Animal domestication has long been recognized for its value in illustrating the effects of selection on anatomical and physiological traits (Belyaev, 1979; Trut et al., 2009). Animals can be bred for traits of particular relevance to humans, such as meat yield, milk yield, egg yield, growth rate, or tameness, and the genetic architecture of such traits can be permanently altered (Jensen and Andersson, 2005). Therefore, domesticated species provide a particularly useful platform for studying the genetic basis of many traits, and they are extensively used in genetics research (Jensen and Andersson, 2005).

In addition to providing a model for genetic studies, domesticated species are useful in investigations of the physiological features that accompany productivity traits (Jackson and Diamond, 1996). The chicken (Gallus gallus domesticus) is a species with a history of domestication that spans at least 8,000 yr (Siegel et al., 1992; Yamashita et al., 1994; Rubin et al., 2010). Selection for meat productivity during the past 100 yr has resulted in a fast-growth breed, the broiler, and a prolific egg layer, the White Leghorn. In this study, we investigated basic cardiovascular physiology in these 3 breeds at 90% of incubation. We aimed to identify breed-specific features of arterial blood pressure and heart rate as well as the physiological mechanisms regulating them. Specifically, we investigated mechanisms mediated by the autonomic nervous system by means of cholinergic and adrenergic receptors. Our overriding hypothesis was that selection for rapid growth would require an acceleration of heart rate and arterial pressure development in broilers compared with White Leghorns and the ancestral breed. The embryonic broiler is characterized by resting relative hypertensive bradycardia, whereas the White Leghorn is hypotensive. All 3 breeds maintained resting arterial pressure and heart rate via a similar β- and α-adrenergic receptor tone; however, cholinergic tone on heart rate was absent in the embryonic White Leghorn. Each breed responded differently to incubation in chronic hypoxic conditions (14% O₂). White Leghorn relied on augmenting cholinergic heart rate tone, and broilers relied on reducing β-adrenergic tone on pressure. We concluded that selection for rapid growth shifts cardiovascular regulatory plasticity to emphasize mechanisms that modulate pressure, and that selection for egg-laying capacity emphasizes mechanisms that modulate heart rate.

Key words: cardiovascular, control, hypoxia, development

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INTRODUCTION

Animal domestication has long been recognized for its value in illustrating the effects of selection on anatomical (Belyaev, 1979; Trut et al., 2009) and physiological traits (Jackson and Diamond, 1996). Animals can be bred for traits of particular relevance to humans, such as meat yield, milk yield, egg yield, growth rate, or tameness, and the genetic architecture of such traits can be permanently altered (Jensen and Andersson, 2005). Therefore, domesticated species provide a particularly useful platform for studying the genetic basis of many traits, and they are extensively used in genetics research (Jensen and Andersson, 2005).

In addition to providing a model for genetic studies, domesticated species are useful in investigations of the physiological features that accompany productivity traits (Jackson and Diamond, 1996). The chicken (Gallus gallus domesticus) is a species with a history of domestication that spans at least 8,000 yr (Siegel et al., 1992; Yamashita et al., 1994; Rubin et al., 2010). Selection for meat productivity during the past 100 yr has rendered a very productive chicken type: the broiler. This breed has an accelerated growth rate (~67.0 g/d) compared with an egg-laying breed, the White Leghorn, which grows at the rate of ~9.0 g/d (Zhao et al., 2004). The broiler is, therefore, a useful model for understanding the physiological traits required for rapid muscle growth. Jackson and Diamond (1996) reported enlarged intestinal mass and high nutrient transporter capacity in broilers in comparison to the Red Junglefowl (Gallus gallus), the ancestor of all domesticated chicken breeds. In juvenile broilers, increased growth rate, muscle mass, and assimilation capacity elevate O₂ demand without compensatory changes in the heart to increase oxygen convective transport (Druyan et al., 2007). As a result,
systemic hypoxemia can spontaneously develop due to the mismatch between cardiac output and growth rate (Wideman and Tackett, 2000; Wideman et al., 2000). Because heart mass does not increase in proportion to overall muscle mass, mechanisms that modulate cardiac output, such as autonomic modulation of heart rate, might be expected to adjust to meet the demands placed on the heart.

