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Expression analysis of bone morphogenetic protein 4 between fat and lean birds in adipose tissue and serum

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ABSTRACT

The objectives of the present study were to characterize the tissue expression of chicken (*Gallus gallus*) bone morphogenetic protein 4 (BMP4) and compare differences in its expression in abdominal fat tissue and serum between fat and lean birds and to determine a potential relationship between the expression of BMP4 and abdominal fat tissue growth and development. The results showed that chicken BMP4 messenger RNA (mRNA) and protein were expressed in various tissues, and the expression levels of BMP4 transcript and protein were relatively higher in adipose tissues. In addition, the mRNA and protein expression levels of BMP4 in abdominal fat tissue of fat males were lower than those of lean males at 1, 2, 5, and 7 wk of age ($P < 0.05$). Furthermore, the serum BMP4 content of fat males was lower than that of lean males at 7 wk of age ($P < 0.05$). BMP4 mRNA expression levels were significantly higher in preadipocytes than those in mature adipocytes ($P < 0.05$), and the expression level decreased during differentiation in vitro ($P < 0.05$). These results suggested that chicken BMP4 might affect abdominal fat deposition through differences in its expression level. The results of this study will provide basic molecular information for studying the role of BMP4 in the regulation of adipogenesis in avian species.

1. Introduction

Bone morphogenetic protein 4 (BMP4) is a member of the bone morphogenetic protein family that exerts pleiotropic effects on cells during development including embryogenesis, organogenesis, and morphogenesis [1]. To date, the role of BMP4 in adipogenesis is poorly understood because previous studies have reported contradictory data.

BMP4 induced the commitment of mouse C3H10T1/2 pluripotent stem cells and human uncommitted precursor cells to the preadipocyte lineage [2–7]. Recent data indicated that the activation of BMP4 signaling might be associated with increased adiposity in humans, indicating that BMP4 might have a positive effect on adipogenesis. In contrast, Noggin, a potent inhibitor of BMP4, induced mesenchymal stem cell adipogenesis [8]. A recent study reported that BMP4 promoted human brown adipocytes differentiation and inhibited the expression of white adipocyte differentiation markers [9], suggesting that BMP4 may be a negative factor in adipogenesis.

The growth and development of adipose tissue is complex, and there are many differences in adipogenesis between birds and mammals [10–12]. Although the role of BMP4 in adipogenesis has been extensively studied in mammals, its functions are unclear in avian species. To address this, the present study was designed to determine

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whether there is relationship between the expression of BMP4 and chicken abdominal fat tissue growth and development by characterizing the tissue expression of BMP4 in chickens and analyzing differences in its expression in abdominal fat tissue and serum between fat and lean birds.

2. Materials and methods

2.1. Experimental birds and management

Animal work was conducted according to the guidelines for the care and use of experimental animals established by the Ministry of Science and Technology of the People's Republic of China (approval number 2006-398) and was approved by the Laboratory Animal Management Committee of Northeast Agricultural University (Harbin, P.R. China). In total, 42 male birds (fat line, n = 21 and lean line, n = 21) from the 14th generation (G_{14}), 176 male birds (fat line, n = 88 and lean line, n = 88) from the 17th generation (G_{17}), and 249 male birds (fat line, n = 126 and lean line, n = 123) from the 18th generation (G_{18}) of Northeast Agricultural University broiler lines divergently selected for abdominal fat content (NEAUHLF) were used in this study. All birds were kept in similar environmental conditions and had free access to feed and water. From hatch to 3 wk of age, all birds received a starter feed (3,100 kcal of ME/kg and 210 g/kg of CP), and from 4 to 7 wk of age, all birds were fed a grower diet (3,000 kcal of ME/kg and 190 g/kg of CP).

2.2. Tissue collection

In total, 42 male birds (3 birds for each broiler line and for each aged 1–7 wk) from G_{14}, and 73 male birds (3 birds for fat broiler line aged 7 wk; 5 birds for each broiler line and for each aged 1–7 wk) from G_{18}, were slaughtered after fasting for 10 h, and the abdominal fat percentage (AFP) was calculated in G_{18} (Fig. 1). At each week of age, the abdominal fat tissue was collected after slaughtering. For birds slaughtered at 7 wk of age, 9 other tissue samples, including cerebrum, liver, kidney, spleen, heart, gizzard, mesentery fat, subcutaneous fat, and gizzard fat, were also collected. After washing with 0.75% NaCl solution, all the tissues were collected, snap-frozen in liquid nitrogen, and stored at −80°C until used for RNA extraction.

