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Effect of early feed restriction on myofibre types and expression of growth-related genes in the gastrocnemius muscle of crossbred broiler chickens

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The purpose of this study was to investigate the immediate and long-term effects of early feed restriction (ER) on morphology and gene expression of lateral gastrocnemius muscle. Newly hatched crossbred broiler chickens were allocated into control and ER groups, the latter being free-fed on alternate days from hatch to 14 days of age (14 d), followed by *ad libitum* feeding as the control group until 63 d. The lateral gastrocnemius muscle was taken at 14 and 63 d, respectively for myofibre typing by both myosin ATPase staining and relative quantification of myosin heavy chain (MyHC) mRNA for slow-twitch (SM), red fast-twitch (FRM) and white fast-twitch (FWM) myofibres. The body weight and lateral gastrocnemius weight were significantly lower in the ER group, accompanied by significantly reduced serum triiodothyronine. The ER group exhibited significantly higher SM and FRM MyHC expression at 14 d, but lower SM expression at 63 d. Myosin ATPase staining revealed a similar pattern. The percentage of SM was higher at 14 d while lower at 63 d in the ER group. These morphological changes were accompanied by changes of mRNA expression for growth-related genes. The ER group expressed lower insulin-like growth factoar I (IGF-I) and higher IGF-I receptor (IGF-IR) at 14 d, yet significantly increased growth hormone receptor and IGF-IR mRNA at 63 d. These results indicate that ER may delay the slow to fast myofibre conversion as an immediate effect, but would result in a lower percentage of slow fibres owing to compensatory growth in the long term, which involves changes of mRNA expression for the growth-related genes in the muscle.

Feed restriction: Myosin heavy chain: Myofibre types: Gene expression: Chicken

Nutritional and metabolic exposure during critical periods of early development can have a long-term programming effect on health in adulthood (Demmelmair *et al.* 2006). This so-called ‘nutritional or metabolic programming’ has been described not only in mammals (de Moura & Passos, 2005; Langley-Evans *et al.* 2005; Wu *et al.* 2006), but also in avian species. A large number of studies have been done in chicken to investigate the long-term effect of early nutritional manipulation on body and organ growth (Deaton, 1995; Kwakkel *et al.* 1998; Lee & Leeson, 2001), digestion (Palo *et al.* 1995*a*,*b*; Pinheiro *et al.* 2004), reproduction (Goerzen *et al.* 1996), immunity (Khajavi *et al.* 2003), lipid metabolism (Zhong *et al.* 1995), and metabolic disorders (Acar *et al.* 1995; Su *et al.* 1999). Early feed restriction (ER) has been reported recently to influence satellite cell mitotic activity (Moore *et al.* 2005*a*,*b*), implying a possible long-term effect of early nutrition on skeletal muscle development in the chicken.

Skeletal muscle accounts for 40–50 % of the body weight and contributes to the regulation of metabolic homeostasis of the body. Skeletal muscle consists of various types of myofibres with different metabolic profiles, contractile properties, as well as biochemical and biophysical characteristics, such as fibre size, colour, glycogen and lipid content (Ashmore & Doerr, 1971). Slow-twitch myofibres (SM) are the smallest, oxidative fibres with red colour, high lipid content and many mitochondria, whereas white fast-twitch fibres (FWM) are the largest glycolytic fibres possessing high glycogen content and few mitochondria. Red fast-twitch myofibres (FRM) are intermediate oxidative-glycolytic fibres that are similar to SM in colour but resemble FWM in their contractile property, possessing both aerobic and anaerobic metabolic capabilities. Recently, myofibre-type composition of skeletal muscle has attracted great attention both in human health and animal production, owing to its close association with insulin sensitivity in mammals (Hickey *et al.* 1995; Korach-Andre *et al.* 2005)

Abbreviations: ER, early feed restriction; FRM, red fast-twitch myofibres; FWM, white fast-twitch myofibres; GH, growth hormone; GHR, growth hormone receptor; IGF, insulin-like growth factor; IGF-IR, type 1 IGF receptor; MyHC, myosin heavy chain; SM, slow-twitch myofibres; T_3, triiodothyronine; T_4, thyroxine.

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and its significance for lean-mass deposition and meat quality in meat-producing animals (Maltin *et al.* 2003), respectively. However, the long-term effect of early nutrition on skeletal muscle growth and myofibre type in poultry has not been reported.

Since myofibres undergo a highly regulated process of fibre conversion and hypertrophy which contributes to lean-mass deposition and metabolic status of the organism in the postnatal period, we hypothesized that early nutritional deficiency would affect myofibre-type composition, and this influence could be carried over through later life after a long term of *ad libitum* feeding. The acting mechanism may involve growth-related genes expressed in the muscle that respond to the early nutritional deficiency and participate in the regulation of myofibre-type conversion and myofibre hypertrophy.

The objectives of the present study were, therefore, to observe the immediate and long-term effects of ER on body and muscle growth, as well as the myofibre composition with both histochemical and molecular typing, and to explore the associated changes of mRNA expression for growth-related genes in the muscle, employing crossbred broiler chickens as the experimental model.

Materials and methods

Animals and experimental design

Newly hatched San Huang chicks (a crossbred local broiler breed) were purchased from a commercial hatchery (Wen's Group, Guangdong, China) and allocated randomly into the control and the ER groups (*n* 40/group). The diets used in the experiment were formulated according to the nutritional requirements of the breed recommended by the breeding company (Table 1). Chickens in the control group were fed *ad libitum* for the whole period of investigation, while the ER group were subjected to feed restriction with feed provided on alternate days from hatch to 14 days of age (14 d), followed by *ad libitum* feeding until the end of the experiment at 63 d when chickens reached the marketing age.

