Available at www.sciencedirect.com

ScienceDirect

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

# Gamma-aminobutyric acid improves oxidative stress and function of the thyroid in high-fat diet fed mice

*Zhenxing Xie* [a], *Shufang Xia* [a], *Guo-Wei Le* [a,*]

[a] *State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, Wuxi, 214122, Jiangsu Province, P.R. China*

ARTICLE INFO

*Article history:*
Received 24 October 2013
Received in revised form 6 March 2014
Accepted 7 March 2014
Available online 31 March 2014

*Keywords:*
Obesity
Oxidative stress
Hypothyroidism
Thyroid stimulating hormone
Thyroid hormone

ABSTRACT

Redox status and thyroid functions of diet-induced obesity (DIO) and DIO-resistant (DIO-R) mice from 20-week high-fat diet-fed (HFD) mice were studied (study I). Whether anti-obesity action of gamma-aminobutyric acid (GABA) in HFD mice was related to its antioxidative action or improvement in thyroid function was also examined (study II) by GABA in drinking water (0.2, 0.12 and 0.06%). In DIO mice, thyroid stimulating hormone (TSH) remarkably increased, free thyroid hormones (THs) decreased, thyroid structures deformed and expressions of THs synthesis-specific and thyroid antioxidative markers, THs receptors β and deiodinases in hypothalamus and liver decreased. DIO-R mice showed normalized TSH, increased THs and its functions. Three GABA treatments normalized TSH, while 0.2% and 0.12% GABA treatments restored redox status, raised THs excretions and functions. Consequently, Down-regulated thyroid and THs functions in DIO mice accounted for obesity. GABA could prevent obesity by ameliorating oxidative stress and HFD-disrupted functions of thyroid and THs.



## 1. Introduction

Obesity is a worldwide epidemic and a key factor for the development of metabolic syndrome and type 2 diabetes (TD2) (Phillips et al., 2013; Whitmore, 2010). Nowadays, high energy diet is an important cause to obesity occurrence for which the endocrine mechanism remains unclear. Recently, many surveys have shown that obesity is closely associated with hypothyroidism (Hari, Verma, Muthukrishnan, & Modi, 2009; Kopylov et al., 2012) exhibiting elevated concentration of plasma TSH, but the results about thyroid hormone (TH) levels are conflicting (Marzullo et al., 2010; Pearce, 2012). It is well-known that THs (triiodothyronine, T3; thyroxine, T4) can increase energy expenditure and can be normally maintained relatively stable by the hypothalamus–pituitary–thyroid (HPT) axis and deiodinases (D: mainly D1 and D2) (St Germain, Galton, & Hernandez, 2009). Thus, elevated TSH levels in obese patients suggest the disorder of the HPT axis. Besides, mice fed HFD presented the resistance of THs signaling pathways (Kim, Crunkhorn, Boes, Bianco, & Patti, 2008) such as down-regulated thyroid hormone receptor (THR) α. Treatment with T3 can counteract hyperglycemia in TD2 model mice and attenuate insulin resistance through the PI3K pathway (Lin & Sun, 2011). Hence, THs disorder or T3 function decline may be the critical point of obesity and TD2 occurrences due to decreased energy expenditure, but the mechanism remains unclear.

On the other hand, obesity is accompanied by the overproduction of reactive oxygen species (ROS) combined with the

* *Corresponding author*. Tel.: +86 510 8591 7789; fax: +86 510 8591 7789.
E-mail address: lgw@jiangnan.edu.cn (G-W. Le).
http://dx.doi.org/10.1016/j.jff.2014.03.003
1756-4646/

decline of antioxidant enzyme activities (Shen, Cai, Tang, & Feng, 2007; Stefanovic, Kotur-Stevuljevic, Spasic, Bogavac-Stanojevic, & Bujisic, 2008). Oxidative stress exerts harmful effects on the organs, but ROS possess dual functions in thyroid. Firstly, THs synthesis requires a certain amount of $H_2O_2$ produced by dual oxidase 2 (Duox2) in the thyroid (Ris-Stalpers, 2006) because iodide is oxidized by $H_2O_2$ under the catalysis of thyroid peroxidase (TPO) after being transported into cells by sodium iodide symporter (NIS) and then incorporated into tyrosyl residues of thyroglobulin (TGB) (Greespan, 2007). Physiologically $H_2O_2$ in the thyroid is eliminated mainly by peroxiredoxin 5 (PRDX5) (Poncin et al., 2008) and glutathione peroxidase 3 (Gpx3) (Cornelia et al., 2007) whose transcriptions are activated by nuclear factor-E2 related factor 2 (Nrf2) (Surh, Kundu, & Na, 2008). Redundant ROS, which are detrimental to iodine uptake (Nadolnik, Niatsetskaya, & Lupachyk, 2008) and even inhibit deiodinase II expression (Lamirand, Pallud-Mothré, Ramaugé, Pierre, & Courtin, 2008) *in vitro*, may disorder thyroid and THs functions and give rise to obesity. However, the assumption has not been verified hitherto. Meanwhile, the effects of functional factors on thyroid should be considered on the basis of antioxidant effects while being used to prevent obesity. Previous studies about the effect of functional components on obesity are seldom involved in thyroid or thyroid hormone functions. To the best of our knowledge, two components have been shown enhancing thyroid function (phytosterol) or thyroid hormone signaling (n-3 polyunsaturated fatty acids) in HFD fed animals, in which phytosterol elevates levels of serum total thyroxine, total triiodothyronine, and free triiodothyronine (Awaisheh et al., 2013) and diet rich with n-3 polyunsaturated fatty acids increase the expression of liver thyroid hormone receptor β (Souza et al., 2010). These effects explain the antilipemic effects apart from their antioxidant abilities (Almazari et al., 2012; Saw, Yang, Guo, & Kong, 2013; Tan & Shahidi, 2013) and also provide the basis for synthesizing of conjugated high-molecular-weight (phytosteryl docosahexaneates) with the antilipemic effect (Tan, Le, Moghadasian, & Shahidi, 2012).

