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Ontogeny of skeletal and cardiac muscle mitochondria oxygen fluxes in two breeds of chicken

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ABSTRACT

From its earliest days of domestication, the domestic chicken (*Gallus gallus domesticus*) has been selectively bred for specific traits. Decades of genetic selection have resulted in significant dissimilarities in metabolism and growth between breeds, in particular fast-growing broilers and highly productive layers. A chicken develops the capacity to elevate metabolism in response to decreases in ambient temperature upon hatching, including well-developed methods of regulating thermogenesis. However, a differential timing between incipient endothermic capacities of broiler and layer strains exists. Although both broiler and layer chicks show the hallmark rapid attainment of endothermic capacity of precocial birds, endothermic capacity of broilers matures faster than that of layers. Here we characterized changes in morphology and mitochondria physiology during the developmental transition as the animals become endothermic. Changes in body mass occurred at a faster rate in broilers, with hatching embryos showing significant increases over embryonic body mass, while layers did not exhibit significant differences in mass until after hatch. Heart and liver both exhibited rapid growth upon hatching that occurred with little change in body mass in both breeds. Skeletal and cardiac mitochondrial respiration capacity in broilers increased from the embryonic stage through hatching. Oxidative phosphorylation was more tightly coupled to ATP production in broilers than layer muscles during external pipping. By selecting for faster growth and higher meat yield, the physiological transition from ectothermy to endothermy was also affected: differences in whole-animal, tissue, and organelle responses are evident in these two divergent breeds of chicken.

1. Introduction

From its earliest days of domestication, > 8000 years ago (Rubin et al., 2010; Siegel et al., 1992), the domestic fowl (considered a subspecies of the extant Red Junglefowl (*Gallus gallus*) from which it was derived), more commonly known as the chicken (*Gallus gallus domesticus*), has been bred for traits such as greater meat and/or increased egg production, ornamentation, ease of care, and aggressive behavior. Broiler strains have been selected for rapid growth and high meat yield, while layer strains have been bred for increased egg production. Decades of genetic selection have resulted in substantial differences in mechanisms of growth and development, which in turn resulted in significant dissimilarities in metabolism between broilers and layers (Druyan, 2010; Sato et al., 2006). These dissimilarities are evident within the first 48 h of embryonic development, continue to manifest after hatching and well into maturity (Druyan, 2010; Ho et al., 2011; Janke et al., 2004; Konarzewski et al., 2000). Inherent differences between domesticated chicken breeds serve to make broiler and layers, with their divergent phenotypes, useful models for investigations of physiological development (Jackson and Diamond, 1996).

In spite of faster growth rates, broiler chickens exhibit lower basal metabolic rates, are more efficient at retaining metabolizable energy for growth, and have lower maintenance energy expenditure than layers (Konarzewski et al., 2000; Swennen et al., 2007). Variations in foraging behavior (Saito et al., 2004; Swennen et al., 2007), protein synthesis, heat production, and lipid metabolism between broiler and layer strains (Muramatsu et al., 1987; Romijn and Lokhorst, 1966; Sato et al., 2006) contribute to these metabolic differences. Crossley and Altimiras (2012) found that selection for rapid growth or egg-laying has resulted in cardiovascular system differences and differing responses to hypoxic exposure during embryonic development. Physiological differences between strains are also evident at the organelle level: studies of isolated skeletal muscle mitochondria of broiler chickens show higher phosphorylation activity and lower proton leak than those of layers, resulting in higher efficiency of oxidative phosphorylation, which presumably may partially account for the lower basal metabolic rate, higher feed efficiency, and faster growth rates seen in broiler strains (Toyomizu et al., 2011).

