2)	0.477
Fasting insulin, mU/L	34 (6–152)	38 (5–186)	3 (28)	0.662
HOMA-IR	11.4 (1.8–47.3)	13.9 (2.5–62.0)	2.1 (11.2)	0.415
Total cholesterol, mmol/L	4.57 (2.55–7.46)	4.43 (3.05–5.86)	−0.14 (0.86)	0.462
LDL-C, mmol/L[a]	2.81 (1.06–5.81)	2.58 (1.42–3.91)	−0.25 (0.72)	0.154
HDL cholesterol, mmol/L	1.03 (0.87–1.32)	1.05 (0.60–1.52)	0.01 (0.16)	0.727
Triglycerides, mmol/L	2.05 (0.80–5.10)	2.03 (0.69–4.00)	0.00 (1.15)	0.995
NAFLD fibrosis score (31)	−1.405 (−3.510 to −0.1900)	−1.549 (−2.500 to −0.090)	−0.1444 (0.6969)	0.366
ALT, IU/L	64 (22–128)	61 (16–126)	−3 (24)	0.618
AST, IU/L	41 (18–86)	37 (13–71)	−4 (13)	0.164
Serum albumin, g/L	44 (39–51)	44 (38–51)	0 (2)	0.804
IHLC, %	13 (8–23)	11 (4–21)	−2 (3)	0.046
VAT volume, mL	3462 (1221–6700)	3323 (1454–6654)	−139 (292)	0.047
SAT volume, mL	3684 (1983–7128)	3553 (1842–6973)	−131 (273)	0.045

Abbreviations: AST, aspartate aminotransferase; HDL, high-density lipoprotein.

[a]Method of measurement (Friedewald or direct measurement) depended on institution.

assessment-estimated insulin resistance (HOMA-IR) after LT4 treatment (Table 1). Although low-dose LT4 did not change HbA1c, improvement in IHLC was correlated with improved HbA1c and HOMA-IR in our patients [Fig. 4(b) and 4(c)]. There was no correlation between improvement in these parameters and decreases in BMI, VAT volume, or SAT volume.

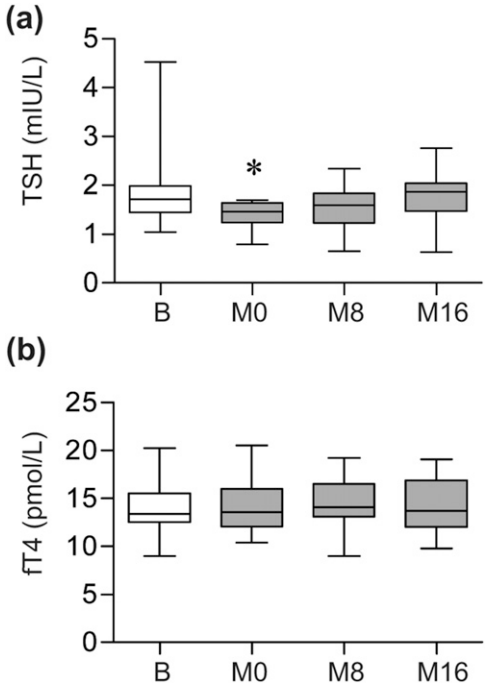

Figure 2. Whisker plots. (a) TSH and (b) fT_4 at baseline (B), after titration (M0), after 8 weeks on maintenance dose (M8), and after 16 weeks on maintenance dose (M16). The whisker plots show minimum, quartile (Q)1, Q2, Q3, and maximum data. *$P < 0.05$ compared with baseline.

Body composition

Body composition was measured at baseline by determining BMI and measuring SAT and VAT volumes by MRI. There were small but significant decreases in body weight (−1.0 kg; 95% CI, −3.0 to 0.0 kg; $P = 0.042$), BMI (−0.4 kg/m^2; 95% CI, −0.7 to 0.0 kg/m^2; $P = 0.044$), VAT volume (−139 mL; 95% CI, −275 to −2 mL; $P = 0.047$), and SAT volume (−131 mL; 95% CI, −259 to −3 mL; $P = 0.045$) after LT4 treatment (Table 1). There were significant correlations between the decreases in BMI, and SAT and VAT volumes (Supplemental Fig. 3). Loss in VAT volume significantly correlated with increased levels of fT_4 after treatment, both after titration and at the end of the study (Supplemental Fig. 4), whereas BMI and SAT volume were not correlated with change in fT_4 levels. Taken together, these data showed that low-dose LT4 decreased BMI, and SAT and VAT volumes proportionally.

