Research Note

Temporal myosin heavy chain isoform expression transitions faster in broiler chickens compared with Single Comb White Leghorns

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ABSTRACT Myosin heavy chain (MyHC), one of the major components in the contractile machinery of skeletal muscle fibers, is found in several isoforms during myogenesis. During chicken development, embryonic, neonatal, and adult MyHC isoforms are expressed. Broiler chickens have been selected for fast and large muscle growth, whereas Single Comb White Leghorn (SCWL) chickens have been selected for egg laying capabilities. This has led to an obvious difference in muscle growth and development with broilers being much larger than SCWL. The objective of this study was to determine if differences in muscle growth and development of SCWL and broilers are associated with differences in temporal expression of MyHC isoforms in skeletal muscle between the 2 breeds. Pectoralis major muscle (PM) was collected from SCWL and broilers at embryonic d 15, 17, and 19 and 1, 5, 11, 20, 27, and 33 d posthatch with n = 3 samples per time point and breed. Western blotting using 3 monoclonal antibodies (EB165, 2E9, and AB8) was performed to compare the expression patterns of embryonic/adult, neonatal, and adult isoforms of MyHC, respectively, for all time points in both SCWL and broiler chickens. Both broiler and SCWL chickens began expressing the neonatal MyHC isoform on d 5; however, SCWL chickens expressed the neonatal isoform much longer than broilers. The SCWL chickens had sustained expression of the neonatal MyHC isoform through d 27, whereas in broiler chickens the neonatal isoform was not expressed at d 20. Pectoralis major tissue from broiler chickens expressed the adult MyHC isoform as early as d 20, whereas the SCWL chickens began expressing the adult isoform later. The rate of transition to neonatal and adult MyHC isoforms in broilers and Leghorns is consistent with the faster maturation and growth of broilers relative to Leghorns. This relationship between faster growth of the PM and the rate of transition of MyHC isoforms within the fast skeletal muscle of the PM may indicate a selection marker for improvement of broiler PM growth.

Key words: muscle development, myosin heavy chain, chicken

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INTRODUCTION

One of the major profitable products produced by the poultry industry is chicken breast meat, or the pectoralis major muscle (**PM**). The PM is such an important product that the poultry industry has selected lines of broiler chickens specifically for their ability to grow very large breast muscle at a much faster rate than that of 40 yr prior (Havenstein et al., 2003a,b; Wick et al., 2003; Reddish et al., 2005b). Eggs are also a major product of the poultry industry, and because of the importance of eggs the industry has selected a separate breed of chickens specifically for their egg laying capabilities. This selection has resulted in the development of the Single Comb White Leghorn (**SCWL**) breed of chicken, which is currently the primary commercial laying chicken. The selection of birds for these 2 different end products has resulted in major differences in both the speed of growth and the PM to BW ratio, or breast yield, between both breeds of bird. The broiler chicken has a greater breast yield than the SCWL.

Myosin heavy chain (**MyHC**) is the major component of the contractile machinery of skeletal muscle fibers. During myogenesis in the SCWL several different isoforms of MyHC are temporally expressed (Tidyman et al., 1997; Wick et al., 2003; Reddish et al., 2005a,b). In the chicken PM the sequence of MyHC isoform expression starts with ventricular MyHC, followed by embryonic, neonatal, and lastly adult MyHC (Tidyman et al., 1997; Bandman and Rosser, 2000; Reddish et al., 2005a). The sequential expression of these MyHC isoforms that is observed during embryonic myogenesis is recapitulated during recovery from injury (d'Albis et al., 1988; Schultz and McCormick, 1994; Bourke et al., 1995; Zhao and Hoffman, 2004; Parise et al., 2006), indicating that the sequential expression of these isoforms is important to muscle growth and development,

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despite the fact that functional diversity of these isoforms is unknown.

The differential temporal expression level of other muscle specific proteins including myogenic markers such as Pax7, which is required for satellite cell specification (Seale et al., 2000) and is a known marker for early satellite cell proliferation (Halevy et al., 2004), has been reported previously. Satellite cells are the source of new mononuclei for myofibers in growing animals (Moss and Leblond, 1971). A report by Shin et al. (2009) showed that at d 1 broiler chickens had only an 87% reduction in Pax7 expression from embryonic age 17, but SCWL chickens had a 96.5% reduction from those levels at embryonic age 17. This difference in Pax7 mRNA expression is indicative of a higher population of differentiating satellite cells in broiler chickens, which allows for a larger rate of hypertrophic growth of the PM in broiler chickens compared with the SCWL (Shin et al., 2009).