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As a precocial species, an embryonic chicken has all the prerequisites to respond appropriately to decreases in ambient temperature upon hatching, including well-developed methods of regulating thermogenesis (Tzschentke and Rumpf, 2011). However, as might be expected with the numerous differences between broilers and layers, differences in developmental timing between incipient endothermic capacities of broiler and layer strains exist. Developmental timing of an endothermic heart rate response to cooling occurred one day earlier in broiler hatchlings than layer hatchlings (Yoneta et al., 2007). Even though young broiler chicks have less mature musculature than layers of the same age and therefore produce less heat per unit mass, they achieved a higher peak metabolic rate due to their overall higher muscle mass (Konarzewski et al., 2000). Despite the paramount role of mitochondrial function in regard to metabolic development, the developmental trajectory of oxidative phosphorylation (OXPHOS) capacity of skeletal and cardiac muscle for either breed type has not been well characterized.

Such differences as previously discussed between chicken strains make this domesticated species a valuable model system to investigate physiological traits underlying differing phenotypes without the complication of phylogeny. Although both breeds have been extensively selectively bred and differ greatly from the extant ancestral type, the Red Junglefowl, the broiler chicken has served as a useful model for understanding physiological traits required for rapid muscle growth (Crossley and Altimiras, 2012). Endothermy and homeothermy initially appear in precocial birds during the first days of post-hatching (Sirsat et al., 2016b; Tzschentke and Rumpf, 2011); therefore, the current developmental study focused on characterizing changes in morphology and mitochondrial physiology during this narrow window of time in which broiler and layer chicken embryos switch from an ectothermic, embryonic phenotype to an endothermic, hatchling phenotype, rather than exploring diverging morphological phenotypes of the breeds as they continue to mature.

2. Methods

2.1. Animals and husbandry

Broiler-type (Cornish X Rock) and layer-type (White Leghorn) eggs were obtained from commercial producers (Broiler—Blanco Industries, McKinney, TX, Layer—Texas A & M Poultry Science Center, College Station, TX and Layer—Red Bluff Farm, Iowa Park, TX) and incubated at 37.5 °C and 60% RH in an upright, cabinet-style incubator (Model 1202, G.Q.F. Mfg., Savannah, GA). Eggs were automatically turned along their polar axis every 4 h. Hatchlings were maintained in a Hatchrite incubator at 35 °C with ad libitum access to water and food on a 12–12 h light cycle. Measurements were conducted on embryos at day 18 of incubation (D18—out of a 21-day total incubation period for both breeds), during internal and external pipping (IP and EP—the two stages of hatching), and on hatchlings 0, 3, and 7 days post-hatching (dph). Eggs and hatchlings were euthanized by exposure to isoflurane to induce anesthesia followed by decapitation. Whole body and organ wet masses, including yolk sac, cardiac ventricles, and liver were measured. Pectoralis/breast (*M. pectoralis pars thoracicus*), femorotibialis/thigh (*M. femorotibialis medialis/intermedialis/lateralis*) and cardiac ventricle samples were dissected in preparation for analysis of mitochondrial respiration. Wet mass of pectoralis/breast (*M. pectoralis pars thoracicus*) and femorotibialis/thigh (*M. femorotibialis medialis/intermedialis/lateralis*) was measured for broilers at all developmental stages mentioned previously, however; mass measurements for these muscles were obtained only at the EP and 3 dph stages in layers.

2.2. Permeabilized fiber mitochondrial respiration

Permabilization of pectoralis/breast (*M. pectoralis pars thoracicus*) and femorotibialis/thigh (*M. femorotibialis medialis/intermedialis/lateralis*) skeletal muscle fibers and heart ventricle cardiomyocytes for mitochondrial respiration was performed as in Sirsat et al. (2016b) and Kuznetsov et al. (2008). Muscle samples were removed from the animal, placed in ice-cold BIOPS solution, and carefully teased apart with fine-tipped forceps. Tissues were then gently shaken for 30 min in 2 ml of BIOPS containing 50 mg ml^{-1} saponin to permeabilize the fibers. Tissues were then washed three times for 10 min each in 2 ml of MiR05 with gentle mixing. Fibers were blotted dry on a Kimwipe, weighed (Analytical XA Balance, Mettler-Toledo, Columbus, OH, USA) and placed in a respiration chamber for analysis. Tissues were maintained at 4 °C during the permeabilize protocol.

