Effects of Glucocorticoids on the Expression of Genes Related to Lipid and Bile Acid Metabolism in Chick Embryo Hepatocytes (Postprint)

Authors: Zhou Huajin, Yang Jiachang, Zhang Hui, Jiguang Wang, Hao Yangyang, Du Peng, Song Zhigang

Date: 2018-12-25T00:00:00+00:00

Abstract

To elucidate the mechanism by which stress induces metabolic abnormalities in poultry liver, this study investigated the effects of the glucocorticoid dexamethasone (DEX) on the expression of genes related to lipid and bile acid metabolism in chicken embryo hepatocytes. SPF (specific pathogen-free) eggs at 19 days of embryonic age were selected, and chicken embryo hepatocytes were primary cultured (37 °C, 5% CO) and treated with 0 (control), 200, 500, or 1,000 nmol/L dexamethasone for 24 h. The results demonstrated that, compared with the control group, high-dose (500, 1,000 nmol/L) dexamethasone treatment significantly decreased the relative mRNA expression levels of lipid metabolism-related genes—fatty acid transport protein 1 (FATP-1), sterol regulatory element-binding protein-1C (SREBP-1C), apolipoprotein B100 (APOB100), liver X receptor (LXR)—and the bile acid uptake-related gene Na+/taurocholate cotransporting polypeptide (NTCP) ($P < 0.05$), while significantly increasing the relative mRNA expression level of the bile acid efflux-related gene bile salt export pump (BSEP) ($P < 0.05$). Low-dose (200 nmol/L) dexamethasone treatment significantly increased the relative mRNA expression levels of bile acid synthesis-related genes—cholesterol 7 -hydroxylase (CYP7A1) and farnesoid X receptor (FXR) ($P < 0.05$), concurrently significantly decreasing the relative mRNA expression level of SREBP-1C ($P < 0.05$) and significantly increasing that of BSEP ($P < 0.05$). These findings indicate that high-dose glucocorticoid exerts inhibitory effects on lipid synthesis, transport, and bile acid uptake in chicken embryo hepatocytes, whereas low-dose glucocorticoid can promote bile acid synthesis and efflux, with some responses exhibiting dose-dependence.

Full Text

Effects of Glucocorticoids on Expression of Lipid and Bile Acid Metabolism-Related Genes in Chicken Embryo Hepatocytes

Zhou Huajin, Yang Jiachang, Zhang Hui, Wang Jiguang, Hao Yangyang, Du Peng, Song Zhigang*

College of Animal Science and Technology/College of Veterinary Medicine, Shandong Agricultural University, Tai' an 271018, China

Abstract

To elucidate the mechanisms underlying stress-induced metabolic abnormalities in avian liver, this study investigated the effects of the glucocorticoid dexamethasone (DEX) on the expression of genes related to lipid and bile acid metabolism in chicken embryo hepatocytes. Primary hepatocytes from 19-day-old specific pathogen-free (SPF) embryos were cultured at 37 °C with 5% CO and treated with 0 (control), 200, 500, or 1,000 nmol/L dexamethasone for 24 hours. Compared with the control group, high-dose dexamethasone (500 and 1,000 nmol/L) significantly reduced the relative mRNA expression levels of lipid metabolism-related genes, including fatty acid transport protein 1 (FATP-1), sterol regulatory element-binding protein-1C (SREBP-1C), apolipoprotein B100 (APOB100), and liver X receptor (LXR) ($P < 0.05$), while also decreasing expression of the bile acid uptake gene Na /taurocholate co-transporting polypeptide (NTCP) ($P < 0.05$) and increasing expression of the bile acid efflux gene bile salt export pump (BSEP) ($P < 0.05$). Low-dose dexamethasone (200 nmol/L) significantly elevated the relative mRNA expression levels of bile acid synthesis genes cholesterol 7 -hydroxylase (CYP7A1) and farnesoid X receptor (FXR) ($P < 0.05$), concurrently reducing SREBP-1C expression ($P < 0.05$) and enhancing BSEP expression ($P < 0.05$). These findings indicate that high-dose glucocorticoids inhibit lipid synthesis, transport, and bile acid uptake in chicken embryo hepatocytes, whereas low-dose glucocorticoids promote bile acid synthesis and excretion, with some responses exhibiting dose-dependent patterns.