The body weight of chickens was recorded weekly after being fasted overnight (*n* 40/group in first 2 weeks and *n* 20/group after 14 d). Feed consumption was recorded weekly. At 14 d and 63 d respectively, 10 chickens were randomly selected from each group and sacrificed for blood and tissue sampling. One side of the lateral gastrocnemius muscle was weighed and rapidly frozen in liquid N_2, then stored at -80°C until RNA isolation, while the other side was taken for measuring the cross-sectional areas and densities of SM, FRM and FWM with myosin ATPase staining.

The experiment was undertaken following the guidelines of the regional animal ethics committee.

Table 1. Nutritional composition of the basal diet

Nutritional composition	Age (weeks) 1–4	5–9
Crude protein (%)	21·5	19·5
Metabolizable energy (MJ/kg)	13·01	13·82
NaCl (%)	0·37	0·33
Ca (%)	0·96	0·85
P (%)	0·45	0·43
Lysine (%)	1·25	1·13
Methionine (%)	0·62	0·56
Methionine + Cysteine (%)	0·95	0·87

Radioimmunoassay for serum hormone levels

The serum concentrations of total triiodothyronine (T_3) and thyroxine (T_4) were measured with RIA using commercial kits purchased from the Shanghai Institute of Biological Products (Shanghai, China). The kits were validated for measuring avian serum samples (Zhao *et al.* 2004*a*).

Histochemical analysis

Myosin ATPase staining was applied to identify myofibre type and to measure myofibre size. Briefly, muscle blocks were excised perpendicularly to the direction of the myofibres. Freshly excised muscles were mounted on corks coated with gum tragacanth, rapidly frozen in liquid N_2, and then stored at -80°C. Serial tissue sections of 10 μm thickness were prepared with a cryostat at -20°C. Sections were washed twice for 1 min each with 18 mmol $CaCl_2$/l and 100 mmol Tris-HCl/l (pH 7·3; pre-rinse solution) and pre-incubated for 5 min at pH 10·4 (100 mmol glycine/l, 1 % $CaCl_2$, 3·7 % formaldehyde) at room temperature. The sections were then washed twice for 30 s each time with pre-rinse solution prior to incubation in 18 mmol $CaCl_2$/l, 20 mmol glycine/l, and 0·025 mmol ATP/l (pH 9·4) for 1 h 45 min at 37°C. Afterwards sections were washed four times in 1 % $CaCl_2$ wash solution and then rinsed in 2 % $CoCl_2$ for 4 min at room temperature. Subsequently sections were washed in distilled water four times for 20 s each then stained in a 1 % Azure Stain for 30 s. The stained sections were finally rinsed continuously under tap water for 10 min and washed in distilled water for 5 min, then dehydrated in ascending series of ethanol concentrations, delipidated in a solution of xylene–absolute ethanol (1: 1, v/v) and coverslipped. Six to seven fascicles (containing about 400–500 myofibres) were randomly selected from five serial sections of each sample using light microscopy (Olympus BH-2, Tokyo, Japan) with camera (JVC, Yokohama, Japan) at magnification 200, and the mean percentages of SM, FRM and FWM myofibres were calculated using image processing software (Image-Pro Plus 4.5, Silver Spring, MD, USA). The fibre type was determined as described by Wegner *et al.* (2000). FWM are stained dark blue, FRM are stained light blue, whereas SM are non-stained (Hoogenraad *et al.* 1979).

RNA extraction and reverse transcription

Total RNA was extracted from the tissue samples with a single-step method of RNA extraction by acid guanidinium thiocyanate–phenol–chloroform (Chomczynski & Sacchi, 1987). Total RNA concentration was then quantified by measuring the absorbance at 260 nm in a photometer (Eppendorf Biophotometer, Hamburg, Germany). Ratios of absorption (260:280 nm) of all preparations were between 1·8 and 2·0. Aliquots of RNA samples were subjected to electrophoresis through a 1·4 % agarose–formaldehyde gel to verify their integrity.

Total RNA (2 μg) was reverse transcribed by incubation at 37°C for 1 h in a 25 μl mixture consisting of 100 U M-MLV RT (Promega, Madison, WI, USA), 8 U RNase inhibitor (Promega), 21 μmol random primers/l (6 bp), 50 mmol Tris-HCl/l (pH 8·3), 3 mmol $MgCl_2$/l, 75 mmol KCl/l, 10 mmol DDT/l and 0·8 mmol each dNTP/l (Promega). The reaction was terminated by heating at 95°C for 5 min and quickly cooling on ice.

