Dexamethasone alters the expression of genes related to the growth of skeletal muscle in chickens (Gallus gallus domesticus)

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Abstract

Glucocorticoids (GCs) are involved in the muscle wasting caused by trauma, inactivity, and stress. In the present study, three experiments were conducted to investigate the effect of GCs on the expression of genes related to muscle development in chickens. Broilers at 7 or 35 days of age were subjected to dexamethasone (DEX) treatment (2 mg/kg body mass (BM)) for 3 or 7 days. The expression levels of genes such as IGF1, IGF1 receptor, MSTN, WW domain containing E3 ubiquitin (UB) protein ligase 1, myogenic determining factor, and myogenic factor 5 were measured. The results showed that BM gain was significantly suppressed by DEX treatment. The plasma level of insulin was increased ($P < 0.05$) by DEX treatment at feeding, whereas IGF1 was decreased ($P < 0.05$). The expression of genes in the IGF1, myostatin, and UB–proteasome (UBP) pathways were altered by DEX treatment in age- and exposure time-related ways. These results suggest that GCs suppress IGF1 and upregulate myostatin and/or activated myostatin and the UBP pathway, which might be the source of the effect of GCs on muscle development.

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Introduction

Glucocorticoids (GCs) are involved in the muscle wasting caused by trauma and inactivity in humans (Ferrando et al. 1999). Hypercortisolism plays a major role in muscular atrophy in Cushing’s disease in humans. Similarly, GCs depress the growth of skeletal muscle in chickens (Lin et al. 2004, Dong et al. 2007). GCs retard the growth of skeletal muscle by suppressing protein synthesis and increasing protein catabolism in chickens (Dong et al. 2007) and mammals (reviewed by Price et al. (2001) and Tsiotra & Tsigos (2006)). In rats, myofibrillar protein breakdown in skeletal muscle progresses through two distinct phases in response to chronic GC administration; an early phase lasting 4–5 days, during which proteolysis increases, and a later phase, during which proteolysis decreases (Kayali et al. 1987, 1990).

Insulin and insulin-like growth factor 1 (IGF1) have extensive anabolic effects in various tissues. In mammals, the insulin/IGF1 signal is mainly mediated by the activation of the phosphoinositide 3-kinase (PI3K)/AKT/mammalian target of rapamycin (MTOR) pathway. The activation of the PI3K/AKT/MTOR pathway promotes protein synthesis and muscle development (Glass 2003). IGF1 can attenuate dexamethasone (DEX)-induced augmented proteolysis (Sacheck et al. 2004). Compared with mammals, the insulin cascade appears to be refractory in the muscle tissue of chickens (Dupont et al. 2004, 2008). However, the PI3K/AKT/MTOR pathway in chicken muscle is activated by refeeding and insulin treatment (Bigot et al. 2003, Duchêne et al. 2008).

Myostatin (MSTN), first discovered as a member of the growth differentiation factor family (GDF8), is a potent inhibitor of muscle growth and is expressed in embryonic and adult skeletal muscle (McPherron et al. 1997). Genetic deletion of MSTN leads to massive hyperplasia and hypertrophy of skeletal muscle in cattle (Kambadur et al. 1997, McPherron & Lee 1997), rats (Nishi et al. 2002), and humans (Schuelke et al. 2004). In chickens, both the proliferation and differentiation of embryonic myoblasts and the proliferation of satellite cells separated from skeletal muscle are inhibited by MSTN (Yang et al. 2003, McFarland et al. 2007). In rats, upregulated MSTN expression has been suggested to be involved in DEX-induced muscle loss (Ma et al. 2003). Whether MSTN is involved in the suppressed muscle development in stressed chickens remains unclear.

Myogenic determining factor (MYOD) and myogenic factor 5 (MYF5) belong to a family of proteins known as myogenic regulatory factors (MRFs). These basic helix–loop–helix (bHLH) transcription factors act
sequentially in myogenic differentiation. MYOD is a tissue-specific MRF that acts as a master transcriptional switch for muscle differentiation and development, whereas MYF5 is the first myogenic regulatory protein expressed in the skeletal muscle lineage. In mammals, either MYOD or MYF5 is required for the formation of skeletal muscle (Rudnicki et al. 1993). In chickens, MYOD and MYF5 are associated with post-hatch chicken myogenesis (Day et al. 2009).

