Biochemical and Biophysical Research Communications xxx (2017) 1–8

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Thyroidectomy stimulates glucagon-like peptide-1 secretion and attenuates hepatic steatosis in high-fat fed rats

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ARTICLE INFO

Article history:
Received 29 August 2017
Accepted 31 August 2017
Available online xxx

Keywords:
GLP-1
Thyroidectomy
β-catenin
Hepatic steatosis

ABSTRACT

Thyroid hormones (THs) as a therapeutic intervention to treat obesity has been tried but the effect of THs on body weight and the mechanistic details of which are far from clear. This study was designed to determine and elucidate the mechanistic details of metabolic action of THs in high-fat diet (HFD) fed Sprague Dawley (SD) rats. Rats were made surgically hypothyroid (thyroidectomy, Thx). Body weights and food and water intake profoundly decreased in HFD fed thyroidectomized group (HN Thx). Results showed that delayed insulin response, increased total cholesterol, high-density lipoprotein, and low-density lipoprotein in HN Thx. Unexpectedly, however, Thx reduced serum and hepatic triglyceride concentrations. Further studies revealed that Thx dramatically increased circulating GLP-1 as well as increased expressions of GLP-1 in small intestine. Diminished hepatic expressions of lipogenic genes, were observed in HN Thx group. Beta-catenin and glutamine synthetase, a known target of β-catenin, were up-regulated in the liver of HN Thx group. The expressions of gluconeogenic genes G6P and PCK were reduced in the liver of HN Thx group. The results may suggest that surgery-induced hypothyroidism increases GLP-1, the actions of which may in part be responsible for the reduction in water intake, appetite and hepatic steatosis.

1. Introduction

Obesity and obesity-related morbidities have ever been climbing over the decades [1,2]. And attempts to develop efficient, affordable, and safe therapeutic intervention devoid of serious adverse effects have been tried with no success. Thyroid hormones (THs) play an essential role in the regulation of whole-body energy balance and metabolism [3]. Thyroid dysfunction is highly prevalent with hypothyroidism being present in 4.6% and hyperthyroidism 1.3% of the population [4]. Hypothyroidism often is associated with weight gain, hypophagia, and decrease in energy expenditure and hyperthyroidism with weight loss, increase in energy expenditure, and hyperphagia [5].

However, the effects of THs on body weight and on weight changes, associations of thyroid status and body weight, and the effects of obesity on thyroid function are inconsistent and unclear. Previous studies described weight loss following levothyroxine treatment in patients with hypothyroidism [6]. A recent study suggested that weight loss after levothyroxine treatment appears to be modest and mediated primarily by loss of water rather than fat [7]. There is conflicting evidence about the effects of thyroidectomy in human on weight. A retrospective study that compared weight change in 102 thyroid cancer patients following thyroidectomy with weight change in euthyroid patients with benign nodules or goiter whose thyroids were not resected found no difference in weight between two groups [8]. In contrast to the previous study, another retrospective study found that the thyroidectomized patients had experienced more weight gain than the matched controls [9].

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http://dx.doi.org/10.1016/j.bbrc.2017.08.154
0006-291X/

Obesity is one of the major risk factor for non-alcoholic fatty liver disease (NAFLD) [10]. NAFLD represents one of the most common chronic disorders of the liver in the Western industrialized nations [11]. Steatosis, the hallmark feature of NAFLD, occurs when the rate of hepatic fatty acid uptake from plasma and *de novo* fatty acid synthesis is greater than the rate of fatty acid oxidation and export as triglyceride. Therefore, an excessive amount of intrahepatic triglyceride represents an imbalance between complex interactions of metabolic events. NAFLD includes a variety of entities ranging from simple fatty liver or hepatic steatosis, to non-alcoholic steatohepatitis (NASH) and cirrhosis of the liver [12]. Thus, NAFLD is a risk factor for the development of hepatocellular carcinoma (HCC) and is associated with the increased necessity of liver transplantation [13]. Increasingly, a correlation between thyroid dysfunction, especially clinical or subclinical hypothyroidism, and NAFLD has been discussed [14], but the results are conflicting. Disturbances in thyroid hormone concentrations may promote hyperlipidemia and obesity, thus contributing to NAFLD [15]. Evidences indicating no association between THs and NAFLD were also reported [16].

With the abovementioned evidence, it is evident that the effect of THs on body weight and hepatic steatosis are surprisingly poorly understood and controversial. Thus, this study was designed to determine and elucidate the mechanistic details of metabolic action of THs in surgery-induced hypothyroid and high-fat diet (HFD) fed Sprague Dawley (SD) rats.

