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Dietary betaine supplementation in hens modulates hypothalamic expression of cholesterol metabolic genes in F1 cockerels through modification of DNA methylation

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ABSTRACT

Betaine is widely used in animal nutrition to promote growth, development and methyl donor during methionine metabolism through nutritional reprogramming via regulation of gene expression. Prenatal betaine exposure is reported to modulate hypothalamic cholesterol metabolism in chickens, yet it remains unknown whether feeding hens with betaine-supplemented diet may affect hypothalamic cholesterol metabolism in F1 offspring. In this study, hens were fed with basal or betaine-supplemented (0.5%) for 30 days, and the eggs were collected for incubation. The hatchlings were raised under the same condition up to 56 days of age. Betaine-treated group showed significantly ($P < 0.05$) higher plasma concentration of total cholesterol and HDL-cholesterol, together with increased hypothalamic content of total cholesterol and cholesterol ester. Concordantly, hypothalamic gene expression of SREBP2, HMGCR, and LDLR was significantly up regulated ($P < 0.05$). Also, mRNA abundances of SREBP1, ACAT1 and APO-A1 were up-regulated, while that of CYP46A1 was significantly down-regulated ($P < 0.05$). These changes coincided with a significant down-regulation of BDNF and CRH, and a significant up-regulation of NPY mRNA expression. Moreover, genes involved in methyl transfer cycle were also modulated. DNMT1 and BHMT were up-regulated ($P < 0.05$) at both mRNA and protein levels, which was associated with significant modifications of CpG methylation on the promoter of SREBP-1, SREBP-2 and APO-A1 genes as detected by bisulfate sequencing. These results indicate that feeding betaine to hens modulates hypothalamic expression of genes involved in cholesterol metabolism and brain functions in F1 cockerels with modification of promoter DNA methylation.

1. Introduction

The formation of the myelin sheath, synapse and dendrites requires cholesterol (Goritz et al., 2005). Cholesterol depletion causes disorder of synaptic vesicle exocytosis in neurons, thus suppresses neuronal activity and neurotransmission (Linetti et al., 2010). Cholesterol in the brain is primarily derived from de novo synthesis, because blood brain barrier prohibits the uptake of lipoproteins from peripheral circulation to the brain (Zhang and Liu, 2015). The primary regulation processes for cholesterol homeostasis in the brain are biosynthesis, conversion, storage, and excretion. Sterol response element binding protein 2 (SREBP2) is the main transcription factor for the activation of 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGCR), the rate-limiting enzyme of cholesterol biosynthesis. The free cholesterol is converted to cholesterol ester by acetyl-CoA cholesterol acyltransferase 1 (ACAT1) for storage in the endoplasmic reticulum, while cholesterol is directly released onto APOA1-containing lipoproteins those are present in cerebrospinal fluid (Koch et al., 2001). Cytochrome P46A1 (CYP46A1), which converts cholesterol to 24S-hydroxycholesterol (Goyal et al., 2014), promotes cholesterol excretion from the brain as 24S-

Abbreviations: ACAT1, acetyl-CoA cholesterol acyltransferase 1; APOA1, apolipoprotein A1; BHMT, betaine homocysteine methyltransferase; BBB, blood brain barrier; CYP46A1, cholesterol 24-hydroxylase A 1; DNMT1, DNA methyltransferase 1; DNMT3A, DNA methyltransferase 3 A; HMGCR, 3-hydroxy-3-methylglutaryl CoA reductase; MeDIP, methylated DNA immunoprecipitation; SAH, S-adenosylhomocysteine; SAM, S-adenosyl methionine; SREBP, sterol regulator element-binding protein

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hydroxycholesterol passes across blood brain barrier much faster than cholesterol itself (Lange et al., 1995). Briefly, functions of above-mentioned genes are academically emphasized at corresponding critical points in the brain cholesterol metabolic pathways.

The hypothalamus plays crucial roles to maintain growth, energy homeostasis, and stress response through various neuropeptides or neurotransmitters (Idriss et al., 2016). Brain-derived neurotrophic factor (BDNF) is associated to neuronal differentiation, protection, survival, as well as synaptic formation and function (Numakawa et al., 2010). Neuropeptide Y is a conserved neurotransmitter that promotes adipogenesis (Zhang et al., 2015) and stimulates food intake (Kalra et al., 1991). Corticotropin-releasing hormone (CRH) plays a major role through stress regulation in chickens (Khan et al., 2015). Also, NPY and CRH are differentially expressed in the hypothalamus of quails divergently selected for low or high feed efficiency (Blankenship et al., 2016). However, evidences linking hypothalamic cholesterol metabolism and neuropeptides expression are lacking in avian species.