Semi-quantitative RT–PCR

The mRNA expression of SM, FRM, FWM, myosin heavy chain (MyHC) and growth hormone receptor (GHR), insulin-like growth factor-I (IGF-I) and type-I IGF receptor (IGF-IR) mRNA was quantified relative to 18S rRNA, using the Quantum RNA 18S Internal Standards kit (catalogue no. 1716, Ambion Inc., Austin, TX, USA), containing primers and competitors, for normalizing variations in pipetting and amplification. RT reaction mix (2 μl) was used for PCR in a final volume of 25 μl containing 0·5 U Taq DNA polymerase (Promega), 5 mmol Tris-HCl/l (pH 9·0), 10 mmol NaCl/l, 0·1 mmol DDT/l, 0·01 mmol EDTA/l, 5 % (w/v) glycerol, 0·1 %(w/v) Triton X-100, 0·2 mmol each dNTP/l, 1·6 mmol $MgCl_2$/l, 0·4 μmol/l each primer pair specific for three types of MyHC, GHR and IGF-IR, respectively, together with 0·4 μmol 18S rRNA primers/l and competitors in proportions optimized for each target gene. Each target gene was co-amplified with 18S rRNA in the same reaction except IGF-I which was amplified separately from 18S rRNA in duplicate. The primers for MyHC and growth-related genes were designed using Primer Premier 5·0 and were synthesized by Yinjun Biotech. Ltd (Shanghai, China). The nucleotide sequences of these primers and the PCR conditions set for respective genes are shown in Table 2. The PCR products from each reaction were sent to Haojia Biotech. Ltd (Shanghai, China) for sequencing to verify the specificity. The reported sequences matched exactly those published in GenBank. Different controls were set to monitor the possible contaminations of genomic DNA and environment DNA at the stage of both RT and PCR. The pooled samples made by mixing equal quantity of total cDNA from all samples were used for optimizing the PCR condition and normalizing the intra-assay variations. The cycles of PCR were determined to ensure that the amplifications were terminated within the linear range for quantification. All samples were included in the same run of RT–PCR and repeated at least three times. Both RT and PCR were performed in a Gene Amp PCR System 9600 (Perkin Elmer, Norwalk, CT, USA).

Quantification of PCR products

An aliquot (10–20 μl) of PCR products was analyzed by electrophoresis on 2 % agarose gels. The gels were stained with ethidium bromide and photographed with a digital camera. The net intensities of individual bands were measured using Kodak Digital Science 1D software (Eastman Kodak Company, Rochester, NY, USA). The ratios of net intensity of target genes to 18S rRNA were used to represent the relative levels of target gene expression.

Statistical analysis

The results were expressed as mean values with their standard errors and differences were considered significant when $P < 0·05$, tested by t test for independent samples. The correlations among the mRNA abundances, body weight and lateral gastrocnemius muscle weight were tested by Pearson's correlation coefficients with correlate, bivariate analysis. All statistical analyses were performed with SPSS 11.0 for windows (StatSoft, Inc., Tulsa, OK, USA).

Results

Growth performance

The growth performance and feed consumption in control and ER groups across the observation period are summarized in Table 3. Chickens in the ER group consumed considerably less feed, the average weekly feed intake being 48·5 and 62·0 % lower compared with that of the control group in the first and second week, respectively. The feed intake in the ER group remained low for 6 weeks thereafter under *ad libitum* feeding. The ER resulted in chickens with significantly lower body weight compared with their control counterparts throughout the experiment. Relative growth rate in the ER group, represented by percentage of daily body weight gain (average daily body weight gain relative to the initial body weight), was much lower under feed restriction in the first two weeks

Table 2. Nucleotide sequences of specific primers and PCR conditions

Target genes	GenBank accession	PCR products (bp)	Primer sequences	PCR conditions
SM	GenBank U85022, U85023	331	F: 5′-aacgccgcaacaacct -3′ R: 5′-ttcttcttcatccgctcc -3′	94°C, 30 s; 57°C, 30 s; 72°C, 30 s 27 cycles
FRM	GenBank NM_204228, M74086	334	F: 5′-atctggtggacaaactgc -3′ R: 5′-aatctatggtctttattctct -3′	94°C, 30 s; 53°C, 30 s; 72°C, 30 s 23 cycles
FWM	GenBank M74087	380	F: 5′-gtgaagggtgtacgcaagt -3′ R: 5′-atagatgacaatgacataaaaaagcaacac-3′	94°C, 30 s; 57°C, 30 s; 72°C, 30 s 19 cycles
GH-R	GenBank NM_001001293	345	F: 5′-ttacttcaacacatcctacacc -3′ R: 5′-tcataatctcttcccatcttca -3′	94°C, 30 s; 53°C, 30 s; 72°C, 30 s 27 cycles
IGF-IR	GenBank AJ223164	397	F: 5′-gtacttcagtgcttcggatgtg -3′ R: 5′-cttcttcagagttggaggtgct -3′	94°C, 30 s; 53°C, 30 s; 72°C, 30 s 27 cycles
IGF-I	GenBank M32791	191	F: 5′-catttcttctaccttggc -3′ R: 5′-tcatccactattcccttg -3′	94°C, 30 s; 53°C, 30 s; 72°C, 30 s 29 cycles

SM, slow-twitch myofibres; FRM, red fast-twitch myofibres; FWM, white fast-twitch myofibres; GHR, growth hormone receptor; IGF-I, insulin-like growth factor I; IGF-IR, type I IGF receptor.

Table 3. Effect of early feed restriction (ER) on body weight, relative body weight gain and average weekly feed intake of crossbred broiler chickens (Values are means with their standard errors)

Age (weeks)	Body weight (g)				Relative daily body weight gain (%)		Average weekly feed intake (g)	
	Control group		ER group					
	Mean	SEM	Mean	SEM	Control group	ER group	Control group	ER group
0	36·0	0·5	35·3	0·4**	–	–	–	–
1	74·8	1·4	50·0	1**	15·4	5·8	83·17	42·5
2	130·3	2·2	68·4	1·5**	10·6	5·4	123·5	46·92
3	255	8	187	7**	13·7	24·8	343·7	322·37
4	390	12	310	8**	7·6	9·4	293·75	244·74
5	540	9	451	13**	5·5	6·5	262·82	228·95
6	732	13	622	15**	5·1	5·4	431·79	374·21
7	988	21	843	18**	5·0	5·1	540·53	517·89
8	1227	38	1062	25**	3·4	3·7	652·63	507·89
9	1402	43	1250	33*	2·0	2·5	458·95	463·16

Mean values were significantly different from those from the control group of the same age: * $P<0.05$, ** $P<0.01$, n 20.

but rose significantly during the first week of the subsequent *ad libitum* feeding demonstrating a tendency of ‘catch up’ growth. However, this increased growth rate lasted only for one week and the relative growth rate declined to a level that is not different from the control group from the week. Accordingly, the weight of the lateral gastrocnemius muscle in the ER group was significantly lower both at 14 and 63 d of age (Fig. 1).

