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Differences in the expression of genes involved in skeletal muscle proteolysis between broiler and layer chicks during food deprivation

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ABSTRACT

Genetic selection results in a higher growth rate and meat yield in broiler chickens than in layer chickens. We herein demonstrated differences in the effects of 24 h of fasting on the expression of genes involved in skeletal muscle proteolysis between broiler and layer chicks. The mRNA levels of proteolysis-related genes were analyzed in the pectoralis major muscle of 14-day-old chicks after 0 or 24 h of fasting. The mRNA levels of ubiquitin ligases such as atrogin-1 and muscle RING finger-1 (MuRF-1) as well as transcription factor forkhead box class O (FOXO) 1 were significantly increased by fasting in broiler and layer chicks, suggesting that the FOXO1-induced ubiquitin–proteasome system, a major proteolytic system in skeletal muscles, was activated by fasting in both chicks. The mRNA levels of atrogin-1 were significantly lower in broiler chicks than in layer chicks after fasting. Furthermore, the mRNA levels of insulin-like growth factor-1 were significantly decreased by fasting in layer chicks, but not in broiler chicks. The mRNA levels of FOXO3 were significantly increased by fasting in layer chicks, but not in broiler chicks. Therefore, the ubiquitin–proteasome system did not appear to have been fully upregulated in broiler chicks. These results suggest that differences in the expression of genes related to the ubiquitin–proteasome system in skeletal muscle proteolysis between broiler and layer chicks during food deprivation are one of the causes of the high growth rate in broiler chickens.

1. Introduction

Skeletal muscle mass is controlled by a balance between protein synthesis and proteolysis (Sandri, 2008, 2013). When the rate of proteolysis exceeds that of protein synthesis, skeletal muscle atrophy occurs. The ubiquitin–proteasome system has been identified as a major proteolytic system in skeletal muscles (Lecker et al., 1999; Sandri, 2008; Gumucio and Mendias, 2013). One of the triggers that activate this system is the expression of E3 ubiquitin ligases such as atrogin-1 and muscle ring-finger protein 1 (MuRF-1). The expression of these muscle specific ubiquitin ligases was previously shown to be increased by fasting (Sandri et al., 2004; Attaix and Baracos, 2010). MuRF-1 catalyzes the breakdown of major myofibril proteins such as actin, myosin heavy chains, myosin light chains, and myosin binding protein C (Glass, 2010; Sandri, 2013), while atrogin-1 catalyzes the breakdown of protein synthesis-related proteins such as MyoD and eIF3-f (Attaix and Baracos, 2010; Sandri, 2013). These findings indicate that atrogin-1 plays an important role not only in the promotion of proteolysis, but also in the inhibition of protein synthesis in skeletal muscles (Attaix and Baracos, 2010; Glass, 2010). Forkhead box class O (FOXO) 1 and FOXO3 play pivotal roles as the transcription factors of atrogin-1 and MuRF-1 (Sandri, 2008; Schiaffino et al., 2013; Sanchez et al., 2014). FOXO3 also has been shown to activate not only the ubiquitin–proteasome system, but also the autophagy–lysosome system, another important proteolytic system in skeletal muscles (Neel et al., 2013; Sandri, 2013; Sanchez et al., 2014). For example, FOXO3 promoted the transcription of several autophagy-related genes such as microtubule-associated protein 1 light chain 3 (LC3), Bcl2/adenovirus E1B 19 kDa-interacting protein 3, and GABA(A) receptor-associated protein (Mammucari et al., 2007; Zhao et al., 2007; Neel et al., 2013; Sanchez et al., 2014). FOXO1 and FOXO3 were both shown to be downregulated by insulin-like growth factor-1 (IGF-1) and upregulated by myostatin (Rommel et al., 2001; Sandri, 2013; Sanchez et al., 2014). In addition, peroxisomal proliferator-activated receptor γ coactivator-1 α (PGC-1α), which acts as a multifunctional transcriptional coregulator in several metabolic pathways such as thermogenesis, mitochondrial biogenesis, and fatty acid metabolism (Handschin and Spiegelman, 2006; Muoio and Koves, 2007; Liu and Lin, 2011), prevented skeletal muscle atrophy by inhibiting FOXO3 transcriptional activity (Sandri et al., 2006; Brault et al., 2010). N^{τ}-methylhistidine, which is neither broken down nor reused for protein synthesis after proteolysis (Young et al., 1972; Long et al., 1975),

Abbreviations: FOXO, forkhead box class O; IGF-1, insulin like growth factor-1; LC3, microtubule-associated protein 1 light chain 3; MuRF-1, muscle RING finger-1; PGC-1α, peroxisomal proliferator-activated receptor γ coactivator-1 α.