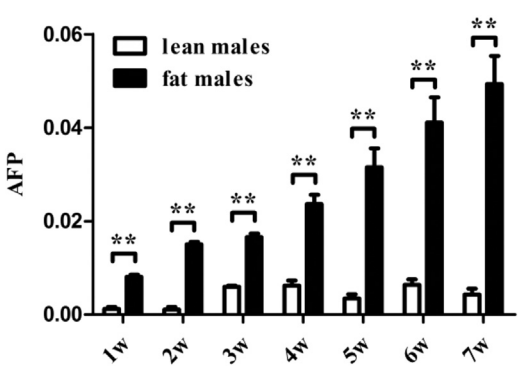

Fig. 1. Comparison of AFP of G_{18} birds used in the study (mean ± SD). $^{**}P < 0.01$. AFP, abdominal fat percentage; G_{18}, the 18th generation; SD, standard deviation.

2.3. Preparation of stromal–vascular cell and fat cell fractions and chicken preadipocyte culture

Chicken stromal–vascular and fat cells were isolated according to the following procedure. First, abdominal fat tissue (3–5 g) was isolated from 12-d old chickens, minced, and incubated with 2 mg/mL of collagenase I (Sigma–Aldrich, St. Louis, MO, USA) with shaking for 1 h at 37°C. The suspension was then passed through a 100- and 600-μm nylon cell strainer (BD Falcon, New York, NY) to remove undigested tissue. The filtrate was centrifuged (200*g* for 10 min at room temperature). The top layer (fat cell fraction) and the pellet (stromal–vascular cell fraction) were collected as chicken fat and stromal–vascular cells, respectively. The separated chicken stromal–vascular cells (chicken preadipocytes) were seeded at a density of 1×10^5 cells/cm^2 in Dulbecco's modified Eagle's medium (DMEM)/F12 medium (Gibco, New York, NY) supplemented with 10% fetal calf serum (Gibco) and maintained at 37°C in a humidified, 5% CO_2 atmosphere.

Until about 70% to 90% confluency (day 3–4), cells were passaged and seeded into 6-well plates at a density of 1×10^5 cells/cm^2. When cells reached 70% to 90% confluency, 160 μ*M* oleate, prepared by dissolving sodium oleate (Sigma–Aldrich) in double distilled water, was added into the medium to induce preadipocyte differentiation.

2.4. Ribonucleic acid isolation and quantitative reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA of tissues (each 100 mg) and cells was extracted using a Trizol reagent kit (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol. Total RNA was treated with DNase I (Takara, Dalian, China), and RNA quality was assessed by visualization of the 18S and 28S ribosomal RNA bands on a denaturing formaldehyde agarose gel. Only RNA with a 28S:18S ratio between 1.8 and 2.1 was used for reverse transcription. In particular, the total RNA of each tissue sample from 3 fat male birds was pooled in equal amounts. The pool of each tissue was used to detect the tissue expression characterization of BMP4 messenger RNA (mRNA). The reverse transcription reactions were performed in a final volume of 20 μL with 1 μg of total RNA, an oligo(dT) anchor primer, and ImProm-II reverse transcriptase (Promega, Madison, WI, USA). Reverse transcription conditions for each complementary DNA amplification were 25°C for 5 min, 42°C for 60 min, and 70°C for 15 min.