Temporal changes in Pax7 expression may also be linked to temporal expression of the MyHC isoforms. Riuzzi et al. (2012) concluded that MyoD, myogenin, and MyHC levels were markers of proliferation to differentiation events. Because Pax7 is a known marker of proliferation, it is hypothesized that reduced expression of Pax7 will be associated with increased expression of neonatal MyHC.

Although the functional diversity of the different MyHC isoforms is not known, it is reasonable to investigate whether the sequential transition of MyHC isoforms may be associated with the different rates of muscle growth and maturation in broilers and SCWL. Due to the major differences in muscle growth and maturation between broiler and SCWL chickens, it is hypothesized that the temporal expression of MyHC isoforms differs between the 2 breeds of chicken. The majority of posthatch muscle growth is generally postulated to occur through hypertrophy. The predominant, and terminal myosin isoform in posthatch poultry is the adult isoform. Previous studies by Reddish et al. (2005b), using fast skeletal muscle MyHC-specific monoclonal antibodies, showed that genetic selection for increased breast yield in broilers resulted in accelerated temporal expression of developmental fast skeletal MyHC isoforms and that this acceleration of development is likely necessary for muscle specific growth, regardless of whole muscle growth. To determine if the differences in growth and maturation of the skeletal muscle between the 2 breeds of chickens are associated with differences in temporal MyHC isoform expression, as well as temporal expression of Pax7, Western blotting protein analysis of the PM was performed using fast skeletal muscle MyHC-specific monoclonal antibodies.

MATERIALS AND METHODS

Experimental Birds

The Ohio State University Institutional Animal Care and Use Committee approved all animal care and handling procedures. Commercial broiler eggs (Ross 708) were incubated and sampled at various developmental time points: embryonic day (**E**) 15, 17, 19 and posthatch day 1, 5, 11, 20, 27, 33. Birds were fed a standard diet ad libitum throughout the trial period. At each time point PM tissue was collected from 4 to 5 chickens and snap-frozen in liquid nitrogen. Tissues were stored at −80°C until protein analysis via Western blotting could be performed.

Sampling Procedure

At posthatch time points, 4 to 5 birds were euthanized via carbon dioxide inhalation and immediately dissected for collection of PM tissue. The PM tissue was placed in a separate tube for each individual bird and snap frozen in liquid nitrogen. At embryonic time points, 4 to 5 eggs were used to collect embryos, which were removed from the egg and euthanized via decapitation. Embryos were immediately dissected and PM tissue was collected, placed into individual tubes, and snap frozen in liquid nitrogen.

Statistical Analysis

Densitometric analysis, using ImageJ software from the National Institutes of Health, was performed for each target protein in Figure 2. These values were then compared with the β-actin level of the corresponding lane to determine the ratio of target protein to β-actin. These ratios were subsequently analyzed using Student's *t*-test for determination of significant differences as well as the calculation of the mean ratio for the target protein for both Leghorns (n = 3) and broilers (n = 3), and the SD for each group. A *P*-value of less than 0.05 was considered significant. Mean ratio and SD are reported along with *P*-value for each time point that demonstrated significant differences.

Western Blot Analysis

Tissue samples were homogenized with a Tissumiser homogenizer (Fisher Scientific, Pittsburgh, PA) in cold 1× lysis buffer, which contained 62.5 m*M* Tris, pH 6.8, and 1% SDS. Samples were then mixed with 2× Laemmli buffer containing 62.5 m*M* Tris, pH 6.8, 1% SDS, 5% 2-mercaptoethanol, 12.5% glycerol, and 0.05% bromophenol blue (Bio-Rad Laboratories, Hercules, CA). Protein separation was performed by 8% SDS-PAGE using a mini-Protean system (Bio-Rad Laboratories). Proteins were then wet-transferred to Immobilon Transfer membranes (Millipore, Billerica, MA). Membranes were blocked in 5% nonfat dry milk in Tris-buffered saline-Tween (**TBST**; 20 m*M* Tris, 150 m*M* NaCl, pH 7.4, plus 0.1% Tween 20) for 30 min at room temperature. Membranes were then incubated with 5 different primary antibodies: EB165 antibody (embryonic and adult 1:50,000), 2E9 antibody (neonatal 1:1,000), AB8 antibody (adult 1:3,000), Pax7 anti-