GABA, an inhibitory neurotransmitter and a functional component (Akiko, Kenji, Eun, Yasushi, & Kozo, 2012; Harnedy & FitzGerald, 2012; Wan-Chung, Chung-Yi, Yuan-Tay, Roch-Chui, & Kuo-Chieh, 2013), is antioxidative *in vitro* (Szydlowska & Kaluza, 1976). Oral GABA administration can improve insulin function, restore blood glucose level and reduce body weight gain in mice fed HFD (Tian et al., 2011). Nevertheless, whether the action of GABA in HFD animals is attributed to its antioxidative activity or the functional recovery of thyroid has not yet been studied. Moreover, intravenous GABA treatment is safe by not changing the basic release of THs from the thyroid (Ahren, 1989). The effect of GABA on the expressions of thyroid synthesis-related genes has not been investigated. In this study, redox status, thyroid and THs functions in DIO and DIO-R mice fed HFD were investigated to clarify the role of thyroid in obesity occurrence. Moreover, the antioxidative capacities of GABA and its effect on HPT were investigated in HFD mice. Thyroid function was characterized by the microscopic structure and the expressions of thyroid-related genes. THR and deiodinase expressions were also quantified by reverse transcription-polymerase chain reaction (RT-PCR).

## 2. Materials and methods

### 2.1. Animal

Two hundred male C57BL/6 mice (6 weeks old) were provided by Shanghai Slac Laboratory Animal Co., Ltd. (Shanghai, China) and were given standard diet in air-conditioned cages at constant temperature (22 ± 2 °C) and humidity (50 ± 10%) in a 12-h light-dark cycle. All the experimental procedures were approved by the Jiangnan University Institutional Animal Use and Care Committee (JN no. 5 2012).

### 2.2. Experimental design and samples preparation

After a week's acclimation, all the mice were distributed into study I and study II.

In study I, 100 mice were randomly divided into a control group (fed control diet, 20 mice), a diet-induced obesity group (DIO, upper one quarter) and a diet-induced obesity-resistant group (DIO-R, lower one quarter) (20 mice per group) selected from the mice fed high fat diet (HFD, 80 mice) according to body weights (Huang, Han, & Storlien, 2003) by the end of the 20-week experiment.

In study II, another 100 mice were randomly divided into a control group (fed control diet, 20 mice), an HFD group (20 mice per group) and other three groups fed with HFD as well as three different doses of GABA (Sigma, St. Louis, MO, USA) by drinking water (0.2, 0.12 and 0.06% g/ml). The former two groups were offered plain water. The control diet (10% fat of energy) and HFD (45% fat of energy) in studies I and II were modified (see supplemental Table S1 in the online version at doi:10.1016/j.jff.2014.03.003) based on the AIN 93 rodent diet composition.

All animals in studies I and II were allowed free access to diet and water in the 20-week experiment. Body weights and water intake were recorded, and fasting blood glucose (FBG) levels of the six groups were measured using a One-Touch ultra blood glucose meter (LifeScan, Milpitas, CA, USA). After fasting for 12 h, the mice were sacrificed after intraperitoneal anesthesia with 2.5 mg/g urethane (Sigma-Aldrich, Gillingham, UK) in the end. Blood from the orbital sinus was collected into anticoagulant tubes. Plasma was separated from blood samples after centrifugation (2500 g for 15 min at 4 °C) and then stored at –20 °C until analysis. The thyroid glands and liver samples of six mice per group were cut out immediately and stored in Trizol solution at –80 °C for RNA extraction.

### 2.3. Plasma lipid status

Plasma total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triacylglycerol (TG) concentrations were analyzed by the corresponding enzymatic colorimetric assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, PR China) according to the instructions of the manufacturer.

### 2.4. ROS and plasma oxidative status

Thiobarbituric acid-reactive substances (TBARS), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (Gpx)