Quantitative RT- PCR was used to analyze gene expression, and the expression of beta actin (β-actin) was used as an internal reference of target gene expression. quantitative RT-PCR was performed using the FastStart Universal SYBR Green Master kit (Roche) on a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Part (1 μL) of each RT reaction product was amplified in a 10-μL PCR reaction system. Reaction mixtures were incubated in an ABI Prism 7500 sequence detection system (Applied Biosystems) programmed to conduct 1 cycle at 95°C for 10 min, 40 cycles at 95°C for 15 s, and at 60°C for 1 min. Dissociation curves were analyzed using Dissociation Curve 1.0 software (Applied Biosystems) for each PCR reaction to detect and eliminate possible primer-dimer artifacts. The relative

expression level of target gene to β-actin was determined using the $2^{-\Delta \text{cycle threshold value (CT)}}$ method, in which $\Delta C_T = C_T$ (BMP4) – C_T (β-actin). The primers used for quantitative RT-PCR were designed to cross introns, and the primer information is shown in Table 1.

2.5. Western blot assay

Tissues were dissected and washed in ice-cold PBS, minced, and homogenized at 4°C in ice-cold radioimmunoprecipitation assay buffer (150-mM NaCl, 50-mM Tris-HCl, pH 7.5, 1% NP-40 lysis buffer, 0.1% SDS, and 0.5% sodium deoxycholate) with the addition of a protease inhibitor phenylmethylsulfonyl fluoride (Sigma), and then centrifuged at 2,300*g* for 20 min at 4°C to obtain total protein. In particular, the total protein of each tissue sample from 3 fat male birds was pooled in equal amounts. The pool of each tissue was used to detect the tissue expression characterization of BMP4 protein. The total protein was added into 6 × denaturing loading buffer, boiled for 5 min, separated by 12% SDS-PAGE and transferred to an Immun-Blot polyvinylidene fluoride membrane (Millipore, Billerica, MA, USA). After incubation with a primary antibody that recognizes chicken BMP4 (1:100; Abmart, Shanghai, China) or chicken β-actin (1:1,000; Beyotime, Beijing, China), a secondary horseradish peroxide-conjugated antibody was added. The Super ECL kit (HaiGene, Harbin, China) was used for detection. Immunoreactive protein levels were determined semiquantitatively by densitometric analysis, using a laboratory imaging and analysis system (CA 91786; UVP, Upland, CA, USA). Results were presented as the relative quantity of BMP4/β-actin.

2.6. Enzyme-linked immunosorbent assay

Male birds from G_{17} and G_{18} of the fat and lean lines were used in the present study. In G_{17}, the AFP of every bird was calculated, and the birds were sorted by AFP. A total of 176 individuals in G_{17} (88 birds with the highest AFP in the fat line and 88 birds with the lowest AFP in the lean line) were selected. In G_{18}, a total of 176 individuals (88 birds from the fat line and 88 birds from the lean line) were selected using the same method as in the G_{17} population (Table 2). Venous blood was collected from the wings of birds without an anticoagulant, and the serum was separated from whole blood by centrifugation at 3,000*g* for 10 min at room temperature. Serum was stored at −20°C. Serum BMP4 content was measured using an ELISA Kit for Chicken Bone Morphogenetic Protein 4 (USCN, Wuhan, China) with 100-μL serum samples according to the manufacturer's instructions, and the intra-assay and interassay coefficients of variation were <10% and <12%, respectively. The results were presented in pg/mL, and the detection limit for BMP4 was 11.7 pg/mL.

Table 1
Primers used for quantitative reverse transcription-PCR.

Gene	Reference	Primers (5′-3′)
BMP4	NM_205237	F:CAGATGTTTGGGCTGCGAAGG R:GCACGCTGCTGAGGTTGAAGAC
β-actin	NM_205518	F: TCTTGGGTATGGAGTCCTG R: TAGAAGCATTTGCGGTGG

Abbreviations: β-actin, beta actin; BMP4, bone morphogenetic protein 4; F, forward; PCR, polymerase chain reaction; R, reverse.

Table 2
Comparisons of AFP between fat and lean males at 7 wk of age for G_{17} and G_{18}.

Generation (n)	AFP (lean males)	AFP (fat males)
G_{17} (88)	0.0062 ± 0.0004[b]	0.0634 ± 0.0004[a]
G_{18} (88)	0.0041 ± 0.0004[b]	0.0589 ± 0.0004[a]

Abbreviations: AFP, abdominal fat percentage; G_{17}, the 17th generation; G_{18}, the 18th generation; LSM, least square mean; SEM, standard error of the mean.
Data are presented as the LSM ± SEM. Superscript letters (a, b) in the same row indicate a significant difference ($P < 0.05$).