Enhanced proteolysis in atrophying muscles derives mainly from general activation of the ubiquitin–proteasome (UBP) pathway. In mammals, there are three enzyme classes involved in the UBP pathway: UB activating enzymes (E1s), UB conjugating enzymes (E2s), and UB ligases (E3s). E3s are responsible for the recognition of substrates and their conjugation with UB (Glickman & Ciechanover 2002, Pickart & Eddins 2004). E3s play an important role in determining the proteins targeted for degradation by the proteasome. In mammals, IGF1 stimulates muscle growth by suppressing protein breakdown and the expression of muscle atrophy-related E3s (Sacheck et al. 2004). In contrast, GCs promote proteolysis by stimulating the UBP pathway (Du et al. 2000, Sacheck et al. 2004). In chickens, WW domain containing E3 UB protein ligase 1 (WWP1), a HECT-type E3, has been suggested to be responsible for chicken muscular dystrophy (Matsumoto et al. 2008).

In broilers, skeletal muscle as a whole accounts for 45–51% of carcass weight (Das et al. 2008). The modern strain of broiler chicken has a fast growth rate, higher breast muscle yield, and higher feeding efficiency, which makes the broiler chicken an interesting model for the development of muscle (Halevy et al. 2000). The rapid growth of the broiler chicken is facilitated by the reduced rate of protein degradation (Hayashi et al. 1985). In heat-stressed broiler chickens, both protein synthesis and breakdown are lower in the M. pectoralis major, and protein synthesis is more affected than breakdown, leading to reduced protein deposition (Temim et al. 2000). Therefore, we hypothesised that the IGF1 and MSTN signalling pathways are associated with the arrest of muscle development in response to GCs.

In the present study, the effects of DEX on muscle development and the transcription of related genes were investigated. The objective was to determine the pathways responsible for the reported suppressive effect of GCs on muscle development in chickens. In experiments 1 and 2, the effect of DEX treatment was measured at different exposure times (3 or 7 days) using starter broilers (7 days of age), which have the highest growth rate. In experiment 3, the effect of DEX treatment was determined in grower broilers (35 days of age), which have the highest absolute growth (Scheuermann et al. 2003).

Materials and methods

Birds and husbandry

Male broiler chicks (Arbor Acres, Gallus gallus domesticus) were obtained from a local hatchery at 1 day of age and reared in an environmentally controlled room. The brooding temperature was maintained at 35 °C (65% relative humidity) for the first 2 days and then decreased gradually to 21 °C (45% relative humidity) until 28 days after the start of the experiment. The temperature was thereafter maintained at 21 °C until the end of the experiment (38 days). The light regime was 23 h light:1 h darkness. All chicks received a commercial starter diet with 20·0% CP and 12·6 MJ ME/kg. All the birds had free access to feed and water during the rearing period. The study was approved by the Shandong Agricultural University and carried out in accordance with the ‘Guidelines for Experimental Animals’ of the Ministry of Science and Technology (Beijing, People’s Republic of China).

Experiment 1

Sixteen 7-day-old broilers (175·2 ± 3·9 g) were assigned to two groups of eight chickens according to body mass (BM), and the chickens were randomly subjected to one of the following two treatments for 3 days: s.c. injection of DEX (2 mg/kg BM) or sham treatment (1 ml saline/kg BM, control) at 0800 h. All the experimental chickens were reared individually and placed in single cages. BM and feed intake were recorded daily.

At the end of the experiment (0800 h, 3 days later), a blood sample was drawn from a wing vein of all the chickens after a 12-h feed withdrawal using a heparinised syringe within 30 s and stored in ice tubes. Plasma was obtained after centrifugation at 400 g for 10 min at 4 °C and was stored at −20 °C for further analysis. Immediately after the blood sample was obtained, the chickens were killed by exsanguination, and breast and thigh muscles were harvested and weighed. A 1 to 2 g sample was obtained from the left M. biceps femoris, cooled down in liquid nitrogen and stored at −80 °C for further analysis.