Serum total thyroid hormone levels

As shown in Fig. 2, both T_3 and T_4 levels decreased with age from 14 d to 63 d, regardless of treatment. Chickens in the ER group exhibited markedly decreased serum concentrations of T_3 and T_4 at 14 d, while at 63 d, serum T_3 remained low in the ER group but T_4 restored to the level that was not different from the control.

Morphology of the lateral gastrocnemius muscle

Fig. 3 shows the morphological changes of the lateral gastrocnemius muscle from myosin ATPase staining. The lateral gastrocnemius muscle of the ER group possessed higher density of SM at 14 d with lower density at 63 d compared with the control group of the same age. The cross-sectional area of both FRM and FWM was significantly smaller in the ER group than that in the control group at 14 d. At 63 d, all three types of myofibres in the ER group were still smaller in size, but only the FWM maintained significantly lower cross-sectional area compared with that in the control (Table 4).

mRNA expression of different types of myosin heavy chain and growth-related genes in the lateral gastrocnemius

As shown in Fig. 4, the ER group expressed significantly higher SM ($P<0.01$), FRM MyHC ($P<0.05$) and IGF-IR ($P<0.01$) but lower FWM MyHC ($P<0.05$) and IGF-I ($P<0.01$) mRNA in the lateral gastrocnemius muscle, compared with their control counterparts at 14 d. At 63 d however, a significant down-regulation was observed in the ER group for SM MyHC expression ($P<0.05$), accompanied by a significantly higher GHR ($P<0.05$) and IGF-IR ($P<0.01$) mRNA expression. Despite trends of decrease for FRM and numeric increase for FWM in the ER group, no significant differences were detected for either FRM or FWM MyHC mRNA expression between two groups (Fig. 5).

Correlations among body weight, the lateral gastrocnemius muscle weight and the expression of myosin heavy chain

The weight of the lateral gastrocnemius muscle significantly correlated with the body weight at both 14 d (r 0·962, $P<0.01$) and 63 d (r 0·821, $P<0.01$). Muscle weight was found to be positively correlated with FWM MyHC expression (r 0·528, $P<0.05$), but negatively correlated with SM MyHC expression (r −0·526, $P<0.05$) at 14 d. A moderate positive correlation (r 0·504, $P<0.05$) was observed between muscle weight and FRM MyHC expression at 63 d.

Discussion

The present experiment demonstrated that nutritional deficiency during early post-hatch development induced permanent negative effect on body weight at slaughter in crossbred broiler chickens, which was in agreement with the previous findings in mice (Widdowson & McCance, 1963) and broiler chickens (Maxwell *et al.* 1991).

The majority of studies in chicken describing the influence of feed restriction of different timing, period, or severity have been focused on growth and carcass characteristics (Camacho *et al.* 2004; Pinheiro *et al.* 2004; Hiramatsu *et al.* 2005). There are scarcely any data concerning the changes of muscle morphology and metabolic properties induced by feed restriction, in addition to changes of muscle weight (Brown *et al.* 1990). It is known that muscle fibre formation is completed late in gestation in mammals and at hatching in avian species; the postnatal muscle growth is determined by myofibre hypertrophy which is accompanied with myofibre-type transformation following the sequence: SM → FRM → intermediate type myofibres → FWM in both mammal (Lefaucheur *et al.* 2004) and avian species (Gauthier *et al.* 1982; Tidyman *et al.* 1997; Rushbrook *et al.* 1998; Bandman & Rosser, 2000).

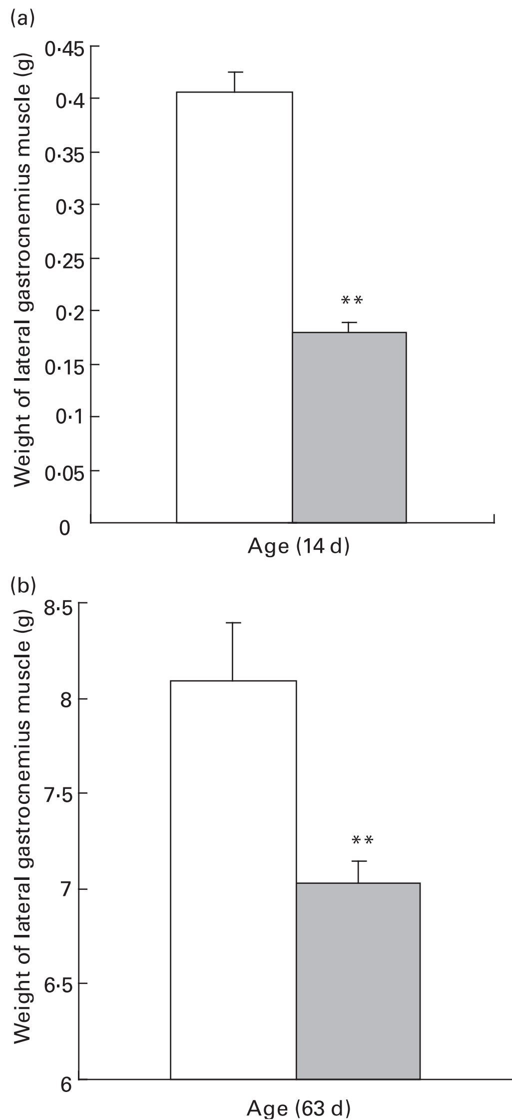

Fig. 1. Effect of early feed restriction (ER) on weight of the lateral gastrocnemius muscle from crossbred broiler chickens at age (a) 14 days and (b) 63 days. Values for the control (□) and the ER (▩) group are given as means with their standard errors represented by vertical bars. Mean value was significantly different from that of the control group: ** $P<0·01$, *n* 10.