Unlike broilers, White Leghorn chickens have been selected for reproductive output (to produce more eggs and larger eggs). In this case, the selective pressures have been placed on liver function and capacity to increase lipid deposition as adults, and therefore there is reduced selection pressure on convective transport capacity.

The growth potential differences between ancestral (Red Junglefowl) and domestic chicken breeds are present early in life. Growth in broiler chickens during embryonic development and within the first few days after hatching is accelerated in comparison to growth of egg-laying breeds (Ohta et al., 2004; Zhao et al., 2004). We have also demonstrated that embryonic organ growth is differentially affected for Red Junglefowl, broilers, and White Leghorns (egg layers) when growth is impaired by low oxygen availability (Lindgren and Altimiras, 2011). Given that embryonic cardiac growth is differentially affected before hatching, selection for differing productivity traits could manifest in divergent patterns of cardiovascular physiological development as well as its regulatory mechanisms. Understanding these differences is relevant to the poultry industry to optimize the efficacy of incubation regimens and conditions to yield animals of high commercial value.

The goal of our study was to investigate embryos of domestic chickens historically selected for increased growth rate or egg-laying capacity to assess how cardiovascular regulatory mechanisms are coupled to these features. Three chicken breeds, broilers, White Leghorns, and Red Junglefowl, were used to assess basal blood pressure and heart rate as well as cholinergic and adrenergic tones on the heart and vasculature. Two highly selected breeds were used to represent the consequences of rapid growth (broilers) or egg-laying capacity (White Leghorns), and the Red Junglefowl represented the ancestral condition. Incubation in low-oxygen conditions was used to evaluate possible developmental differences in regulatory plasticity triggered by a metabolic challenge. Our overriding hypothesis was that selection for rapid growth would require an acceleration of heart rate and arterial pressure development in broilers compared with White Leghorns and the ancestral breed. Further, given the high convective transport demands placed on the cardiovascular system of broilers in adulthood, we expected the phenotypic plasticity for cardiovascular regulation to be reduced in this breed. Overall, we found a difference in baseline cardiovascular function, cholinergic tone, and sensitivity to chronic hypoxic incubation between the breeds.

**MATERIALS AND METHODS**

**Source of Embryonic Chickens**

Eggs of 2 domesticated chicken breeds, White Leghorn and Ross 308 breed broiler, were obtained from Gimrånäs AB (Herliljunga, Sweden) and Lantmännens Svehatch AB (Väderstad, Sweden), respectively. Eggs of Red Junglefowl chickens were obtained from the experimental captive population kept in Sweden since 1993 (first at Götala Research Station, Swedish Agricultural University, Skara, and currently at Vreta Agricultural School, Linköpings universitet, Linköping). Freshly laid eggs were transported to the Department of Biology at the University of Linköping, placed in cold storage (≈15°C), and turned automatically once every hour until the initiation of the study (within 2 wk). At the onset of the study, eggs were weighed to the nearest 0.1 g, numbered, and randomly placed in an incubator (Masallés model 25 HS, Barcelona, Spain) set at either 21% \( (N_{21}) \) or 14% \( (H_{14}) \) oxygen. All eggs were incubated at 37.8 ± 0.5°C with a relative humidity of 45%, and eggs were automatically turned 90° on the long axis every hour. In all cases, the \( N_{21} \) treatment was considered as the control conditions for all breeds.

**Control of Ambient Oxygen**

During Incubation

We maintained 14% \( O_2 \) in the hypoxic incubator by mixing air and nitrogen using a gas mixer (model 3500 HL, Sechrist Industries Inc., Anaheim, CA) at a flow rate of 9 L/min. Oxygen levels in the incubators were continuously monitored using a galvanic oxygen sensor (DD103, Pico Technology, UK), and the data was stored using a PC-based software package (PicoLog, PicoTechnology, UK).

**Surgical Procedures**

Total incubation time for chickens is 21 d. During d 19 of incubation, eggs were taken from incubators, weighed to the nearest 0.1 g, candled to locate a tertiary chorioallantoic artery, the location was marked, and the egg was placed in a custom-made temperature-controlled surgical chamber constructed of Plexiglass, with the chamber temperature set at 37.8 ± 0.5°C. A portion of the eggshell was removed, and an occlusive catheter was inserted into the previously located artery under a dissection microscope (Wild M38, Heerbrugg, Switzerland) using a heat-pulled polyethylene tube (PE-90) filled with 0.9% heparinized saline, as previously described (Altimiras and Crossley, 2000). When catheterization was completed, the catheter was fixed to the shell with cyanoacrylic glue and the egg was placed blunt end up in a water-jacketed temperature-controlled experimental chamber set at 38 ± 0.5°C.