Experiment 2

Twenty-four 7-day-old broilers (175·0 ± 3·0 g) were allocated into three groups of eight chickens according to BM, and the chickens were randomly subjected to one of the following three treatments for 7 days: a s.c. injection of DEX (1 mg/kg BM, twice/day), sham treatment (1 ml saline, control), or sham treatment with pair-fed treatment to keep the feed consumption consistent with the DEX-treated chickens in the previous day (Urdaneta-Rincon & Leeson 2002). All the experimental chickens were reared individually.
and placed in single cages. BM and feed intake were recorded daily. At the end of the experiment, blood and muscle samples were obtained as described above.

**Experiment 3**

Forty-eight 35-day-old broilers (1730±41 g) were allocated to three groups of 16 chickens according to BM. The experimental chickens were randomly subjected to one of the following three treatments for 3 days: a s.c. injection of DEX (1 mg/kg BM, twice/day), sham treatment (1 ml saline, control), or sham treatment with pair-fed treatment. All the experimental chickens were reared individually and placed in single cages. BM and feed intake were recorded daily.

At the end of the experiment, half of all the experimental chickens were sampled in the feeding state, and the other half were sampled in the fasting state after a 12-h feed withdrawal.

**Measurements**

Plasma concentrations of glucose, urate, and total amino acids were measured spectrophotometrically with commercial diagnostic kits (Hitachi High-Technologies Corp.; Jiancheng Bioengineering Institute, Nanjing, People’s Republic of China).

Plasma insulin was measured by RIA with guinea pig anti-human IGF1 serum (Tianjing Nine Tripods Medical & Bioengineering Co., Ltd, People’s Republic of China). $^{125}$I-labelled human IGF1 competes with chicken IGF1 for sites on insulin–porcine antibodies. The IGF1 in this study is referred to as immunoreactive IGF1. All samples were included in the same assay to avoid interassay variability. The intra-assay CV was 7.0%.

Gene expression was measured using real-time reverse transcription (RT)-PCR. Briefly, total RNA from BF was extracted using Trizol (Invitrogen). The quantity and quality of the isolated RNA were determined by biophotometer (Eppendorf, Hamburg, Germany) and agarose gel electrophoresis, respectively, and RT was carried out using RT reactions (10 ml) consisting of 500 ng of total RNA, 5 mmol/l MgCl$_2$, 1 µl RT buffer, 1 mmol/l dNTP, 2.5 µl U avian myeloblastosis virus reverse transcriptase, 0.7 nmol/l oligo d(T), and 10 U ribonuclease inhibitor (TaKaRa Biotechnology, Co., Ltd, Dalian, People’s Republic of China). cDNA was amplified in a 20 µl PCR reaction system containing 0.2 µmol/l of each specific primer (Sangon Biological Engineering Technology & Service Co., Ltd, Shanghai, People’s Republic of China) and SYBR green master mix (TaKaRa Biotechnology, Co., Ltd, Dalian, People’s Republic of China). Each cycle consisted of a denaturation step at 95°C for 10 s, an annealing step at 95°C for 5 s, and an extension step at 60°C for 34 s. Glyceraldehyde-3-phosphate dehydrogenase

**Table 1** Primer sequences

<table>
<thead>
<tr>
<th>GenBank no.</th>
<th>Orientation</th>
<th>Primer sequences (5’→3’)</th>
<th>Product size (bp)</th>
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<td></td>
<td>Reverse</td>
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<td>158</td>
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<tr>
<td></td>
<td>Reverse</td>
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</tr>
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<td>CAT2</td>
<td>BU492799</td>
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<td>86</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
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<td></td>
</tr>
<tr>
<td>MSTN*</td>
<td>NM_204214</td>
<td>GCCCTTGTGAGACTGAGGAG</td>
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<td></td>
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<tr>
<td></td>
<td>Reverse</td>
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<td></td>
<td>Reverse</td>
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</tr>
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<td></td>
<td>Reverse</td>
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<tr>
<td>GAPDH</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>GGGGAGACAGAAGGAGACAGGA</td>
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</table>

CAT2, cationic amino acid transporter; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IGF1, insulin-like growth factor 1; IGF1R, IGF1 receptor; MSTN, myostatin; and WWP1, WW domain containing E3 ubiquitin protein ligase 1.