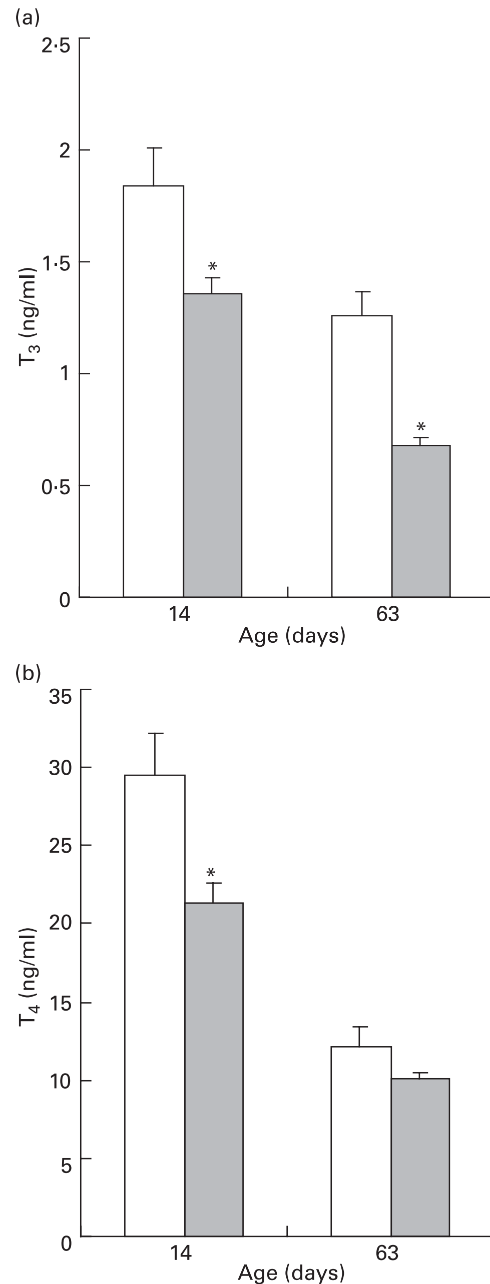

Fig. 2. Effect of early feed restriction (ER) on serum thyroid hormone (T_3 (a) and T_4(b)) levels of crossbred broiler chickens. Values for the control (□) and the ER (▩) group are given as means with their standard errors represented by vertical bars. Mean value was significantly different from that of the control group: * $P<0·05$, *n* 10.

Traditionally myofibre typing of the skeletal muscle is achieved by using a morphological method (Hoogenraad *et al.* 1979; Wegner *et al.* 2000). Based on the knowledge that different types of myofibres express different MyHC that are encoded by respective genes (Chen *et al.* 1997; Rushbrook *et al.* 1998), a multiplex RT–PCR technique was developed for investigating myofibre-type composition based on the fibre-specific expression of different MyHC isoforms (Tanabe *et al.* 1998). This technique was employed in recent studies for typing porcine and bovine skeletal muscles (Muroya *et al.* 2002; Zhao *et al.* 2004*b*). The relative percentage of a specific myofibre type can be estimated by quantification of corresponding MyHC mRNA expressed in that muscle (Tidyman *et al.* 1997).

The transition of myofibre types depends on intrinsic programming related to the myoblast lineage from which muscle fibres develop and is further regulated by extrinsic influences such as neural, hormonal, dietary and mechanical factors including muscle activity (Vadaszova *et al.* 2004). It is reported that the conversion from embryonic myofibres to neonatal myofibres and adult myofibres would be delayed by muscular dystrophy (Tidyman *et al.* 1997). In the present study both morphological and molecular typing pointed to higher oxidative fibres (SM and FRM) and lower glycolytic fibres (FWM) in the ER group compared with their control counterparts immediately after feed restriction at 14 d,

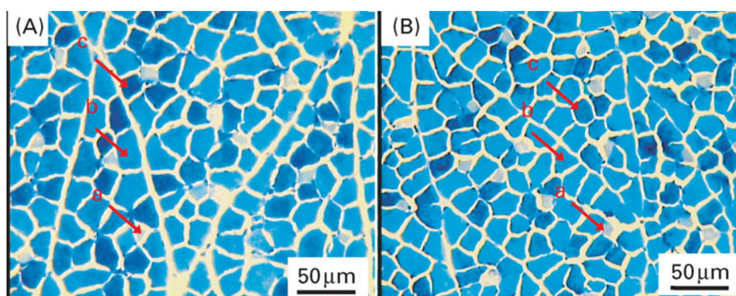

Fig. 3. Representative images of histochemical ATPase staining at magnification 200 for the lateral gastrocnemius muscle of crossbred broiler chickens from (A) the control group and (B) the early feed restriction group at 14 days of age showing a, slow-twitch myofibre; b, red fast-twitch myofibre, and c, white fast-twitch myofibre.

suggesting delayed or arrested conversion from SM to fast-twitch myofibre by feed restriction, even though the molecular typing by relative quantification of mRNA expression of three different MyHC did not completely match the result of morphological study due to differences in resolution and specificity of the two methods.

