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The effects of dietary betaine supplementation on fatty liver performance, serum parameters, histological changes, methylation status and the mRNA expression level of Spot14α in Landes goose fatty liver

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ABSTRACT

We evaluated the effects of betaine supplementation on liver weight, liver/body weight, serum parameters and morphological changes. Compared with the control and overfed groups, the geese that were fed the betaine diet showed increased liver weight and decreased abdominal adipose tissue weight compared with the overfeeding groups. Betaine treatment also significantly increased ChE, HDL, LDH and ALT levels ($P<0.01$ or $P<0.05$). Decreased macrovesicular steatosis and increased microvesicular steatosis were observed in the betaine-treated group, and the lipid was well-distributed in the betaine supplement group. The expression of S14α mRNA in the livers of the betaine-treated geese was higher than that in the control or the overfed geese. We performed sodium bisulfite sequencing of the individual alleles of this region (between +374 and −8 base pairs relative to the transcription start site), containing 33 CpG dinucleotides. In the overfed group expressing higher S14α transcripts, the average methylation at the 33 CpGs sites was 87.9%. This contrasted with 69.6% in the control group that showed lower expression of the S14α gene ($P<0.01$). However, no significant change in methylation in the transcription start site was found between the betaine-treated geese (82.6%) and the overfed geese (87.9%). These results indicate that the DNA methylation pattern in the S14α gene transcription start site may not be related to the expression of S14α transcript in response to betaine supplementation.

1. Introduction

Fatty goose liver (foie gras) is a well-known delicacy with a delicate texture and delicious flavor due, in part, to high levels of unsaturated fatty acids. Consumers worldwide enjoy foie gras, and there is a large international market. China is a principal producer of foie gras, as there are large numbers of geese flocks in production. Even so, little is known about the relationship between the effective production of these animals and the expression of lipogenic genes. A previous report showed the effects of different breeds and different bulk feeds on the development of geese fatty liver (Fournier et al., 1997; Hermier et al., 1994), and a variety of papers exist that describe the imbalances between hepatic lipogenesis and lipid secretion that are involved in the susceptibility of ducks and geese to hepatic steatosis (Fournier et al., 1997; Hermier et al., 1994, 1999, 2003; Davail et al., 2003). Thus, the susceptibility to geese fatty liver was partly due to a genetic effect (Mourot et al., 2000) and its relationship to the imbalance of lipid metabolism and imbalance resistance.

Betaine is formed by the oxidation of choline and is present in most living organisms (Barak et al., 1996). It was initially introduced to the feed industry as a replacement for methionine and choline in poultry and fish diets, where it is presumed to act both as a methyl donor and as an osmoprotectant (Kidd et al., 1997). In addition, betaine enhances the synthesis of methylated compounds including carnitine and phospholipids (Carter et al., 1995; Chiang et al., 1996). Thus, betaine might be integrally involved in lipid metabolism via its role in phosphatidylcholine synthesis and in FA oxidation, because carnitine is required for transport of long-chain FAs into mitochondria, where they are degraded via β-oxidation (Carter et al., 1995). Moreover, betaine has been accepted as a hepatoprotective agent against alcoholic (Barak et al., 1997) and non-alcoholic steatosis (Neuschwander-Tetri, 2001). Therefore, betaine can be used to enhance the resistance to imbalance between lipid synthesis (increased) and secretion (reduced) due to its hepatoprotective effect.

The thyroid hormone-responsive Spot14 (S14) gene, which encodes a small acidic protein, is localized in hepatic nuclei and acts to transduce hormone-and nutrient-related signals to genes involved in lipid metabolism. Spot14α has been demonstrated to decrease the expression of a cascade of enzymes in the lipogenic pathway in

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Table 1
Oligo-nucleotide primer pairs for lipogenic genes.

Gene symbol	Primer sequence (5′–3′)	Anneal. T (°C)	Amplicon size	GenBank accession number
β-actin	F: 5′-ACCACCGGTATTGTTATGGACT-3′ R: 5′-TTGAAGGTGGTCTCGTGGAT-3′	65	398 bp	M26111
S14α	F: 5′-GAGGAACGTCCTCTGTGACC-3′ R: 5′-GAGGCTTTGCATTTTATTTCAG-3′	63	314 bp	DQ227766
Bisulfite-1	F: 5′-TTGGGGTTATGGAGTAGTATTT-3′ R: 5′-TCTACTCCAAAATCTAACTATACCTC-3′	54	382 bp	EU710582

hepatocytes transfected with Spot14α antisense oligonucleotides such as ATP-citrate lyase, fatty acid synthase, and malic enzyme (Kinlaw et al., 1995; Brown et al., 1997; Cunningham et al., 1998; Zhu et al., 2005). Meanwhile, the Spot14α gene model has been used to study hepatic gene regulation by carbohydrates and hormones in lipid metabolism (Ota et al., 1997) and in lipogenic tissues.

The goose S14 gene was cloned and found to share a similar gene organization to that of chickens, ducks and mammals (Su et al., in press). However, little is known regarding the transcriptional level of the goose S14α gene in response to overfeeding in Landes goose livers. Meanwhile, the DNA sequence of the S14α gene is characterized by a large CpG island in the region of the transcriptional start site. Moreover, covalent modification of DNA by methylation of cytosine residues in CpGs is a heritable and reversible epigenetic process that is involved in the regulation of a diverse range of biological processes.

As betaine is involved in both the resistance to hepatic imbalances and lipid metabolism, we designed this study to determine the effect of dietary betaine on body and liver parameters, serum parameters, histological changes, and the transcriptional level and methylation status of the thyroid hormone-responsive Spot14 (S14) gene, which is involved in lipid metabolism by transducing hormone-and nutrient-related signals.