Betaine is obtained either from diet or choline oxidation (Lever et al., 2010). Betaine supplementation also improves the growth performance and carcass characteristics in domestic animals (Eklund et al., 2005). It plays as methyl donor to convert homocysteine to methionine through betaine homocysteine methyltransferase (Villamor et al., 2012). Then methionine conveys its methyl group to S-adenosylmethionine (SAM), the universal methyl donor for various methylation reactions catalyzed by different methyltransferases (Mudd et al., 2012). Betaine has been reported to protect mouse embryos preimplantation against increased osmolarity in vitro (Anas et al., 2008). Betaine, as a methyl donor, sustains methionine synthesis under conditions of impaired B-vitamin status in human (Holm et al., 2007). It is shown to improve the adverse effects of alcohol on the liver, particularly on fatty liver (Barak et al., 1997; Barak et al., 1996; Barak and Tuma, 1983), and to prevent the development of steatosis (Kharbanda et al., 2007; Kharbanda et al., 2009). In case of NAFLD, betaine enhance liver function, including reduction of aminotransferases and liver steatosis in human (Abdelmalek et al., 2009). Hepatic cholesterol is altered by prenatal betaine administration in piglets (Cai et al., 2014). Betaine is documented to regulate hepatic cholesterol metabolism in chicken (Hu et al., 2015). Which involves some modifications of epigenetic marks, including DNA and histone methylation. Recently, we reported that in ovo injection of betaine modulates hypothalamic expression of cholesterol metabolic genes in chicks (Idriss et al., 2016). However, it remains unknown whether feeding hens with betaine may affect hypothalamic cholesterol metabolism in offspring (F1) cockerels.

Therefore, the present study was aimed to investigate the effect of maternal betaine exposure on hypothalamic cholesterol content and the expression of cholesterol metabolic genes in male F1 offspring chickens. Hypothalamic expression of BDNF, NPY and CRH was detected to relate with the cholesterol metabolism. Furthermore, expression of genes involved in methionine cycle and methyl transfer was determined in association with methylation analysis on the promoter of affected genes.

2. Materials and methods

2.1. Ethics statement

The Animal Ethics Committee in Nanjing Agricultural University approved the experimental protocol, with the project number 31672512. The sampling procedures complied with the “Guidelines on Ethical Treatment of Experimental Animals” (2006) No. 398 set by the Ministry of Science and Technology, China.

2.2. Animals and treatment

Rugao Yellow chicken breeder hens kept in Poultry Institute of Yangzhou, Jiangsu, were obtained and randomly divided into Control and Betaine groups, fed respectively with basal or betaine-supplemented (0.5%) diet (Table 1) for 30 days. Eggs were collected from both groups and placed together in the incubator. The incubator was set according to our previous publication (Ahmed et al., 2014). Newly hatched chicks (F1) were raised under the same standard condition up to 56 d of age when all the chickens were weighed and sacrificed for sampling, following a previously described procedure (Haussmann et al., 2012). Blood samples were taken from the jugular vein into heparinized tubes and centrifuged at 3000 × *g*, 4 °C for 10 min to prepare plasma. Hypothalami were dissected according to a previous publication (Yuan et al., 2009) and rapidly frozen in liquid nitrogen, then stored at − 70 °C for further analysis.

Table 1
Composition and nutrient content of the experimental diet.

	Control	Betaine
Ingredient %		
Corn	65.00	65.00
Soybean meal	24.67	24.67
Shell powder	6.70	6.70
Limestone	2.03	2.03
Salt	0.30	0.30
Dicalcium phosphate	0.83	0.83
Zeolite	0.01	0.01
Choline chloride	0.17	0.17
Methionine	0.12	0.12
Vitamin premix[a]	0.03	0.03
Minerals premix[b]	0.10	0.10
Betaine	0	0.5

[a] The vitamins premix contain (per kg): Vitamin D3: 9,000,000 IU; Vitamin K: 35,000,000 IU; Vitamin B1: 10 g; Vitamin B2: 28 g; Vitamin B6: 12 g; Vitamin B12: 80 mg; Vitamin E: 140 g; Vitamin K3: 9 g; D-biotin: 5.60 g; D-pantothenic acid: 36 g; folic acid: 3.5 g; Niacinamide: 100 g; ethoxyquin:1.65 g.

[b] The minerals premix contain (per kg): Cu: 6.4 g; Fe: 72 g; Zn: 64 g; Mn: 72 g; Se: 240 mg; I: 480.

2.3. Total cholesterol, cholesterol ester, and free cholesterol content

Total cholesterol (Tch, E1015) and free cholesterol (E1016) contents in the hypothalamus were measured by using commercial cholesterol assay kits purchased from Applygen Technologies (Beijing, China). These kits were previously validated for detecting cholesterol contents in chicken serum and liver samples (Hu et al., 2015). Cholesterol ester content was calculated by subtracting the free cholesterol content from the total Tch content. Briefly, approximately 15 mg of ground hypothalamic sample was homogenized in 0.3 mL ice-cold RIPA lysis buffer (18 mmol/L Tris, pH 7.5, 300 mmol/L mannitol, 50 mmol/L EDTA, and 0.1 mmol/L PMSF) with a Polytron homogenizer (PT1200E, Brinkman Instruments, Littau, Switzerland), and used in the assay following the manufacturer's instructions.

2.4. Plasma concentration of total cholesterol, HDL-C, and LDL-C

Plasma concentration of total cholesterol was measured using a commercial cholesterol assay kit (E1005; Applygen Technologies, Inc.). Plasma levels of LDL-cholesterol (LDL-C) and HDL-cholesterol were measured with respective assay kits (006340 and 006328) purchased from Beijing BHKT Clinical Reagent Co., Ltd., China.

2.5. Total RNA isolation and quantitative real-time PCR

Total RNA was isolated from 20 mg ground hypothalamic samples with TRIzol reagent (Invitrogen, USA). Two micrograms of total RNA were treated with RNase-free DNase and reverse-transcribed to cDNA using a random hexamer primer (Promega, USA). Two microliters of diluted cDNA (1:25, vol/vol) were used for real-time PCR that was performed with a Mx3000P Real-Time PCR System (Stratagene, USA). The technical variations were normalized using β-actin as an internal