Comparative Biochemistry and Physiology, Part B 192 (2016) 30–37

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part B

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

Expression of mitochondria-related genes is elevated in overfeeding-induced goose fatty liver

Rashid H. Osman [a,b,1], Dan Shao [a,1], Long Liu [a], Lili Xia [a], Xiaoxian Sun [a], Yun Zheng [a], Laidi Wang [a], Rui Zhang [a], Yihui Zhang [a], Jun Zhang [a], Daoqing Gong [a,*], Tuoyu Geng [a,*]

[a] College of Animal Science and Technology, Yangzhou University, Yangzhou, Jiangsu 225009, China
[b] College of Veterinary Science, West Kordofan University, El Nuhud 20, Sudan

ARTICLE INFO

Article history:
Received 8 July 2015
Received in revised form 16 November 2015
Accepted 20 November 2015
Available online 25 November 2015

Keywords:
Fatty liver
Goose
Mitochondrion
Overfeeding

ABSTRACT

Mitochondrion, the power house of the cell, is an important organelle involving in energy homeostasis. Change in mitochondrial mass and function may lead to metabolic disorders. Previous studies indicate that mitochondrial mass loss and dysfunction are associated with non-alcoholic fatty liver disease (NAFLD) in human and mouse. However, it is unclear whether mitochondrial genes are involved in the development of goose fatty liver. To address this, we determined the response of goose mitochondrial genes to overfeeding and other fatty liver-related factors (e.g., hyperinsulinemia, hyperglycemia, and hyperlipidemia). We first employed RNA-seq technology to determine the differentially expressed genes in the livers from normally-fed vs. overfed geese, followed by bioinformatics analysis and quantitative PCR validation. Data indicated that a majority of mitochondrial genes in the liver were induced by overfeeding. To understand how these genes are regulated in the context of fatty liver, we treated goose primary hepatocytes with high levels of glucose, fatty acids and insulin. The results indicated that these factors had an influence on the expression of some mitochondria related genes. Together, these findings suggest that the induction of mitochondrial gene expression by overfeeding is required for the development of goose fatty liver, and this induction is partially attributable to hyperglycemia, hyperlipidemia and hyperinsulinemia.

1. Introduction

Geese, as a descendant of migrant birds, have an excellent capacity to deposit fat in the liver. This capacity reflects an adaptation that meets the need for long-distance migration (Hermier et al., 1991). In goose industry, this capacity has been utilized for fatty liver production for long time. Goose fatty liver, produced by overfeeding in a short period of time, is a delicious and nutritious food (Zhu et al., 2011). As a specialized breed for fatty liver production, the French Landes Grey Goose is a famous breed for its high capacity for fatty liver production, and its fatty liver may increase up to 10-times normal liver weight or 10% of body weight (Hermier et al., 1994). Recent findings indicate this marvelous capacity is related to dramatic up-regulation of genes involved in *de novo* lipogenesis (e.g. SCD) (Strable and Ntambi, 2010). Similar to goose fatty liver, non-alcoholic fatty liver disease (NAFLD) in human and mouse is also associated with overeating of energy-rich food (e.g. high sugar or fat diet). This similarity suggests that goose fatty liver may share some mechanisms with NAFLD in mammals. Indeed, genes involved in *de novo* lipogenesis are also upregulated in patients or mice with NAFLD (Horton et al., 2002; Ferre and Foufelle, 2010).

Previous studies indicate that NAFLD in humans or mice is usually accompanied with reduced mitochondrial gene expression, mass loss and dysfunction (Sanyal et al., 2001; Caldwell et al., 2004; Nakamuta et al., 2005), which suggests the important role of mitochondria in the development of NAFLD. It is well known that mitochondria are important organelles involving multiple biological process, including energy homeostasis, oxidative stress, cell death, and many diseases (Day and James, 1998) as mitochondria are the site of Krebs cycle, cellular respiration and oxidative phosphorylation, and the major producer of reactive oxygen species (ROS). Modulation of mitochondrial mass and function has emerged as a major adaptive response to changes in energy balance arising from glucose availability or oxygen deficiency among other nutrient stresses. For example, nutrient-sensitive changes in peroxisome proliferator-activated receptor-γ coactivator-1 (PGC-1) activity alter expression of genes required for mitochondrial biogenesis, in addition to genes required for fatty acid metabolism (Dominy et al., 2010; Lin and Accili, 2011).

