

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* ($\rho = -0.289$, $P = 0.031$) / *BTG2* ($\rho = -0.500$, $P < 0.001$) in pectoralis,

and *BTG1* ($\rho = -0.462$, $P < 0.001$) 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

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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

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gene/transducer of *BTG/TOB* family (Bradbury et al., 1991; Fletcher et al., 1991; Mauxion 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**) (Vadgama 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 (Kamaid 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 β 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 glucose homeostasis 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. HNND20191201). 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 ¹	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
NaHCO ₃	1.20
Methionine	0.15
Lysine	0.30
Premix ²	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 D, 3,400 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).

Statistical Analysis

All statistical analyses were performed with the statistical software SAS v9.4. (SAS Institute, Cary, NC). Normal distribution and homogeneity of variances were checked by the Shapiro-Wilk and Levene's test, respectively. One-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons (normal distribution) was performed to elucidate a potential

Table 2. Primers used for qPCR.

Gene	Accession No	Primer	Sequence (5'-3')
<i>BTG1</i> ¹	NM_205350.2	Reverse	AGCACAGCGG ATTGGATTGA
		Forward	ACAGCACACAGA TTGAGCCA
<i>BTG2</i>	XM_418053.7	Reverse	CGCACTGTGC TTGCACTTAC
		Forward	ACACTTGAACGT GAGGCTCC
<i>β-actin</i>	NM_205518.1	Reverse	GTCCACCGCAA ATGCTTCTAA
		Forward	TGCGCATTATGG GTTTTGTT

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 \pm 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 concentrations 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

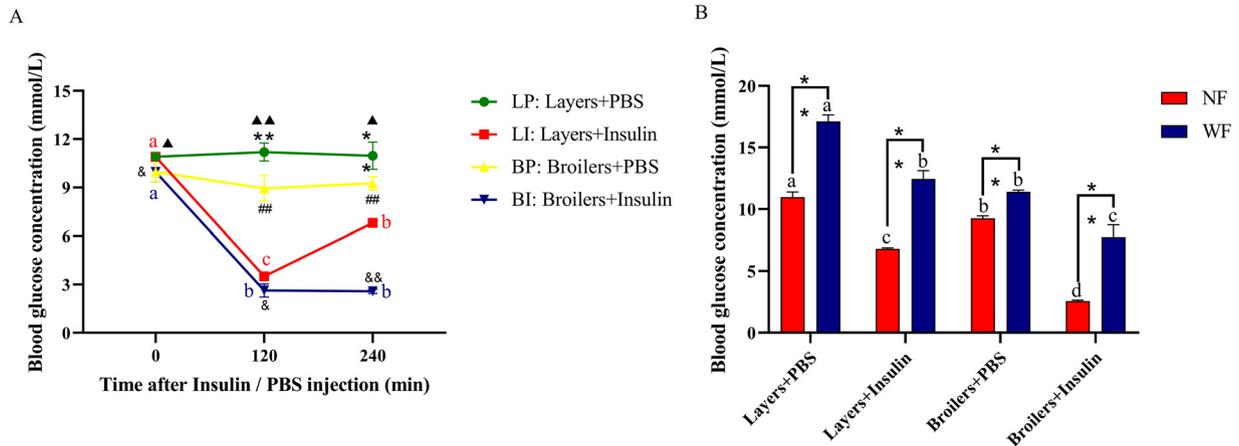


Figure 1. Effect of exogenous insulin on blood glucose concentration. (A) Chickens were in a fasting state. Different letters across time indicate $P < 0.05$ in the same treatment group. Between the LP and LI group at the same time-point, * indicates $P < 0.05$, ** $P < 0.01$. Between the BP and BI groups at the same time points, # indicates $P < 0.05$, ## $P < 0.01$. Between the LP and BP groups at the same time points, ▲ indicates $P < 0.05$, ▲▲ $P < 0.01$. Between the LI and BI groups at the same time points, & indicates $P < 0.05$, && $P < 0.01$. Absence of a letter or symbol or the same letter indicates $P > 0.05$. (B) Blood glucose concentrations of chickens at 240 min after insulin or PBS injection. “NF”: no feed was provided after 120 min. “WF”: feed was provided after 120 min. * indicates $P < 0.05$, ** $P < 0.01$. Different letters indicate $P < 0.05$ in different treatment groups under NF or WF conditions. Absence of a letter or symbol 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.