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is released by the degradation of myofibrillar proteins. Thus, E3 ubiquitin ligases, FOXOs, IGF-1, myostatin, PGC-1α, and N^{τ}-methylhistidine have been used as indicators of protein metabolism in skeletal muscles.

Chicken meat and eggs are important protein sources for human nutrition worldwide. Broilers, which are meat-type chickens, have been genetically selected for their high growth rate and meat yield (Arthur and Albers, 2003). On the other hand, egg-type chickens, layers, have been bred for traits such as the high production of eggs per hen housed and egg weight (Arthur and Albers, 2003). Previous findings suggested that the high growth rate of broiler chickens resulted from lower skeletal muscle proteolysis than that in layer chickens (Maruyama et al., 1978; Maeda et al., 1984; Hayashi et al., 1985; Kang et al., 1985; Saunderson and Bryan, 1985; Saunderson and Leslie, 1988). However, the molecular mechanisms underlying low skeletal muscle proteolysis in broiler chickens have not yet been fully elucidated.

Starvation induces skeletal muscle proteolysis in order to provide amino acids for hepatic gluconeogenesis or energy production, leading to the loss of skeletal muscle mass (Mitch and Goldberg, 1996). Previous studies reported the effects of fasting on the expression of genes involved in skeletal muscle proteolysis in chickens. For example, atrogin-1 mRNA levels increased in response to fasting in layer chickens (Nakashima et al., 2006). The expression of atrogin-1 and MuRF-1 mRNA was elevated in food-deprived broiler chickens (Li et al., 2011; Ohtsuka et al., 2011). These findings suggest that atrogin-1 and MuRF-1 play important roles in fasting-induced skeletal muscle proteolysis in chickens as well as in mammals. Furthermore, atrogin-1 mRNA levels were found to be lower in broiler chickens than in layer chickens under *ad libitum* feeding conditions (Nakashima et al., 2009). These findings raised the possibility that the regulatory mechanism underlying the gene expression of ubiquitin ligases in skeletal muscle differs between broiler and layer chickens, resulting in low skeletal muscle proteolysis in broiler chickens.

In the present study, the effects of fasting on the expression of skeletal muscle protein metabolism-related genes were compared in skeletal muscle in broiler and layer chickens.

2. Materials and methods

2.1. Animals and feed

Day-old male broiler (chunky) and layer (White Leghorn) chicks were purchased from local hatcheries (Ishii Poultry Farming Cooperative Association, Tokushima, Japan; Ghen Co., Gifu, Japan, respectively). They were given free access to water and a commercial chicken starter diet (23.5% crude protein and 3050 kcal/kg, Nippon Formula Feed Mfg. Co. Ltd., Kanagawa, Japan). This study was approved by the Institutional Animal Care and Use Committee (Permission number: 25-08-01) and carried out according to the Kobe University Animal Experimental Regulation.

2.2. Sampling and preparation

Ohtsuka et al. (2011) and Nakashima et al. (2006) previously reported the effects of 24 h of fasting on skeletal muscle proteolysis in 2-week old chicks. Therefore, we employed the same fasting time (24 h) and age (14-day-old) in this study. Sixteen 14-day-old male broiler and layer chicks were each weighed and allocated based on body weight to two cages (eight birds in each cage). After 0 or 24 h of fasting, body weights were measured, and chicks were sacrificed by decapitation. Blood was collected from the carotid artery. Plasma was separated immediately by centrifugation at 3000 *g* for 10 min at 4 °C, and the plasma concentrations of glucose, non-esterified fatty acids (NEFA), and insulin were measured using a commercial kit (Labassay™ Glucose and Labassay™ NEFA, Wako Pure Chemical Industries, Ltd., Osaka, Japan; Rat Insulin ELISA KIT (TMB), Shibayagi, Gunma, Japan). Plasma N^{τ}-methylhistidine concentrations were measured as described previously (Ibuki et al., 2014). The left pectoralis major muscle, abdominal adipose tissue, and liver were excised and weighed. Protein content in the pectoralis major muscle was determined by the method of Lowry et al. (1951). The pectoralis major muscle was frozen by liquid nitrogen and stored at −80 °C for real-time PCR analysis.