2.7. Statistical analysis

Comparison between 2 groups was performed by Student's *t* test. In particular, statistical differences of serum BMP4 content between fat and lean birds were analyzed using JMP version 7.0 (SAS Institute, Cary, NC, USA) with the following model:

$$Y = \mu + L + F(L) + D(F, L) + BW_7 + e \quad [1]$$

where Y is the phenotypic value of serum BMP4 content, μ is the population mean, L is the fixed effect of the line, $F(L)$ is the random effect of family within line, D (F, L) is the random effect of dam within family and line, body weight at 7 wk of age (BW_7) is treated as a covariate, and e is the random residual effect. Significant differences between the least squares means of phenotype of the fat and lean lines were calculated. Statistical analysis among more than 2 groups were performed using the SAS software system version 9.2 (SAS Institute) with the PROC GLM procedure followed by Tukey's HSD test, with the following model:

$$Y = \mu + T + e \quad [2]$$

model [2] was used for cell samples, in which Y is the phenotypic value of BMP4 expression level, μ is the population mean, T is the fixed effect of time point during preadipocyte differentiation, and e is the random residual effect. Differences were considered significant at $P < 0.05$ unless otherwise indicated.

3. Results

3.1. Characterization of the tissue expression of BMP4

Real-time RT-PCR and Western blot results showed that BMP4 mRNA and protein were expressed in all of the tissues tested from 7-wk old fat male birds of G_{18} (Figs. 2 and 3). In addition, the expression level of BMP4 transcript was relatively higher in spleen, abdominal fat, mesentery fat and subcutaneous fat; medium in gizzard fat and liver; and lower in kidney, heart, cerebrum, and gizzard (Fig. 2). The expression level of BMP4 protein was relatively higher in abdominal fat and subcutaneous fat; medium in mesentery fat, gizzard fat, liver and spleen; and lower in kidney, heart, cerebrum, and gizzard (Fig. 3A, B).

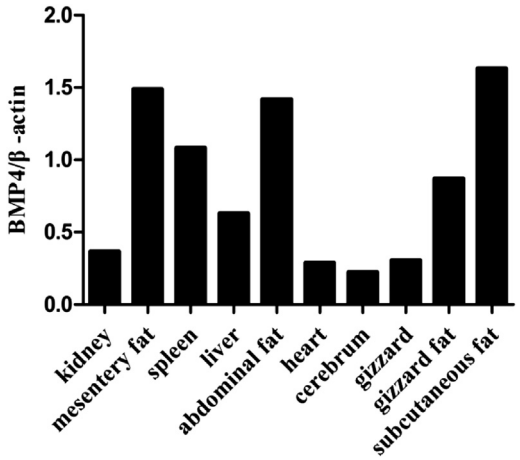

Fig. 2. Characterization of the tissue expression of chicken BMP4 mRNA in 7-wk old fat male broilers of G_{18}. β-actin, beta actin; BMP4, bone morphogenetic protein 4; G_{18}, the 18th generation; mRNA, messenger RNA.

3.2. Comparison of BMP4 mRNA expression levels in abdominal fat tissue between fat and lean males

Real-time RT-PCR was used to detect BMP4 mRNA expression levels in abdominal fat tissue. BMP4 transcript expression levels in G_{14} lean males were higher than those in fat males at 1 and 2 wk of age ($P < 0.05$; Fig. 4). In G_{18}, there was a significantly higher expression of BMP4 mRNA in abdominal fat tissue of lean males compared with fat males at 1, 2, 5, and 7 wk of age ($P < 0.05$; Fig. 5).