The experimental chamber consisted of a stainless steel water-jacketed trough fitted with a lid containing...
4 circular openings. Eggs were placed blunt end up on a small amount of vermiculite within the openings, resulting in the egg resting within the trough below the level of the lid. Each cell was fitted with a clear lid with small openings to externalize the arterial catheter and to pass gas mixtures. Room air (21% O2) saturated with water vapor was passed through the chamber at 200 mL/min. Before the gas mixture entered the experimental chamber containing the embryo, it was warmed by passing through a 1-m coil of polyethylene tubing (PE-50) lining the inside of the water-jacketed chamber. During the course of the study, chamber temperature was continuously monitored with a thermometer (Bat 12, Bailey Instruments Inc., Saddle Brook, NJ).

**Signal Recording and Calibration**

Each catheter was attached to a pressure transducer (DP6100, Peter von Berg, Germany) connected to a 4CHAMP amplifier (Somedic AB, Sweden), and the output was connected to a Powerlab data acquisition system (ADInstruments Inc., Colorado Springs, CO) connected to a laptop computer (Macintosh Powerbook G4). Data acquisition software (Chart 6) was used to collect arterial pressure continuously (P<sub>mean</sub>) at a frequency of 400 Hz. Heart rate (f<sub>H</sub>) was instantaneously calculated from the pressure signal. In all cases, reference zero pressure was set at the top of the experimental bath. At the completion of the study, egg position from the top of the chamber was determined, and pressure values were corrected as previously described (Altimiras and Crossley, 2000).

**Experimental Protocol**

Prior to experimental manipulation, P<sub>mean</sub> and f<sub>H</sub> were allowed to stabilize for at least 45 min. Embryos that failed to stabilize were eliminated from the experiment. In all cases, all breeds and experimental groups were studied in the experimental chamber under normoxic conditions. The study consisted of serial bolus injections of antagonists to block the cholinergic and adrenergic receptors that are involved in cardiovascular control. Pharmacological agents were injected into each embryo via a T connector in the arterial catheter. A 0.9% saline flush of twice the antagonist injection volume followed each drug treatment. Injection volumes were adjusted for embryonic mass to not exceed 5% of the total estimated blood volume (Altimiras and Crossley, 2000; Crossley and Altimiras, 2000; Crossley et al., 2003; Lindgren et al., 2011). Drugs used in the study included atropine (1 mg/kg, Sigma-Aldrich, St. Louis, MO) to characterize vagal tone by targeting cholinergic receptors, propranolol (3 mg/kg, Sigma-Aldrich) to characterize β-adrenergic receptors, and phenolamine (1 mg/kg, Sigma-Aldrich) to characterize the role of α-adrenergic receptors by targeting α-adrenergic receptors. Following the injection of a drug, blood pressure and heart rate were recorded until they stabilized, which occurred approximately 20 min postinjection. For the purposes of this study, “tone” was defined as continuous stimulation of a receptor type maintaining basal cardiovascular function. In each case, the values for P<sub>mean</sub> and f<sub>H</sub> just before each antagonist injection were used as the control for a given injection within an individual embryo.

After completing the experimental protocol, embryos were euthanized with an arterial injection of pentobarbital (50 mg/kg) and saturated KCl. The embryo was then removed from the egg to determine yolk-free embryonic wet mass with a precision of 0.1 g and heart mass with a precision of 1 mg. This protocol was approved by the local ethical board (Linköpings djurförsöksnämnd) under permit Dnr.45–03 to J. Altimiras.