Keywords: glucocorticoids; chicken embryo hepatocytes; lipid metabolism; bile acid synthesis; gene expression

---

Stress induces abnormal energy metabolism in poultry, and elucidating the pathways through which glucocorticoids influence hepatic lipid and bile acid synthesis is crucial for understanding stress mechanisms and developing prevention strategies. Glucocorticoids participate in lipid metabolism, with elevated glucocorticoid levels observed in obese diabetic mouse models and insulin-resistant patients, correlating with fatty liver and hyperglycemia development. Both syn-

thetic and natural glucocorticoids potently regulate hepatic lipid metabolism; studies in mouse primary hepatocytes have shown that dexamethasone promotes protein-dependent fatty acid uptake and increases triacylglycerol and sphingolipid accumulation. Additionally, glucocorticoids are involved in bile acid metabolism—hepatic-specific glucocorticoid receptor knockout in mice reduces bile acid content in the gallbladder, and glucocorticoids regulate bile acid synthesis in rat hepatocytes by inducing cholesterol 7 -hydroxylase (CYP7A1) activity. In broiler chickens, dexamethasone administration in drinking water suppresses the tricarboxylic acid cycle and fatty acid oxidation while promoting hepatic de novo fatty acid synthesis. Current research on stress regulation of avian liver metabolism has primarily relied on in vivo experiments, yielding results reflecting combined effects of glucocorticoids, insulin, and other factors. Therefore, in vitro studies are needed to investigate the direct effects of glucocorticoids. This study employed different concentrations of dexamethasone to treat chicken embryo hepatocytes and used reverse transcription PCR (RT-PCR) to quantify genes related to lipid and bile acid metabolism, thereby revealing the isolated effects of glucocorticoids and establishing a foundation for further investigation into stress-induced metabolic alterations in poultry.

1.1 Experimental Materials

Dexamethasone injection (5 mg/mL) was purchased from Shandong Lukang Chenxin Pharmaceutical Co., Ltd. RNA extraction kits were obtained from CW Biotech (CW0584), reverse transcription kits (cat. no. 4897030001) from Roche, and real-time PCR kits (cat. no. 4913914001) from Roche. Primer sequences for sterol regulatory element-binding protein-1C (SREBP-1C), liver X receptor (LXR), apolipoprotein B100 (APOB100), fatty acid transport protein 1 (FATP-1), CYP7A1, farnesoid X receptor (FXR), bile salt export pump (BSEP), and Na /taurocholate co-transporting polypeptide (NTCP) are listed in and were synthesized by Shanghai Sangon Biotech. The 2 $\Delta\Delta$CT method was used to quantify relative mRNA expression levels of target genes, normalized to -actin as the reference gene.

1.2 Experimental Design

Primary chicken embryo hepatocytes were isolated from 19-day-old SPF eggs and seeded at 1 $\times$ 10 cells/mL in 6-well plates (one well per replicate). Cells were cultured under standard conditions (37 °C, 5% CO) with medium changes every 24 hours. After 72 hours of culture, cells were treated with 0 (control), 200, 500, or 1,000 nmol/L dexamethasone according to experimental groups. Following 24 hours of treatment, cell supernatants and hepatocytes were collected for RT-PCR analysis.

1.3 Gene Quantification

Reverse transcription was performed at 55 °C for 30 minutes, followed by inactivation at 85 °C for 5 minutes. Two-step quantitative PCR conditions were:

Step 1, denaturation at 95 °C for 10 seconds (1 cycle); Step 2, 40 cycles of 95 °C for 5 seconds and 60 °C for 34 seconds.

1.4 Statistical Analysis

Data were analyzed using one-way ANOVA with the GLM procedure in SAS 9.0 software. Means were compared using Tukey' s HSD test, with $P < 0.05$ considered statistically significant.