3.3. Comparison of BMP4 protein expression levels in abdominal fat tissue between fat and lean males

The expression of BMP4 protein levels was detected only from 4 to 7 wk of age in G_{14} because there was insufficient sample for Western blot analysis at 1, 2, and

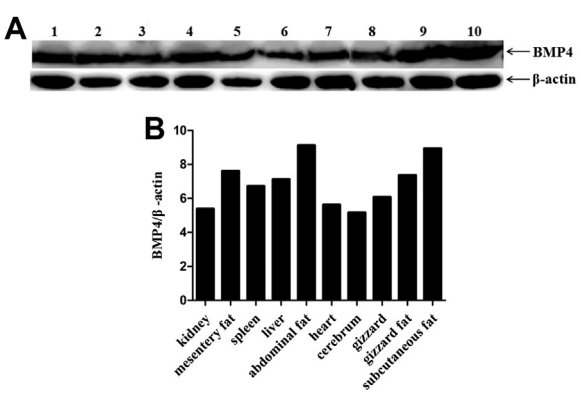

Fig. 3. Characterization of the tissue expression of chicken BMP4 protein in 7-wk old fat male broilers of G_{18}. (A) Western blot of BMP4 protein in chicken various tissues of G_{18}. Lane 1, kidney; lane 2, mesentery fat; lane 3, spleen; lane 4, liver; lane 5, abdominal fat; lane 6, heart; lane 7, cerebrum; lane 8, gizzard; lane 9, gizzard fat; lane 10, subcutaneous fat. (B) Analysis of BMP4 protein expression levels in chicken various tissues of G_{18}. β-actin, beta actin; BMP4, bone morphogenetic protein 4; G_{18}, the 18th generation.

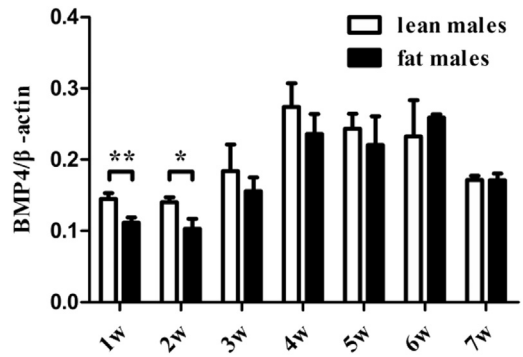

Fig. 4. Analysis of BMP4 mRNA expression levels in abdominal fat tissue between fat and lean males of G_{14} (mean ± SD). $^{*}P < 0.05$ and $^{**}P < 0.01$. β-actin, beta actin; BMP4, bone morphogenetic protein 4; G_{14}, the 14th generation; mRNA, messenger RNA; SD, standard deviation; w, wk of age.

3 wk of age. The results showed that the BMP4 protein expression level in G_{14} lean birds was higher than that in G_{14} fat birds at 4, 5, and 7 wk of age ($P < 0.05$) but was lower in lean birds compared with fat birds at 6 wk of age ($P < 0.05$; Fig. 6A, B). In G_{18}, the BMP4 protein expression levels were higher in lean birds than those in fat birds at 1, 2, 5, and 7 wk of age ($P < 0.05$; Fig. 7A, B).

3.4. Comparison of BMP4 levels in serum between fat and lean males

Enzyme-linked immunosorbent assay was used to detect serum BMP4 content. There was a significantly higher concentration of BMP4 in the serum of lean males compared with fat males at 7 wk of age in birds derived from G_{17} and G_{18} (Table 3).

3.5. Characterization of BMP4 expression during preadipocyte differentiation

Chicken preadipocytes (stromal–vascular cell fraction) and mature adipocytes (fat cell fraction) were isolated from the abdominal adipose tissues of broilers, and BMP4 mRNA

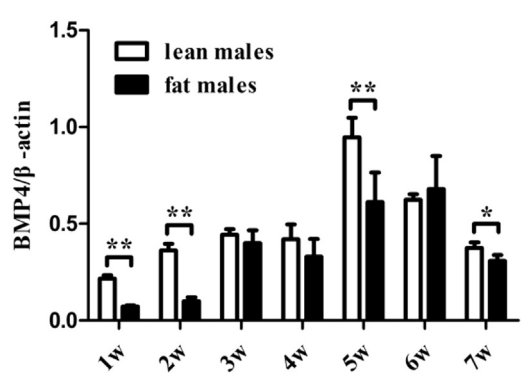

Fig. 5. Analysis of BMP4 mRNA expression levels in abdominal fat tissue between fat and lean males of G_{18} (mean ± SD). $^{*}P < 0.05$ and $^{**}P < 0.01$. β-actin, beta actin; BMP4, bone morphogenetic protein 4; G_{18}, the 18th generation; mRNA, messenger RNA; SD, standard deviation; w, wk of age.