BTG1 and *BTG2* extensively expressed in both chicken breeds, while they exhibited unique and overlapping expression patterns with relatively abundant expression in the skeletal muscles of broilers. The *BTG1* expression was the highest in the heart, followed by the skeletal muscles, including the pectoralis and leg muscles, and was the lowest in the ileum (Figure 2A); whereas the *BTG2* expression was the highest in the skeletal muscles, including the pectoralis and leg muscles, followed by the pancreas, and was the lowest in the brain (Figure 2C).

We further compared the *BTGs* mRNA level in heart, pectoralis, and pancreas between 2 breeds. It showed that there were no significant differences for both *BTG1* and *BTG2* expression between 2 breeds in the corresponding tissues (Figures 2B and 2D). The expression pattern of *BTG1* was similar in the layers and broilers, in which it was mainly expressed in the heart, where its expression was significantly higher than that in the pectoralis or pancreas ($P < 0.05$; Figure 2B). However, the expression of *BTG2* was the highest in the pectoralis of the broilers, where it was significantly higher than that in the pancreas and heart ($P < 0.05$; Figure 2D). Of note, there were no significant differences in *BTG2* expression among three tissues of the layers.

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

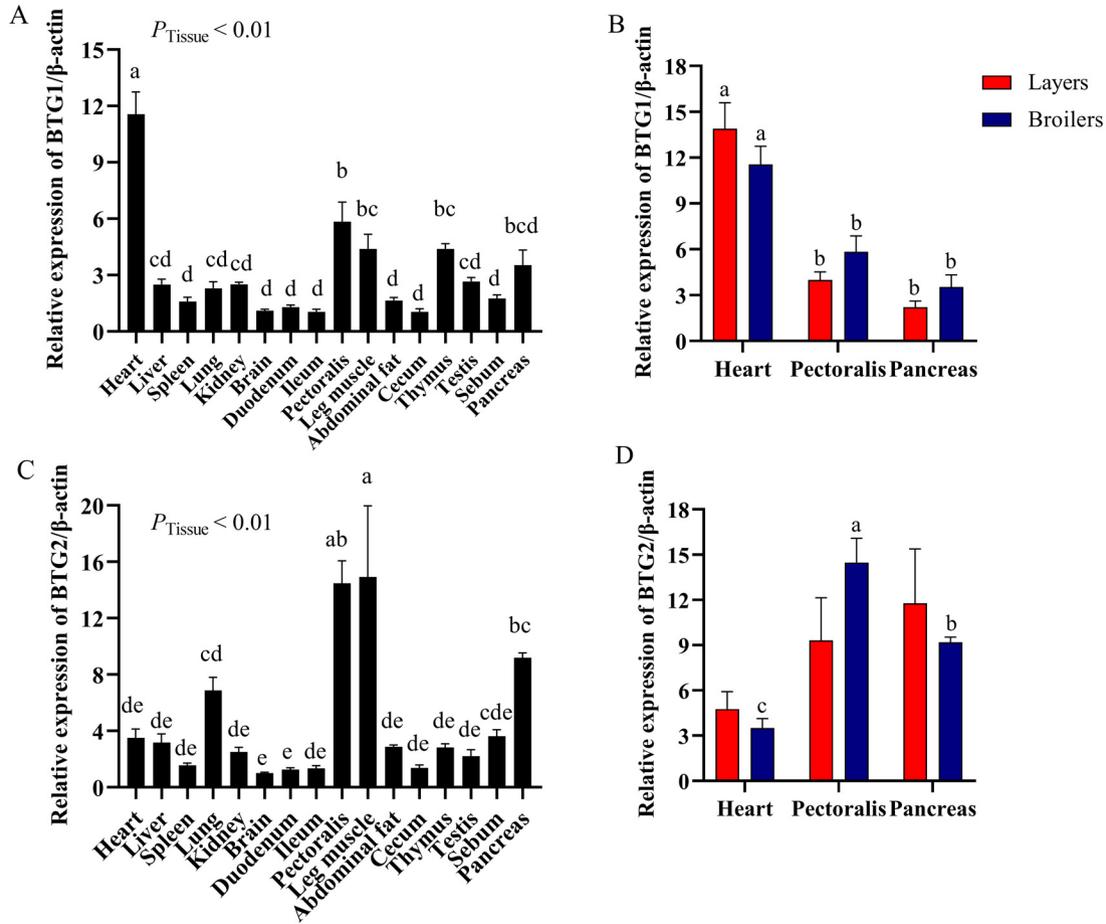


Figure 2. *BTG1* and *BTG2* mRNA levels. Chickens were in a fasting state. (A and C) Relative abundances of *BTG1* and *BTG2* mRNAs in different tissues in the basal state of broilers. Chickens were slaughtered after fasting for 16 h (n = 4 per breed). (B and D) Levels of *BTG1* and *BTG2* mRNAs in insulin-sensitive tissues in the basal state. Different letters across tissues indicate $P < 0.05$ in the same breed. Absence of letter or the same letter indicates $P > 0.05$.

