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The role of endoplasmic reticulum stress and insulin resistance in the occurrence of goose fatty liver

Tuoyu Geng [a, b, *, 1], Lili Xia [a, 1], Fuyuan Li [a], Jing Xia [a], Yihui Zhang [a], Qianqian Wang [a], Biao Yang [a], Sean Montgomery [c], Hengmi Cui [a, b], Daoqing Gong [a, **]

[a] College of Animal Science and Technology, Yangzhou University, Yangzhou, 225009, PR China
[b] Institute of Epigenetics and Epigenomics, Yangzhou University, Yangzhou, 225009, PR China
[c] Department of Botany, University of British Columbia, 6270 University Boulevard, British Columbia, V6T 1Z4, Canada

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ABSTRACT

In mammals, insulin resistance (IR) is required for the occurrence of non-alcoholic fatty liver disease, and endoplasmic reticulum stress (ERS) contributes to IR. As geese have physiological and metabolic characteristics different from mammals, it is unclear whether these mechanisms also underlie the occurrence of goose fatty liver. To address this, 70-day-old geese were treated with an ERS inducer or overfed, and variables associated with ERS or IR were subsequently determined. The data indicated that the group of geese treated with the ERS inducer for 20d appeared to be more intolerant to blood glucose than the control group, and their livers showed features of hepatic steatosis, suggesting ERS can induce IR and hepatic steatosis in geese. In contrast, overfeeding did not induce ERS, probably due to the upregulated expression of fatty acid desaturases, but induced higher fasting/postprandial blood glucose as well as glucose intolerance in geese, which was accompanied by a dramatic increase of liver weight. Taken together, these findings delineated the role of ERS and IR in the occurrence of goose fatty liver.

1. Introduction

As obesity is globally prevalent, non-alcoholic fatty liver disease (NAFLD) constitutes a great burden on the public's health, now becoming an epidemic disease. It is estimated that the incidence of NAFLD affects 15–30% of the human population [1]. A notable feature of NAFLD is hepatic steatosis, a liver condition involving the deposition of a large amount of triglyceride (TG). Goose fatty liver shares this feature with human NAFLD. However, unlike mammals, geese have an excellent ability to naturally recover from hepatic steatosis, which suggests that geese may have a defensive mechanism protecting their livers from pathological change. Investigation of the mechanism underlying goose fatty liver may provide a therapeutic approach to treating human NAFLD. Unfortunately, compared to human NAFLD, the mechanism underlying goose fatty liver is almost unknown.

Epidemiological studies indicate that NAFLD is closely related to metabolic syndromes including obesity, diabetes, dyslipidemia, insulin resistance (IR), and hypertension [2]. Plenty of evidence suggests that IR plays a pivotal role in the occurrence of NAFLD [3,4]. Insulin resistance means that the body needs more insulin to maintain normal glycemia in the face of reduced peripheral insulin sensitivity. Clinically, higher fasting/postprandial blood glucose and glucose intolerance in a glucose tolerance test (GTT) may reflect insulin resistance, which is often used in animal studies as well [5]. Data indicate that lipotoxicity in tissues is a cause of insulin resistance [6]. Lipotoxicity-induced insulin resistance is mediated by inflammation, reactive oxygen species (ROS), ceramides, or ERS [7–10]. Among these mediators, ERS contributes to IR through its three signaling pathways: double-stranded RNA-activated kinase-like ER kinase (PERK), activating transcription factor 6 (ATF6), and inositol requiring ER -to-nucleus signal kinase 1 (IRE1/XBP1) [11–14]. ERS has recently been considered a central player in metabolic syndromes, including IR [7,15,16].

Abbreviations: IR, insulin resistance; NAFLD, non-alcoholic fatty liver disease; ERS, endoplasmic reticulum stress; TG, triglyceride; GTT, glucose tolerance test.

* Corresponding author. College of Animal Science and Technology, Yangzhou University, Yangzhou, 225009, PR China.
** Corresponding author.
E-mail addresses: tygeng@yzu.edu.cn (T. Geng), yzgong@163.com (D. Gong).
[1] These authors contributed to this work equally.

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In humans, NAFLD is often associated with overeating energy-rich diets (e.g. high fat or carbohydrate diets). Similarly, fatty liver is also observed in feed-satiated (or overfed) duck and goose. However, several lines of evidence support the notion that the physiology of avian species is unique in some respects. In comparison to mammals, avian species have higher blood glucose levels, use the liver as an important deposit site for fat, and synthesize about 95% of endogenous fatty acids in the liver through *de novo* lipogenesis, and obese chickens regulate insulin-signaled lipogenesis and body fat deposition differently than obese mammals. Therefore, a degree of caution must be exercised when applying findings from mammalian studies to poultry science.

Goose fatty liver is a nutritious food produced by overfeeding geese with an energy-rich diet over a short period of time. We and others previously demonstrated overfeeding could induce dramatic upregulation of genes for *de novo* lipogenesis in goose liver [17,18], which is consistent with the notion that the liver is the major site of *de novo* lipogenesis in avian species. Insulin induces strong effects on lipid accumulation, expression of genes for lipogenesis, fatty acid oxidation, and very low density lipoprotein (VLDL)-triacylglycerol (TG) assembly and secretion in primary goose hepatocytes [19,20], and hyperinsulinemia is closely linked to insulin resistance. These findings suggest that IR also plays an important role in the overfeeding-induced goose fatty liver. However, this has not been confirmed in overfed geese yet. In this study, we hypothesize that ERS, which could result from the lipotoxicity of saturated fatty acids, underlie the occurrence of IR and fatty liver in overfed geese. To test this, we employed pharmacological and overfeeding approaches to determine the role of ERS and IR in goose fatty liver. Findings from this study may not only provide an insight into the mechanism underlying IR and goose fatty liver, but also identify mechanistic differences between human and goose fatty liver, thus expediting the development of therapeutic treatments for human disease.