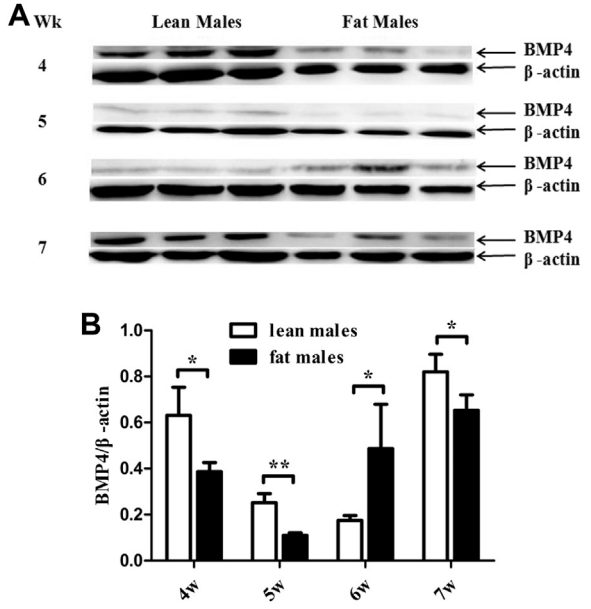

Fig. 6. Protein expression levels of BMP4 in abdominal fat tissue between fat and lean males of G_{14}. (A) Western blot of BMP4 protein in chicken abdominal fat tissue between fat and lean males of G_{14}. (B) Analysis of BMP4 protein expression levels in chicken abdominal fat tissue between fat and lean males of G_{14} (mean ± SD). $^{*}P < 0.05$ and $^{**}P < 0.01$. β-actin, beta actin; BMP4, bone morphogenetic protein 4; G_{14}, the 14th generation; SD, standard deviation; w, wk of age.

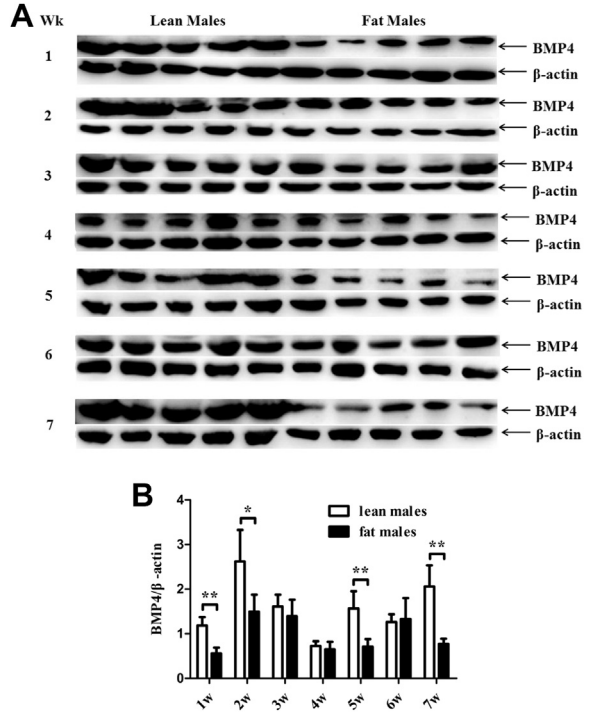

Fig. 7. Protein expression levels of BMP4 in abdominal fat tissue between fat and lean males of G_{18}. (A) Western blot of BMP4 protein in chicken abdominal fat tissue between fat and lean males of G_{18}. (B) Analysis of BMP4 protein expression levels in chicken abdominal fat tissue between fat and lean males of G_{18} (mean ± SD). $^{*}P < 0.05$ and $^{**}P < 0.01$. β-actin, beta actin; BMP4, bone morphogenetic protein 4; G_{18}, the 18th generation; SD, standard deviation; w, wk of age.

expression levels were analyzed using real-time RT-PCR. BMP4 was expressed in both chicken preadipocytes and mature adipocytes, and its expression was significantly higher in preadipocytes than that in mature adipocytes ($P < 0.01$; Fig. 8A). In addition, BMP4 expression level decreased from 0 to 60 h but increased at 72 h during preadipocyte differentiation induced by oleate in vitro (Fig. 8B).