2. Materials and methods

2.1. Animals and experimental design

Four wk-old male Sprague-Dawley (SD) rats were obtained from Central Laboratories (Seoul, Korea) and acclimated for one week. Rats were individually housed and allowed *ad libitum* access to standard rat chow and water. All animal experiments were approved by the Animal Care Committee of Keimyung University, Deagu, Korea, in accordance with the institutional guidelines for care and use of laboratory animals (KM-2013-40). After one week of acclimation, rats were divided into three groups; rats that were fed normal chow throughout the experimental period with sham operation at 14 wk were named as NN Sham, rats that were fed high-fat diet (HFD; 34.3% fat by weight) and then normal chow diet (5.8% fat by weight) after sham operation at 14 wk were name HN Sham, and rats that were fed HFD and then normal chow diet after thyroidectomy at 14 wk were name HN Thx. Rats were euthanized 12 wks After surgery and blood and liver tissues were harvested and stored at −80 °C.

2.2. Intraperitoneal glucose tolerance test (PGTT)

PGTT was performed 1 wk before the operation and 10 wks after the surgery. Rats were administrated with 1 g/kg glucose (D-(+)-glucose, Sigma-Aldrich, St. Louis, MO, USA) by peritoneal injection after an overnight fast. Blood samples were collected from the tip of the tail and measured using a glucometer (Accu-Chek Active, Roche, Switzerland).

2.3. Surgical procedures

Surgical hypothyroidism was induced first by making vertical skin incision in the neck. The sternohyoid and the sternothyroid muscles were separated using a blunt probe. After the trachea was exposed, the overlying thyroid glands were gently teased and removed using a blunt forceps. The skin incision was closed with non-absorbable suture material. For the sham operations, a midline vertical skin incision in the neck was performed, and then closed. Operative time was prolonged to produce a similar degree of anesthetic stress to those rats that underwent thyroidectomy. Rats were anesthetized by intraperitoneal injection of zoletil (5–10 mg/kg) and xylazine (10 mg/kg) mixture (1:1). Rats were fasted overnight before and after surgery, and were fed the same standard chow *ad libitum* after the operation.

2.4. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted with Trizol (Invitrogen, USA) and reverse transcribed. qPCR analysis was performed using the SYBR Green PCR Master Mix (TOYOBO, Japan). The relative expression of each gene was normalized against β-actin. The samples were assayed on a Light Cycler 480 (Roche, Germany) instrument and the concentration was calculated as copies per microliter using the standard curve.

2.5. Western blot analysis

Harvested tissues were subjected to SDS-PAGE on appropriate resolving gels and immunoblotted. Briefly, tissues were lysed in ice-cold lysis buffer containing protease inhibitors cocktail solution. Proteins were separated by 10% SDS-PAGE and transferred to nitrocellulose membrane (Amersham, USA). The membrane was blocked in 5% skim milk in TBS-T before incubation for overnight at 4 °C with primary antibody of anti-PCK1 (1: 1,000, Abcam, UK), anti-Glutamine Synthetase (1: 1000, Abcam, UK), anti-G6Pase (1: 1000, Santa Cruz Biotechnology, USA) and anti-β-catenin (1: 1000, Cell Signaling, USA). Beta-actin (1: 2000, Sigma-Aldrich, USA) was used as an internal control. Membrane was then washed in TBS-T and incubated with horseradish peroxidase-conjugated secondary antibodies (1: 1000, SC-2005, Santa Cruz Biotechnology, USA). Protein bands were detected using enhanced chemiluminescent reagents (Thermo Fisher scientific, USA). Membrane detected was detected using LAS-3000 (Fujifilm, Japan).

2.6. Immunohistochemistry (IHC)

Liver was fixed with 10% formalin and embedded in paraffin, and sectioned. The sections were stained with IHC for light microscopic examination. Sections were permeabilized in PBS, incubated in 10 mM sodium citrate buffer followed by incubation with goat polyclonal anti-PCK1 (1: 300, Abcam, UK), anti-Glutamine Synthetase (1: 2000, Abcam, UK), anti-GLP-1 (1: 1000, Abcam, UK) and anti-β-catenin (1: 50, Cell Signaling, USA). Sections were then incubated with secondary antibody (1: 200, Santa Cruz Biotechnology, USA), followed by staining with diaminobenzidine chromogen (Vector Laboratories, USA) and counterstaining with hematoxylin (Dako, Denmark). The stained sections were examined under microscopy (x 400) and all histological assessments (Nikon, Japan) were made by a pathologist.

2.7. Oil red O stain

Liver frozen section slides were fixed with 10% formalin for 10 min. Next, the slides were stained for 10 min at room temperature in freshly diluted Oil Red O solution, after which they were washed for 1 min with distilled water. And counterstaining with hematoxylin (Cat# S3309, Dako, Denmark), after which they were washed for 2 min with distilled water. The stained sections were examined under microscopy (x 400) and all histological assessments (Nikon, Japan) were made by a pathologist.

Please cite this article in press as: J.Y. Kang, et al., Thyroidectomy stimulates glucagon-like peptide-1 secretion and attenuates hepatic steatosis in high-fat fed rats, Biochemical and Biophysical Research Communications (2017), http://dx.doi.org/10.1016/j.bbrc.2017.08.154