Exogenous insulin promotes the expression of B-cell translocation gene 1 and 2 in chicken pectoralis

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ABSTRACT B-cell translocation genes (BTG) have been proved to play important roles in carbohydrate metabolism through modifying insulin homeostasis and glucose metabolism. This study, therefore, was conducted to investigate the effects of exogenous insulin on the expression of BTG1 and BTG2 in chickens. Twenty-four-day-old broilers and layers were fasted for 16 h and randomly assigned to insulin treatment group (subcutaneously injected with 5 IU/kg body weight) or control group (received an equivalent volume of phosphate-buffered saline). Blood glucose concentration was measured, and it showed that the blood glucose concentrations in the layers were significantly (P < 0.05) higher than that in the broilers under fasting state. Response to exogenous insulin, the blood glucose concentrations were greatly reduced in both breeds. Of note, the blood glucose concentration restored to 62% of the basal state at 240 min (P < 0.05) after insulin stimulation in layers, whereas it was still in low level until 240 min in broilers (under fast state). Tissue profiling revealed that both BTG1 and BTG2 were abundantly expressed in the skeletal muscles of broilers. A negative correlation was observed between blood glucose and BTG1 (r = -0.289, P = 0.031) / BTG2 (r = -0.500, P < 0.001) in pectoralis, and BTG1 (r = -0.462, P < 0.001) in pancreas. As blood glucose decreased due to exogenous insulin administration (under fast state), the expression of both BTG1 and BTG2 notably upregulated in birds’ pectoralis at 120 min and/or 240 min, meanwhile pancreas BTG1 was also upregulated. Re-feeding at 120 min elevated the blood glucose and reduced the expression of BTG genes in pectoralis generally. In addition, the change of BTG1 and BTG2 expression showed distinct difference between layers and broilers at 120 min and 240 min after insulin stimulation in pectoralis, pancreas and heart tissue; even after re-feeding at 120 min, BTG2 expression at 240 min after insulin injection was downregulated in the pectoralis of layers, while it was upregulated in that broilers. Collectively, these results indicated that response to exogenous insulin, chicken blood glucose exhibited breed-specific dynamic change, and meanwhile the expressions of both BTG1 and BTG2 genes in chickens were significantly altered by exogenous insulin in a breed- and tissue-specific manner. BTG1 and BTG2 genes may negatively regulate bird’s blood glucose by promoting the glucose uptake corporately in pectoralis, and through regulating the insulin secretion in pancreas (especially BTG1).

Key words: insulin, blood glucose, BTGs, chicken

INTRODUCTION

Insulin is an important peptide hormone produced by pancreatic β-cells (Moore and Cooper, 1991), which is the master regulator of glucose metabolism (Norton et al., 2022). It functions through cooperatively regulating the expression of related genes in insulin-sensitive tissues, such as skeletal muscle, heart, liver, and pancreas in tissue-specific manner (Iliadis et al., 2011; Petersen and Shulman, 2018). Chickens have higher blood glucose concentration than mammals and exhibit insulin-resistant (Akiba et al., 1999). Chickens from lines artificially selected for juvenile low and high body weight differed in glucose homeostasis and pancreas physiology (Sumners et al., 2014). That is, birds with different genetic background have distinct response to exogenous insulin. The outcomes from our previous study found that compared with high-weight broilers, low-weight black-bone chickens had better glucose regulation ability in response to exogenous insulin disturbances (Ji et al., 2020).