2.3. Real-time PCR analysis

Total RNA was extracted from the pectoralis major muscle using Sepazol-RNA I (Nacalai Tesque, Inc., Kyoto, Japan). First-strand cDNA was synthesized from total RNA using ReverTra Ace® qPCR RT Master Mix with gDNA Remover (Toyobo Co. Ltd, Osaka, Japan). The complementary DNAs of IGF-1, myostatin, and atrogin-1 were amplified with primers as previously described (Ibuki et al., 2013, 2014). The complementary DNAs of MuRF-1 (GenBank accession no. XM_424369), FOXO1 (GenBank accession no. NM_204328), FOXO3 (GenBank accession no. XM_001234495), and PGC-1α (GenBank accession no. AB170013) were amplified with the following primers: MuRF-1 sense, 5′-tgg aga ttg agc aag gct at-3′; antisense, 5′-gcg agg tgc tca aga ctg act-3′; FOXO1 sense, 5′-tct ggt cag gag gga aat gg-3′; antisense, 5′-gct tgc agg cca ctt tga g-3′; FOXO3 sense, 5′-ggg aag agc tcc tgg at-3′; antisense, 5′-ggg cgc ctt gcc aac t-3′; and PGC-1α sense, 5′-gag gat gga ttg cct tca ttt a-3′; antisense, 5′-gcg tca tgt tca ttg gtc aca-3′. As an internal standard, the cDNA of 18S ribosomal RNA (GenBank accession no. AF173612) was also amplified with the following primers: sense, 5′-tgc atg gcc gtt ctt agt tg-3′; antisense, 5′-tgc cag agt ctc gtt cgt tat c-3′. The level of mRNA was quantified in duplicate using the Thermo Scientific PikoReal Real-Time PCR System (Thermo Fisher Scientific Oy, Vantaa, Finland) and THUNDERBIRD™ SYBR® qPCR Mix (Toyobo Co. Ltd., Osaka, Japan) according to the supplier's recommendations.

2.4. Western blot analysis

A Western blot analysis was performed as previously described (Ibuki et al., 2014). Briefly, protein lysates were prepared as described by Duchêne et al. (2008). Muscle lysates were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting using the HorizeBlot (ATTO Co., Tokyo, Japan) according to the supplier's recommendations. Bands were detected using ECL™ Prime Western Blotting Detection Reagent (GE Healthcare Ltd, Buckinghamshire, UK), visualized with the Lumicube (Liponics Inc., Tokyo, Japan) and quantified using CS Analyzer (ATTO Co., Tokyo, Japan). Anti-Akt (#9272), anti-p-Akt (S473) (#9271), anti-FOXO1 (#9454), anti-p-FOXO1 (S256) (#9461), anti-FOXO3 (#12829), anti-p-FOXO3 (S253) (#9466), anti-LC3 (#12741), and horseradish peroxidase-conjugated anti-rabbit IgG (#7074) were purchased from Cell Signaling Technology (Beverly, MA, USA). As an internal standard, anti-vinculin (#V4139) was purchased from Sigma-Aldrich Co. LLC (St. Louis, MO, USA). After the detection of bands, membranes were rinsed with Restore™ Plus Western Blot Stripping Buffer (Thermo Fisher Scientific Inc., Rockford, IL, USA), and used for reprobing with appropriate antibodies.

2.5. Statistical analysis

Data were analyzed using a two-way ANOVA with the main effect of fasting time (Fasting) and chicken type (Type). If a significant interaction was observed in the two-way ANOVA analysis, the Tukey–Kramer test was performed to analyze the difference among the groups. All statistical analyses were performed using Excel 2013 (Microsoft, USA) with the add-in software Statcel 3 (OMS, Tokyo, Japan).

3. Results

The results obtained for body weight, tissue weights, muscle protein content, and plasma concentrations of glucose, NEFA, N^{τ}-methylhistidine,