**Statistical Analysis**

A 2-way ANOVA, with chicken breed and incubation treatment (N21 or H14) as independent factors, was used to determine significant differences (P < 0.05) in the mass parameters (embryo, heart, and initial egg mass) control mean arterial pressure (P<sub>mean</sub>) and f<sub>H</sub>. These data are presented in Table 1. A paired Student’s t-test was used to determine if the injection of a drug significantly altered P<sub>mean</sub> or f<sub>H</sub> from pre-injection values for each breed and incubation condition. All embryos within each protocol were treated identically (that is, they received the identical sequence of drugs), and therefore, the paired t-test is appropriate. The results of these tests are presented in Table 2.

Differences in the intensity of the response to the drugs between the breeds and under different oxygen conditions were assessed using a 2-way ANOVA on the arcsine-transformed percent changes in P<sub>mean</sub> and f<sub>H</sub>. Normalization and transformation of the data was necessary to restrict the comparison to the effect of the drug, due to the large changes in P<sub>mean</sub> and f<sub>H</sub> recorded after the injection of the different drugs. In all cases, Fisher’s least significant difference post hoc comparison was used to isolate significant differences between breed and treatment (P < 0.05).

All statistical tests were conducted with the software package Statistica (version 5, Statsoft, Tulsa, OK). All data are presented as mean ± SE. Sample size for each drug treatment for White Leghorn (W), the broiler (B), the Red Junglefowl (J) from each incubation condition are as follows: atropine; N21 W (n = 9), B (n = 15), J (n = 12) and H14 W (n = 8), B (n = 10), J (n = 9), propranolol; N21 W (n = 9), B (n = 15), J (n = 12) and H14 W (n = 8), B (n = 10), J (n = 8), phenolamine; N21 W (n = 9), B (n = 8), J (n = 6) and H14 W (n = 8), B (n = 8), J (n = 6).
RESULTS

Hypoxic Incubation Impairs Embryonic Growth

As expected, H14 impaired embryonic growth in all breeds, as indicated by a reduction in wet mass by 6.7% in White Leghorn, 10.2% in broiler, and 11.5% in Red Junglefowl (Table 1). Heart wet mass was also reduced by 10.5% in White Leghorn, 18.5% in broiler, and 14.2% in Red Junglefowl. The relative heart size in broiler was significantly larger than in White Leghorn and Red Junglefowl, but the reduction in relative heart size due to H14 was not different between breeds.

Baseline Blood Pressure and Heart Rate Vary Among Breeds and Is Affected by Hypoxia

Baseline Pmean and fH in White Leghorn and broiler embryos studied in normoxic conditions were in general agreement with those in prior studies (van Golde et al., 1996, 1997; Höchel et al., 1998; Crossley and Altimiras, 2000; Yoneta et al., 2006), and values for Red Junglefowl fell within a range between values for White Leghorn and Red Junglefowl. The relative heart size in broiler was significantly larger than in White Leghorn and Red Junglefowl, but the reduction in relative heart size due to H14 was not different between breeds.

Table 1. Initial egg mass, embryonic mass, heart mass, and heart-to-body mass percentage of White Leghorn (W), broiler (B), and Red Junglefowl (J) embryos studied chronically incubated under normoxia (N21) or hypoxia (H14) at d 19 of incubation1

<table>
<thead>
<tr>
<th>Variable</th>
<th>W</th>
<th>H14</th>
<th>B</th>
<th>H14</th>
<th>J</th>
<th>H14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial egg (g)</td>
<td>60.8 ± 1.0a</td>
<td>59.0 ± 0.6a</td>
<td>68.8 ± 1.4b</td>
<td>68.4 ± 1.5b</td>
<td>38.7 ± 0.8c</td>
<td>38.7 ± 0.7c</td>
</tr>
<tr>
<td>Embryonic (g)</td>
<td>28.3 ± 0.8a</td>
<td>26.4 ± 0.4a</td>
<td>36.1 ± 0.9b</td>
<td>32.4 ± 0.7b</td>
<td>20.0 ± 0.4c</td>
<td>17.7 ± 0.3c</td>
</tr>
<tr>
<td>Heart (g)</td>
<td>0.19 ± 0.01a</td>
<td>0.17 ± 0.01a</td>
<td>0.27 ± 0.01b</td>
<td>0.22 ± 0.01b</td>
<td>0.120 ± 0.003c</td>
<td>0.103 ± 0.004c</td>
</tr>
<tr>
<td>Heart/embryonic (%)</td>
<td>0.65a</td>
<td>0.63a</td>
<td>0.70b</td>
<td>0.73b</td>
<td>0.61a</td>
<td>0.58a</td>
</tr>
</tbody>
</table>

a–cDiffering lettering indicates significant (P < 0.05) differences between embryonic strains in each condition. 
1Data are presented as mean values ± SE.
*Indicates a significant difference within strain due to incubation conditions.

