Effects of hypoxia on the mechanical properties of the aortic wall in the 19 days old chicken embryo (*Gallus gallus*)

Matin Hansen

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Author: Martin Hansen

Sammanfattning
Abstract:

Stress during prenatal development leads to effects on the cardiovascular system. Rouwet et al. (2002) stated that White Leghorn chicken embryos incubated under hypoxic conditions (15 % O_2) developed aortic hypertrophy, seen as a decrease in lumen diameter and an increase in wall/lumen ratio. In this study four different chicken strains were used: two broiler strains (Swedish, Linköping and Dutch, Maastricht) and White Leghorn and the wild close relative of the domestic chicken, the red jungle fowl. The chicken embryos were incubated under normoxic (21 % O_2) and hypoxic (14 % O_2) conditions, sampled at day 19 and processed by use of regular histological techniques to estimate the dimensions of the aorta. Wall/lumen ratio was not significantly different between hypoxia and normoxia in any of the four strains: 0.71±0.23 vs. 0.67±0.11 in normoxic and hypoxic broilers (Linköping) respectively, 0.93±0.22 versus 0.91±0.16 in broilers (Maastricht) respectively, 0.90±0.20 vs. 0.73±0.27 in jungle fowl and 0.62±0.15 vs. 0.70±0.12 in White Leghorns. However, wall thickness was significantly different in jungle fowl (0.33±0.04 vs. 0.27±0.03, normoxic and hypoxic respectively, $P<0.05$), and lumen diameter in White Leghorns (0.53±0.06 vs. 0.46±0.05, $P<0.05$) embryos. The effect of hypoxia on wall elasticity in broiler embryos was tested. No difference was found. In conclusion, no evidence of aortic hypertrophy was found, but this cannot exclude alterations in the composition of the aortic wall which will be the subject of further studies.

Nyckelord
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Hypertrophy, hypoxia, elasticity, pressure-diameter loops, aorta.

Content

1 Abstract

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Keywords:
Hypertrophy, hypoxia, elasticity, pressure-diameter loops, aorta.

2 Introduction

Chronic stress during embryonic development leading to a low birth weight is associated with an increased risk to develop coronary heart disease, hypertension, and non-insulin dependent diabetes later in life, a phenomenon widely known as the fetal origins hypothesis (Barker's hypothesis, Barker 1993). The most common cause of embryonic growth retardation is placental insufficiency, which is a combination of both malnutrition and chronic hypoxia (Ruijtenbeek et al. 2003b). The effect of these pathologies is thought to be linked to alterations caused by hypertrophy in the heart and arteries (Rouwet et al. 2002).

The walls of large blood vessels comprise three layers: tunica adventitia, tunica media and tunica intima. The adventitia is the limiting fibrous outer coat that consists almost entirely of collagen (Braun et al. 2003). The media or middle layer consists of circular and longitudinal smooth muscle cells separated by thick elastic lamellae (Braun et al. 2003). The intima or inner layer, closest to the lumen, is composed of endothelial cells and elastic fibers. Arteries are more muscular than veins and therefore have a thicker tunica media. Arteries close to

the heart (e.g. aorta) are more elastic and have a thicker intima and the elasticity of the aortic wall decreases with increasing distance from the heart.

The oscillations in blood pressure and flow generated by contractions of the heart are dampened in the arterial system by the elasticity of the arterial walls. As blood is injected into the arterial system, pressure rises and the vessel expands. As the heart relaxes, blood flow to the periphery is maintained by the elastic recoil of the vessel walls, resulting in a reduction in arterial volume.

The viscoelastic properties of mature arteries are determined largely by the relative proportions of elastin, collagen, and vascular smooth muscle cells and each of the main components of the arterial wall contributes uniquely to the overall mechanical properties of the tissue (Wells 1999, Speckmann and Ringer 1966). Although elastic, arteries become progressively stiffer with increasing distention. As a result they are easily distended at low pressures but, resist further expansion at high pressures. The transfer of stress from elastin to collagen with increasing strain (stress) has been attributed to the progressive engagement of collagen fibers that are crimped at low strains. Thus low-modulus elastin dominates wall mechanics at low stress, whereas high-modulus collagen dominates at high stress (Braun et al. 2003, Wells 1999). Although arterial elastic properties are determined by elastin and collagen, arterial wall viscosity is thought to be mainly a result of the presence of smooth muscle cells (Wells 1999).