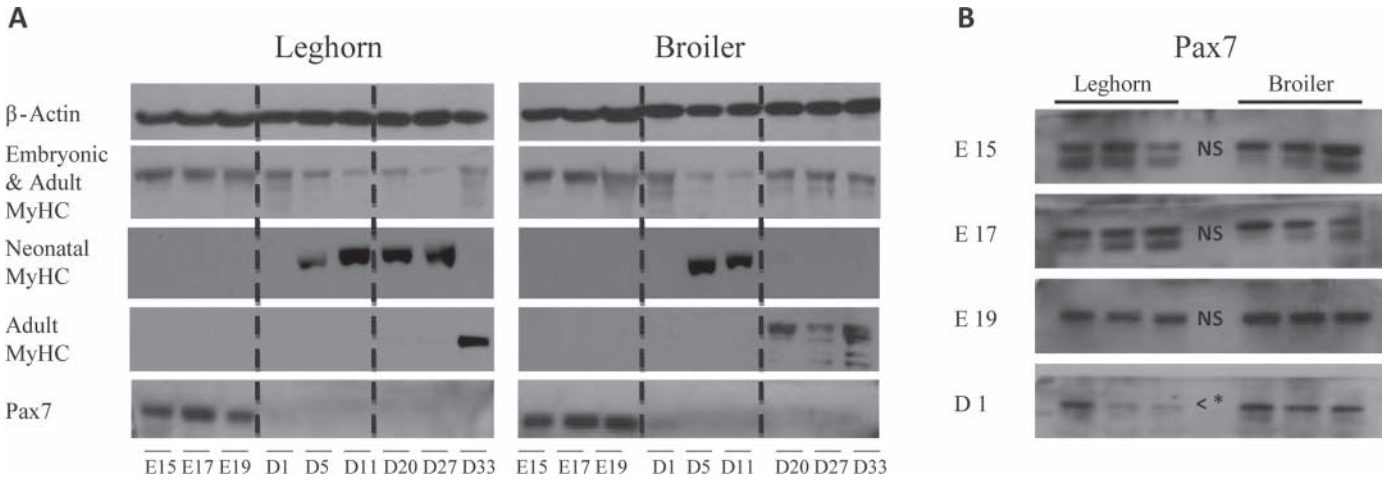

Figure 1. Individual expression of the different isoforms of myosin heavy chain (MyHC) and breed comparison of Pax7 expression between broilers and Single Comb White Leghorns (SCWL). A. Time point comparison between layer and broiler chickens for embryonic d (E) 15, 17, 19, and d (D) 1, 5, 11, 20, 27, and 33 posthatch. n = 1 for each time point. B. Expression of chicken Pax7 early myogenesis factor. Each lane represents one individual bird. n = 3 per time point and breed. $^*P < 0.05$.

body (1:500; Developmental Studies Hybridoma Bank), or β-actin antibody (1:2,000 Santa Cruz Biotechnology Inc., Santa Cruz, CA) at room temperature for 1 h. The EB165, 2E9, and AB8 antibodies were a gift from Everett Bandman (University of California, Davis). The specificities of the chicken fast skeletal myosin heavy chain specific monoclonal antibodies have been detailed elsewhere (Moore et al., 1992). Membranes were washed 6 to 10 times in TBST and subsequently incubated in horseradish peroxidase-conjugated anti-mouse IgG (1:5,000 dilution; Cell Signaling Technology, Boston, MA) or anti-goat IgG (1:5,000 dilution; Santa Cruz Biotechnology Inc.) in TBST for 1 h at room temperature. The membranes were washed again 6 to 10 times with TBST. Proteins were detected using ECL plus (GE Healthcare, Piscataway, NJ) and were exposed using Amersham Hyperfilm ECL (GE Healthcare).

RESULTS AND DISCUSSION

We first examined the differences in MyHC isoform expression between individual broiler and SCWL chickens across all the time points (n = 1; Figure 1A) and then compared the breeds at each time point (n = 3) (Figure 1B and 2). As shown in Figure 1A, the EB165 antibody, which is specific for both the embryonic and adult MyHC isoforms, reacted with MyHC in SCWL from embryonic d 15 with sustained expression through d 5. Detection of embryonic and adult MyHC decreased on d 11 and remained low through d 33. In comparison, the EB165 antibody detected embryonic or adult MyHC isoform expression in broilers from embryonic d 15 through d 1, with expression decreasing on d 5 and increasing again on d 20 with continued expression through d 33. The breed comparison in Figure 2A supports these results, showing that expression of embryonic and adult MyHC decreases greatly on d 5, increases on d 20, and is sustained through d 33 in broilers, whereas SCLW embryonic and adult MyHC expression decreases later than in broilers and increases on d 27. These results indicate that the PM matures faster in the broiler chicken compared with the SCWL.