4. Discussion

BMP4, a member of the transforming growth factor β superfamily originally identified based on its ability to induce ectopic bone formation [13], is now implicated in embryogenesis, organogenesis, and morphogenesis by controlling the differentiation of numerous cell types [14–17]. Previous reports showed that BMP4 was present in various tissues and had effects on the growth and development of many tissues in mammals [18–22]. Interestingly, the present study demonstrated that chicken BMP4 mRNA and protein were expressed in various tissues, suggesting BMP4 might have roles in multiple tissues growth and development in chickens.

For decades, adipose depots were regarded as an inert mass for energy storage. However, this concept has been radically revised in the past 20 yr. It is now known that adipose tissue not only serve as a fat depot but also act as a secretory and/or endocrine organ with a central role in the regulation of energy balance and thermoregulation. An increase in adipose tissue mass is due to an increase in the number and size of adipocytes [23–25]. Adipocyte number is regulated by the commitment of mesenchymal stem cells to the adipocyte lineage and by the proliferation and apoptosis of preadipocytes, whereas adipocyte size is regulated by the differentiation of preadipocytes, and the limited expandability of adipose tissue can lead to inappropriate adipocyte expansion [7,26–29]. A variety of hormones, growth factors, and cytokines are involved in the differentiation program of adipocytes [30,31]. Among these factors, BMP4 has important effects on the mammalian adipose tissues growth and development [2,3,32,33]. BMP4 null animals exhibited no mesoderm induction, and gastrulation was impaired [16]. Many tissues including adipose tissue arise from the mesoderm, suggesting BMP4

Table 3
Comparisons of serum BMP4 content between fat and lean males at 7 wk of age for G_{17} and G_{18}.

Generation (n)	Serum content (pg/mL)	Lean males	Fat males
G_{17} (88)	BMP4	166.56 ± 10.39^{a}	70.69 ± 9.88^{b}
G_{18} (88)	BMP4	110.95 ± 5.39^{a}	93.01 ± 5.28^{b}

Abbreviations: BMP4, bone morphogenetic protein 4; G_{17}, the 17th generation; G_{18}, the 18th generation; LSM, least square mean; SEM, standard error of the mean.
Data are presented as the LSM ± SEM. Superscript letters (a, b) in the same row indicate a significant difference ($P < 0.05$).

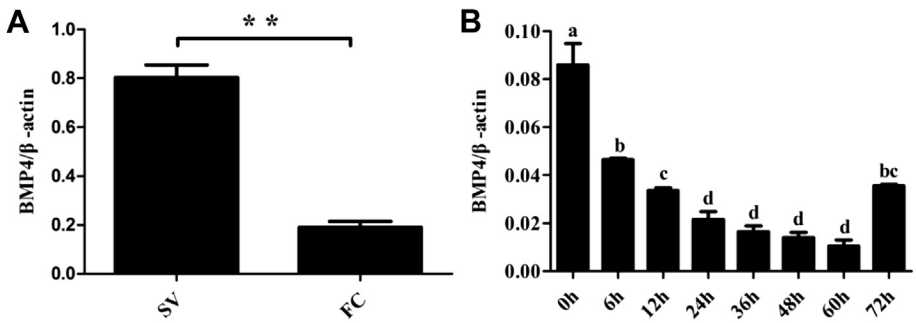

Fig. 8. Expression of BMP4 during chicken preadipocyte differentiation. (A) Analysis of BMP4 mRNA expression level in chicken preadipocytes (SV) and mature adipocytes (FC; mean ± SD). $^{**}P < 0.01$. (B) BMP4 expression pattern during chicken preadipocyte differentiation (mean ± SD). $^{a–d}$ The different lowercase letters above columns indicate significant differences among various time points ($P < 0.05$). β-actin, beta actin; BMP4, bone morphogenetic protein 4; FC, fat cell fraction; SD, standard deviation; SV, stromal–vascular cell fraction.

may be involved in the regulation of adipose tissue development. In the present study, the expression levels of BMP4 transcript and protein were relatively higher in adipose tissues, indicating BMP4 might play a critical role in chicken adipose tissue growth and development.