B-cell translocation gene 1 (BTG1) and BTG2 (also called PC3 or TIS21) belong to the B-cell translocation
gene/transducer of $BTG/TOB$ family (Bradbury et al., 1991; Fletcher et al., 1991; Mauzion et al., 2009; Winkler, 2010), they were highly conservative among species (Rouault et al., 1993). $BTG$ family participates to regulate gene transcription and cellular differentiation and inhibits proliferation. Limited researches showed that $BTGs$’ expression dynamically changed in gene-specific manner, and could be regulated by exogenous stimulation. The mRNA for $BTG1$, $BTG2$, and $BTG3$ presented differed temporal expression patterns in the rat ovary during the periovulatory period after human chorionic gonadotropin ($hCG$) treatment, and they were highly induced both in rat ovaries and granulosa cells (Li et al., 2009). IGF-I significantly elevated $BTG1$ mRNA levels in human MCF-7 breast cancer cells, and $BTG1$ mRNA was inhibited by inhibitors of PI3/Akt kinase and mitogen-activated protein kinase (MAPK) (Vaglam et al., 2006). The expression profiles of bird’s $BTG1$ and $BTG2$ displayed dynamic and differential expression patterns during early embryonic development by in situ hybridization (Kamai and Giráldez, 2008). $BTG2$ and $BTG3$ presented distinct tissue expression features in lean and fatty genotype pigs, and $BTG3$ mRNA level dynamically expressed in skeletal muscle of fetuses’ different stages in genotype-specific manner (Feng et al., 2007). Although few information were searched out about the effect of insulin on $BTG$ gene’s expression in animal, several studies noticed there were some links between the expression of $BTG1$ or $BTG2$, insulin homeostasis, and glucose metabolism (Hwang et al., 2012; Hwang et al., 2013; Kim et al., 2014; Xiao et al., 2016). It was reported that $BTG1$ could regulate the insulin sensitivity of the mouse liver by promoting c-JUN expression (Xiao et al., 2016). $BTG2$ mediates glucagon-like peptide-1 to stimulate insulin secretion by inducing the expression of pancreatic duodenal homeobox-1 in pancreatic $\beta$ cells (Hwang et al., 2013). Besides, the overexpression of $BTG2$ enhances the transcription and translation of glucose 6-phosphatase, phosphoenolpyruvate carboxykinase, and cAMP-response element binding protein, thereby significantly increasing hepatic glucose production (Hwang et al., 2012). Kim et al. (2014) reported that $BTG2$ up-regulated the orphan nuclear receptor NUR77, which in turn regulated hepatic hormoneostasis in a diabetic mouse model. These suggested that $BTG$ family may participate in the insulin signaling pathway and insulin may regulate $BTG$ genes’ expression level in animal.

In addition, long-term artificial selection of chickens for meat and egg production resulted in 2 highly divergent genetic breeds: faster growth (Arbor Acres broilers) and slower growth (Hy-Line layers). There are various differences in energy metabolism (Saunderson and Leslie, 1988) and basal metabolic rate (Kuenzel and Kuenzel, 1977) in 2 breeds of birds, which was possibly explained by the difference of insulin sensitivity (Shiraishi et al., 2011). We hypothesized that 2 divergent breeds display differential glucose metabolism feature under insulin stimulation, meanwhile $BTGs$’ mRNA level could be regulated by insulin in a breed- and tissue-specific manner. Wherefore, the aim of present work was to investigate the effects of exogenous insulin on the blood sugar of different breeds of chickens, and the dynamic response of the $BTG$ genes in various insulin-sensitive tissues (heart, breast muscle, and pancreas) to exogenous insulin manipulation. These results would extend our understanding of the regulatory roles of the insulin-responsive $BTGs$ and the functions of $BTGs$ in glucose metabolism.

**MATERIALS AND METHODS**

**Animals**

All procedures carried out were approved by Henan Agricultural University Institutional Animal Care and Use Committee (approval No. HNNDD20191201). One-day-old male Arbor Acres broilers ($n = 120$) and Hy-Line layers ($n = 120$) were reared in stainless steel cages in a climate-controlled facility. The light schedule was 23L:1D throughout the trial. In addition, all birds were free access to feed and water. The diet (Table 1) was formulated according to the Chinese Feeding Standard for Chicken (2004). The initial ambient temperature set at 33 to 35°C in the first week, and followed by the temperature was gradually reduced based on normal management practices to 22°C by 20 d. The birds were vaccinated at first day of age against Newcastle Disease and Infectious Bronchitis at the hatchery facilities. At 18 d of age the vaccination against Newcastle Disease was repeated by spraying.