The mechanisms underlying the association of impaired mitochondria with NAFLD include loss/inactivation of mitochondrial proteins/enzymes, reduced mitogenesis, mitochondrial DNA mutation, oxidative stress, mitophagy, and apoptosis (Wei et al., 2009; Hipkiss, 2010).

* Corresponding authors.
E-mail addresses: yzgong@163.com (D. Gong), tygeng@yzu.edu.cn (T. Geng).
[1] These authors contribute to the work equally.

http://dx.doi.org/10.1016/j.cbpb.2015.11.006
1096-4959/

Impaired mitochondria do not only lead to imbalanced fat metabolism in the liver, but also promote the production of reactive oxygen species (ROS) (Kowaltowski et al., 2001; Begriche et al., 2013) and cell death by mitochondrial permeability transition pore (PTP) opening (Crompton, 1999). It is known that many factors influence oxidative stress or apoptosis (Begriche et al., 2006; Mantena et al., 2008; Serviddio et al., 2011). For example, saturated fatty acids (SFA) increase oxidative stress, apoptosis and mitochondrial dysfunction, while unsaturated fatty acids can suppress this (Hardwick et al., 2009). Therefore, SFA may be a strong risk factor causing mitochondrial impairment in NAFLD. We recently found a number of fatty acid desaturases that were induced by overfeeding in goose fatty liver in addition to SCD, and goose fatty liver contains more unsaturated fatty acids (USFA) than SFA (Gutierrez et al., 2004). We thus speculated that mitochondria in goose liver could be protected from the harmful effect of severe steatosis due to high USFA content. Moreover, goose and other migratory birds, unlike mammals, do not only deposit energy at adipose tissue, but also use their livers as a major depot of fat. Thus, lipid metabolism in goose may be different from mammals. However, it is unclear whether mitochondria in the liver contribute to the difference in lipid metabolism between goose and mammals.

Based on the key role of mitochondria in mammalian fatty liver and the difference in lipid metabolism between goose and mammals, we hypothesized that goose liver mitochondria were involved in the development of fatty liver in a different way. To test this, Landes geese were normally fed or overfed, and their livers were used for transcriptome analysis with RNA-sequencing (RNA-seq) technology. The differentially expressed genes resulted from this analysis were subjected to further bioinformatics analysis. Indeed, mitochondrial genes were extremely enriched among the differentially expressed genes, and a majority of mitochondria-related genes were up-regulated in overfed geese compared to normally fed geese, which is different from the loss of mitochondrial mass in mammalian NAFLD. This result was verified by quantitative PCR. To understand how the mitochondrial genes were upregulated in the context of fatty liver, we treated goose primary hepatocytes with fatty liver-related factors, including high levels of glucose, fatty acids and insulin. Our data suggest that hyperglycemia, hyperlipidemia and hyperinsulinemia likely regulate the expression of these genes. Together, the present study suggests that the elevation of mitochondrial gene expression is required for the development of goose fatty liver, and revealing the mechanism underlying the difference between goose and mammalian liver mitochondria in the context of fatty liver may provide a therapeutic approach to human NAFLD.

2. Material and methods

2.1. Experimental animals and design

A total of 8 healthy 60-day-old Landes geese were provided from Wu Wang Farm (Chuzhou, China). Geese were randomly divided into a control group and an overfeeding group (n = 4 each group). The overfeeding was performed by a skilled worker. First, the mouth of an individual goose was opened, the tube part of a funnel was then put in the mouth, and finally the diet was force-fed by a electronic machine into the body of the geese through the funnel. Two dietary regimes were used: the geese in the overfeeding group were overfed with a carbohydrate diet consisting of boiled maize (maize boiled for 5 min, supplemented with 1% plant oil and 1% salt), while the geese in the control group were allowed *ad libitum* feeding of the boiled maize. Before overfeeding, one-week pre-overfeeding was conducted in these experimental geese to increase their adaption to overfeeding. In this week, feed intake was gradually increased from 100 g to 300 g per day. At 68 days of age, overfeeding began. The overfeeding protocol was as follows: in the first 5 days the daily feed intake (3 meals a day) reached 500 g, followed by 1200 g of daily feed intake (5 meals a day) for two weeks. All geese were kept in cage with free access to water. The routine husbandry management was carried out through the experiments. At 86 days of age, the geese were fasted overnight with free access to water. The next morning the geese were weighed and killed with an electrolethaler before harvesting any tissues. The livers of all the geese were harvested and rapidly frozen in liquid nitrogen as well as stored at −70 °C until use. Animal protocols were approved by the Yangzhou University Animal Ethics Committee.