*The primer sequences are according to Guernec et al. (2004).
GAPDH was amplified as an internal control to normalise the differences in individual samples. The primer sequences for chicken IGF1 and IGF1 receptor (IGFIR), CAT2, MSTN, MYOD, MYF5, WWP1, and GAPDH are listed in Table 1.

The PCR products were verified by electrophoresis on a 0.8% agarose-gel and by DNA sequencing. Standard curves were generated using pooled cDNA from the samples being assayed, and the comparative cycle threshold (C_T) method (2^-ΔΔC_T) was used to quantitate mRNA levels, according to Livak & Schmittgen (2001). All samples were run in duplicate, and the primers were designed to span an intron to avoid genomic DNA contamination.

Statistical analysis

All the data were subjected to one-way ANOVA using Statistical Analysis Systems statistical software package (Version 8e, SAS Institute, Cary, NC, USA), and the main effect of DEX treatment was evaluated with an individual chicken as a replicate. Duncan’s multiple range tests were conducted to compare the effect among different treatments. A value of $P<0.05$ was considered as significantly different.

Results

In the present study, the effect of DEX treatment on skeletal muscle development was evaluated in three experiments. The effects of both short-term (3 days) and long-term (7 days) DEX treatment were investigated. Growth performance and the expression of genes related to muscle development and muscle wasting were measured.

Experiment 1

In experiment 1, 7-day-old chickens were exposed to 3 days of DEX treatment. DEX treatment significantly ($P<0.0001$) increased feed intake and decreased BM gain (Fig. 1A). DEX-treated chickens tended to have higher breast muscle mass ($P=0.058$) and thigh muscle mass ($P=0.097$) than control chickens.

DEX treatment significantly ($P<0.01$) increased plasma levels of urate and total amino acids (Table 2). Plasma concentrations of glucose and IGF1 were not significantly ($P>0.05$) affected by DEX treatment. However, plasma insulin levels tended to be higher ($P=0.062$) in DEX-treated chickens than in control chickens.

The expression levels of CAT2, IGF1, IGFIR, MSTN, WWP1, MYOD and MYF5 were all significantly ($P<0.01$) downregulated by 3 days of DEX treatment compared with the control treatment (Fig. 2A).

Figure 1 Effects of dexamethasone treatment (DEX, 1 mg/kg BM) on feed intake (g/day), body mass gain (BM, g/day), breast muscle mass and thigh muscle mass (g/g BW %) of broilers. (A) Experiment 1: 7-day-old broilers were subjected to DEX treatment for 3 days. (B) Experiment 2: 7-day-old broilers were subjected to DEX treatment for 7 days. (C) Experiment 3: 35-day-old broilers were subjected to DEX treatment for 3 days. Values are means ± s.e.m. ($n=8$). a,bMeans are significantly different, $P<0.05$. 
DEX treatment, however, significantly increased plasma expression of MSTN, an inhibitory effect of mild feed restriction on the chickens than in control chickens, which indicates was significantly lower in both DEX-treated and pair-fed MSTN compared with either control or pair-fed treatment groups.

In experiment 3, 35-day-old chickens were subjected to DEX treatment for 7 days. DEX treatment significantly decreased feed intake (P=0.015) and BM gain (P<0.01) compared with the control group (Experiment 2, Fig. 1B). However, DEX-treated chickens had similar feed intake but lower (P<0.01) BM gain compared with their pair-fed counterparts. The relative masses of breast and thigh muscle were not significantly influenced by DEX treatment. Plasma concentrations of glucose, total amino acids and IGF1 were not significantly affected by DEX treatment (Table 3). DEX treatment, however, significantly increased plasma urate (P<0.05) and insulin (P<0.0001).