**Table 1.** Composition and nutrient levels in the basal diets (dry matter basis).

| Ingredients and analysis | 1−49 d |
|--------------------------|--------|
| **Ingredients, %**        |        |
| Corn grain                | 33.65  |
| Soybean meal, 43% CP      | 6.50   |
| Soybean meal, 46% CP      | 13.65  |
| Corn protein feed         | 16.50  |
| Corn gluten meal          | 1.50   |
| Wheat middling and reddog | 24.00  |
| Stone powder              | 1.53   |
| NaHCO₃                    | 1.20   |
| Methionine                | 0.15   |
| Lysine                    | 0.30   |
| Premix²                   | 1.02   |
| **Total**                 | 100.0  |
| AME, MJ/kg                | 11.28  |
| CP                        | 14.94  |
| Dig Lysine                | 0.87   |
| Dig Methionine            | 0.68   |
| Ca                        | 1.00   |
| **Total P**               | 0.60   |
| **Available P**           | 0.42   |

1Abbreviations: AME, apparent metabolism energy; Ca, calcium; CP, crude protein; Dig, digestibility; P, phosphorus.

2Provided per kilogram of diet: Cu (CuSO₄·5H₂O), 8 mg; Fe (FeSO₄·7H₂O), 80 mg; Zn (ZnSO₄·7H₂O), 80 mg; Mn (MnSO₄·H₂O), 80 mg; Se (NaSeO₃), 0.3 mg; I (KI), 0.7 mg; vitamin A, 2,700 IU; vitamin D₃, 4,400 IU; vitamin E, 10 IU; thiamine, 2 mg; riboflavin, 5 mg; pyridoxine, 3 mg; vitamin B₁₂, 0.007 mg; calcium pantothenate, 10 mg; folate, 0.5 mg; biotin, 0.1 mg; niacin, 30 mg; choline, 750 mg.
**Insulin Sensitivity Test**

At 24-day-old, broilers (800 ± 57 g) and layers (200 ± 15 g) with body weight close to the population average value were selected and fasted for 16 h, then randomly divided into 2 groups: insulin (n = 12 per breed) or control (n = 12 per breed). Insulin treated birds were injected subcutaneously insulin (insulin aspart, NovoRapid, China) with 5 IU/kg body weight (based on the preliminary test), while control birds received an equivalent volume (calculated by 5 IU/kg body weight insulin solution/chicken) of phosphate-buffered saline (PBS). Re-feeding was initiated at 120 min immediately in half insulin- and PBS- treated chickens, and meanwhile the rest birds continued fast. Blood glucose concentration was measured with a hand-held glucometer (Accu-Chek Performa, Roche, Germany) at 0, 120, and 240 min via wing veins after insulin/PBS injection.

The mRNA Expression of BTG1 and BTG2

At 0, 120, and 240 min after the injection of insulin or PBS, the birds used in the insulin sensitivity test (IST) were euthanized, and the following tissues, including heart, liver, spleen, lung, kidney, brain, duodenum, ileum, pectoralis, leg muscle, abdominal fat, cecum, thymus, testis, sebum, and pancreas were immediately removed and snap frozen in liquid nitrogen until analysis. Tissue samples were snap frozen in liquid nitrogen, and stored at −80°C until analysis. Total RNA was extracted from all samples with Trizol Reagent (Sigma-Aldrich, China). The concentration and quality of the RNA were analyzed with agarose gel electrophoresis and spectrophotometry (Nanodrop, Thermo Scientific, Shanghai, China), respectively. The cDNA was synthesized with the PrimeScript RT Reagent Kit with gDNA Eraser (Vazyme Biotech Co., Ltd, Nanjing, China) in a 10 μL reaction containing 1,000 ng of total RNA and primers, according to the manufacturer’s instructions. The qPCRs were performed in triplicate for each sample, with the following cycling parameters: initial denaturation at 95°C for 30 s, followed by 40 cycles of 95°C for 10 s, 60°C for 30 s, and 95°C for 15 s; with a final elongation step at 65°C for 5 s. Primers for **BTG1**, **BTG2**, and the **β-actin** genes were designed using Primer3 Input (version 0.4.0; https://bioinfo.ut.ee/primer3-0.4.0/) and presented in Table 2. The melting curves of the qPCR were analyzed to ensure the specificity of amplification. Relative gene expression was quantified by normalizing to the expression of **β-actin** (Rao et al., 2013).