The individual comparison of neonatal MyHC isoform (Figure 1A) showed that expression began on d 5 in SCWL with sustained expression through d 27, dropping off greatly by d 33. Expression in broilers began on d 5 and continued through d 11, dropping off greatly by d 20. In the breed comparison shown in Figure 2B, neonatal MyHC was detected in both SCWL and broilers as early as d 5 posthatch. Expression was sustained in both breeds on d 11, but on d 20 and 27 broilers showed a significant ($P < 0.05$; d 20: Leghorn 1.55 ± 0.50 vs. broiler 0.20 ± 0.10; d 27 Leghorn 1.20 ± 0.30 vs. broiler 0.19 ± 0.29) decrease in expression compared with SCWL. The SCWL expression of neonatal MyHC decreased after d 27. These results are consistent with the decrease in MyHC detection by EB165 beginning on d 11 and continuing through d 27 for SCWL and the decrease in MyHC detection by EB165 from d 5 to 11 in broilers.

Comparison of individual expression of the adult MyHC isoform (Figure 1A) showed expression beginning on d 33 in SCWL, whereas expression began on d 20 and continued through d 33 in broilers. In the breed comparison shown in Figure 2C, expression of adult MyHC was detected as early as d 11 in broilers, with a significant increase in expression on d 20 ($P < 0.001$; Leghorn 0 ± 0 vs. broiler 0.48 ± 0.03) and d 27 ($P < 0.01$; Leghorn 0 ± 0 vs. broiler 9.14 ± 4.94) compared with SCWL. Broilers maintained expression of adult MyHC through d 33. In SCWL, expression was not detected until d 33. The initial expression of the adult MyHC isoform was consistent with the significant ($P < 0.05$; d 20: Leghorn 1.55 ± 0.50 vs. broiler 0.20 ± 0.10; d 27 Leghorn 1.20 ± 0.30 vs. broiler 0.19 ± 0.29)

