Research Article

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Thyroid-stimulating hormone regulates hepatic bile acid homeostasis via SREBP-2/HNF-4α/CYP7A1 axis

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Background & Aims: Bile acids (BAs) play a crucial role in dietary fat digestion and in the regulation of lipid, glucose, and energy metabolism. Thyroid-stimulating hormone (TSH) is a hormone produced by the anterior pituitary gland that directly regulates several metabolic pathways. However, the impact of TSH on BA homeostasis remains largely unknown.

Methods: We analyzed serum BA and TSH levels in healthy volunteers under strict control of caloric intake. Thyroidectomized rats were administered thyroxine and injected with different doses of TSH. *Tshr*$^{-/-}$ mice were supplemented with thyroxine, and C57BL/6 mice were injected with *Tshr*-siRNA via the tail vein. The serum BA levels, BA pool size, and fecal BA excretion rate were measured. The regulation of SREBP-2, HNF-4α, and CYP7A1 by TSH were analyzed using luciferase reporter, RNAi, EMSA, and CHIP assays.

Results: A negative correlation was observed between the serum levels of TSH and the serum BA levels in healthy volunteers. TSH administration led to a decrease in BA content and CYP7A1 activity in thyroidectomized rats supplemented with thyroxine. When *Tshr* was silenced in mice, the BA pool size, fecal BA excretion rate, and serum BA levels all increased. Additionally, we found that HNF-4α acts as a critical molecule through which TSH represses CYP7A1 activity. We further confirmed that the accumulation of mature SREBP-2 protein could impair the capacity of nuclear HNF-4α to bind to the *CYP7A1* promoter, a mechanism that appears to mediate the effects of TSH.

Conclusions: TSH represses hepatic BA synthesis via a SREBP-2/HNF-4α/CYP7A1 signaling pathway. This finding strongly supports the notion that TSH is an important pathophysiological regulator of liver BA homeostasis independently of thyroid hormones.

Keywords: Thyroid-stimulating hormone; Bile acids; SREBP-2; HNF-4α; CYP7A1.
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Abbreviations: BA, bile acid; TSH, thyroid-stimulating hormone; TSHR, thyroid-stimulating hormone receptor; TH, thyroid hormone; TR, thyroid hormone receptor; FXR, farnesoid X receptor; SHP, small heterodimer partner; LRH-1, liver receptor homologue-1; PXR, pregnane X receptor; CYP7A1, cholesterol 7α-hydroxylase; CYP8B1, sterol 12α-hydroxylase; SREBP-2, sterol regulatory element-binding protein 2; HNF-4α, hepatocyte nuclear factor 4α; 25-HC, 25-hydroxycholesterol.

Introduction

Thyrotropin (thyroid-stimulating hormone, TSH) is produced by the anterior pituitary gland, the main function of which has been widely considered to be in the regulation of thyroid hormone (TH) synthesis and release in the thyroid [1]. However, extra-thyroidal effects of TSH have now been reported. For example, TSH has been shown to stimulate leptin secretion through its effects on adipocytes via TSH receptors (TSHRs) expressed in adipose tissue [2,3]. TSH has also been reported to be a negative regulator of skeletal remodeling [4]. Our results also demonstrate that TSH can directly increase hepatic triglyceride and cholesterol content [5,6]. Together, these findings clearly suggest that the

direct regulation of metabolic pathways is an important aspect of TSH function. However, the precise role of TSH in metabolism appears to be highly complex, and there remains much to be learned about this process. In this paper, we present evidence supporting a direct role of TSH in bile acid (BA) homeostasis, a surprising example of a previously undescribed metabolic function of TSH.

BAs have long been considered to be little more than emulsifiers of lipids, and thus, they have not generated much excitement or research interest among hepatologists. However, it now appears that BAs have important and intriguing signaling functions, and numerous researchers from academia and industry have begun to investigate this emerging field [7,8]. BAs rapidly activate nuclear receptors and are known to play an important role in the regulation of lipid, glucose, and energy metabolism [9]. BAs also control hepatic *de novo* lipogenesis, very low density lipoprotein-triglyceride export, and plasma triglyceride turnover via farnesoid X receptor (FXR) and its downstream targets, including small heterodimer partner (SHP) [10]. In addition, BAs can activate TGR5, stimulate energy metabolism, and improve glucose tolerance and insulin sensitivity [11,12]. Notably, recent evidence has suggested that BAs may be a new potential therapeutic target for diabetes and obesity [13].

Maintaining BA homeostasis is essential to human health. Excess BAs are cytotoxic to the liver and can cause pancreatic damage [14–16]. They have even been reported to promote the development of gastrointestinal cancer [17]. Decreased BA synthesis has also been associated with adverse health outcomes in humans, including hypercholesterolemia and atherosclerosis [18], which result in cardiovascular diseases. BAs are vital for the evolution of mammalian longevity and for healthy aging [19]. Because of its tremendous importance, BA homeostasis is a tightly regulated process that requires further investigation.

