Response of Atrogin-1/MAFbx Expression in Various Skeletal Muscles to Fasting in Broiler Chickens

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The effects of fasting on expression of atrogin-1/MAFbx in various skeletal muscles of broiler chickens were investigated. Real-time PCR analyses revealed that mRNA expression of chicken atrogin-1/MAFbx was higher in the soleus, gastrocnemius, and biceps femoris than in the pectoralis superficialis under normal nutritional conditions. Compared to normal nutritional conditions, fasting (24h) induced a 5-, 11-, 9-, and 7-fold increase in atrogin-1/MAFbx mRNA in the pectoralis superficialis, biceps femoris, gastrocnemius, and soleus muscles, respectively. These results indicate that the effects of fasting on expression of the atrogin-1/MAFbx gene vary among various skeletal muscles of broiler chickens.

Key words: atrogin-1/MAFbx, broiler, chicken, fasting, skeletal muscle

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Introduction

Selective breeding of broiler chickens for meat production has led to the generation of strains with accelerated growth rates, in particular which exhibit enhanced growth of the pectoralis (breast) muscle (Griffin and Goddard, 1994, Emmerson, 1997, Konarzewski et al., 2000, Berri et al., 2001). The accumulation of muscle protein, a key step in muscle growth, is determined by the balance between the rates of protein synthesis and degradation. The high rate of accumulation of breast muscle protein in rapidly growing broiler chickens appears to be achieved almost entirely by a marked decrease in the fractional rate of protein degradation (Maeda et al., 1984, Hayashi et al., 1985). However, the mechanism underlying the reduced protein degradation rate in the pectoralis muscle of broiler chickens has not been determined.

In skeletal muscles, cell mass is determined by the balance between the rates of protein synthesis and protein degradation. Studies of experimental animals have consistently demonstrated that protein degradation by the ubiquitin-proteasome system was increased in muscles undergoing atrophy (Mitch and Goldberg, 1996, Dehoux et al., 2003, Dehoux et al., 2004, Lecker et al., 2004, Skurk et al., 2005). Proteolysis in catabolic conditions was also primarily due to activation of the ubiquitin-proteasome proteolytic pathway (Kettelhut et al., 1994, Price et al., 1996, Lecker et al., 2004). In this way, proteins destined to be degraded are linked to a chain of ubiquitin molecules, which targets them for rapid breakdown by the proteasome (Glickman and Ciechanover, 2002). Evidence suggested that atrogin-1, an E3 ubiquitin ligase also referred to as MAFbx (muscle atrophy F-box), plays a pivotal role in muscle atrophy (Bodine et al., 2001, Gomes et al., 2001). Its expression is increased in catabolic conditions that result in muscle atrophy (Bodine et al., 2001, Gomes et al., 2001, Dehoux et al., 2003, Dehoux et al., 2004). Atrogin-1/MAFbx played a critical role in the development of muscle proteolysis and its gene expression was a reliable index of muscle protein degradation (Ohtsuka et al., 2011). However, it is not clear how atrogin-1/MAFbx expression is regulated in different skeletal muscles of broiler chickens.

In the present study, we compared the effects of fasting on expression of atrogin-1/MAFbx mRNA in various skeletal muscles (pectoralis superficialis, biceps femoris, gastrocnemius and soleus) of growing broiler chickens.

Materials and Methods

Animal Preparation and Experimental Protocol

One-day-old male broiler chicks (Chunky strain) were reared in an electrically-heated battery brooder and were provided with water and a commercial starter diet (crude protein 23%, metabolizable energy 3050 kcal/kg diet; Toyohashi Feed Co Ltd, Aichi, Japan) ad libitum until 11 days of age. Thereafter, six chicks with a body weight of

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325 g were subjected to muscle sampling. Skeletal muscle samples (pectoralis superficialis, biceps femoris, gastrocnemius and soleus) were frozen in liquid nitrogen and stored at −80°C until RNA was extracted.

At 11 days old, twelve birds of a similar body weight were selected and housed in wire-bottomed aluminum cages. They were given free access to a commercial starter diet and water for 3 days. Thereafter, 14-day-old chicks were divided into two groups: the fed group and the fasted group. Fed chicks were maintained as described above. Fasted chicks had no access to food for 24 h before they were killed. The pectoralis superficialis, biceps femoris, gastrocnemius, and soleus muscles was rapidly excised, weighed, frozen in liquid nitrogen, and stored at −80°C.

All experimental procedures were conducted in accordance with the guidelines of the Animal Care and Use Committee of the NARO Institute of Livestock and Grassland Science.

Real-Time PCR

Total RNA was extracted using TRIzol Reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the manufacturer’s directions. Complementary DNA was synthesized from total RNA using random hexamer primers (TaKaRa, Tokyo, Japan) and ReverTra Ace (TOYOBO, Tokyo, Japan). The sequences of the primers were as follows: chicken atrogin-1/MAFbx (NM_001030956), forward: 5′-CCA ACA ACC AGA CCT GT-3′ and reverse: 5′-GGA GCT TCA CAC GAA CAT GA-3′; and chicken 18S rRNA (AF173612), forward: 5′-AAA CGG CTA CCA CAT CCA AG-3′ and reverse: 5′-CCT CCA ATG GAT CCT CGT TA-3′. mRNA levels were measured by real-time PCR analysis using a LightCycler instrument (Roche Diagnostics, Mannheim, Germany) and the QuantiTect SYBR Green PCR system (Qiagen, Venlo, Netherlands). The 18S rRNA level was measured as an internal control.