In vivo, BMP4 mRNA expression was increased 2-fold in the inguinal fat of obese/obese (ob/ob) mice compared with their lean counterparts [34]. In contrast, the expression of BMP4 mRNA and protein in inguinal adipose tissues was significantly lower in ob/ob mice compared with controls (C57BL/6 mice) [35]. In the present study, there was a significant difference in mRNA and protein expression levels of BMP4 in abdominal adipose tissue between fat and lean male birds. Compared with lean males, the BMP4 expression level in fat males was much lower at 1, 2, 5, and 7 wk of age in G_{18}. Of note, there was a significant difference in AFP between fat and lean males from 1 to 7 wk of age. Thus, the differential expression of BMP4 might be associated with fat deposition in chickens. White adipose tissue (WAT) is an energy storage depot and brown adipose tissue is specialized for energy dissipation. A previous study showed BMP4 increased brown-specific marker uncoupling protein 1 expression in primary human adipose stem cells and decreased expression of the white-specific marker transcription factor 21 [36,37]. It was previously shown that the forced expression of BMP4 in white adipocytes of transgene mice reduced white adipocyte size and WAT mass and that BMP4-deficient mice enlarged the size of white adipocytes, which implied a correlation between small adipocyte size and high expression levels of BMP4 [38]. Moreover, the level of expression of BMP4 in human WAT was inversely correlated to adiposity [38]. Therefore, we speculated that a high expression level of BMP4 in abdominal adipose tissue of lean male birds might reduce adipocyte size and lead to a decrease of abdominal fat mass deposition.

In the present study, the serum BMP4 content of the lean male birds was higher than that of the fat male birds, which is consistent with a previous study in humans showed that serum BMP4 levels were inversely correlated with obesity in patients with type 2 diabetes [39]. In contrast, the expression of BMP4 in the serum was significantly higher in ob/ob mice compared with controls (C57BL/6 mice) [35]. In addition, a previous report of humans showed that serum BMP4 levels were positively associated with obesity in nondiabetic individuals [40]. Therefore, it was speculated that BMP4 may have different roles in different situations of adiposity.

Although there were significant differences in AFP between fat and lean males from 1 to 7 wk of age, the expected significant differences in the expression of BMP4 in the abdominal adipose tissues between fat and lean males were not detected at some time points. The growth and development of adipose tissue is complicated and is related to multiple genes and pathways. The results of a comprehensive analysis of chicken adipose tissue gene expression profile revealed that many genes are involved in adipogenesis and were differentially expressed in adipose tissue between fat and lean lines [41]. BMP4 probably partially contributes to the deposition of chicken abdominal fat tissue. In the present study, the mRNA expression levels of BMP4 in abdominal adipose tissues were not accompanied by parallel changes in its protein expression level in G_{14} birds. It was speculated that the expression of chicken BMP4 is related to posttranscriptional modification.

A previous study showed that BMP4 was overexpressed in mouse A33 preadipocyte line [3]. The present study also shows that BMP4 mRNA was expressed at a higher level in chicken preadipocytes compared with mature adipocytes. Moreover, chicken BMP4 had a decreased expression pattern during preadipocyte differentiation induced by oleate, which is consistent with previous reports in mice that BMP4 was dynamically decreased during adipogenesis in 3T3-L1 cell line [42,43]. However, a previous study showed that BMP4 increased during the differentiation of human stromal–vascular cells to mature adipocytes, with a cocktail consisting of insulin, dexamethasone, isobutyl methylxanthine, and pioglitazone [7]. These findings indicate a difference in the regulation of adipogenesis between birds and humans, alternatively, the opposite result may be due to the use of the different differentiation media.

In summary, the expression of BMP4 was associated with chicken abdominal fat tissue growth and development. However, the precise mechanisms of how BMP4 regulates the growth and development of chicken abdominal fat tissue need to be investigated further.

Acknowledgments

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