Compared with control or pair-fed groups, the mRNA levels of IGF1 were significantly downregulated by DEX treatment (Fig. 2B). In contrast, the gene expression of IGF1R, MYOD and MYF5 was not significantly influenced by 7 days of DEX treatment. The expression of WWP1 was upregulated (P<0.01) by DEX treatment compared with either control or pair-fed treatments. In contrast, the mRNA level of MSTN was significantly lower in both DEX-treated and pair-fed chickens than in control chickens, which indicates an inhibitory effect of mild feed restriction on the expression of MSTN.

### Experiment 3

In experiment 3, 35-day-old chickens were subjected to DEX treatment for 3 days. Compared with the control group, DEX-treated chickens consumed less feed. Moreover, DEX-treated chickens had a negative BM gain, whereas their control and pair-fed counterparts exhibited a positive BM gain during the 3-days experimental period (Fig. 1C).

Under the feeding state, DEX treatment significantly upregulated the gene expression of CAT2 (P<0.01), IGF1R (P<0.001), MYOD (P<0.0001) and WWP1 (P<0.01) compared with either the control or pair-fed chickens (Fig. 3A). Although the expression of MYF5 was increased in the DEX-treated chickens, there was no significant difference between the DEX and pair-fed chickens. The mRNA level of IGF1 in the pair-fed treatment group was higher (P<0.05) than in the control group, while there was no significant (P>0.05) difference between the DEX and control treatments. In contrast, the gene expression of MSTN was significantly downregulated in the pair-fed treatment group compared with either the control or DEX treatment groups.

In the fasting state, the expression levels of CAT2, IGF1, MYF5, WWP1 and MSTN were not significantly affected by DEX treatment (Fig. 3B). Compared with the pair-fed group, the expression of MYOD (P<0.05) and IGF1R (P=0.066) were upregulated by DEX treatment. In contrast, there was no significant difference between the DEX and control treatments.

### Discussion

#### Effect of DEX treatment on muscle development

In line with previous studies in mammals (Bowes et al. 1996) and chickens (Dong et al. 2007), DEX treatment suppressed BM gain and skeletal muscle development (Fig. 1). This result was supported by the increased circulating urate and TAA in DEX-treated chickens compared with their pair-fed counterparts (Table 3), which indicates enhanced protein catabolism.

The present results indicate that skeletal muscle development was arrested by DEX treatment in pace with BM. Savary et al. (1998) reported that GC induces a significant decrease in protein synthesis in fast-twitch glycolytic and oxidative glycolytic muscles of rats. In our previous studies (Lin et al. 2006, Dong et al. 2007),

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Table 2 Effects of 3 or 7 days of dexamethasone treatment (DEX, 1 mg/kg BM) on plasma parameters of broilers at 7 days of age. Values are means ± S.E.M. (n=8)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DEX</th>
<th>Control</th>
<th>Pair-fed</th>
<th>P value</th>
</tr>
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<tr>
<td>3 days of treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>12.0±0.3</td>
<td>11.4±0.2</td>
<td>–</td>
<td>NS</td>
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<tr>
<td>Urate (mg/l)</td>
<td>95.8±7.8a</td>
<td>61.5±6.4b</td>
<td>–</td>
<td>0.0014</td>
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<tr>
<td>Insulin (µIU/ml)</td>
<td>29.7±1.6a</td>
<td>7.5±1.0pb</td>
<td>–</td>
<td>0.0623</td>
</tr>
<tr>
<td>T-AA (µmol/l)</td>
<td>34.7±1.8a</td>
<td>28.6±7.7pb</td>
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<td>0.0014</td>
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<tr>
<td>IGF1 (ng/ml)</td>
<td>7.45±0.81</td>
<td>7.22±0.73</td>
<td>–</td>
<td>NS</td>
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<tr>
<td>7 days of treatment</td>
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<tr>
<td>Glucose (mmol/l)</td>
<td>11.6±0.3</td>
<td>10.9±0.2</td>
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<tr>
<td>Urate (mg/l)</td>
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<td>60.2±5.2b</td>
<td>54.49±5.6b</td>
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<td>Insulin (µIU/ml)</td>
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<td>T-AA (µmol/l)</td>
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<td>IGF1 (ng/ml)</td>
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<td>5.83±0.56</td>
<td>6.02±0.64</td>
<td>NS</td>
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</table>