2.1 Effects of Dexamethasone on Relative mRNA Expression Levels of Lipid Metabolism-Related Genes in Chicken Embryo Hepatocytes

As shown in [Figure 1: see original paper]-A, C, and D, treatment with 500 and 1,000 nmol/L dexamethasone significantly reduced the relative mRNA expression levels of FATP-1, APOB100, and LXR compared with the control group ($P < 0.05$). [Figure 1: see original paper]-B demonstrates that dexamethasone at 200, 500, and 1,000 nmol/L all significantly decreased SREBP-1C mRNA expression ($P < 0.05$).

2.2 Effects of Dexamethasone on Relative mRNA Expression Levels of Bile Acid Metabolism-Related Genes in Chicken Embryo Hepatocytes

[Figure 2: see original paper]-A and B show that 200 nmol/L dexamethasone significantly increased the relative mRNA expression levels of CYP7A1 and FXR ($P < 0.05$). As depicted in [Figure 2: see original paper]-C, 500 and 1,000 nmol/L dexamethasone significantly reduced NTCP mRNA expression ($P < 0.05$). [Figure 2: see original paper]-D reveals that dexamethasone at 200, 500, and 1,000 nmol/L all significantly elevated BSEP mRNA expression ($P < 0.05$).

3.1 Glucocorticoids and Lipid Metabolism in Chicken Embryo Hepatocytes

In avian species, fatty acid synthesis follows the same pathways as in mammals, occurring primarily in the liver and regulated by multiple proteins. Glucocorticoids are closely associated with lipid metabolism; rats administered dexamethasone at 0.05 mg/kg daily for 7 weeks exhibited decreased hepatic fat content and reduced mRNA expression of lipid synthesis-related genes including fatty acid-binding protein-1 (FABP-1), peroxisome proliferator-activated receptor (PPAR), and glycerol-3-phosphate acyltransferase (GPAT), suggesting an inhibitory effect of dexamethasone on lipid synthesis. In the liver, fatty acids cross the plasma membrane via active transport mediated by the fatty acid transport protein (FATP) family, with FATP-1 facilitating long-chain fatty acid flux into and out of cells. Additionally, FATP-1 influences intracellular fatty acid metabolism and lipid accumulation by regulating fatty acid acylation. In this study, high-dose dexamethasone significantly reduced FATP-1 mRNA expression in chicken embryo hepatocytes, indicating that glucocorticoids suppress hepatocellular fatty acid synthesis.

LXR and SREBP-1C also play critical roles in lipid synthesis. LXR acts as a cholesterol sensor, regulating genes involved in cholesterol absorption, transport, efflux, and excretion, with overexpression markedly enhancing transcription of lipid synthesis-related enzymes. Studies in Landes geese have shown that increased LXR mRNA expression promotes hepatic fat deposition. In triglyceride synthesis, fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) serve as rate-limiting enzymes whose expression is regulated by SREBP-1C. When intracellular cholesterol levels are high, LXRs activate SREBP-1C, which increases oleic acid synthesis; the resulting unsaturated fatty acids esterify excess cholesterol, promoting cholesterol storage. Conversely, reduced LXR activation inhibits SREBP-1C transcription, decreasing lipogenesis and triglyceride synthesis. In this study, high-dose dexamethasone significantly reduced mRNA expression of both LXR and SREBP-1C, further demonstrating the inhibitory effect of glucocorticoids on lipid synthesis.

APOB is a structural component of very low-density lipoprotein (VLDL) and serves as a scaffold protein for lipoprotein synthesis, secretion, and transport, significantly influencing energy transport and metabolism. VLDL delivers triglycerides from liver to peripheral tissues, and excessive hepatic APOB synthesis elevates plasma VLDL and low-density lipoprotein (LDL) levels, potentially causing atherosclerotic cardiovascular disease and hepatic lipid accumulation leading to fatty liver. Furthermore, the chicken APOB gene SNP (T123G) significantly affects abdominal fat and fat percentage. In this study, high-dose dexamethasone significantly downregulated APOB100 mRNA expression in hepatocytes, suggesting that glucocorticoids inhibit lipoprotein assembly and transport.