**Table 2. Primers used for qPCR.**

| Gene   | Accession No | Primer     | Sequence (5’-3’) |
|--------|--------------|------------|-----------------|
| **BTG1** | NM_205350.2  | Reverse    | AGCACACCGG      |
|        |              |            | ATTGATTGA       |
|        |              | Forward    | ACTGACACGAGA    |
|        |              |            | TTGACGCTCAGC    |
| **BTG2** | XM_418053.7  | Reverse    | CGCAGTTGCG      |
|        |              |            | TTGACACTTTTAC   |
| **β-actin** | NM_205518.1 | Reverse    | GTCCACCCCGAA    |
|        |              |            | ATGCCTTCTAAA    |
|        |              | Forward    | TGGCCCATTTATTGG  |
|        |              |            | GTTTTGTG        |

1**BTG1**, B-cell translocation gene 1.

Response by exogenous insulin stimulation on blood glucose concentrations and the expression of **BTG** genes. The correlations of serum glucose with the expression of **BTG** genes were analyzed with spearman correlation with two-tailed tests. The results were shown as means ± standard error of the mean. *P* < 0.05 was considered statistically significant.

**RESULTS**

**Dynamic Changes in Blood Glucose Concentrations After Exogenous Insulin**

Response to the injection of insulin, the blood glucose concentrations greatly reduced in both breeds of chickens. The layers exhibited stronger blood glucose recovery than the broilers. The blood glucose concentration at 120 min was only 32% of that at 0 min in the layers (*P* < 0.05), and was only 26% (*P* < 0.05) of that at 0 min in the broilers (Figure 1A), followed by their blood glucose concentration increased to 62% of the basal state at 240 min in the layers (*P* < 0.05), whereas the blood glucose concentration still stay at a low level by 240 min after the injection of insulin in the broilers (under the fast state, Figure 1A).

We further investigated the effect of re-feeding on the blood glucose recovery at 240 min after insulin injection in 2 breeds. At 240 min, the blood glucose concentration of the chickens provided feed at 120 min (WF) was significantly higher than that of the chickens with no feed (NF) in each group (*P* < 0.01), and both failed to restore the normal level in insulin treated groups (Figure 1B). Overall, the layers had higher blood glucose concentration at 240 min than the broilers after exogenous insulin stimulation, regardless of the provision of feed (*P* < 0.05; Figure 1B).

**Tissue Expression Patterns of BTG1 and BTG2**

Considering the tissue expression patterns of genes could reflect the potential function of genes in some degree, so we first explored the tissue expression profiling of **BTG1** and **BTG2**. It showed that both chicken
**Effects of Exogenous Insulin on the Expression of BTG1 (Under Fast State)**

Heart is the prominently expressed organ for chicken BTG1. After insulin injection, the expression level of BTG1 decreased at 120 min, but had largely recovered to the basal level by 240 min in the heart tissues of the layers and broilers. The mRNA level of BTG1 in the broilers at 120 min was significantly lower than that at 0 min or at 240 min ($P < 0.05$; Figure 3A). Insulin stimulation reduced the levels of BTG1 mRNA in the hearts of the layers and broilers at 120 min when compared with that PBS control, but only the difference in the broilers was statistically significant ($P < 0.01$; Figure 3A). No significant correlation was observed between birds’ blood glucose and heart BTG1 mRNA level (Table 3).