The viscoelasticity of arteries greatly influences cardiovascular function, since it determines the interrelationship among blood pressure, blood flow, and vascular dimensions. The viscoelastic properties of the aorta and its major branches are particularly important, because they determine the input impedance that the arterial system presents to pulsatile ventricular outflow, and therefore they are important determinants of cardiac workload (Wells 1998). The proximal part of the aorta acts as a buffering chamber storing part of the ventricular stroke volume during systole. During diastole, elastic recoil of the aortic wall propels this volume to the periphery, thereby maintaining a continuous peripheral blood flow (Wells 1999).

A particular important phase of arterial remodeling occurs during the later phase in embryonic development. During this time, there are large changes in cardiovascular function, including closure of the foramen ovale and ductus arteriosus, redistribution of systemic blood flows, and substantial changes in central arterial pressure and cardiac output, and large changes in arterial dimensions and wall constituents accompany these changes in function (Wells 1999, Altimiras and Crossley 2000, Crossley et al. 2003).

It has previously been illustrated by Rouwet et al. (2002) that White Leghorn chicken embryos incubated under hypoxic (15 % O_2) conditions had smaller embryonic mass and expressed alterations in aortic morphology by a decrease in lumen diameter, leading to a high wall/lumen ratio.

The aim of this study was to investigate the effect of hypoxia on the mechanical properties of the aortic wall in the 19 days old broiler chicken embryo. The broiler chicken has been breed for fast growth and a high body mass, which in turn means a lot of strain to the cardiovascular system during development. The hypothesis therefore is that not only will the broiler chicken embryo show aortic hypertrophy but also effects on wall elasticity.

3 Materials and methods

3.1 Incubation conditions

Four different chicken strains: Broiler Linköping (fast-growing strain Ross 308), broiler Maastricht, White Leghorn and the wild close relative of the domestic chicken, the red jungle fowl were used in this study. The broiler eggs were obtained from Kläckeribolaget (Väderstad, Sweden), the jungle fowls eggs were obtained from Götala Research Station (SLU, Skara), the White Leghorns eggs were obtained from (`t Anker, Ochten, The Netherlands**)**, broiler Maastricht were obtained from a commercial supplier in the Netherlands. All eggs were stored at 18 ˚C for up to seven days and turned twice a day before incubation.

Incubation was started at 15:00. A lag time of approximately 6 h was estimated before the real onset of incubation due to the thermal inertia of the eggs and therefore embryonic development was assumed to start at 21:00. The ages used for the broilers Linköping was 19 and 20 days (externally pipped), for the jungle fowl and the White Leghorns 19 days. Normally the eggs were internally pipped (ip) on the morning of day 20 and externally pipped (ep) in the afternoon of day 20.

The incubator used (model 25 HS, Masalles Comercial, Spain) was set at two different oxygen conditions normoxic (21 % O_2) and hypoxic (15 % O_2) for the 19 days old broiler Maastricht and the White Leghorns and normoxic (21 % O_2) and hypoxic (14 % O_2) for the 19 days old broiler Linköping and 19 days old jungle fowl. The incubators were set 37.8 ˚C and 45 % relative humidity except for three incubations with 19 days old broilers Linköping which were treated with: Normoxic (21 % O_2) plus 25 % relative humidity, normoxic (21 % O_2) plus 70 % relative humidity and hypoxic (14 % O_2) plus 25 % relative humidity. During the whole incubation the eggs were turned automatically once every hour.

3.2 Handling of embryos

Embryos were euthanized by an injection of 0.5 mL of sodium pentobarbital (60 mg mL^{-1}, Apoteket AB) or by decapitation. The eggs were set in the refrigerator after euthanasia for half an hour up to a maximum of 4 h previous to dissection. All eggs were weighed before and after incubation to the nearest tenth of a gram and the total length and width were measured with Vernier callipers to the nearest millimetre.

All procedures were approved by the local ethical committee (diary number 48-04).

3.3 Histological sampling

3.3.1 Dissection and fixation

The aortic arch from 19 days old foetuses was dissected and the brachiocephalic arteries and left right and ductus arteriosus were tied off.

Pictures of the aortic arch were taken with a digital camera (Nikon coolpix 990) to estimate the dimensions of the aorta before fixation. A sapphire ball (3.15 mm in diameter) was placed next to the aortic arch as a reference.