2. Materials and methods

2.1. Experimental animals

Fifteen healthy 60-day-old Yangzhou geese and sixteen healthy 70-day-old Landes geese were provided by Ruinong Science and Technology Limited Company (Yangzhou, Jiangsu, China), and used for the experiments. They were raised under the same husbandry conditions prior to the experiments. Yangzhou geese were randomly divided into two groups, with 10 geese treated with tunicamycin, the ERS inducer, and 5 geese used as a control group. For tunicamycin treatment, 10 geese were fed forage supplemented with tunicamycin (2 mg/kg, Qcbio Science & Technologies (Shanghai) Co., Ltd) for 20 days, and geese foraged without tunicamycin were used as a control. In the overfeeding experiment, 16 Landes geese were randomly divided into an overfeeding group (N = 10) and a control group (N = 6). The geese were either fed boiled maize *at libitum* for control or overfed a carbohydrate diet consisting of boiled maize supplemented with 1% plant oil and 0.8–1% salt for treatment. The average weight of feed eaten each day by those fed *ad libitum* was approximately 350 g vs. 800 g for those force-fed. We determined basic, fasting, and/or postprandial blood glucose levels, and performed a glucose tolerance test (GTT) at the indicated time points. At the end of treatment, all the geese were slaughtered and their livers were immediately harvested and frozen in liquid nitrogen. Liver samples were then transferred and stored at −80 °C till use. Animal protocols were approved by the Yangzhou University Animal Ethics Committee.

2.2. Glucose tolerance test (GTT)

For the glucose tolerance test (GTT), geese were fasted overnight and 1 g glucose per kg of body weight was injected intravenously. Blood was collected before injection and 5, 30 and 60 min post-injection. We measured glucose levels using a glucometer (Sinocare; ChangSha, Inc.).

2.3. Quantitative PCR analysis

Total RNA was isolated from liver with TRIzol (Takara Biotechnology Co., Ltd). Reverse transcription of total RNA was carried out according to manufacturer's instructions using Prime Script™ RT reagent kit Perfect Real time kit(Takara Biotechnology Co., Ltd). The expression levels of *Grp78*, *Xbp1*, *Fads1*, *Fads2* and *Fads6* genes in the livers were performed on a real-time PCR machine. SYBR Premix Ex Taq™ (Perfect Real Time) kit was also purchased from Takara Biotechnology Co., Ltd. Goose Gapdh was used as a house keeping gene for normalization. Its primers are as follows: 5′-GCCATCAATGATCCCTTCAT-3′, 5′-CTGGGGTCACGCTCCTG-3′. The 20 μL PCR reaction system was composed of 10 μL 2 × UltraSYBR Mixture, 0.4 μL ROX, 0.4 μL of each primer, 1 μL cDNA and 7.8 μL ddH_2O. The PCR profile was performed under the following conditions: cDNA was denatured at 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s and 60 °C for 34 s, and all samples were done in triplicate. The other primers are listed in Table 1. The message RNA abundance of genes of interest was calculated using $2^{-\Delta\Delta Ct}$ and presented as fold change over control.

2.4. Statistical analysis

All data are presented as the means ± standard error of the means (SEM). The statistical significance of differences was evaluated with the Student's t-test or one-way ANOVA. $P < 0.05$ was considered statistical significance.

3. Results

3.1. Tunicamycin induced hepatic ERS, systematic glucose intolerance, and fatty liver in goose

In mammals, ERS contributes to IR. To test whether this mechanism is applicable to geese, we treated Yangzhou geese with the ERS inducer, tunicamycin. Quantitative PCR analysis showed that, in contrast to the control, the expression level of *Grp78* in the liver, a marker gene of ERS, was induced by tunicamycin (Fig. 1). The expression of *Xbp1* was also consistently induced by tunicamycin (Fig. 1), suggesting one of the three signaling pathways in ERS was activated. We thus concluded that tunicamycin could induce ERS in goose liver, at least partially via the XBP1 pathway. To test if ERS induced by tunicamycin contributes to systematic IR, we

Table 1
List of primer sequences.

Primer name	Sequence (5′to 3′)	Amplicon(bp)
Grp78-forwad	CGAGTAGAGCCACCGACAAG	240
Grp78-reverse	ACTCAGACGGGAAGTGGAGA	
XBP1-forwad	CGTGATGGAATCACAAGTGG	200
XBP1-reverse	GGGTCCAGACTGTCCAGAAA	
FADS1-forwad	AGAGGGTTCTTTGGGACT	171
FADS1-reverse	TTTGCCTACCACATTTCA	
FADS2-forwad	CACTTGCAACATCGAGCAGT	152
FADS2-reverse	GGACTCCGTACTTGGCACAT	
FADS6-forwad	GGGCTGTCTTCTTCATCGAG	146
FADS6-reverse	GACGTAGCGGTTCAGGAAAG	