Lipid profile

We measured serum lipid profiles at baseline and after low-dose LT4 treatment. There were no significant changes in serum total cholesterol, LDL-C, high-density

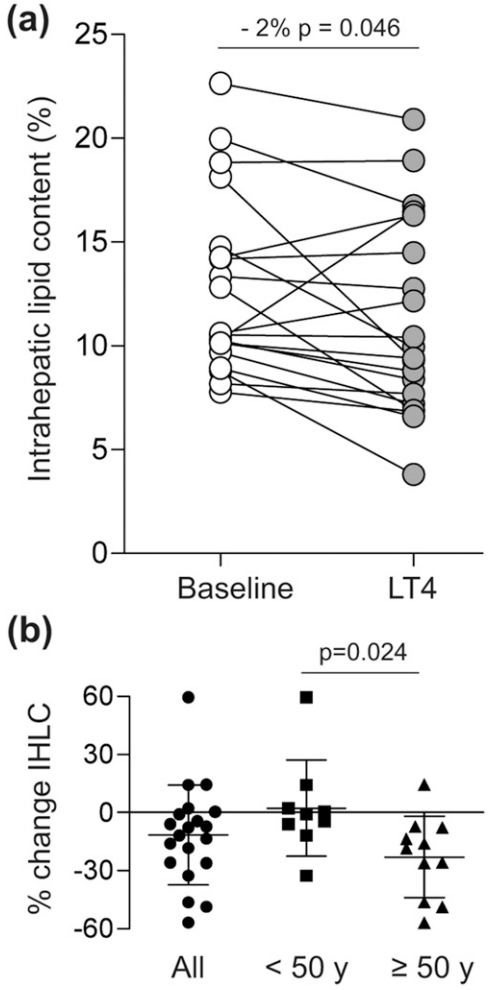

Figure 3. IHLC measured by ^{1}H-MRS. (a) IHLC at baseline and after 16 weeks of LT4 treatment of all patients. (b) Scatter plot of relative decrease of IHLC in all patients (data points indicated by black circles), patients <50 years old (n = 9; data points indicated by black squares), and patients ≥50 years old (n = 11; data points indicated by black triangles). Data are given as mean ± SD.

lipoprotein cholesterol, and triglyceride levels (Table 1). Patients with higher baseline LDL-C and triglyceride levels had greater decreases in LDL-C level after LT4 treatment (Supplemental Fig. 5). However, in all patients with T2DM, there was no significant improvement in lipid profile.

Adverse events

The safety data were analyzed for every patient receiving at least one dose of the study drug. There was no significant increase in heart rate during the study (2 ± 10 bpm; P = 0.289; Table 1). No serious adverse events were reported in the study. A total of 35 adverse events were reported in 14 of 29 patients receiving at least one dose of the study drug [grade 1 (mild), n = 29; grade 2 (moderate), n = 6]. Three patients discontinued the study medication during the titration phase because of adverse effects. One patient experienced palpitations, headache, diarrhea, and vomiting before deciding to discontinue treatment. Another patient experienced diarrhea, vomiting, dizziness, abdominal pain, coughing, bloating, and fatigue before discontinuing treatment. Yet another patient experienced pruritis before discontinuing treatment. In the patients taking the study drug for 16 weeks, there were three events of mild chest discomfort that resolved without taking medication and were classified as grade 1. All adverse events are shown in Supplemental Table 1.

Discussion

To date, it has not been shown in humans if TH can reduce steatosis, although reduced TH action has been implicated in the progression of NAFLD. In our study, we observed reduced intrahepatic concentration of TH in a rodent model of NAFLD. Previously, decreased intrahepatic TH levels were suggested by the observation that deiodinase 1 protein was reduced in the human NAFLD liver (21). We also observed reduced hepatic deiodinase 1 mRNA expression in conjunction with normal serum T_3 levels in our rat model of NAFLD. These findings suggest that intrahepatic TH levels may be reduced in patients with NAFLD despite patients having normal serum TH levels. We thus conducted this clinical study to investigate the potential efficacy of TH supplementation to increase intrahepatic concentration of TH in patients with NAFLD and T2DM. We found that low-dose LT4 moderately decreased IHLC, with more prominent effects occurring in older patients. LT4 treatment did not

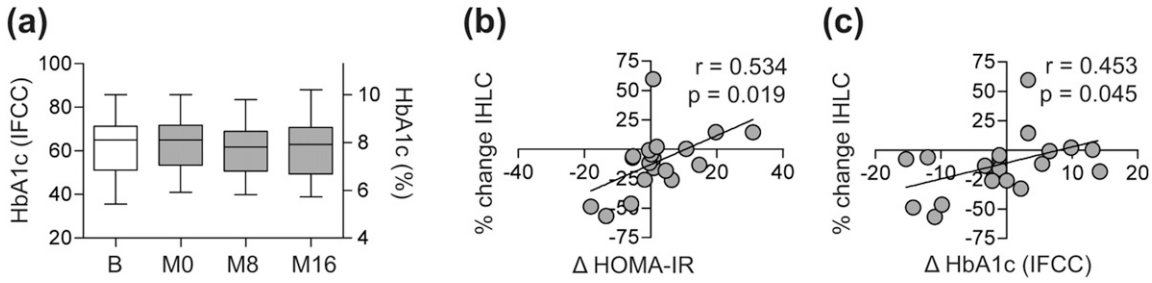

Figure 4. HbA1c and HOMA-IR data plots. (a) HbA1c at baseline (B), after titration (M0), after 8 weeks on maintenance dose (M8), and after 16 weeks on maintenance dose (M16). Whisker plot showing minimum, 25%, 50%, 75%, and maximum data. (b, c) Correlations between relative change in IHLC and (b) change in HOMA-IR during the study and (c) change in HbA1c during the study. IFCC, International Federation of Clinical Chemistry.

change glycemic control, although greater reduction of IHLC was associated with improved glycemic control and less insulin resistance.

