Research Article

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Thyrotropin increases hepatic triglyceride content through upregulation of SREBP-1c activity

Fang Yan[1,3], Qi Wang[2,4], Ming Lu[1,3], Wenbin Chen[2], Yongfeng Song[1,3], Fei Jing[1,3], Youfei Guan[5], Laicheng Wang[2], Yanliang Lin[2], Tao Bo[2], Jie Zhang[2], Tingting Wang[3], Wei Xin[2], Chunxiao Yu[1,3], Qingbo Guan[1,3], Xinli Zhou[1,3], Ling Gao[2,3], Chao Xu[1,3,*], Jiajun Zhao[1,3,*]

[1]*Department of Endocrinology and Metabolism, Shandong Provincial Hospital affiliated to Shandong University, Jinan, China;* [2]*Scientific Center, Shandong Provincial Hospital affiliated to Shandong University, Jinan, China;* [3]*Institute of Endocrinology, Shandong Academy of Clinical Medicine, Jinan, China;* [4]*Institute of Pharmacology of Shandong University, Jinan, China;* [5]*Department of Physiology and Pathophysiology, Shenzhen University Health Science Center, Shenzhen, China*

Background & Aims: Hallmarks of non-alcoholic fatty liver disease (NAFLD) are increased triglyceride accumulation within hepatocytes. The prevalence of NAFLD increases steadily with increasing thyrotropin (TSH) levels. However, the underlying mechanisms are largely unknown. Here, we focused on exploring the effect and mechanism of TSH on the hepatic triglyceride content.

Methods: As the function of TSH is mediated through the TSH receptor (TSHR), *Tshr*$^{-/-}$ mice (supplemented with thyroxine) were used. Liver steatosis and triglyceride content were analysed in *Tshr*$^{-/-}$ and *Tshr*$^{+/+}$ mice fed a high-fat or normal chow diet, as well as in *Srebp-1c*$^{-/-}$ and *Tshr*$^{-/-}$*Srebp-1c*$^{-/-}$ mice. The expression levels of proteins and genes involved in liver triglyceride metabolism was measured.

Results: Compared with control littermates, the high-fat diet induced a relatively low degree of liver steatosis in *Tshr*$^{-/-}$ mice. Even under chow diet, hepatic triglyceride content was decreased in *Tshr*$^{-/-}$ mice. TSH caused concentration- and time-dependent effects on intracellular triglyceride contents in hepatocytes *in vitro*. The activity of SREBP-1c, a key regulator involved in triglyceride metabolism and in the pathogenesis of NAFLD, was significantly lower in *Tshr*$^{-/-}$ mice. In *Tshr*$^{-/-}$*Srebp-1c*$^{-/-}$ mice, the liver triglyceride content showed no significant difference compared with *Tshr*$^{+/+}$*Srebp-1c*$^{-/-}$ mice. When mice were injected with forskolin (cAMP activator), H89 (inhibitor of PKA) or AICAR (AMPK activator), or HeG2 cells received MK886 (PPARα inhibitor), triglyceride contents presented in a manner dependent on SREBP-1c activity. The mechanism, underlying TSH-induced liver triglyceride accumulation, involved that TSH, through its receptor TSHR, triggered hepatic SREBP-1c activity via the cAMP/PKA/PPARα pathway associated with decreased AMPK, which further increased the expression of genes associated with lipogenesis.

Conclusions: TSH increased the hepatic triglyceride content, indicating an essential role for TSH in the pathogenesis of NAFLD.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the non-alcohol-induced accumulation of extra fat in the liver, resulting in diseases ranging from benign steatosis to advanced cirrhosis and cancer. Importantly, people with NAFLD have an increased risk of developing cardiovascular problems, such as heart attack and stroke [1]. The prevalence of NAFLD ranges from 9 to 36.9% of the population in different regions of the world [2]. Approximately 20% of the United States population suffers from NAFLD, and the prevalence of this condition is increasing [3]. The exact cause of NAFLD remains unknown. However, endocrine disorders such as obesity, insulin resistance, and hypothyroidism likely play a strong role in the pathology of this disease [4,5].