3.2 Glucocorticoids and Bile Acid Metabolism in Chicken Embryo Hepatocytes

Bile acids are synthesized from cholesterol in hepatocytes, with CYP7A1 acting as the rate-limiting enzyme. Research has demonstrated that glucocorticoids exert dose-dependent stimulatory effects on bile acid synthesis; treatment of rat hepatocytes with 50 nmol/L dexamethasone increased bile acid synthesis 3-fold and 7-fold on days 2 and 3, respectively, while 1 mol/L dexamethasone elevated synthesis 2.2-fold after 2 days. Glucocorticoids regulate bile acid synthesis in rat hepatocytes by inducing CYP7A1 activity. Combined dexamethasone and thyroid hormone treatment significantly increased total bile acid synthesis in human and rat hepatocytes, raising cholic acid content by 23% and dihydroxycholic acid by 77% while enhancing CYP7A1 activity. In cultured hepatocytes, 27-hydroxylase mRNA is not normally expressed but appears after 72 hours of dexamethasone treatment, indicating that dexamethasone upregulates both CYP7A1 and sterol 27-hydroxylase activities to modulate bile acid synthesis. Consistent with these findings, low-dose dexamethasone in this study increased CYP7A1 mRNA expression, confirming that glucocorticoids promote bile acid synthesis.

NTCP, located on the basolateral membrane of hepatocytes, is the primary

transporter for bile acid uptake. Dexamethasone has been reported as a potent inducer of BSEP in rat liver, with rat hepatocytes cultured in 100 nmol/L dexamethasone for 4 days exhibiting strong BSEP functional activity, which aligns with our observation of dexamethasone-induced BSEP mRNA upregulation. Studies have shown that CYP7A1 is negatively regulated by FXR through feedback inhibition; in the liver, FXR activation suppresses CYP7A1 expression via downstream target gene SHP, reducing bile acid synthesis. Additionally, hepatic FXR activation by bile acids downregulates NTCP expression while upregulating the bile acid efflux transporter BSEP. In this study, 200 nmol/L dexamethasone increased CYP7A1 and FXR mRNA expression, whereas 500 and 1,000 nmol/L dexamethasone inhibited NTCP expression and enhanced BSEP expression, suggesting that low-level glucocorticoids promote bile acid synthesis and excretion while high-level glucocorticoids suppress bile acid uptake in chicken embryo hepatocytes.

4 Conclusion

High-dose glucocorticoids inhibit lipid synthesis, transport, and bile acid uptake in chicken embryo hepatocytes, whereas low-dose glucocorticoids promote bile acid synthesis and excretion, with some responses exhibiting dose-dependent patterns.

References

[1] ROSE A J,HERZIG S.Metabolic control through glucocorticoid hormones:an update[J].Molecular and Cellular Endocrinology,2013,380(1/2):65-78.
[2] LIVINGSTONE D E,GRASSICK S L,CURRIE G L,et al.Dysregulation of glucocorticoid metabolism in murine obesity:comparable effects of leptin resistance and deficiency[J].Journal of Endocrinology,2009,201(2):211-218.
[3] HARASIM-SYMBOR E,KONSTANYNOWICZ-NOWICKA K,CHABOWSKI A.Additive effects of dexamethasone and palmitate on hepatic lipid accumulation and secretion[J].Journal of Molecular Endocrinology,2016,57(4):261-273.
[4] ROSE A J,DÍAZ M B,REIMANN A,et al.Molecular control of systemic bile acid homeostasis by the liver glucocorticoid receptor[J].Cell Metabolism,2011,14(1):123-130.
[5] PRINCEN H M,MEIJER P,HOFSTEE B.Dexamethasone regulates bile acid synthesis in monolayer cultures of rat hepatocytes by induction of cholesterol 7 -hydroxylase[J].Biochemical Journal,1989,262(1):341-348.
[6] LV Z P,PENG Y Z,ZHANG B,et al.Glucose and lipid metabolism disorders in the chickens with dexamethasone-induced oxidative stress[J].Journal of Animal Physiology and Animal Nutrition,2018,102(2):e706-e717.
[7] LIVAK K J,SCHMITTGEN T D.Analysis of relative gene expression data using real-time quantitative PCR and the $2-\Delta\Delta CT$ method[J].Methods,2001,25(4):402-408.
[8] WU T,YANG L,JIANG J G,et al.Chronic glucocorticoid treatment induced circadian clock disorder leads to lipid metabolism and gut microbiota alter-