A needle (BD Microlance^{TM} 3) with a diameter of 0.9 mm was connected to a polyethylene tube (Intramedic® I.D 0.86 mm). The needle was inserted into the proximal end of the aortic arch and tied of between the right and left brachiocephalic arteries. The aortic arch was cut off right after the right ductus arteriosus joins with the thoracic aorta leading into the abdominal aorta.

The aorta was then perfused with 2 mL of saline (0.9 % NaCl) to clean the aorta from blood cells. Then the aorta was perfused with buffered formalin (10 % of formalin, pH 7.4) for ten minutes at an intravascular pressure representative of *in vivo* mean pressure values (between 2.7 - 3.0 kPa) as shown by Altimiras and Crossley (2000). The pressure was calibrated against a static water column and measured with a disposable pressure transducer (DPT100 Peter von Berg). The transducer was connected to a 4 channel pressure amplifier (4CHAMP, Somedic AB).

The aortas from the 19 days old broiler embryos were kept in phosphate buffered formalin (10 % formalin) between one to too weeks, and the aortas from the broiler S, broiler P, White Leghorns and jungle fowl were kept in buffered formalin for 24 hours. The shrinkage effect of fixation has previously been shown for Bouin fixative (Enell 2004). The shrinkage effect of formalin was considered in relation to long versus short fixation time.

3.3.2 Dehydration, paraffin inclusion and sectioning

Following the fixation procedure all aortas were washed in 70 % ethanol several times and then dehydrated in 95 % ethanol for two hours and 99.5 % ethanol for three hours. After this the aortas were cut right after the left brachiocephalic artery to simplify later sectioning. Following this the aortas was further dehydrated in 1:1 99.5 % ethanol/xylene for one hour and then in xylene for one hour and finally impregnated with paraffin overnight before embedding.

A paraffin microtome **(**Jung AG, Heidelberg**)** was used to make 10 µm transverse sections of the aorta. The 20 first sections were discarded to prevent edge effects. Subsequent sections were placed on glass slides coated with albumin, with 20 sections on each glass.

Deparaffination was conducted in subsequent changes in xylene and ethanol (99.5 %, 95 %, 70 %) baths and finally in distilled water. The sections were thereafter stained with Ehrlich haematoxylin-eosin and mounted whit DPX. Finally, the sections were scanned using a digital camera (SPOT RT Slider, Diagnostic instruments inc.) connected to a light microscope (Nikon eclipse E400).

3.4 Pressure-diameter loops

The aortic arch from the 19 days old broiler (Linköping) embryos incubated under normoxic and hypoxic conditions was dissected and the brachiocephalic arteries were tied off. Pictures of the aortic arch were taken as described in the previous section. Two needles (BD Microlance^{TM} 3) with a diameter of 0.9 mm were connected to a polyethylene tube (Intramedic® I.D 0.86 mm) each. The first needle was inserted into the proximal end of the aortic arch and tied of between the right and left brachiocephalic arteries.

A cut was made where the right ductus arteriosus joins with the thoracic aorta and the second needle connected to a polyethylene tube was inserted. The needle connected to the proximal end of the aorta was then attached to a 50 µL syringe filled with Krebbs-Ringer Bicarbonate buffer (KRB) (contained in g L^{-1}: NaCl, 6.728; NaAcetate, 0.820; KCL, 0.373; $CaCl_2 \cdot 2H_2O$, 0.368; $MGCl_2 \cdot 6H_2O$, 0.102; $NaH_2PO_4 \cdot H_2O$, 0.110; $NaH_2PO_4 \cdot 12H_2O$, 0.072; $NaHCO_3$, 2.100; glucose 1,495) (from Qin and Nishimura, 1998) and the other needle was attached to a disposable pressure transducer (DPT100 Peter von Berg). The transducer was connected to a 4 channel pressure amplifier (4CHAMP, Somedic AB) calibrated against a static water column. The aorta was placed in a KRB bath, which was heated to 37 ˚C.

The aorta was then stretched under physiological pressures corresponding to the specific stage of development (between 2.7 – 3.0 kPa) as measured by Altimiras and Crossley (2000) for half an hour. After this the pressure was relieved and certain volume of ringer was perfused (0.5 µL 1.0 µL or 2.0 µL depending on the vessel) and the increase in aortic diameter was photographed by a video camera (CCD C4200, Hamatsu Photosonic K.K.) connected to a stereoscopic zoom microscope (Olympus SZH-ILLD, Olympus Optical CO. Ltd.), and the corresponding pressure was recorded.