Subclinical hypothyroidism (SCH), characterized by elevated thyrotropin (TSH) and normal free thyroxine (T4) levels, has recently been demonstrated to be a risk factor for NAFLD [6,7]. The prevalence of NAFLD gradually increased with rising TSH levels [6]. Moreover, a prospective case-control study showed that

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* Corresponding authors. Addresses: Shandong Provincial Hospital affiliated to Shandong University, Institute of Endocrinology, Shandong Academy of Clinical Medicine, 324, Jing 5 Rd., Jinan, Shandong 250021, China. Tel.: +86 531 68776094; fax: +86 531 87068707 (C. Xu). Tel.: +86 531 68776375; fax: +86 531 87068707 (J. Zhao).
E-mail addresses: doctorxuchao@163.com (C. Xu), jjzhao@medmail.com.cn (J. Zhao).
Abbreviations: NAFLD, non-alcoholic fatty liver disease; SCH, subclinical hypothyroidism; TSH, thyrotropin; T4, free thyroxine; TG, triglyceride; SREBP-1c, sterol regulatory element-binding protein-1c; PPARα, peroxisome proliferator-activated receptor α; AMPK, AMP-activated protein kinase; *Tshr*$^{-/-}$, TSH receptor knockout mice; *Tshr*$^{+/+}$, TSH receptor wild type mice; TSHR, thyrotropin receptor; SREBP-1a, sterol regulatory element-binding protein-1a; ACC1, acetyl-CoA carboxylase 1; FASN, fatty acid synthase; SCD1, stearoyl-CoA desaturase 1; FABP1, fatty acid-binding protein 1; GPAT, glycerol phosphate acyltransferase; Cpt1α, carnitine palmitoyltransferase 1α; ApoB, apolipoprotein B; MTP, microsomal triglyceride transfer protein; VLDL, very low-density lipoprotein.

SCH is an independent factor that predicts the development of NAFLD, with a hazard ratio (95% CI) of 2.21 (1.42–3.44) after adjustment for indicators of the metabolic syndrome [7]. Moreover, clinical studies indicated a positive association between TSH and serum TG level [8,9]. As TSH is the only thyroid function component affected in SCH, we endeavoured to investigate whether TSH induces the accumulation of extra fat in liver cells in the context of NAFLD development and aimed to determine the underlying molecular mechanism. Notably, no related studies have been reported to date.

Accumulation of triglycerides within hepatocytes is the hallmark of NAFLD and the liver is a central organ in the regulation of triglyceride metabolism [2,10]. Moreover, sterol regulatory element binding protein 1c (SREBP-1c) is a key lipogenic transcription factor, which directly activates the expression of more than 30 genes, dedicated to fatty acid uptake and triglyceride synthesis [11]. Increased SREBP-1c levels were found in patients with histologically diagnosed NAFLD [12]. SREBP-1c was regulated at multiple levels, such as the proteolytic cleavage of SREBP-1c precursors and post-translational modification of mature SREBP-1c [13]. Studies have reported that SREBP-1c expression decreased in peroxisome proliferator-activated receptor α (*PPARα*)-null mice compared with wild type, suggesting the PPARα-dependent induction of hepatic fatty acid synthesis and SREBP-1c activation [14,15]. Moreover, it is known that PPARα agonists enhance the activity of the *Srebp-1c* promoter through direct binding with the DR1 motif [16], and AMP-activated protein kinase (AMPK) induced SREBP-1c phosphorylation at Ser372 associated with the accumulation of nuclear SREBP-1c [17].

We hypothesized that TSH might play a novel role in the regulation of the triglyceride content in liver, which, at least partially, involves the development of NAFLD. Therefore, in the present study, we characterized the effect and mechanism of TSH on the hepatic triglyceride content in TSH receptor knockout ($Tshr^{-/-}$), Srebp-1c knockout ($Srebp\text{-}1c^{-/-}$) and $Tshr^{-/-}Srebp\text{-}1c^{-/-}$ double knockout mice. Compared with wild type ($Tshr^{+/+}$) mice, $Tshr^{-/-}$ mice exhibited a relatively low degree of liver steatosis. Moreover, TSH increased the hepatic triglyceride content via the thyrotropin receptor (TSHR), in which SREBP-1c activation induced by the cAMP/PKA/PPARα signalling pathway, associated with declining AMPK activity, played an indispensable role. These findings highlight a novel physio-pathological role for TSH in the regulation of triglyceride metabolism in the liver and suggest that TSH or hepatic TSHR might have key therapeutic importance in preventing fatty liver disease.

Materials and methods

See Supplementary Materials and methods section.

Results

The function of TSH is mediated through the highly specific TSHR [18]. In a previous study, we demonstrated the presence of functional TSHRs in hepatocytes [19]. To examine the effect of TSH on liver steatosis, we generated a *Tshr* knockout mouse model.