In the pectoralis, birds’ BTG1 mRNA level presented a negative relationship with blood glucose ($\rho = -0.289, P = 0.031$). With the blood glucose reduce after insulin injection, the expression of BTG1 greatly increased over time, and was significantly higher at 240 min than that at 0 min or 120 min ($P < 0.05$) in both layers and broilers (Figure 3B). Comparing with PBS control, the levels of BTG1 mRNA at 240 min were significantly upregulated by insulin stimulation in both layers ($P < 0.01$) and broilers ($P < 0.05$; Figure 3B). Furthermore, the relative abundance of BTG1 mRNA was significantly upregulated in the pectoralis of layers at 120 min after insulin injection ($P < 0.01$) and was higher than that of broilers ($P < 0.01$; Figure 3B).

Analogous to the response in pectoralis, the expression of BTG1 in pancreas was negatively correlated with blood glucose ($\rho = -0.462, P < 0.001$). The BTG1 mRNA level increased with time in the pancreas after the injection of insulin (Figure 3C). The pancreatic BTG1 mRNA levels were significantly higher at 240 min than that at 0 min in both layers and broilers ($P < 0.05$; Figure 3C). It is worth noting that comparing with that PBS control, the pancreatic BTG1 mRNA level was significantly upregulated at 240 min after insulin injection in broilers ($P < 0.05$; Figure 3C).

**Effects of Exogenous Insulin on the Expression of BTG2 (Under Fast State)**

With the dynamic change of blood glucose in birds, heart BTG2 level was also changed under insulin
stimulation. Comparing with PBS control, at 240 min after insulin injection, the BTG2 mRNA level was significantly downregulated in the hearts of layers \((P < 0.01; \text{Figure 4A})\). In addition, the expression of BTG2 gradually increased with time in the hearts of PBS control layers, and was significantly higher at 240 min than that at 0 min or 120 min \((P < 0.01; \text{Figure 4A})\). However, no significant correlation was observed between heart BTG2 mRNA level and blood glucose level \((\text{Table 3})\).

With the dynamic change of blood glucose by exogenous insulin, the expression of BTG2 significantly changed in the pectoralis in a breed-specific manner. There was a negative correlation between BTG2 mRNA level and blood glucose \((\rho = -0.500, P < 0.001)\). The BTG2 mRNA level in the pectoralis of the layers was sharply elevated at 120 min and then fell at 240 min, and BTG2 mRNA was significantly higher at 240 min than that at 0 min or 120 min \((P < 0.01; \text{Figure 4A})\). However, no significant correlation was observed between heart BTG2 mRNA level and blood glucose level \((\text{Table 3})\).

Effects of Feeding on the Expression of BTG1 and BTG2 in Chickens After Insulin Injection

Considering re-feeding at 120 min significantly improved the recovery of bird’s blood glucose \((\text{with layers’ higher than broilers, Figure 1B})\), we further investigated re-feeding on the mRNA level of BTG genes. Consistent with the finding about the potentially negative regulation function of BTG genes on birds’ blood glucose \((\text{under fasting state})\) in pectoralis, we observed that the expression of BTG1 was generally downregulated by re-feeding in the pectoralis of layers and broilers no matter under insulin or PBS injection.
The expression of BTG1 presented similar change in the pectoralis tissues of layers and broilers at 240 min, where BTG1 mRNA was significantly increased by insulin stimulation, regardless of whether feed was provided or not after 120 min (P < 0.05; Figures 5A and 5B); While this was only true in the pancreas tissues of the broilers (Figure 5B).

We also observed with the rise of blood glucose by re-feeding, BTG2 mRNA level at 240 min was significantly downregulated in the pectoralis tissues of insulin-treated broilers (P < 0.05; Figure 5C), and in the pectoralis of insulin or PBS injected broilers (P < 0.05; Figure 5D). In addition, Re-feeding significantly downregulated BTG2 mRNA level in the hearts of PBS control layers (P < 0.05; Figure 5C) and upregulated BTG2 mRNA level in the pancreas of PBS control layers (P < 0.05) (Figure 5D). Meanwhile, we observed the change of bird’s BTG2 genes mRNA level exhibited clear breed heterogeneity under insulin stimulation after re-feeding. Regardless of whether feed was provided or not after 120 min, BTG2 mRNA was significantly increased by exogenous insulin in the pectoralis tissues of broilers at 240 min (P < 0.05; Figure 5D); Reversely, it was significantly reduced by insulin stimulation in the pectoralis tissues of layers after re-feeding (P < 0.01; Figure 5C).