The pathway mediating the effect of nutritional deficiency on myofibre type transformation is not completely elucidated, yet reduced serum concentration of thyroid hormones was suggested to be involved in the mechanism (Gunning & Hardeman, 1991; Dewil *et al.* 1999). Hypothyroidism leads to preferential expression of slower fibre types while hyperthyroidism enhances the content of fast fibres in rats (Vadaszova *et al.* 2004). Indeed, we detected significant reduction both for T_3 (−26 %) and T_4 (−28 %) serum concentrations in the ER group at 14 d (Fig. 2). In addition, morphological study revealed reduced cross-sectional area of all myofibre types in the ER group at 14 d compared with the control group, although the difference of slow fibres did not reach the statistically significant level. This result demonstrated that ER stunted the hypertrophy of myofibres in general regardless of fibre types, which may eventually result in reduced lateral gastrocnemius muscle weight and decreased body weight. Furthermore, higher proportion of slow fibres would contribute to lower muscle weight in the ER group, as indicated by the correlation analysis.

The influence of ER on the myofibre-type composition is long-lasting. To our surprise, however, the difference between the ER and the control group in myofibre-type composition at 63 d was in contrast to that observed at 14 d. Both morphological and molecular typing showed lower SM in the ER group at 63 d, with reduced cross-sectional area of all myofibre types in the ER group at 63 d compared with the control group, although only FWM reached the statistically significant level. This result demonstrated that ER may induce an accelerated myofibre hypertrophy in the long term.

The postnatal events contributing to myofibre hypertrophy comprise satellite cell proliferation, differentiation and protein turnover. Many studies indicate a possible role of a growth hormone (GH)/IGF-I system in the regulation of postnatal muscle growth (Oksbjerg *et al.* 2004). The feed restriction-induced reduction in body weight in chickens was accompanied by a significant fall in circulating IGF-I (Scanes & Griminger, 1990; Morishita *et al.* 1993; Leili *et al.* 1997) and a rise in plasma GH (Krestel-Rickert *et al.* 1986; Kuhn *et al.* 1991), which are restored to the normal levels by refeeding (Kita *et al.* 1996; Maxwell *et al.* 1999). A recent study provided evidence that the *in vivo* effects of GH on muscle mass and strength are primarily mediated by activation of the IGF-I receptor in skeletal muscle, since mice lacking IGF-I function specifically in skeletal muscle, due to the over expression of a dominant-negative IGF-I receptor in this tissue (MKR mice) exhibited no effects of GH on the cross-sectional area of myofibres and the proliferation of satellite cells (Kim *et al.* 2005). The role of the GH–IGF-I axis in the regulation of avian muscle growth is more vague and still under debate (Buyse & Decuypere, 1999). It is suggested that in the chicken, hepatic expression of IGF-I mRNA is GH-dependent after hatching, whereas IGF-I expression in other tissues, including muscle, is independent

Table 4. Effect of early feed restriction (ER) on morphology of the lateral gastrocnemius muscle
(Values are means with their standard errors)

	14 d				63 d			
	Control		ER		Control		ER	
Parameters	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Density of slow-twitch myofibres (%)	17·5	1·9	24·4	4·1*	25·4	2·9	18·0	2·2*
Density of red fast-twitch myofibres (%)	53·0	4·6	47·6	6·1	23·2	1·7	27	11
Density of white fast-twitch myofibres (%)	29·6	4·3	28·0	4·8	51·4	3·4	55	11
Average cross-sectional area of slow-twitch myofibres (μm^2)	142	50	118	15	1550	340	1020	310
Average cross-sectional area of red fast-twitch myofibres (μm^2)	408	56	246	56*	2118	407	1690	180
Average cross-sectional area of white fast-twitch myofibres (μm^2)	405	96	232	36*	2960	410	2160	160*

Mean values were significantly different from those from the control group of the same age: * $P < 0·05$, *n* 6.