Permeabilized skeletal and cardiac muscle fibers weighing between 3 and 5 mg wet mass were placed into 2 ml of MiR05 respiration medium containing catalase (280 units/ml) in an Oroboros Oxygraph-2k microrespirometer (Oroboros, Innsbruck, Austria). A hyperoxygenated environment in the respiration chambers was produced by additions of 2–5 µl H_2O_2 (200 mM) to avoid potential limitations in O_2 diffusion into the permeabilized fibers (Pesta and Gnaiger, 2012). Upon stabilization of oxygen consumption, malate (0.5 mM) and glutamate (10 mM) were added to stimulate non-phosphorylating 'resting' mitochondrial respiration in the absence of adenylates ($LEAK_N$). This was followed by addition of 5 mM ADP to stimulate oxidative phosphorylation through complex I ($OXPHOS_{CI}$). Then, 10 µM cytochrome *c* was provided to test for mitochondrial membrane integrity. Because cytochrome *c* cannot cross an intact outer mitochondrial membrane, any substantial increase in oxygen flux after addition of cytochrome *c* indicates damaged mitochondria. Oxygen flux measurements that exhibited an increased flux > 15% after addition of cytochrome *c* were not included in data analysis. Succinate (20 mM) was then provided to stimulate oxidative phosphorylation through complexes I and II ($OXPHOS_{CI+CII}$). Rotenone (1 µM), an inhibitor of Complex I that halts NADH oxidation, was added to assess oxidative phosphorylation via complex II alone ($OXPHOS_{CII}$) followed by addition of antimycin A (2.5 µM), which inhibits the passing of electrons from Complex III, resulting in termination of oxidative phosphorylation. Tetramethylphenylenediamine (TMPD, 0.5 mM), in the presence of ascorbate (2 mM), acts as an artificial substrate for reducing cytochrome *c*; therefore, addition following antimycin A inhibition serves as a measure of Complex IV activity.

Temporal changes in permeabilized fiber oxygen flux, including $LEAK_N$, $OXPHOS_{CI}$, $OXPHOS_{CI+CII}$, $OXPHOS_{CII}$, and cytochrome oxygenase (Complex IV) capacity of broiler D18 and EP embryos as well as 1 and 3 dph (day post-hatching) hatchlings were examined to establish a general developmental trajectory of the chicken. To explore effect of breed type, permeabilized fiber oxygen flux was also measured in muscle samples from the layer breed during EP and 3 dph.

2.3. Solutions

Solutions were made according to Pesta and Gnaiger (2012). Biopsy preservation solution (BIOPS) contained 10 mM CaK_2-EGTA, 7.23 mM K_2-EGTA, 20 mM imidazole, 20 mM taurine, 50 mM K-MES, 0.5 mM dithiothreitol, 6.56 mM $MgCl_2 \cdot 6H_2O$, 5.77 mM ATP and 15 mM phosphocreatine adjusted to pH 7.1. Mitochondrial respiration medium (MiR05) was composed of 0.5 mM EGTA, 3 mM $MgCl_2 \cdot 6H_2O$, 60 mM lactobionic acid, 20 mM taurine, 10 mM KH_2PO_4, 20 mM HEPES, and 110 mM D-sucrose, and 1 g l^{-1} BSA adjusted to pH 7.1. Solutions were filtered through a nitrocellulose membrane via a Merck Millipore system to remove contaminants before experimental use and storage. All chemicals were purchased from Sigma Aldrich (St. Louis, MO).

2.4. Statistical analysis

Differences in yolk-free body mass, skeletal muscle mass, heart mass, liver mass, and fractional heart and liver mass between broiler and layer breeds were examined by two-way ANOVA with breed and

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