2. Materials and methods

2.1. Animals

A total of 18 healthy male Landes geese (*Anser anser*; BW = 4.0 ± 0.01 kg), obtained from Xingyun Jiangsu, were fed a commercial diet to the age of 10 weeks. From 10 to 12 weeks, the feed restriction was progressively released to increase the volume of the digestive tract and to initiate the metabolic adaptation to overfeeding (500 g/d). At 13 weeks (85 d), the geese were divided into three groups (Table 1). Geese in the first group ($n = 6$) continued the control diet and were allowed to feed *ad libitum* (150 g/d) on a diet containing 2600 kcal and 138 g/kg protein and up to 500 g/kg grass. The remaining geese ($n = 12$) were switched to a overfeeding diet (420 g/d), which consisted of two-thirds salted and boiled maize (3370 kcal/kg, 90 g protein/kg and 4.5 g fat/kg) to which 0.4% waterfowl fat was added and one-third (by volume) of water. Geese fed the overfeeding diet were fed six meals per day for three weeks (with the overfeeding diet without betaine). Of the geese that were fed the overfeeding diet, six were also fed betaine (Genetime Biotech Co., Nanjing, China) as a dietary supplement (1 g/d/goose). At week 15, the geese were slaughtered, and blood samples were collected approximately 24 h prior to slaughter. All individuals were weighed at slaughter, and the liver and abdominal adipose tissue were also weighed after dissection. The animals were cared for and slaughtered according to the practices approved by the Nanjing Agricultural University Animal Ethics Committee.

2.2. Measurement of serum parameters and liver triiodothyronine

Serum was separated by centrifugation at 3000 g for 15 min and stored at −20 °C. The measurements of alanine transaminase (ALT; EC 2.6.1.2) (kinetic method, Daiichi Pharmaceutical Co. Ltd, Japanese), γ-glutamyl transpeptidase (GGT; EC 2.3.2.2) (Szasz method, Shanghai Forum Long March Medical Science Co. Ltd, China), cholinesterase (ChE; EC 3.1.1.8) (P-hydroxyl-benzoic acid choline; Nakamura Dental Mfg Co. Ltd, Japan), triglyceride (TG) (GPO-PAP, Biosino Biotechnology Company Ltd, China), high-density lipoprotein cholesterol (HDL) (Direct Assay Method, Daiichi Pharmaceutical Co. Ltd, Japanese), low-density lipoprotein cholesterol (LDL) (Direct Assay Method, Daiichi Pharmaceutical Co. Ltd, Japanese), and lactate dehydrogenase (LDH; EC 1.1.1.27) (Colorimetric Method, Shanghai DF Biochemical Technology Co. Ltd, China) were determined using an automatic multi-function-biochemical analyzer (Hitachi Ltd, Japan).

2.3. Histological studies

The livers were immediately fixed in neutral buffered formalin, embedded in paraffin wax, cut into 5-μm thick sections, and stained with hematoxylin and eosin (H&E) as described by Ji and Kaplowitz (2003). To analyze liver lipid infiltration, hepatocytes were stained with Sudan Yellow in all three groups of geese ($n = 6$ per group). The livers were fixed in neutral 10% formalin for 24 h, rinsed in 70% ethanol and immersed in Herxeheimer's solution (5% Sudan IV in ethanol and acetone) at room temperature for 15 min (Del Boccio et al., 1990). After transferring the tissues into 80% ethanol for 20 min and washing in running water, lipid infiltration into the livers was evaluated by calculating the area of stain (Henry and Bentley, 1981).

2.4. RNA isolation and RT-PCR

Total RNA was isolated from tissues using TRIZOL reagent (Invitrogen, USA) according to the manufacturer's instructions, and the quality of the total RNA was determined using a spectrophotometer at 260/280 nm (OD260/OD280 = 1.8–2.0). The integrity of the ribosomal RNA bands was confirmed on agarose gels. Single-strand cDNA synthesis was carried out from 1 μg of total RNA by reverse transcription. After denaturation at 70 °C for 10 min, the RNA samples were incubated in 1× PCR buffer, 0.5 mM deoxynucleoside triphosphate mix, 4 μM oligo (dT) primer, 32 U RNase and 200 U M-MLV reverse transcriptase (Promega) in a final volume of 25 μL. This

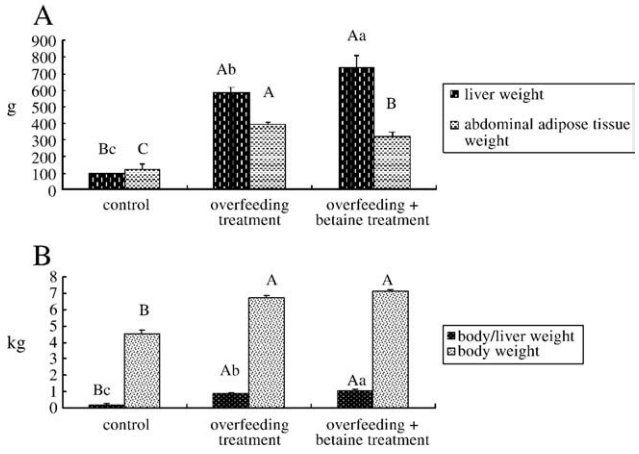

Fig. 1. The effects of overfeeding and betaine supplementation on body weight parameters in geese. (A) Liver mass (g) and abdominal adipose mass (g). (B) Liver/body weight and body mass (kg). Different superscript letters indicate significant differences (capital letters: $P<0.01$; lower-case letters: $P<0.05$). Each value represents the mean of six observations ± standard error.