a,bMeans within the same row are significantly different, P<0.05.
breast and thigh muscle mass (% BM) were more severely retarded than BM in broilers subjected to long-term corticosterone treatment (7 or 10 days) around 35 days of age. Moreover, it was observed that breast muscle was more sensitive to the detrimental effect of corticosterone (Dong et al. 2007). Hence, these results suggest that the retardation of skeletal muscle development by GCs is exposure time- and age-dependent.

**Effects of DEX treatment on the expression of IGF1, MSTN and UBP**

IGF1 is a key regulator of muscle development and metabolism in birds and other vertebrate species. A single injection of IGF1 can influence muscle protein synthesis in young chicks (Conlon & Kita 2002). In the present study, the suppression of circulating IGF1 by DEX treatment was detected in the feeding state and not in the fasting state. Guernec et al. (2004) reported that circulating IGF1 is decreased by feed deprivation in chickens. In rats, overnight fasting suppresses rates of protein synthesis in skeletal muscle (Bark et al. 1998). The current results imply that DEX may suppress the activating effect of feeding on the release of IGF1.

The downregulated *IGF1* (Fig. 2B) and upregulated *MSTN* mRNA levels (Fig. 3A) in DEX-treated chickens indicate that IGF1 and MSTN are involved in the regulation of muscle development by DEX. In line with these results, there is a positive relationship between muscle *IGF1* mRNA levels and the post-hatch muscle growth of chickens (reviewed by Duclos (2005)). However, both proliferation and differentiation were found to be inhibited by myostatin in chick embryonic myoblasts (Yang et al. 2003). The different observation regarding IGF1 and MSTN is in line with the result in mammals that myostatin expression is not influenced by IGF1 deletion (Miyake et al. 2007), suggesting that no strong correlation exists between IGF1 and MSTN. Moreover, the effect of DEX treatment on the expression of IGF1 and MSTN seems to be different from that of feed restriction. In rats, the suppressed muscle growth caused by underfeeding may be mainly attributed to reduced serum IGF1 and partially to reduced *IGF1* mRNA, rather than to a change in myostatin gene expression (Yamaguchi et al. 2006). However, significantly decreased expression of *MSTN* was also observed with 3 days of DEX treatment (Fig. 2A). Smith et al. (2010) found that DEX injection and fasting are associated with reduced *Mstn* mRNA levels in rats, but MSTN protein levels were found to be unchanged after treatment with DEX and increased after fasting. Further research is required to understand how MSTN is involved in the effect of GCs on broiler chickens.

In mammals, there is accumulating evidence indicating the involvement of the UBP pathway in muscle wasting during pathophysiological conditions. In the present study, the expression of WWP1 was significantly upregulated after 7 days of DEX treatment (Fig. 2B), suggesting that UBP may be involved in the suppression of muscle development by DEX treatment. According to previous studies in mammals, GCs promote proteolysis by stimulating the UBP pathway (Du et al. 2000, Sacheck et al. 2004). The muscle-specific E3 ligases atrogin-1/MAFbx and Murf1 are both upregulated upon GC administration to the skeletal muscles of rats (Bodine et al. 2001). Adrenalectomised rats fail to increase protein breakdown in muscle and show 50% lower levels of UB–protein conjugates than starved control animals (Wing et al. 1995). In chickens, WWP1 has been suggested to be involved in muscular dystrophy (Matsumotoa et al. 2008). The present results suggest that UBP is involved in DEX-induced muscle retardation and, in turn, muscular dystrophy.
However, the mRNA levels of WWP1 were significantly suppressed in 3-day-old chickens exposed to short-term DEX treatment (Fig. 2A), whereas no significant difference was found in fasting 35-day-old chickens, reflecting the age- and feeding state-related effects of DEX on the activation of the UBP pathway. The underlying mechanism needs to be investigated further.