Cholinergic Tone Is Similar Between Breeds but Absent in N21 White Leghorns

Cholinergic blockade with atropine significantly increased fH in broiler (Δ19 ± 4 min⁻¹ on average) and Red Junglefowl (Δ17 ± 2 min⁻¹) in both N21 and H14 embryos (Figure 2B). For White Leghorn, only H14-incubated embryos responded to the atropine injection with a significant increase in fH (Δ23 ± 2 min⁻¹) similar in intensity to that of the other breeds. Atropine had no significant effect on Pmean in any of the breeds or in either oxygen condition (Figure 2A).

β-Adrenergic Tone on Blood Pressure Is Abolished in H14 Broilers

β-Adrenergic blockade altered Pmean and fH in all breeds (Table 2). In N21 embryos, β-blockade produced a significant increase in Pmean for all breeds. Specifically, Pmean increased by Δ0.6 ± 0.1 kPa for White

Table 2. The significance (P < 0.05) changes in arterial pressure (Pmean) and heart rate (fH) from control values after injections of atropine (ATROP), propranolol (PROP), and phentolamine (PHENTO) in White Leghorn (W), broiler (B), and Red Junglefowl (J)1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Drug</th>
<th>W N21</th>
<th>H14</th>
<th>B N21</th>
<th>H14</th>
<th>J N21</th>
<th>H14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pmean</td>
<td>ATROP</td>
<td>NS (9)</td>
<td>NS (8)</td>
<td>NS (15)</td>
<td>NS (10)</td>
<td>NS (12)</td>
<td>NS (9)</td>
</tr>
<tr>
<td>fH</td>
<td>ATROP</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Pmean</td>
<td>PROP</td>
<td>* (9)</td>
<td>* (8)</td>
<td>* (15)</td>
<td>NS (10)</td>
<td>* (12)</td>
<td>* (8)</td>
</tr>
<tr>
<td>fH</td>
<td>PROP</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Pmean</td>
<td>PHENTO</td>
<td>* (9)</td>
<td>* (8)</td>
<td>* (8)</td>
<td>* (8)</td>
<td>* (6)</td>
<td>* (6)</td>
</tr>
<tr>
<td>fH</td>
<td>PHENTO</td>
<td>*</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

1Variables that were unaffected by a given drug treatment are indicated as NS. The number of embryos studied from each breed is indicated in parentheses.
*Indicates a significant difference from control values in the normoxic (N21) and 14% O2 (H14) embryos at 19 d of incubation.
Leghorn, $\Delta 0.7 \pm 0.1$ kPa for broiler, and $\Delta 0.7 \pm 0.1$ kPa for Red Junglefowl (Table 2; Figure 3A). The H14 White Leghorn and Red Junglefowl embryos responded to $\beta$-blockade with a change in $P_{\text{mean}}$ that was similar to that in the N21 groups; however, $P_{\text{mean}}$ was unaffected in H14 broiler embryos (Figure 3A).

$\beta$-Adrenergic blockade significantly ($P < 0.05$) decreased $f_H$, which ranged from 32 ± 3 to 39 ± 3 min$^{-1}$ ($\Delta 14\%$), in both N21- and H14-incubated groups in all breeds (Table 2; Figure 3B). The intensity of the $f_H$ response to $\beta$-blockade was independent of breed and oxygen condition.

### α-Adrenergic Chronotropic Tone Is Absent in Broiler Chickens

In both oxygen conditions and for all breeds, α-adrenergic blockade with phentolamine decreased $P_{\text{mean}}$ significantly, by an average of 23% (Table 2; Figure 4A).

The $f_H$ response to $\alpha$-blockade was significant ($P < 0.05$) for White Leghorn ($-\Delta 34 \pm 3$ min$^{-1}$) and Red Junglefowl H14 group only ($-\Delta 30 \pm 9$ min$^{-1}$), as shown in Table 2 and Figure 4B. Phentolamine had no effect on $f_H$ in broiler, and H14 did not alter the $P_{\text{mean}}$ response to the blockade in any breed.