Diet and exercise are the cornerstones for the treatment of NAFLD, because no drug treatment currently is registered for the treatment of NAFLD (32). When these lifestyle measures have been used alone or together for up to 1 year, significant decreases in ALT levels and IHLC were observed (33). The moderate beneficial effect on IHLC by LT4 that we observed in our study (−12%) was comparable to the effect observed in patients undergoing the currently advised exercise regimen of 30 to 60 minutes five times per week for 16 weeks (−10%) (28). Therefore, LT4 alone or in combination with diet and exercise can reduce IHLC significantly, perhaps even in patients resistant to lifestyle modifications.

Our study shows the efficacy of TH treatment in NAFLD in euthyroid men. It has been shown that TH is beneficial for reducing the prevalence of steatosis measured by ultrasonography in patients with overt hypothyroidism (34). The moderate effect shown in our study of euthyroid men could be improved by better selection of patients for treatment, setting a lower TSH target, and increasing treatment duration. Our study suggests that older patients benefit more from treatment with LT4, which could be due to lower hepatic deiodinase 1 levels in the aged group causing even lower intrahepatic T_3 levels (35). Furthermore, patients with higher baseline TSH values, such as those occurring in subclinical hypothyroidism, may exhibit greater decreases in IHLC. Concerning the TSH target, we observed only a moderate decrease of serum TSH (pretreatment TSH level, 1.86 ± 0.76 mIU/L; post-titration TSH level, 1.41 ± 0.25 mIU/L) in our patients. Patients whose TSH level was within our TSH range had the lowest prevalence of NAFLD in an association study; however, it currently is not known what the optimal serum TSH threshold is for intervention (18, 19). Finally, longer treatment could possibly increase the effect size. However, in patients with subclinical hypothyroidism or euthyroidism, the beneficial effects of longer treatment with TH on IHLC need to be weighed against long-term clinical adverse effects such as atrial tachycardia, arrhythmias, and loss of bone density. These adverse effects are mainly due to activation of the TH receptor-α, whereas in the liver, the TRβ is the predominant isoform. The recent development and use of TRβ-receptor–specific or liver-specific thyromimetics could produce beneficial effects on NAFLD, but with fewer adverse effects than TH (36). In this connection, animal studies showed that TRβ-specific thyromimetics decreased IHLC in various nutritional or genetic rodent models of NAFLD (23–25). Recently, a glucagon and TH conjugate that mainly targets the liver decreased hepatic steatosis and ALT concentration in a mouse model of NAFLD (26). In humans, MGL-3196, a TRβ-agonist, is being investigated in a phase 2 trial for biopsy-proven nonalcoholic steatohepatitis (ClinicalTrial.gov identifier: NCT 02912260). The current study is important for establishing the effect of the natural ligand of the receptor in light of development of these compounds for the treatment of NAFLD.

We did not observe glycemic deterioration from TH-induced gluconeogenesis as previously described in a TRβ-agonist and thyrotoxicosis (25, 37). However, our study showed improvements in HbA1c and HOMA-IR with decreasing IHLC. There is a strong link between hepatic steatosis and insulin resistance, and correction of steatosis reverses insulin resistance in man (9–11). LT4 treatment of NAFLD in patients with T2DM, therefore, may reduce insulin resistance.

We cannot exclude any placebo effect(s) due to participation in this study. However, patients were recommended to maintain their baseline exercise and dietary regimen during LT4 treatment and did not receive any additional consultations on lifestyle modifications, including alcohol consumption. Of note, difficulties in patient recruitment prevented us from enrolling enough patients to meet our target sample size calculation (n = 33; power, 80%) to detect changes in IHLC of 3%. Nonetheless, we observed a significant effect on IHLC with the 20 patients included in this analysis. The sample size for our study was relatively small for correlation analysis, so we may not have had the adequate power to identify other less robust correlations between clinical phenotypes and outcomes. Placebo-controlled, randomized controlled trials with a larger number of patients from different ethnicities and sexes need to be performed to confirm the current findings.

In summary, we showed that low-dose LT4 decreased IHLC in euthyroid male patients with T2DM. Although the long-term effects of moderate reduction of IHLC on morbidity and mortality are currently not known, improvement of insulin resistance and possible prevention of fibrosis by TH, as recently shown in the lung, will have important clinical significance (38). Finally, our studies provide a strong rationale for further investigation, development, and testing of THs and/or TH analogs for the treatment of NAFLD in patients with T2DM.

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