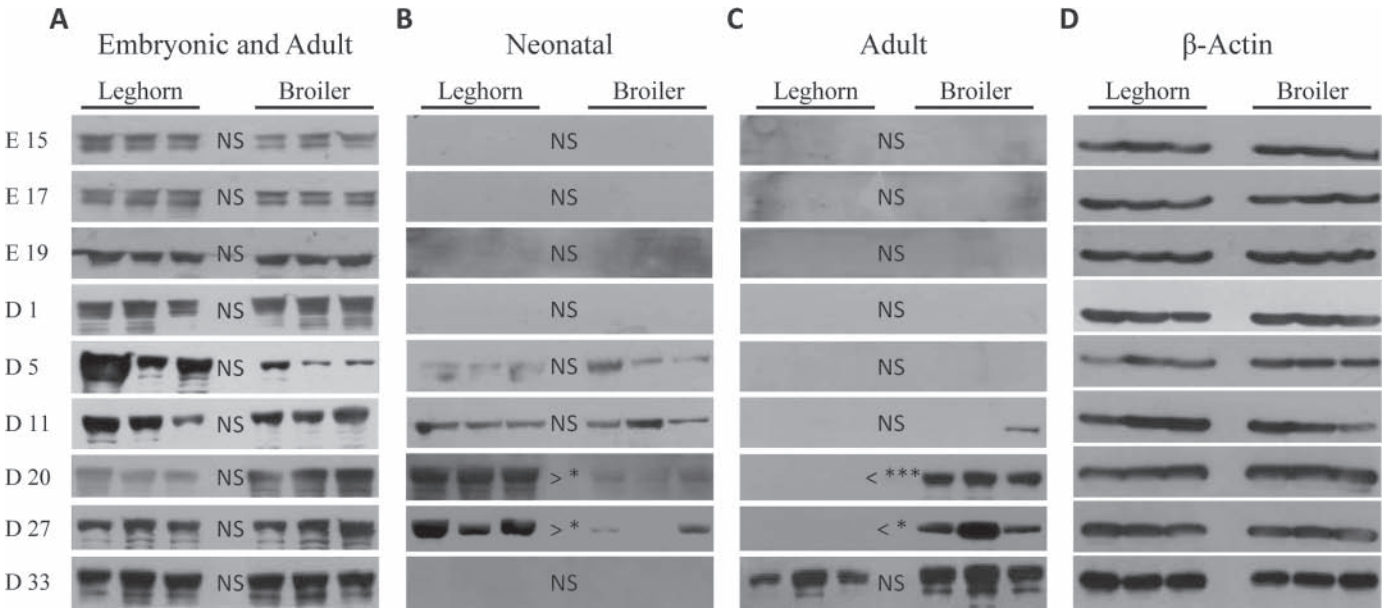

Figure 2. Breed comparison of the different myosin heavy chain (MyHC) isoforms between broilers and Single Comb White Leghorns (SCWL). A. Expression of embryonic and adult chicken MyHC isoforms using EB165 monoclonal antibody. Each lane represents one individual bird. n = 3 per time point and breed. B. Expression of neonatal chicken MyHC isoform using 2E9 monoclonal antibody. Each lane represents one individual bird. n = 3 per time point and breed. C. Expression of adult chicken MyHC isoform using AB8 monoclonal antibody. Each lane represents one individual bird. n = 3 per time point and breed. D. β-actin. Each lane represents one individual bird. n = 3 per time point and breed. $^*P < 0.05$, $^{***}P < 0.001$. D = posthatch day; E = embryonic day.

decline in neonatal isoform expression in both breeds as well as the increase in MyHC detection by EB165. These results further indicate that muscle fibers of the PM are maturing faster in the broiler chicken.

When individual expression of Pax7 (Figure 1A) was compared between the 2 breeds there was little difference in the expression levels from embryonic d 15 to 19, although broilers did show slight continued expression of PAX 7 through d 1. The breed comparison shown in Figure 1B shows little difference in expression level from embryonic d 15 to 19 with significantly higher expression of Pax7 in broilers at d 1 ($P < 0.05$; Leghorn 0.37 ± 0.23 vs. broiler 0.79 ± 0.12) posthatch. This suggests that broilers may undergo myogenesis longer than SCWL, which could lead to a larger number of myofibers.

Genetic selection has had a profound and visible effect on muscle growth and development in all the domestic poultry species resulting in extreme differences in muscling phenotypes. Because of these differences, the effects of this selection has been widely investigated in chickens (Aberle and Stewart, 1983; Scheuermann et al., 2004; Shin et al., 2009), turkeys (Velleman et al., 2003), and quail (Campion et al., 1982; Ye et al., 1999). It has been reported that broiler chicken muscle cells have a faster rate of proliferation (Blunn and Gregory, 1935; Moss, 1968), myofibers with a larger diameter (Smith, 1963), a faster rate of radial hypertrophy of myofibers (Aberle and Stewart, 1983), a larger number of myofibers (Scheuermann et al., 2004), and thus, greater muscle mass (Mizuno and Hikami, 1971) than SCWL chickens.

This study found differences in temporal expression of MyHC isoforms between the 2 breeds of chicken. Broilers transitioned from embryonic MyHC to neonatal MyHC on d 5 posthatch and then from neonatal MyHC to adult MyHC on d 20 posthatch. The SCWL transitioned from embryonic MyHC to neonatal MyHC on d 5 posthatch also. The SCWL transitioned from neonatal MyHC to adult MyHC on d 27 posthatch, 7 d later than broilers. These data indicate that the neonatal to adult MyHC transition is occurring faster in broilers than SCWL, indicating earlier maturation and terminal differentiation of the myofibers in PM in broilers. This shows that broilers not only have larger muscle growth at faster rates than SCWL, but they also have more mature muscle fibers than SCWL at the same age. Broilers are not a specific breed and there are many lines of chickens that are called broilers that have different growth characteristics. It is likely that the broiler line which expresses adult MyHC isoform earliest may also be the line that has the fastest PM growth.

Broilers displayed significantly higher expression of Pax7 at embryonic d 19 and d 1 posthatch than SCWL. These data are consistent with the report of higher mRNA expression of Pax7 in broiler chickens by Shin et al. (2009), which demonstrated significantly ($P < 0.05$) higher mRNA levels of Pax7 in broiler chickens than SCWL at d 1 and a significant decrease in Pax7 mRNA in the broiler, but not in the SCWL, at d 5. Although the transition to neonatal MyHC in the present study appears to coincide with the downregulation of Pax7 demonstrated by Shin et al. (2009), differences in ex-

pression of neonatal MyHC between SCWL and broilers were not present at d 5. This indicates that there is no association between the downregulation of Pax7 in broilers at d 5 and neonatal MyHC expression. Despite the lack of an association between Pax7 expression and neonatal MyHC expression, these data do suggest that broilers may have a lager satellite cell population than SCWL due to significantly higher expression of Pax7 at embryonic d 19 and d 1. An increased satellite cell population in broilers would allow greater potential for hypertrophic growth in broilers than SCWL.

The functional diversity of the MyHC isoforms is still not known; however, the data in this report indicate that there is a relationship between faster growth of the PM and the rate of transition of MyHC isoforms within the fast skeletal muscle of the PM. This knowledge advances our understanding of the differences between broiler and SCWL muscle growth and development, and may indicate a selection marker for improvement of broiler PM growth.

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