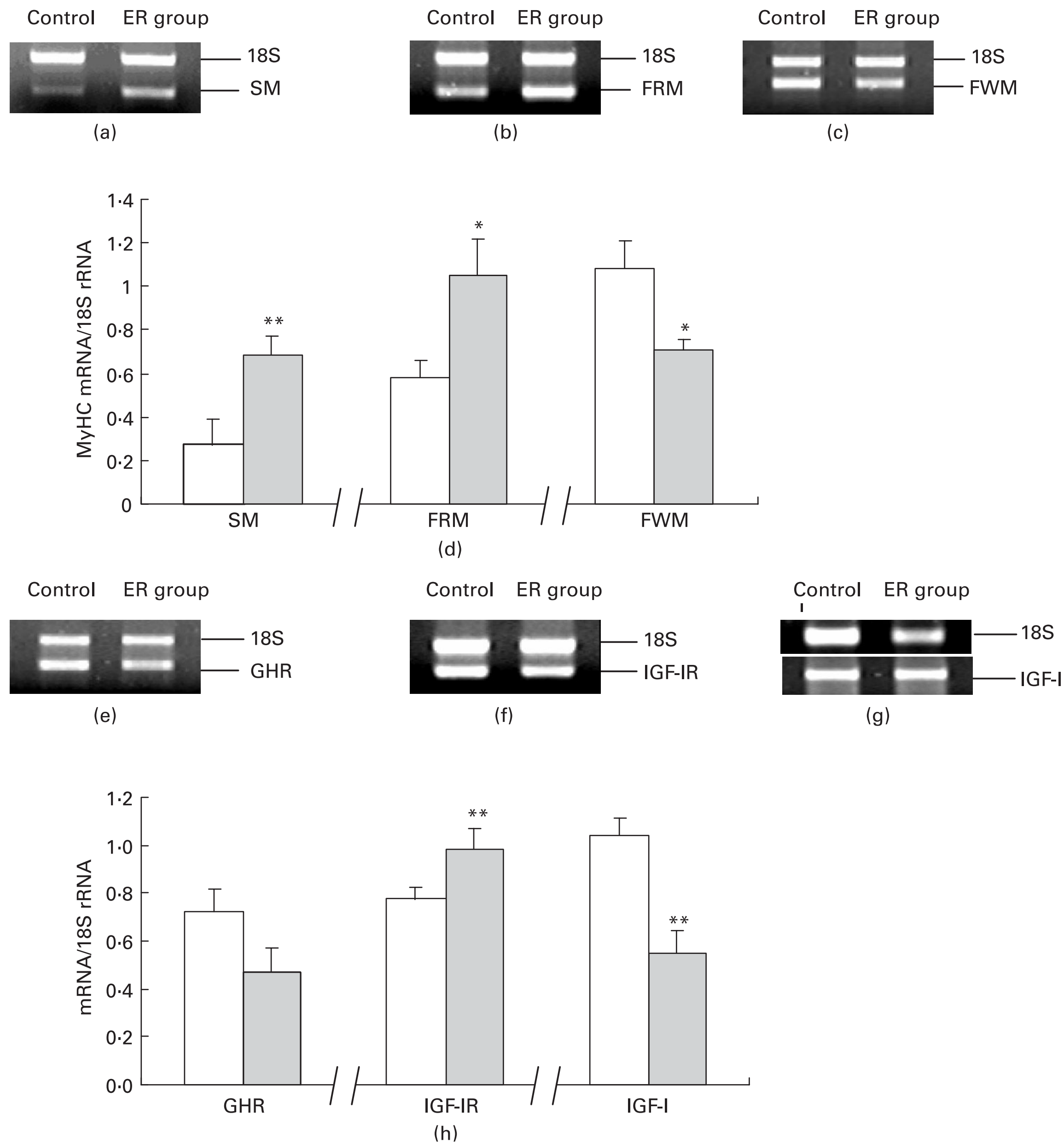

Fig. 4. Effect of early feed restriction (ER) on mRNA expression of myosin heavy chain (MyHC) and growth-related genes in the lateral gastrocnemius muscle of 14 d old crossbred broiler chickens. (a)–(c) Representative electrophoresis photos of RT–PCR products for slow-twitch myofibre (SM), red fast-twitch myofibre (FRM) and white fast-twitch myofibre (FWM) MyHC mRNA, co-amplified with 18S rRNA, respectively. (d) Results of statistical analysis for abundance of SM, FRM and FWM MyHC mRNA in the control (□) and the ER (▩) group. (e)–(g) Representative electrophoresis photos of RT–PCR products for growth hormone receptor (GHR) and type 1 insulin-like growth factor receptor (IGF-IR), co-amplified with 18S rRNA, as well as insulin-like growth factor I (IGF-I) mRNA, amplified separately from 18S rRNA, respectively. (h) Results of statistical analysis for abundances of GHR, IGF-IR and IGF-I mRNA. mRNA levels of target genes are expressed as arbitrary units relative to 18S rRNA in the control (□) and the ER (▩) group. Values are given as means with their standard errors represented by vertical bars. Mean value was significantly different from that of the control group: * $P<0·05$, ** $P<0·01$, *n* 10.

of GH and GHR (Tanaka *et al.* 1996). The absence of a functional GHR in the dwarf chicken is associated with a greater decline in DNA synthesis but no alterations were observed in IGF-I mRNA or peptide in muscle (Goddard *et al.* 1996). In avian skeletal muscle satellite cells, GHR gene expression was regulated by cGH in a biphasic manner which correlated with the GH effect on cell proliferation: 2–10 ng hormone/ml increased GHR mRNA and DNA synthesis, whereas higher concentrations attenuated these effects (Halevy *et al.* 1996). In the present experiment, delayed conversion from SM to fast-twitch myofibre and higher percentage of slow fibres in the ER group at 14 d was associated with significantly higher IGF-IR mRNA expression but lower IGF-I mRNA in the gastrocnemius muscle, while the lower percentage of SM and retarded hypertrophy in the ER group observed at 63 d was accompanied by significantly elevated GHR and IGF-IR mRNA expression. The higher muscle IGF-IR mRNA

