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Long-term effect of dietary overload lithium on the glucose metabolism in broiler chickens

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ABSTRACT

Lithium, like insulin, activates glycogen synthase and stimulates glucose transport in rat adipocytes. To investigate the effect of dietary overload lithium on glucose metabolism in broiler chickens, one-day-old chicks were fed a basal diet supplemented with 0 (control) or 100 mg lithium/kg (overload lithium) for 35 days. Compared to controls, glucose disappearance rates were lower ($p = 0.035$) 15–120 min after glucose gavage, and blood glucose concentrations were lower ($p = 0.038$) 30 min after insulin injection in overload lithium broilers. Overload lithium decreased ($p < 0.05$) glycogen and glucose-6-phosphate concentrations in liver, but increased ($p < 0.05$) their concentrations in pectoralis major. Overload lithium increased ($p < 0.05$) mRNA expression of glucose transporter (GLUT) 3 and GLUT9 in liver, and GLUT1, GLUT3, GLUT8, and GLUT9 in pectoralis major, but decreased ($p < 0.05$) cytosolic phosphoenolpyruvate carboxykinase (PEPCK) in liver and mitochondrial PEPCK in pectoralis major. These results suggest that dietary overload lithium decreases glucose tolerance and gluconeogenesis, but increases insulin sensitivity and glucose transport in broiler chickens.

1. Introduction

The use of lithium salts in the treatment of bipolar disorder has generated interest in their effects on several metabolic pathways (Luca et al., 2016). Studies have found that lithium stimulates glucose uptake and glycogen production in diaphragm tissue and adipocytes isolated from rats (Bosch et al., 1992; Chen et al., 1998). Insulin-like effects of lithium salt have also been reported in rat hepatocytes (Cheng et al., 1983), but the results from in vivo studies have been contradictory. Administration of lithium to healthy rats has been shown to lead to both impairment (Fontela et al., 1986) and improvement of responses during glucose tolerance tests (Vendsborg, 1979). In patients with type II diabetes mellitus, lithium administration causes a slight decrease in glycemia without altering serum insulin levels (Jones et al., 1983). Lithium has also been shown to increase whole glucose uptake and muscle glycogen content in healthy rats (Macko et al., 2008). These results seem to indicate that lithium has insulin-like effect on glucose metabolism in vivo, but the precise mechanisms by which these effects are exerted are unknown.

Compared to mammals, avian species are considered hyperglycemic and typically have fasting blood glucose (BG) concentrations that are twice that of non-diabetic humans (Scanes and Braun, 2012). In addition, relative insulin resistance is common among birds, including chickens, despite having “normal” plasma insulin concentrations (Simon et al., 2011). In mammals, chronic hyperglycemia is almost always associated with serious deleterious effects on the body; in contrast, even older chickens do not appear to exhibit any ill effects of prolonged hyperglycemia (Scanes and Braun, 2012). Given the growing concern about hyperglycemia and insulin resistance in humans, and the fact that obesity is often associated with impaired glucose metabolism, chickens have recently been recognized as a potential model for studying diabetes (Datar and Bhonde, 2011).

Lithium is an inhibitor of serine/threonine kinase glycogen synthase kinase-3 beta (GSK-3β) (Luca et al., 2016), which is involved in the regulation of phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase), two key enzymes in gluconeogenesis

Abbreviations: ANOVA, analysis of variance; AUC, area under the curve; BCAAs, branched-chain amino acids; BG, blood glucose; BW, body weight; C/EBPβ, CCAAT/enhancer binding protein; ELISA, enzyme-linked immunosorbent assay; G6P, glucose-6-phosphate; G6Pase, glucose-6-phosphatase; GLM, general linear model; GLUT, glucose transporter; GSK-3β, serine/threonine kinase glycogen synthase kinase-3 beta; InsR, insulin receptor; LS, least square; PBS, phosphate buffered saline; PEPCK, phosphoenolpyruvate carboxykinase; PEPCK-C, cytosolic PEPCK; PEPCK-M, mitochondrial PEPCK; PPARγ, peroxisome proliferator-activated receptor-γ; SEM, standard error of the mean; SREBP, sterol regulatory element-binding transcription factor; T3, triiodothyronine; T4, tetraiodothyronine

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(Lochhead et al., 2001). GSK3β can also facilitate the glycogen synthesis process through activation of glycogen synthase (Beurel et al., 2015). In a previous study, we found that long-term overload lithium decreased adipogenesis in the abdominal fat tissue of broiler chickens (Bai et al., 2017). However, to our knowledge, there have been no studies on the influence of long-term overload lithium on glycogen concentrations or gluconeogenesis in the tissues of broiler chickens. Therefore, the goal of the present work was to determine the effect of dietary overload lithium on glucose metabolism and expression of glucose regulatory genes in the liver and pectoralis major of broiler chickens.

2. Materials and methods

2.1. Materials

Lithium chloride (purity: 99.9%), glucose (purity: 99.5%), human insulin (purity: 100%), 10% neutral buffered formalin, paraffin wax (purity: 99%), hematoxylin (purity: 99%), esosin (purity: 99%), and K_2CO_3 (purity 99.9%) were purchased from Sigma Aldrich (St. Louis, MO, USA). Ethanol (purity: 99.9%), perchloric acid (purity: 99.9%), and formaldehyde (purity: 99.9%) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Agarose gel was purchased from Biol-Rad Laboratories Inc. (Hercules, CA, USA). Pure α-amyloglucosidase (from *Aspergillus niger*, 70 units/mg protein), and the ELISA kits for plasma insulin and glucose assay were purchased from Sigma Aldrich (Shanghai, China). The enzyme-linked immunosorbent assay (ELISA) kits for plasma triiodothyronine (T_3) and tetraiodothyronine (T_4) assay were purchased from Nanjing Jiancheng Biological Institute (Nanjing, China). The TRIzol reagent for tissue RNA extraction and the kit for mRNA reverse transcription were purchased from Thermo Fisher Scientific (Waltham, MA, USA). The SYBR Green PCR Mix was purchased from TaKaRa (Dalian, China).