### DISCUSSION

The present study shows that subcutaneous administration of insulin has a differential effect on blood glucose and BTGs expression pattern in layer chicks and broiler chicks. Specifically, layers exhibited stronger blood glucose recovery than the broilers no matter what in the fast state and refeed state under insulin stimulation. At 240 min, the hypoglycemic effect of insulin was weakened with time in layers, but the blood glucose still in a low level in broilers. The differential dynamic change pattern between layers and broilers by insulin was similar to the reports between selected low weight and high weight broilers (Sumners et al., 2014), and between broilers and Silkie (Ji et al., 2020). It showed that it’s a common phenomenon that birds with low

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**Table 3.** Spearman correlation between blood glucose and BTGs mRNA level in different tissues.

|          | Pectoralis | Heart | Pancreas |
|----------|------------|-------|----------|
|          | BTG1       | BTG2  | BTG1     | BTG2  | BTG1 | BTG2 |
| P        | -0.289*    | -0.500** | 0.142   | 0.294* | -0.462** | -0.100 |
| P value  | 0.031      | < 0.001 | 0.295  | 0.028  | < 0.001  | 0.941 |

Note: **, P < 0.01; *, P < 0.05.

Data was analyzed based on two-tailed test. ρ, spearman correlation coefficient. N = 56.
body weight had relative rapid glucose recovery ability, which may be related with that low body weight chickens had more abundant insulin receptor (Shiraishi et al., 2011) and stronger insulin homeostasis ability (Ji et al., 2020).

In mammals, more than 70% of glucose was cleared by skeletal muscles in the insulin-stimulated state (Kahn, 1992). The basal glucose uptake also varied greatly among tissues/organs of chicks, with high level in heart, and low level in pectoralis and pancreas (Tokushima et al., 2005). Insulin could significantly improve the glucose uptake in skeletal muscles while had no significant effect on the glucose uptake in heart and pancreas in chicks (Tokushima et al., 2005).

Here we observed that BTG1 and BTG2 were insulin sensitive in birds and responded to insulin stimulation in breed- and tissue-specific manner. Tissue profiling showed that both of them were abundantly expressed in the skeletal muscles of birds. In addition, chicken BTG1 and BTG2 are also commonly expressed in the myotome of the early embryonic stage (Kamaid and Giráldez, 2008). It suggested the potential function of BTGs on glucose uptake in skeletal muscles.

As far as the relationship between BTG and insulin concerned, it was well-established that BTG family genes had been linked with insulin sensitivity, and glucose metabolism (Hwang et al., 2012, 2013; Kim et al., 2014; Xiao et al., 2016). BTG2 regulates glucose homeostasis via upregulation of Nur77 in diabetic mice (Kim et al., 2014). Overexpression of BTG2 increased the blood glucose output and subsequently impaired glucose and insulin tolerance (Kim et al., 2014). Reversely, BTG1 could significantly decreased levels of blood glucose and serum insulin, and improved insulin sensitivity by regulating the hepatic insulin sensitivity in mice via c-Jun (Xiao et al., 2016). Here we also observed both BTG1 and BTG2 mRNA level in bird’s pectoralis was negatively correlated with blood glucose. After insulin stimulation, the blood glucose dramatically decreased, meanwhile both BTG1 and BTG2 mRNA level in bird’s pectoralis were upregulated in gene- specific manner. It should be specially noted that the distinct dynamic expression patterns of BTG2 in the pectoralis of broilers and layers matched well with the dynamic blood glucose change features of broilers and layers under exogenous insulin stimulation (r = −0.500, P < 0.001). It suggested that birds’ BTG1 and BTG2 may be involved in the regulation of glucose metabolism in insulin-dependent manner in pectoralis, thereinto BTG2 may function more important role. In addition, our outcomes also showed that the change of both BTG1 and BTG2 presently clear breed heterogeneity in response to insulin stimulation in pectoralis, where both BTG1 and BTG2 of layers had higher level and greater change for layers at 120 min after insulin stimulation, and the change pattern of 2 genes at 240 min of 2 genes were also differed, which may affect the glucose uptake in pectoralis of birds and result to the difference in the recovery ability of blood.