Statistical Analysis

Data were analyzed using the Student’s t-test. A p value of <0.05 was considered statistically significant. Results are expressed as the mean±standard error.

Results and Discussion

Real-time PCR analyses were performed to examine the expression of chicken atrogin-1/MAFbx in various skeletal muscles. Expression of chicken atrogin-1/MAFbx was higher in the soleus, gastrocnemius, and biceps femoris than that in the pectoralis superficialis (Fig. 1). As a result, they exhibit a higher proportion of muscle, especially pectoralis muscle, relative to body weight compared to other skeletal muscles. In the present study, we showed that atrogin-1/MAFbx expression is lower in the pectoralis superficialis muscle than in other skeletal muscles (biceps femoris, gastrocnemius, and soleus muscles, respectively). Atrogin-1/MAFbx expression in skeletal muscle is selectively induced by fasting and is coordinately upregulated during fasting at the molecular level. Fasting also stimulates expression of atrogin-1/MAFbx mRNA in rodent skeletal muscles (Dehoux et al., 2004, Lecker et al., 2004). We previously reported that fasting stimulated atrogin-1/MAFbx expression in pectoralis superficialis and gastrocnemius muscles of chickens (Nakashima et al., 2006, Ohtsuka et al., 2011). Li et al. (2011) reported that fasting stimulated atrogin-1/MAFbx expression at the mRNA and protein levels in skeletal muscle of broiler chickens. In the current study,

![Fig. 1. Expression level of atrogin-1/MAFbx mRNA in various skeletal muscles of broiler chickens.](image-url)
fasting stimulated expression of atrogin-1/MAFbx mRNA in pectoralis superficialis, biceps femoris, gastrocnemius and soleus muscles of broiler chickens. The effects of fasting on atrogin-1/MAFbx mRNA expression in different skeletal muscles (muscle types) of chickens have not been reported. The present study provides the first evidence that the response of atrogin-1/MAFbx gene expression to fasting differs among different types of skeletal muscles (pectoralis superficialis, biceps femoris, gastrocnemius and soleus) in broiler chickens. During fasting, expression of atrogin-1/MAFbx is increased prior to skeletal muscle weight loss, and its mRNA expression level is high when protein degradation is rapid (Gomes et al., 2001). We previously reported that mRNA expression of atrogin-1/MAFbx in chicken pectoralis superficialis and gastrocnemius muscles drastically increases during fasting is related to the rate of protein degradation and the muscular size of chickens (Nakashima et al., 2006, Ohtsuka et al., 2011). In the current study, expression of atrogin-1/MAFbx mRNA in various skeletal muscles (pectoralis superficialis, biceps femoris, gastrocnemius, and soleus) of chicken was increased by fasting.

The difference in atrogin-1/MAFbx expression following fasting among different muscles may be associated with the types of muscle fiber. Gastrocnemius (fast-twitch muscle) is more sensitive to fasting than soleus (slow-twitch muscle), and muscle atrophy upon fasting is more severe in fast-twitch muscles than in slow-switch muscles (Li and Goldberg, 1976, Frayn and Maycock, 1979). During fasting, atrogin-1/MAFbx expression was higher in the gastrocnemius than in the soleus whereas it was higher in the biceps femoris (fast-twitch muscle) than in the gastrocnemius muscle. Although the pectoralis superficialis is a fast-twitch muscle, expression of atrogin-1/MAFbx during fasting was lower in this muscle than in the other muscles (biceps femoris, gastrocnemius and soleus). Selective breeding of broiler chickens for meat production has led to the generation of strains with accelerated growth rates, in particular which exhibits enhanced growth of the pectoralis (breast) muscle (Griffin and Goddard, 1994, Emmerson, 1997, Konarzewski et al., 2000, Berri et al., 2001). The high rate of accumulation of breast muscle protein in rapidly growing broiler chickens appears to be achieved almost entirely by a marked decrease in the fractional rate of protein degradation (Maeda et al., 1984, Hayashi et al., 1985). In this context, the expression of atrogin-1/MAFbx in different types of muscle fibers (slow- and fast-twitch muscles) and in broiler chickens requires further investigation.

In conclusion, we demonstrated that the pectoralis superficialis muscle of broiler chickens expresses low levels of atrogin-1/MAFbx, the muscle-specific ubiquitin ligase, in comparison to other muscles (biceps femoris, gastrocnemius and soleus). Furthermore, expression of atrogin-1/MAFbx in broiler chickens in response to fasting differed among skeletal muscles.

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Fig. 2. Effects of fasting on the mRNA level of atrogin-1/MAFbx in various skeletal muscles of broiler chickens. The results of RNA quantification are expressed as ratios to relative to the 18S rRNA levels in fed chicken, whose expression level was taken to be 1. Data are means±standard error (n=5–6). *, p<0.05; **, p<0.01. White bars, fed chicks; black bars, fasted chicks.
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