2.2. Animals and diets

All animal protocols were approved by the Animal Health and Care Committee in Sichuan Agricultural University. A total of three experiments were conducted using one batch of male broiler chickens (Daheng strain) obtained from a local hatchery for each experiment. Five one-day-old birds were randomly allotted into ten cages per treatment group. Dietary treatments consisted of a basal diet supplemented with 0 (control) or 100 mg lithium/kg from lithium chloride (overload lithium). Birds were housed in electrically heated and thermostatically controlled cages (40 × 50 × 30 cm), and were given ad libitum access to feed and water. The ambient temperature was gradually decreased by 0.5 °C per day from 32 °C on experimental day 1–25 °C on day 14, and was kept at 25 °C until completion of the study on day 35. The basal diet (metabolizable energy = 12.59 MJ/kg; crude protein = 21.50%; methionine = 0.50%; lysine = 1.10%; calcium = 1.00%; non-phytate phosphorus = 0.45%) was formulated to meet the requirements of chickens. The concentration of lithium was chosen based on previous studies in poultry in which dietary 100 mg lithium/kg drastically decreased body weight gain of animals (Scott et al., 1973; Bai et al., 2017). The control diet contained no detectable lithium, whereas the overload lithium diet contained 100.80 mg lithium/kg as analyzed using an atomic absorption spectrometer (ContrAA 700, Analytik Jena AG, Jena, Germany).

2.3. Experiment 1: oral glucose tolerance test

At 35 days of age, overload lithium and control broilers were fasted for 16 h. Two birds per replicate cage were selected according the average body weight (BW) for either glucose or vehicle treatment (n = 10 per treatment). Glucose-treated broilers received a glucose bolus (2 g/kg BW; 20% w/v H_2O) by oral gavage. Vehicle-treated broilers received an equivalent volume of 1 × phosphate buffered saline (PBS). BG and blood insulin concentrations were measured in glucose-treated broilers, and only BG concentration was measured in vehicle-treated broilers at 0, 5, 15, 30, 60, 120, and 240 min after treatment via small brachial blood vessels. BG concentrations were determined using a handheld glucometer (Sinocare Inc., Changsha, China). Blood insulin concentrations were analyzed using the ELISA kit (Sigma-Aldrich). Area under the curve (AUC) and glucose clearance rates (15–120 min) were calculated as described by Gilbert et al. (2011). Data of BG or blood insulin were analyzed by analysis of variance (ANOVA). The model for BG included the main effects of diet, glucose gavage, time, and their interactions. The model for blood insulin included the main effects of diet, time, and their interaction.

2.4. Experiment 2: insulin sensitivity test

At 35 days of age, overload lithium and control broilers were fasted for 16 h. Two bird per replicate cage were selected according the average BW for either insulin or vehicle treatment (n = 10 per treatment). Insulin-treated broilers received 80 μg/kg BW human insulin (Sigma-Aldrich) diluted in 1 × PBS via intraperitoneal (i.p.) injection. Vehicle-treated birds received an equivalent volume of 1 × PBS. BG concentrations were measured at 0, 30, 60, and 120 min in both insulin- and vehicle-treated broilers, as described in Section 2.3. Re-feeding was initiated immediately following the 120-min sampling time point in insulin-treated broilers. BG concentrations were then measured 180 min after treatment (after 1 h re-feeding). Data for insulin- and vehicle-treated broilers were analyzed separately. Two analyses were performed. The first included BG concentrations at 0, 30, 60, and 120 min, and the second analysis involved the difference in BG concentration between overload lithium and control broilers at 180 min after treatment. The first analysis was by two-way ANOVA, and the effects in the statistical model included diet, time, and their interaction. The second analysis was by one-way ANOVA.

2.5. Experiment 3: glucose metabolism in liver and pectoralis major

2.5.1. Samples collection

At 35 days of age, BW of broilers in each replicate cage were measured (n = 10). Two birds per replicate cage were selected according to average BW. For one broiler, 5 mL of blood was taken from the brachial vein 12 h after feed withdrawal. Blood samples were collected in heparinized tubes and centrifuged (3000g for 20 min at 4 °C) to collect plasma (n = 10). After blood collection, the broilers were euthanized and the samples of left lobe of liver, kidney and pancreas were collected for histopathological analysis (n = 6). A second bird per replicate cage was euthanized for tissue sample collection (n = 10). Approximately 2.0 g samples of left lobe of liver and pectoralis major were collected and stored at −20 °C for later measurement of glycogen and glucose-6-phosphate (G6P). Separate 0.2 g samples of left lobe of liver and pectoralis major were collected and snap-frozen in liquid nitrogen and stored at −80 °C until further analysis.

2.5.2. Blood characteristics

Plasma insulin and glucose concentrations were analyzed using the ELISA kits from Sigma-Aldrich, and plasma T_3 and T_4 concentrations were measured using the ELISA kits from Nanjing Jiancheng Biological Institute according to the manufacturers' recommendations. Plasma free amino acid concentrations were measured using an amino acid analyzer (L-8900, Hitachi, Tokyo, Japan) as described by Shimbo et al. (2009).

2.5.3. Histology of liver, kidney, and pancreas

Samples for histological analysis were fixed in 10% neutral buffered formalin for 24 h, rinsed with 70% ethanol, dehydrated in serial dilutions of ethanol, and embedded in paraffin wax. Paraffin-embedded