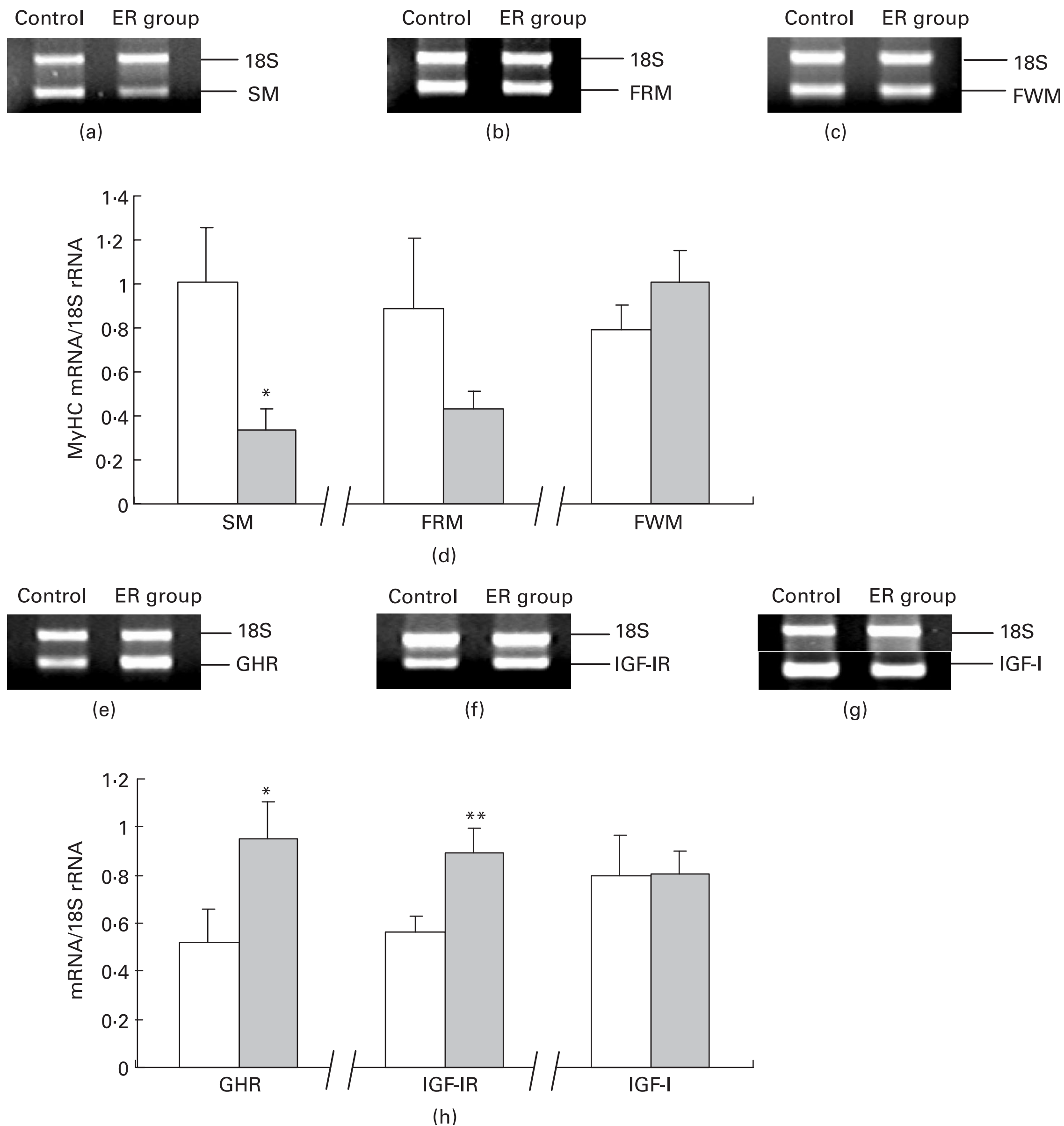

Fig. 5. Effect of early feed restriction (ER) on mRNA expression of myosin heavy chain (MyHC) and growth-related genes in the lateral gastrocnemius muscle of 63 d old crossbred broiler chickens. (a)–(c) Representative electrophoresis photos of RT–PCR products for slow-twitch myofibre (SM), red fast-twitch myofibre (FRM) and white fast-twitch myofibre (FWM) MyHC mRNA, co-amplified with 18S rRNA, respectively. (d) Results of statistical analysis for abundances of SM, FRM and FWM MyHC mRNA in the control (□) and the ER (▩) group. (e)–(g) Representative electrophoresis photos of RT–PCR products for growth hormone receptor (GHR) and type 1 insulin-like growth factor receptor (IGF-IR), co-amplified with 18S rRNA, as well as insulin-like growth factor I (IGF-I) mRNA, amplified separately from 18S rRNA, respectively. (h) Results of statistical analysis for abundances of GHR, IGF-IR and IGF-I mRNA. mRNA levels of target genes are expressed as arbitrary units relative to 18S rRNA in the control (□) and the ER (▩) group. Values are given as means with their standard errors represented by vertical bars. Mean value was significantly different from that of the control group: * $P<0{\cdot}05$, ** $P<0{\cdot}01$, *n* 10.

expression at 14 d could be the consequence of decreased circulating and local level of IGF-I, as indicated by significantly decreased abundance of IGF-I mRNA in the muscle. The up-regulated expression of both GHR and IGF-IR mRNA in the muscle at 63 d may reflect the physiological response of the muscle to the ER for a compensatory growth via accelerated myofibre hypertrophy. This adaptation mechanism failed to achieve the complete growth compensation by the end of the experiment, probably due to the limited time allowed for the 'catch-up' growth. However, it seems unlikely that the alterations in muscle morphology and metabolic properties as reflected by the change in myofibre-type composition would be restored even if chickens in the ER group attained normal body weight. This life-long effect of ER may thus induce changes in metabolic status and compromise meat quality in later life.

In conclusion, ER delays the myofibre conversion from slow-tonic to fast-twitch types, resulting in retarded muscle growth that may account for the decreased body weight. The influence of ER on myofibre types is long-lasting, significant differences still seen after 7 weeks when chickens reach the marketing age. Changes in muscle expression of

GHR and IGF-IR mRNA may be involved in the acting mechanism.

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