In this study, a series of *in vivo* and *in vitro* experiments confirmed that TSH is indeed a novel intrinsic regulator of BA homeostasis under both normal and pathological conditions. Our data also demonstrate that the effects of TSH are independent of THs. Interestingly, these results contradict the traditional concept of TSH and contribute to a better understanding of the pathophysiological effects of TSH outside of the thyroid gland.

Materials and methods

See the Supplementary materials.

Results

Serum TSH and total BA levels are negatively correlated in healthy volunteers

We performed a clinical study to determine the relationship between serum TSH and total BA levels in humans. Because of the well-known influence of diet on the total BA concentration in serum [20], a 12h study of 15 healthy male volunteers was conducted while the participants were under strict dietary control of total caloric intake (Supplementary Table 3). Individual total BA concentrations fluctuated with food intake over the 12 h. In most cases, the peaks were observed half an hour after meals, at 12:30 and 18:30 (Fig. 1A). Serum TSH levels changed in a manner that was opposite of that observed for total serum BA levels (Fig. 1B). The changes observed relative to the initial value at 8:30 for circulating levels of TSH and total BAs evolved with almost opposite phases (Fig. 1C). Notably, the minimal levels of TSH and the peak levels of total BAs appeared simultaneously half an hour after meals. In contrast, TH (free T_3 and free T_4) showed no significant changes with diet (Fig. 1D). Moreover, serum C4 concentrations also showed changes that were similar

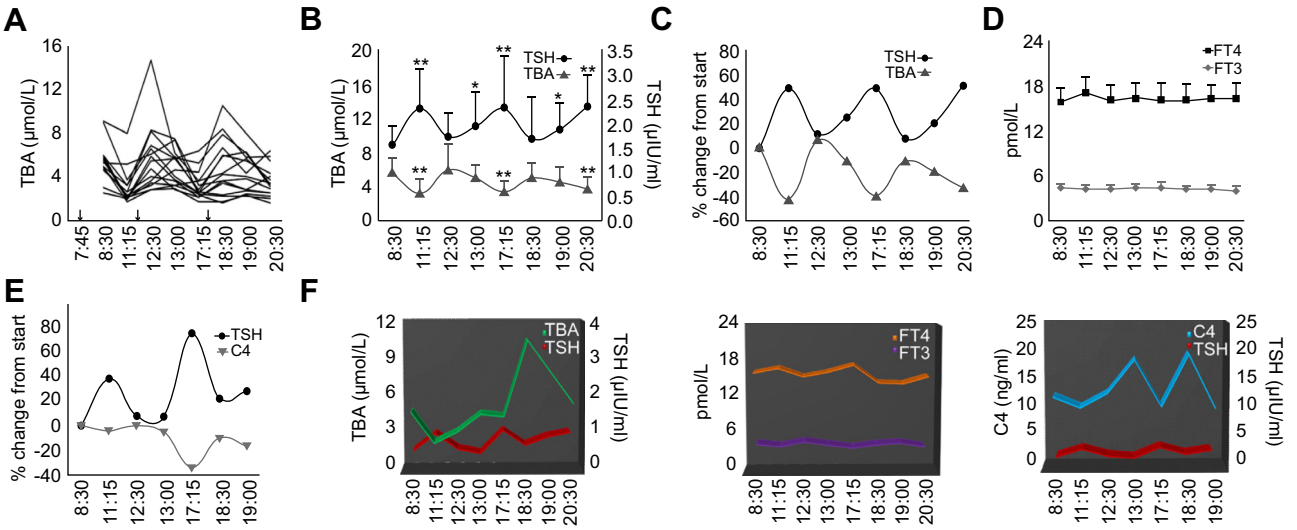

Fig. 1. Serum TSH and total BA levels negatively correlated in healthy volunteers. (A) Diurnal variation of total BA concentrations in the fifteen healthy subjects recruited for this study. Arrows indicate standard meals that were ingested at 7:45, 11:45, and 17:45. (B–D) Each symbol represents the mean value from the fifteen subjects. (B) Diurnal fluctuation of serum TSH (circles) and total BA (triangles) levels. (C) Diurnal changes of serum TSH (red) and total BAs (green). Data are expressed as the percent change from the initial morning value at 8:30. (D) Serum levels of free T_4 (FT_4) and free T_3 (FT_3). (E) Diurnal fluctuation of the serum levels of TSH (circles) and C4 (triangles), which represents the CYP7A1 activity in 8 healthy volunteers. Data are expressed as the percentage change relative to the initial morning value that was observed at 8:30. Each symbol represents the mean value from the 8 subjects. (F) Serum TSH and total BA (left) levels, FT_3 and FT_4 levels (middle) and C4 levels (right) from a representative individual (No. 14) are shown. $^{*}p$ <0.05, $^{**}p$ <0.01 *vs.* the value at 8:30.