The procedures were performed in four consecutive runs, each run consisting of 10 to 16 volume infusions depending on the vessel. After each volume infusion the vessel was left alone for two minutes after which the corresponding pressure was recorded and the next insertion was made. The first run for each vessel was used as a stretch training protocol but was not considered in the analysis.

3.5 Data analysis

For morphometric analysis of the digital pictures taken from the sections a custom made program using the LabView programming environment (LabView version 6.1, IMAQ vision v.5.1, National Instruments) was used, which allowed the quantification of total area, lumen area of the vessel and wall thickness.

The basic procedure consisted of a user controlled threshold routine that binarized the image in black and white. An automatic particle recognition algorithm allowed the detection of all particles in the image (in white). From this, the area of the largest particle (in pixels) corresponding to the total outline of the aorta could be obtained. Subsequently, the program traced the lumen of the aorta using the inverted binarized signal to locate the area within the aorta with lowest pixel values. Wall thickness was computed as the average o the smallest distance from all points of the lumen to the outer aortic wall.

From the area values in pixels, total vessel diameter (TD) and lumen diameter (LD) were calculated assuming that the areas defined perfect circles and using a micrometer scale as absolute reference (Ernst Leitz 2 mm). Values for the absolute wall thickness (WT) were calculated using the same reference micrometer. This method was validated in a former study (Enell 2004).

3.6 Statistics

All data, except for pressure-diameter loops, were analyzed with independent sample t-test and Levene's Test for Equality of Variances using SPSS for windows version 6.2. Results are presented as mean ± SD throughout the thesis unless otherwise stated (Curran-Everett and Benos 2004). The fiduciary level of significance was set to $p=0.05$.

4 Results

4.1 Egg morphology

Outer dimensions and total egg mass for the eggs of the different strains used are given in Table 1. The two broiler strains used had the largest eggs with an egg mass of 68.3 ± 4.8 g and 68.3 ± 3.3 g for broiler Linköping and broiler Maastricht respectively and outer dimensions egg length of 6.0 ± 0.2 cm and egg width of 4.53 ± 0.11 cm and egg length 6.0 ± 0.3 cm and width 4.6 ± 0.3 cm for broiler Linköping and broiler Maastricht respectively. The jungle fowl embryos had the smallest eggs 36.5 ± 3.8 g and an egg length of 4.83 ± 0.20 cm and an egg width of 3.70 ± 0.13 cm.

Table 1. Morphometric data of the eggs used for the different chicken strains.

Strain	Egg mass (g)	Egg length (cm)	Egg width (cm)	n
Broiler Linköping	68.3 ± 4.8	6.0 ± 0.2	4.5 ± 0.1	32
Broiler Maastricht	68.3 ± 3.3	6.0 ± 0.3	4.6 ± 0.3	16
White Leghorn	59.0 ± 2.7	5.7 ± 0.1	4.3 ± 0.3	16
Jungle fowl	36.5 ± 3.8	4.8 ± 0.2	3.7 ± 0.1	15

4.2 Effects of incubation

Hypoxic treated embryos were significantly smaller than the control in three out of four cases as shown in Table 2. The significant ones were Broiler Linköping with 35.4 ± 3.6 g and 21.9 ± 2.3 g ($t_{(10)}$= 7.76; $p<0.05$) for the control and hypoxic treatments respectively, White Leghorn with 27.2 ± 1.1 g and 23.5 ± 1.2 g ($t_{(14)}$=6.37; $p<0.05$) respectively, and the jungle fowl with 21.7 ± 3.5 g and 17.2 ± 2.5 g ($t_{(13)}$=2.91; $p<0.05$) respectively.

The amount of water lost during incubation was significantly different between the controls and the 70 % relative humidity treated broiler Linköping embryos with 12.6 ± 1.6 % and 6.2 ± 0.8 % ($t_{(14)}$=10.69; $p<0.05$) respectively, and between the control and hypoxic treated White Leghorns with 13.0 ± 1.4 % and 10.6 ± 1.2 % ($t_{(14)}$=3.73; $p<0.05$) respectively.

Table 2. Embryonic mass of 19 days old chicken embryos and amount of water lost during incubation. Values shown as mean ± SD. The normoxic treatment for each strain is considered as control. Significant values set as p=0.05.