stimulation. Comparing with PBS control, at 240 min after insulin injection, the *BTG2* mRNA level was significantly downregulated in the hearts of layers ($P < 0.01$; 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 ($P < 0.01$; Figure 4A). However, no significant correlation was observed between heart *BTG2* mRNA level and blood glucose level (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 120 min than at 0 min or 240 min ($P < 0.05$) under the exogenous insulin stimulation (Figure 4B). Whereas, in the pectoralis of the broilers, the expression of *BTG2* stably increased with time after insulin injection, and *BTG2* mRNA was significantly higher at 240 min than at 0 min or 120 min ($P < 0.05$; Figure 4B). At 120 min, *BTG2* mRNA level of insulin-treated layers was significantly higher than that of layer’s PBS control ($P <$

0.01) and that of broilers’ insulin group ($P < 0.01$; Figure 4B). However, at 240 min, *BTG2* mRNA of insulin-treated broilers was significantly higher than its PBS control ($P < 0.05$) and that of layers’ insulin group ($P < 0.05$; Figure 4B).

Unlike *BTG1*, *BTG2* mRNA level in pancreas was not significantly changed by insulin stimulation, but the expression of *BTG2* showed a decreasing trend after insulin injection, especially in broilers (Figure 4C).

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 (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 (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

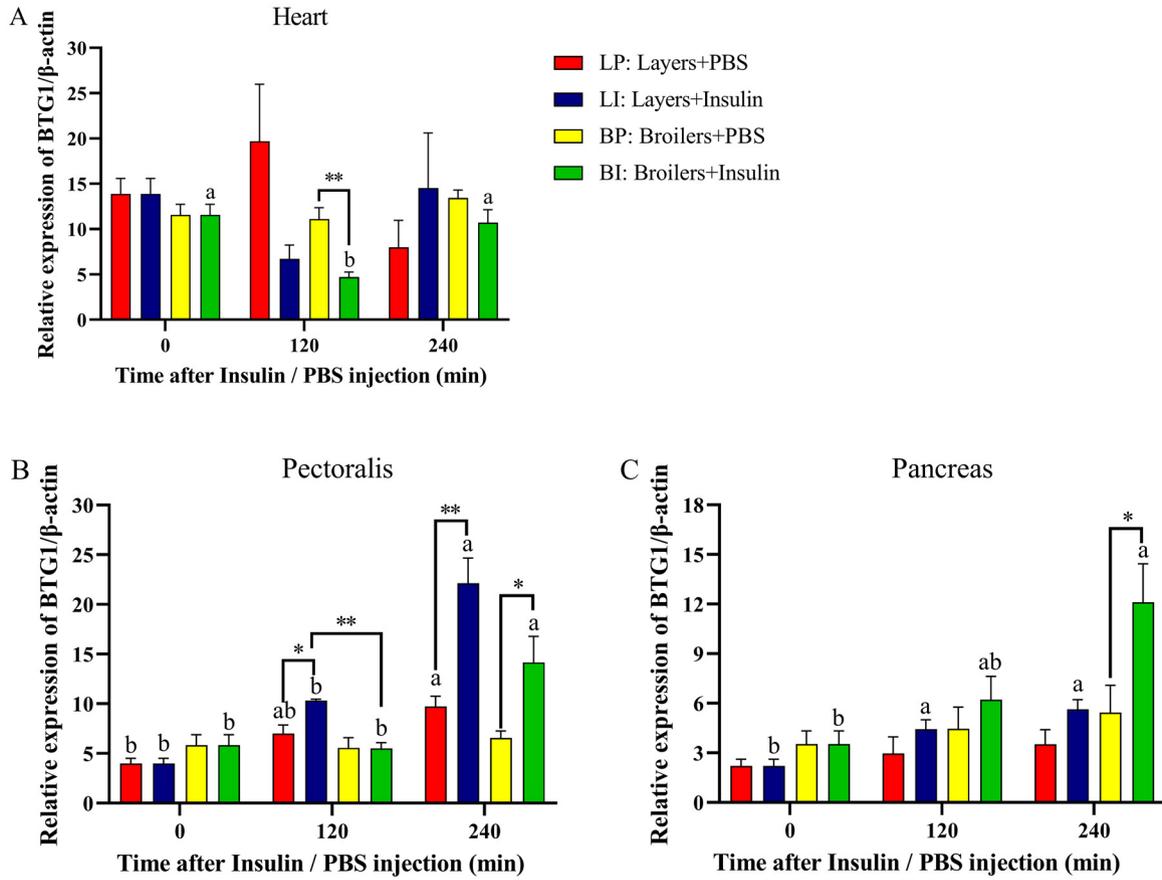


Figure 3. Expression of *BTG1* at different time points after injection of exogenous insulin. Chickens were in a fasting state. Different letters across times indicate $P < 0.05$ in the same treatment group. * indicates $P < 0.05$. ** indicates $P < 0.01$. Absence of letter or * or the same letter indicates $P > 0.05$. Abbreviations: BI, insulin-treated broilers; BP, PBS-treated broilers; LI, insulin-treated layers; LP, PBS-treated layers.

(Figures 5A and 5B). 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 of the insulin-treated layers ($P < 0.05$; 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 ($P < 0.05$).

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

	Pectoralis		Heart		Pancreas	
	<i>BTG1</i>	<i>BTG2</i>	<i>BTG1</i>	<i>BTG2</i>	<i>BTG1</i>	<i>BTG2</i>
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.

(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 (Summers et al., 2014), and between broilers and Silkies (Ji et al., 2020). It showed that it's a common phenomenon that birds with low

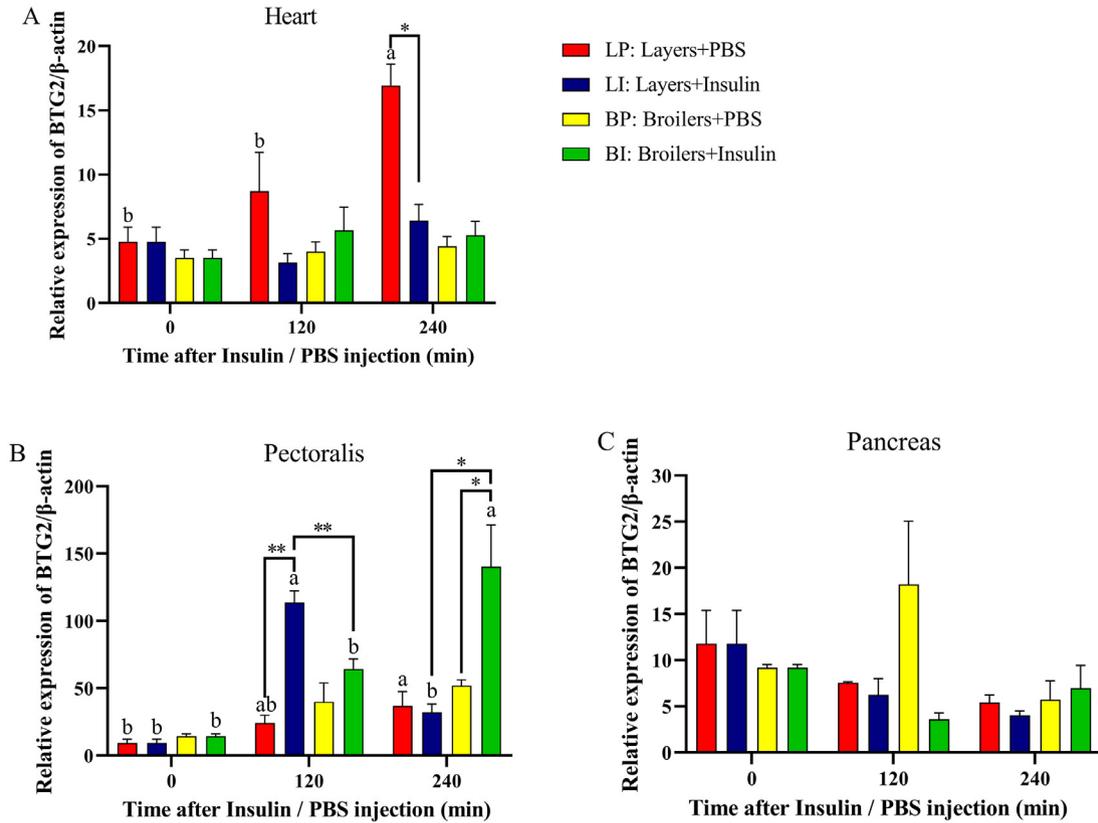


Figure 4. Expression levels of *BTG2* at different time points after injection of exogenous insulin. Chickens were in a fasting state. Different letters across time points indicate $P < 0.05$ in the same treatment group. * indicates $P < 0.05$. ** indicates $P < 0.01$. Absence of letter or * or the same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