Liver steatosis is attenuated in $Tshr^{-/-}$ mice and TSH promotes triglyceride accumulation

First, we fed mice with a high-fat diet. After 20 weeks, $Tshr^{-/-}$ mice were generally lean compared with $Tshr^{+/+}$ mice (Supplementary Fig. 1A). $Tshr^{+/+}$ mice exhibited a uniformly pale yellow fatty liver, while $Tshr^{-/-}$ mice had a relatively normal liver (Fig. 1A and Supplementary Fig. 1B). Importantly, the hepatic triglyceride content (Fig. 1B) and serum total triglyceride levels (Supplementary Fig. 1C) were decreased in $Tshr^{-/-}$ mice. Correspondingly, $Tshr^{-/-}$ mice showed reduced fat accumulation in the hepatic intracellular vacuoles (Fig. 1C and D). Notably, both THRβ protein expression and mRNA levels of genes that are under direct control of thyroid hormones (deiodinase iodothyronine type 1 [*Dio1*] and the thyroid hormone-inducible hepatic protein [*Spot14*] proved not to be detectably changed between $Tshr^{+/+}$ and $Tshr^{-/-}$ mice (Supplementary Fig. 1D and E). These results indicated that the absence of TSHR effectively improved high-fat diet-induced obesity and liver steatosis independent of thyroid hormones.

We next explored the role of TSH in liver triglyceride metabolism in $Tshr^{-/-}$ and $Tshr^{+/+}$ mice under chow diet. Similarly, less

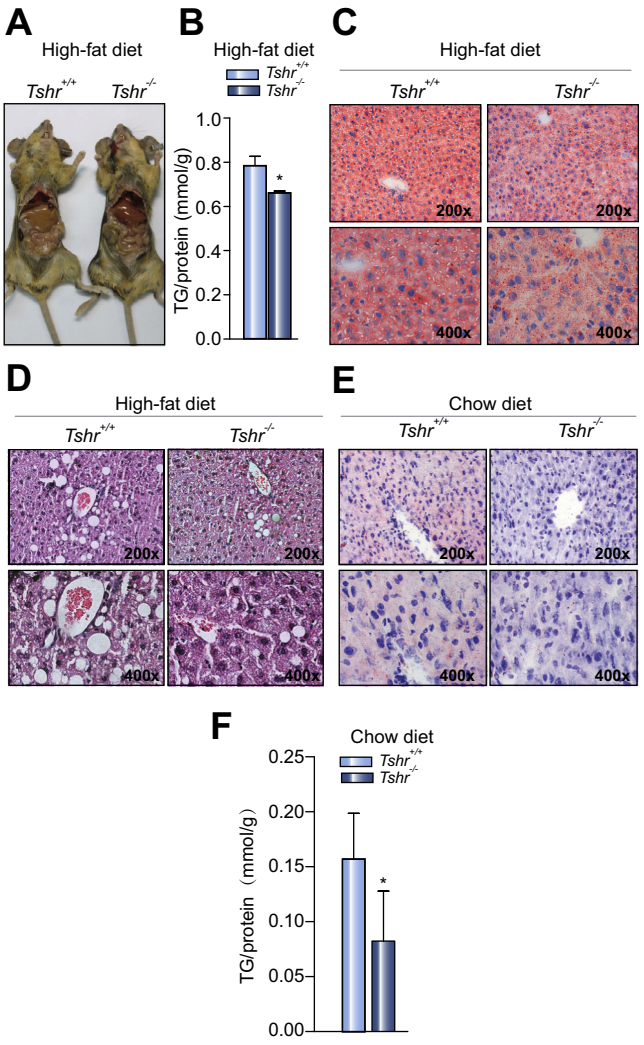

Fig. 1. Liver steatosis is attenuated in $Tshr^{-/-}$ mice and TSH promotes triglyceride accumulation. (A–D) Mice fed a high-fat diet. (A) Representative gross morphology of the livers. (B) Liver triglyceride (TG) content assay. Liver tissue sections were stained using oil red O (C), and H&E (D). (E–F) Mice fed a chow diet. (E) Representative images of Oil Red O staining of the liver sections and (F) the liver TG content assay. The data were presented as the mean ± SD. $^{*}p$ <0.05, *vs.* $Tshr^{+/+}$ mice (n = 3–5). TG content was normalized by total protein contain in the same sample.