2.2. Preparation of goose primary hepatocytes

Hepatocytes were isolated from Landes goose embryos at 23 days of incubation. The protocol for hepatocyte isolation is briefly described as follows: a goose embryo was placed in a sterilized plate, body cavity was opened, the liver was moved out from the embryo, the liver was rinsed with physiological saline and minced in a sterilized vial, and the minced liver was digested with 3–5 volumes of digestive solution containing 0.1% type IV collagenase (Cat. No. LS004186, Worthington, USA) at 37 °C for 40 min (shake the mixture every 10 min to disperse hepatocytes), followed by adding equal volume of complete culture medium that contains high glucose DMEM (Cat. No. C11995500BT, GIBCO, USA), 100 IU/mL penicillin (Cat. No. P1400, Sigma, USA), 100 μg/mL streptomycin (Cat. No. P1400, Sigma, USA), 2 mM glutamine (Cat. No. ST083, Beyotime, USA), 10% fetal bovine serum (Cat. No. 10099141, Clark, Australia), and 0.02 mL/L EGF (Cat. No. 315-09-100, Peprotech, UK). Subsequently, the mixture was filtered with sterile nylon net (200 meshes) to discard undigested tissues, spin down hepatocytes from the collected liquid at 22.4 ×*g* (r = 80 mm) for 8 min at normal temperature, 3 volumes of red blood cell lysis buffer (Cat. No. R1010, Solarbio, China) was added to the cell pellet, which was suspended and incubated on ice for 10 min, followed by spinning the mixture at 22.4 ×*g* (r = 80 mm) for 5 min at normal temperature, the supernatant was discarded and the cell pellet was rinsed with culture medium, which was repeated twice, finally, the cell pellet was suspended again with complete culture medium, the number of cells was counted, and the cells were then diluted with culture medium to 1×10^6 cells/mL and plated in 12-well dishes and incubated in 5% CO_2 incubator at 38 °C until treatment. The media was renewed at first 6 h of incubation.

2.3. Treatment of cultured cells with glucose, fatty acids and insulin

After 28 h of incubation at 38 °C, the old culture medium was removed, and the isolated goose primary hepatocytes were rinsed with phosphate buffered saline (PBS) for later treatment. For glucose treatment, the stock solution of 500 mM glucose (Cat. No. G7021, Sigma, USA) was made with complete culture medium at room temperature, followed by mixing certain amount of the stock solution with complete culture medium to make a final solution containing 100 mM or 200 mM glucose, which was used to treat the cells for 14 h. The control was the cells treated with complete culture media alone. For palmitate treatment, the stock solution of 100 mM potassium palmitate (Cat. No. P5585, Sigma, USA) was made in water supplemented with 10 μL/mL dimethyl sulfoxide (DMSO) (Cat. No. O7501, Solarbio, China) at room temperature, then a working solution of 2.5 mL potassium palmitate was made by mixing 1 μL of stock solution with 39 μL complete culture media that contained 2% fatty acid-free bovine serum albumin (BSA) (Cat. No. HWG23861, Beijing HWRK Chemical Co., Ltd.). Using this working solution, a final solution of culture medium containing BSA-conjugated 0.25 mL or 0.50 mL potassium palmitate was made for treating the cells for 14 h. The control was the cells treated with complete culture media containing 2% BSA plus 10 μL/mL DMSO alone. Similarly, a final solution of culture medium containing BSA-conjugated 0.25 mL or 0.50 mM sodium oleate (Cat. No. O7501, Sigma, USA) was made for oleate assays. For insulin treatment, 1 mM insulin solution as stock solution was made by dissolving insulin powder (Cat. No. I5500, Sigma, USA) in water (2% hydrogen chloride was added to the water until the insulin powder was completely dissolved). A certain amount