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 ($\rho = -0.500$, $P < 0.001$). It suggested that birds' *BTG1* and *BTG2* may be involved in the regulation of glucose metabolism in insulin-dependent manner in pectoralis, therinto *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

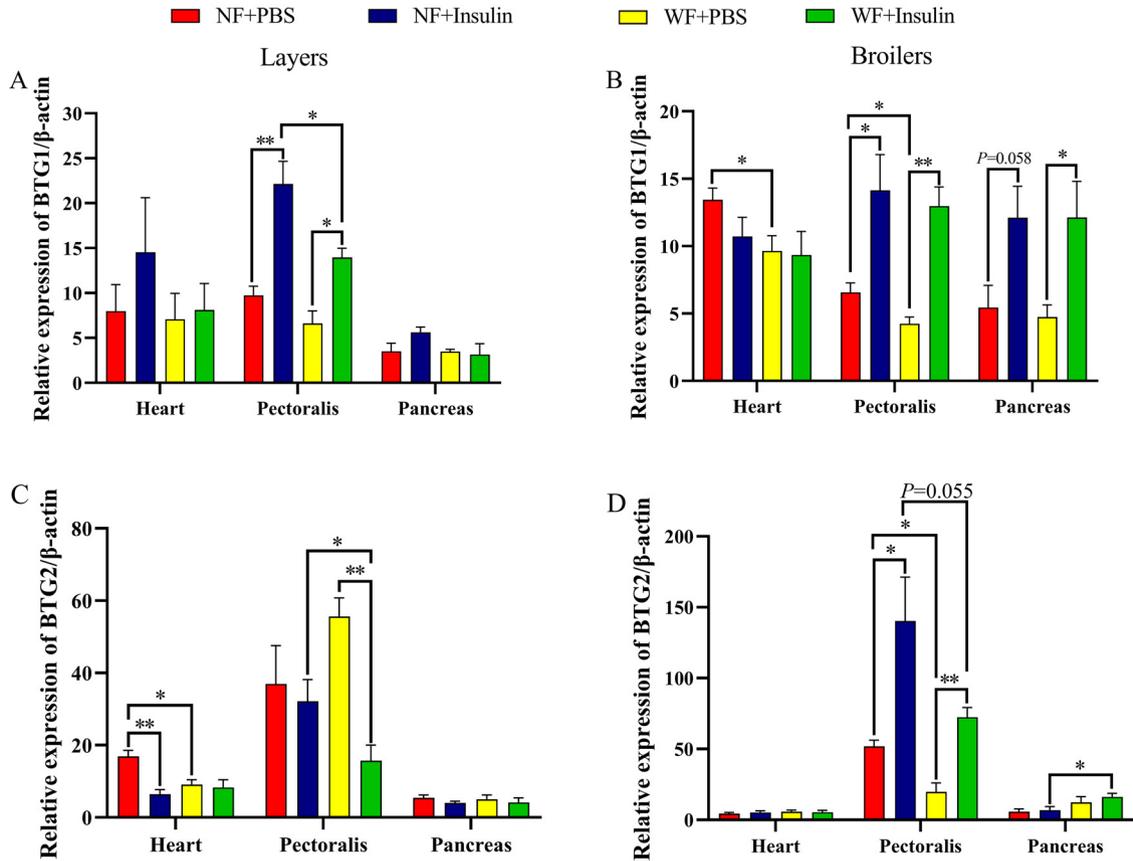


Figure 5. Effects of feeding chickens (or not) at 240 min after insulin injection on their *BTG1* and *BTG2* expression. Expression of *BTG1* in layers (A) and broilers (B). Expression of *BTG2* in layers (C) and broilers (D). Chickens were fed or continued fasting at 120 min after injection with insulin or PBS. “WF + PBS/Insulin”: indicates feed was provided to the chickens and they were treated with PBS or insulin. “NF + PBS/Insulin” indicates that feed was not provided to the chickens and they were treated with PBS or insulin. * indicates $P < 0.05$. ** indicates $P < 0.01$. Absence of * indicates $P > 0.05$.

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 ($\rho = -0.462$, $P < 0.001$; Table 3), and its expression in broilers was more sensitive response to exogenous insulin. It may be related with that the 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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