Effects of DEX treatment on myogenic factors

In the present study, the effects of DEX on the expression of myogenic factors were investigated. MYOD, MYF5 and a number of MRFs initiate and maintain the expression of muscle-specific genes during embryogenesis and postnatal muscle growth (Bailey et al. 2001). In rats, the loss of MRFs leads to reduced body size (Knapp et al. 2006). In the present study, upregulated transcription of MYOD and MYF5 in response to DEX treatment was observed in 35-day-old chickens (Fig. 3). During the proliferative phase, DEX treatment dose-dependently reduces the proliferation rate of C2C12 cells, increases Myod, Myf5 and Mrf4 and reduces myogenin mRNA levels (te Pas et al. 2000). In long-term DEX-treated male rats (14 days), increased expression levels of Myod and Myf5 have been detected (te Pas et al. 1999). Recently, it was reported that GC activates the N-terminal ubiquitination pathway for MYOD degradation in myotubes to promote muscle protein catabolism (Sun et al. 2008). Hence, the increased transcription of MYOD and MYF5 in response to DEX treatment seems to be the feedback effects of augmented MYOD degradation. It is also possible that these regulators could be activated in an attempt to repair the loss of MYOD (Musaro & Rosenthal 2003). However, the suppression of MYOD and MYF5 expression by DEX was detected at 3 days of treatment and diminished at 7 days of treatment in 7-day-old

<table>
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<th></th>
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<td>10.51 ± 0.52b</td>
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<tr>
<td>Insulin (µU/ml)</td>
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<td>10.68 ± 1.63b</td>
<td>11.47 ± 1.50b</td>
<td>0.0460</td>
</tr>
<tr>
<td>T-AA (µmol/ml)</td>
<td>51.11 ± 5.13a</td>
<td>31.17 ± 1.36b</td>
<td>28.80 ± 2.56b</td>
<td>0.0002</td>
</tr>
<tr>
<td>IGF1 (ng/ml)</td>
<td>6.46 ± 0.39b</td>
<td>8.28 ± 0.30b</td>
<td>8.24 ± 0.56b</td>
<td>0.0120</td>
</tr>
<tr>
<td><strong>Fasting state</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>12.28 ± 1.58</td>
<td>9.93 ± 0.49</td>
<td>9.96 ± 0.30</td>
<td>NS</td>
</tr>
<tr>
<td>Urate (mg/l)</td>
<td>55.39 ± 5.94a</td>
<td>25.58 ± 2.26b</td>
<td>35.15 ± 4.27b</td>
<td>0.0004</td>
</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td>13.83 ± 1.43a</td>
<td>8.77 ± 1.17b</td>
<td>8.89 ± 1.03b</td>
<td>0.0125</td>
</tr>
<tr>
<td>T-AA (µmol/ml)</td>
<td>67.40 ± 1.15a</td>
<td>47.15 ± 1.07b</td>
<td>46.99 ± 1.17b</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IGF1 (ng/ml)</td>
<td>7.92 ± 0.81</td>
<td>7.25 ± 0.54</td>
<td>7.77 ± 0.41</td>
<td>NS</td>
</tr>
</tbody>
</table>

Means within the same row are significantly different, P < 0.05.
chickens (Fig. 2). This result may imply that the effect of DEX on the expression of MRFs is dependent on the developmental stage of muscle. Indeed, in mammals, the abundance of MYOD, MYF5 and myogenin all decline within 1 week of birth (reviewed by Stewart & Rittweger (2006)).

In conclusion, the effect of GCs on skeletal muscle development was investigated in broiler chickens. The results indicate that DEX suppresses the growth of BM and skeletal muscle. DEX treatment decreased circulating IGF1 levels in broiler chickens. For the first time, it was shown that the expression levels of IGF1, MSTN and the UBP pathway are altered by DEX treatment in both age- and exposure time-dependent manners. The results suggest that suppressed IGF1, upregulated MSTN and/or an activated UBP pathway are involved in the retardation of muscle development by GCs.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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