ations in rats[J].Life Sciences,2018,192:173-182.
[9] QI R L,FENG M,TAN X,et al.FATP1 silence inhibits the differentiation and induces the apoptosis in chicken preadipocytes[J].Molecular Biology Reports,2013,40(4):2907-2914.
[10] MARTIN G,POIRIER H,HENNUYER N,et al.Induction of the fatty acid transport protein 1 and acyl-CoA synthase genes by dimer-selective rexinoids suggests that the peroxisome proliferator-activated receptor-retinoid X receptor heterodimer is their molecular target[J].Journal of Biological Chemistry,2000,275(17):12612-12618.
[11] 韩春春, 黄晓宇, 王继文. 鹅 LXR 基因的克隆及填饲对其 mRNA 水平的影响 [J]. 畜牧兽医学报,2009,40(9):1405-1409.
[12] AOKI N,YOSHIDA D,ISHIKAWA R,et al.A single daily meal at the beginning of the active or inactive period inhibits food deprivation-induced fatty liver in mice[J].Nutrition Research,2014,34(7):613-622.
[13] 张晾, 张燕燕, 叶玲敏, 等. 多不饱和脂肪酸饮食对载脂蛋白 E 缺失小鼠含载脂蛋白 B100 脂蛋白代谢的影响 [J]. 生物化学与生物物理进展,2015,42(9):843-849.
[14] 张森, 石慧, 李辉. 鸡 apoB 基因 T123G 多态位点与生长和体组成性状的相关性研究 [J]. 畜牧兽医学报,2006,37(12):1264-1268.
[15] 高广亮, 张庆秋, 李辉, 等. 鸡肝细胞中 L-BABP 基因表达对脂类代谢基因及甘油三酯和总胆固醇的影响 [J]. 畜牧兽医学报,2015,46(1):32-40.
[16] LI T G,CHIANG J Y L.Bile acid signaling in metabolic disease and drug therapy[J].Pharmacological Reviews,2014,66(4):948-983.
[17] WARSKULAT U,KUBITZ R,WETTSTEIN M,et al.Regulation of bile salt export pump mRNA levels by dexamethasone and osmolarity in cultured rat hepatocytes[J].Biological Chemistry,1999,380(11):1273-1279.
[18] ELLIS E,GOODWIN B,ABRAHAMSSON A,et al.Bile acid synthesis in primary cultures of rat and human hepatocytes[J].Hepatology,1998,27(2):615-620.
[19] WANG L,LEE Y K,BUNDMAN D,et al.Redundant pathways for negative feedback regulation of bile acid production[J].Developmental Cell,2002,2(6):721-731.
[20] KERR T A,SAEKI S,SCHNEIDER M,et al.Loss of nuclear receptor SHP impairs but does not eliminate negative feedback regulation of bile acid synthesis[J].Developmental Cell,2002,2(6):713-720.
[21] ZOLLNER G,WAGNER M,FICKERT P,et al.Role of nuclear receptors and hepatocyte-enriched transcription factors for Ntcp repression in biliary obstruction in mouse liver[J].American Journal of Physiology-Gastrointestinal and Liver Physiology,2005,289(5):G798-G805.
[22] PLASS J R M,MOL O,HEEGSMA J,et al.Farnesoid X receptor and bile salts are involved in transcriptional regulation of the gene encoding the human bile salt export pump[J].Hepatology,2002,35(3):589-596.

Note: Figure translations are in progress. See original paper for figures.