### DISCUSSION

Domesticated species are valuable model organisms that can be used to investigate the physiological traits underlying differing phenotypes without the complication of phylogeny (Jackson and Diamond, 1996; Zhao et al., 2004; Zoer et al., 2009). In this study, we investi-
gated the cardiovascular regulatory mechanisms that support productivity traits in embryonic chickens. Our findings indicate that selection for rapid growth or egg-laying capacity in chickens alters the tonic contribution of adrenergic and cholinergic autonomic mechanisms to the regulation of blood pressure and heart rate. Selection for somatic growth in broiler chickens favors mechanisms that elevate vascular resistance and blood pressure during embryonic development, coupled to an increased plasticity for β-adrenergic vascular tone. We also found that selection for reproductive output in an egg-laying breed, the White Leghorn, is coupled to a greater plasticity in cholinergic tone, with changes in cardiac output predominantly achieved through changes in heart rate rather than vascular resistance, without concurrent effects on blood pressure.

**Blood Pressure Differs Between the Ancestral Red Junglefowl and Commercial Breeds**

Differences in embryonic blood pressure were observed and correlated with selection for productivity traits. P_mean in both selected breeds differed from that of the ancestral Red Junglefowl. Although White Leghorn embryos were relatively hypotensive, broiler embryos were relatively hypertensive (Figure 1A).
extension, the embryonic response to chronic hypoxic incubation of all 3 breeds illustrates an ancestral and domesticated phenotype.

When facing a reduction in oxygen availability, the ancestral response is hypotension, as indicated by the response of Red Junglefowl. Broiler embryos have the same response, with hypoxic hypotension due to a decrease in vascular resistance combined with an increase in vascular density. Prior studies examining the effects of chronic hypoxic incubation of chicken embryos have demonstrated both an increase in chorioallantoic membrane (CAM) vascular density and a decrease in structural vascular resistance (Adair et al., 1987, 1988; Dusseau and Hutchins, 1989; Le Noble et al., 1993). A parallel vascular response is shown by adult hypertensive rats subjected to chronic hypoxic conditions (Jakoubek et al., 2008; Vilar et al., 2008). Concurrent with the increased vascular density in chickens, there is an increased vasodilatory tone in chronic hypoxia that contributes to the hypoxic hypotension seen in this study (Lindgren et al., 2011).

Interestingly, hypoxia did not affect resting blood pressure of White Leghorn, indicating a reduced sensitivity to limitations of oxygen availability (Figure 1A), which was also indicated by the lesser effects of hypoxia on organ growth in White Leghorn compared with the effects of hypoxia on organ growth in other breeds (Lindgren and Altimiras, 2011). Such breed differences in cardiovascular resting variables can be explained by changes in the autonomic tones on the heart and vasculature, as shown by the cholinergic and adrenergic blockade responses (Figures 2, 3, and 4).

**Cardiovascular Regulation in Broiler Embryos**

The baseline hypertension in broilers is likely due to an elevated stroke volume (Berne and Levy, 1997), given that there was no difference in adrenergic tone between the control broiler and ancestral Red Junglefowl. Using heart mass as a metric of stroke volume during development as previously proposed (Hillman, 1976; Walsberg et al., 1986; Birchard and Reiber, 1996; Gamperl et al., 2002), the larger relative heart mass in broilers (Table 1) would cause such an increased stroke volume. The reduction in heart rate could also be coupled to a difference in baroreflex function between the breeds, resulting in a relative bradycardia in the broiler breed due to increased baroreceptor activity.

We also observed that chronic hypoxia blunted the β-adrenergic tone on blood pressure in broilers (Figure 3A). Because the loss of β-adrenergic tone on blood pressure does not occur in White Leghorns or Red Junglefowl, it is likely that the mechanism is coupled to the selection for somatic growth in broilers (Figure 3A).