Figure 4. Expression levels of BTG2 at different time points after injection of exogenous insulin. Chickens were in a fasting state. Different letters across time points indicate P < 0.05 in the same treatment group. * indicates P < 0.05. ** indicates P < 0.01. Absence of letter or * or the same letter indicates P > 0.05. Abbreviations: BI, insulin-treated broilers; BP, PBS-treated broilers; LI, insulin-treated layers; LP, PBS-treated layers.
glucose between 2 breeds at the later stage after insulin injection.

The endocrine pancreas of birds contains 3 islet types and releases glucagon, insulin, somatostatin, where glucagon release can be stimulated by insulin in vivo (Dupont et al., 2015). It has been reported that BTG2 positively regulates insulin secretion via induction of pancreatic duodenal homeobox-1 in mouse pancreatic β-cells (Hwang et al., 2013). In the current study, we observed that pancreas BTG2 was relatively stable under insulin stimulation, while pancreas BTG1 gene was negatively related with the blood glucose (r = -0.462, P < 0.001; Table 3), and its expression in broilers was more sensitive response to exogenous insulin. It may be related with that impaired homeostasis of blood glucose and insulin in broilers (Ji et al., 2020), which resulted in more BTGs (especially BTG1) participating in the regulation of the insulin secretion in pancreas under exogenous insulin stimulation.

Heart is the highly expressed tissue for bird’s BTG1. Unlike that in pectoralis and pancreas, the mRNA levels of BTG1 and BTG2 were generally downregulated by exogenous insulin. However, there were no significant correlation between blood glucose and the expression of both BTG1 in bird’s heart (Table 3). It seemed that BTGs in heart and pectoralis of birds may function in a separate way under insulin stimulation. It has been observed that insulin acted on heart and skeletal muscle glucose uptake in a completely different way in weightlifters and endurance athletes (Takala et al., 1999).

Feeding could reduce the BTG2 genes’ expression in mouse liver (Kim et al., 2014). Xiao et al. (2016) reported that overexpressing BTG1 (via tail vein) in mice significantly decreased levels of blood glucose and serum insulin in both fed and unfed conditions. We observed insulin upregulated pectoralis BTG1 mRNA level at 240 min in both re-feeding and fast conditions, while bird’s BTG2 mRNA level exhibited clear breed heterogeneity under insulin stimulation after re-feeding, which may contribute to the faster recovery of blood glucose in layers after insulin injection.

Overall, the expression of both BTG1 and BTG2 genes in chickens is responsive to insulin, and the expression of chicken BTG genes was significantly altered by exogenous insulin in a breed- and tissue-specific manner, which shows the complexity of gene expression regulation in the body. In this study, the exogenous insulin down-regulated the blood glucose levels, meanwhile both BTG1 and BTG2 mRNA levels were generally upregulated in the pectoralis of layers and broilers, which implies that BTG1 and BTG2 corporately participate in the regulation of pectoralis glucose homeostasis. We also observed that BTG genes’ mRNA level (especially in pectoralis) showed clear breed heterogeneity
response to insulin, which may contribute to the difference of the blood glucose recovery ability between broilers and layers partly.

CONCLUSIONS

In summary, the outcomes of the study showed that response to exogenous insulin, chicken blood glucose exhibited breed-specific dynamic change, layers showed stronger blood glucose recovery than the broilers; chicken BTG1 and BTG2 have unique and overlapping expression patterns, with relatively high expression in the skeletal muscle tissues; and meanwhile the expressions of both BTG1 and BTG2 genes in chickens were significantly altered by exogenous insulin in a breed- and tissue-specific manner; expressions of BTG genes (BTG1 and BTG2) in pectoralis and pancreatic BTG1 are negatively correlated with blood glucose in chickens.

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DISCLOSURES

The authors declare no competing interests.

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