Prior studies have established that cardiac contractility in chronic hypoxia is maintained by β-adrenergic stimulation associated with receptor sensitization (Lindgren and Altimiras, 2009) and doubling of circulating catecholamine levels (Mulder et al., 2000; Lindgren et al., 2011). We estimate that, in control conditions, the adrenergic effect keeps contractility at 10% of its maximum, decreasing to 34% of maximum levels in hypoxic conditions. Therefore, β-adrenergic blockade has a substantial effect on cardiac output due to a decrease in heart rate (Figure 3B) and a decrease in the force of contraction in the hypoxic embryos. However, there is also a concurrent elimination of a vascular vasodilatory effect, shown by the increased sensitivity of femoral arterial rings to β-dependent vasodilation without affecting the response of chorioallantoic arteries in hypoxic-incubated embryos (Lindgren and Altimiras, 2009; Lindgren et al., 2011). Therefore, the blunted β-adrenergic tone on blood pressure in the hypoxic broiler embryos is likely attributed to a significant change in cardiac output, eliminating the change in blood pressure following β-adrenergic blockade.

**Cardiovascular Regulation in Embryos of the Egg-Laying Breed**

Control White Leghorn embryos were hypotensive relative to the ancestral Red Junglefowl (Figure 1A). However, relative heart mass was similar in White Leghorn and Red Junglefowl, although embryonic mass differed (Table 1). This, combined with the equivalent β- and α-adrenergic tone on \(P_{\text{mean}}\) in the White Leghorn and Red Junglefowl, suggests that the relative hypotension may again be the result of increased vascularization, particularly of the CAM, which receives approximately 50% of the cardiac output (Mulder et al., 1998). Interestingly, the controls and chronic hypoxic White Leghorn embryos did not differ in either resting cardiovascular parameters or the adrenergic tone on the system (Figures 3A and 4A). Overall selection for reproductive capacity in White Leghorn resulted in inadvertent selection for an animal that is relatively hypotensive and exhibits relatively less sensitivity to reduced oxygen. This difference from the Red Junglefowl was also evident in the role of cholinergic tone on the cardiovascular system.

White Leghorns diverged from the ancestral Red Junglefowl pattern in that cholinergic tone on the \(f_H\) in White Leghorns was absent under control conditions (Figure 2B), as previously reported for this breed (Tazawa et al., 1992; Altimiras and Crossley, 2000; Crossley and Altimiras, 2000; Crossley et al., 2003). Thus, we suggest that the presence of a cholinergic tone is an ancestral feature. White Leghorn exhibited a lower resting \(f_H\) in the absence of cholinergic tone under control conditions (Figure 2B) and maintained the same \(f_H\) in chronic hypoxic conditions by activating cholinergic tone (Figure 2B). Prior studies of fetal sheep subjected to bouts of hypoxic exposures have demonstrated an augmentation of cholinergic tone on fetal \(f_H\) to offset the accompanied elevation in β-adrenergic stimulation
CARDIOVASCULAR REGULATORY PLASTICITY IN CHICKEN BREEDS

(Parer, 1983). Although both baseline values and adrenergic tone on f_1 were similar to the control White Leghorn embryos measured in normoxia, prior studies have documented an elevation in circulating catecholamines during chronic hypoxia (Mulder et al., 2000; Lindgren et al., 2011). This elevation may increase the stimulatory adrenergic tone on the White Leghorn embryonic heart before d 19, inducing the onset of cholinergic tone. This represents an alternative strategy to that of broilers and may reflect a limited capacity to increase O_2 diffusion via increased CAM vascularization in the White Leghorn embryos. Thus, activating cholinergic tone would maintain constant f_1 under conditions in which catecholamine levels are elevated, increasing chronotropic stimulation in an embryonic breed with limited capacity to offset the pressor actions of this stimulus.

In conclusion, our results indicate that selection for rapid growth or egg laying in chickens has modified the capacity of the species to adjust the cardiovascular system to respond to environmental alterations during embryonic incubation. White Leghorns preferentially respond to hypoxic incubation with changes in heart rate regulation to modulate cardiac output via the early onset cholinergic tone. Broilers, on the other hand, alter β-adrenergic regulation of vascular tone when challenged with hypoxia during incubation, a reduction that may limit embryonic cardiovascular performance in the broiler, which will be exacerbated in the animal after hatching. Although comparative studies of adult White Leghorns, broilers, and Red Junglefowl have not been conducted, our study indicates that changes in cardiovascular performance between breeds may be apparent early in life and may have important implications for the poultry industry.

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