Strain	treatment	embryo mass	significance	% of control	water loss in %	significance	n
Broiler	Normoxic	35.4 ± 3.6		100	12.6 ± 1.6		6
(Linköping)	Hypoxic	21.9 ± 2.3	$t_{(10)}$=7.76	62	11.0 ± 1.0	ns	6
	25 % relative humidity	32.9 ± 4.0	ns	93	14.0 ± 2.3	ns	10
	70 % relative humidity	34.5 ± 3.7	ns	97	6.2 ± 0.8	$t_{(14)}$=10.69	10
Broiler	Normoxic	31.9 ± 4.7		100	11.4 ± 2.3		8
(Maastricht)	Hypoxic	28.0 ± 2.7	ns (p=0,059)	88	11.7 ± 2.1	ns	8
White	Normoxic	27.2 ± 1.1		100	13.0 ± 1.4		8
Leghorn	Hypoxic	23.5 ± 1.2	$t_{(14)}$=6.37	87	10.6 ± 1.2	$t_{(14)}$=3.73	8
Jungle	Normoxic	21.7 ± 3.5		100	14.8 ± 4.7		7
fowl	Hypoxic	17.2 ± 2.5	$t_{(13)}$=2.91	79	14.7 ± 4.2	ns	8

4.3 Effects of fixation

The shrinkage effect was significantly larger for those aortas that were in formalin fixative for one to two weeks, longer fixation resulted in a shrinkage of 20.4 ± 12.7 % in comparison with 11.8 ± 7.8 % ($t_{(72)}$= 3.58; $p<0.05$) for those aortas that were in fixation for a total time of 24 hours (Figure 1).

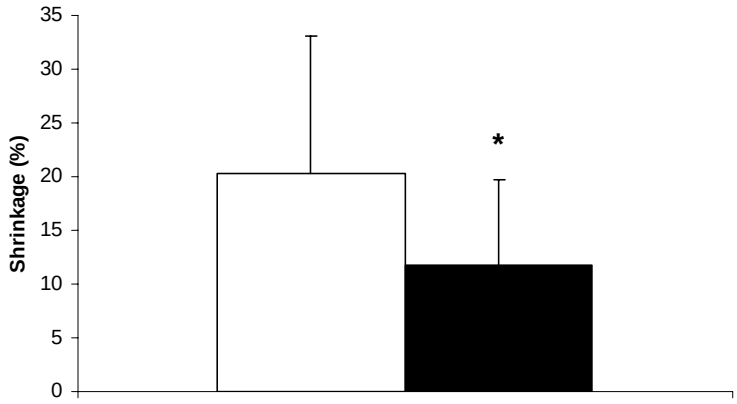

Figure 1.Effects of long versus short fixation. The white column is aortas fixed under a longer period (1-2 weeks) n= 28, and the black column is aortas fixed for a short period of time (24 hours) n= 46.

4.4 Effects of hypoxia on aortic morphology

There were no significant changes in total diameter for fresh or fixed aortas between the normoxic and hypoxic treatments in any of the four strains as shown in Table 3. There was a significant difference between the normoxic and hypoxic White Leghorn in the lumen diameter 0.53 ± 0.06 mm and 0.46 ± 0.05 mm ($t_{(14)}$= 2.25; $p<0.05$); respectively as shown in Figure 2. There was also a significant difference in wall thickness in the jungle fowl between the normoxic and the hypoxic treatment 0.33 ± 0.04 and 0.27 ± 0.03 ($t_{(13)}$=3.19 ;$p<0.05$) respectively. No significance could be found on wall/Lumen ratio between treatments.

Table 3. Effect of hypoxia on total diameter fresh as well as fixed. The normoxic treatment for each strain is considered as control.

Strain	Treatment	Fresh total diameter	Fixed total diameter	n
Broiler Linköping	Normoxic	1.58 ± 0.12	1.32 ± 0.21	6
	Hypoxic	1.45 ± 0.05	1.26 ± 0.12	6
Broiler Maastricht	Normoxic	1.40 ± 0.07	1.30 ± 0.06	8
	Hypoxic	1.31 ± 0.10	1.21 ± 0.12	8
White Leghorn	Normoxic	1.30 ± 0.13	1.20 ± 0.08	8
	Hypoxic	1.34 ± 0.10	1.14 ± 0.12	8
Jungle fowl	Normoxic	1.19 ± 0.07	1.08 ± 0.07	7
	Hypoxic	1.13 ± 0.09	1.00 ± 0.08	8

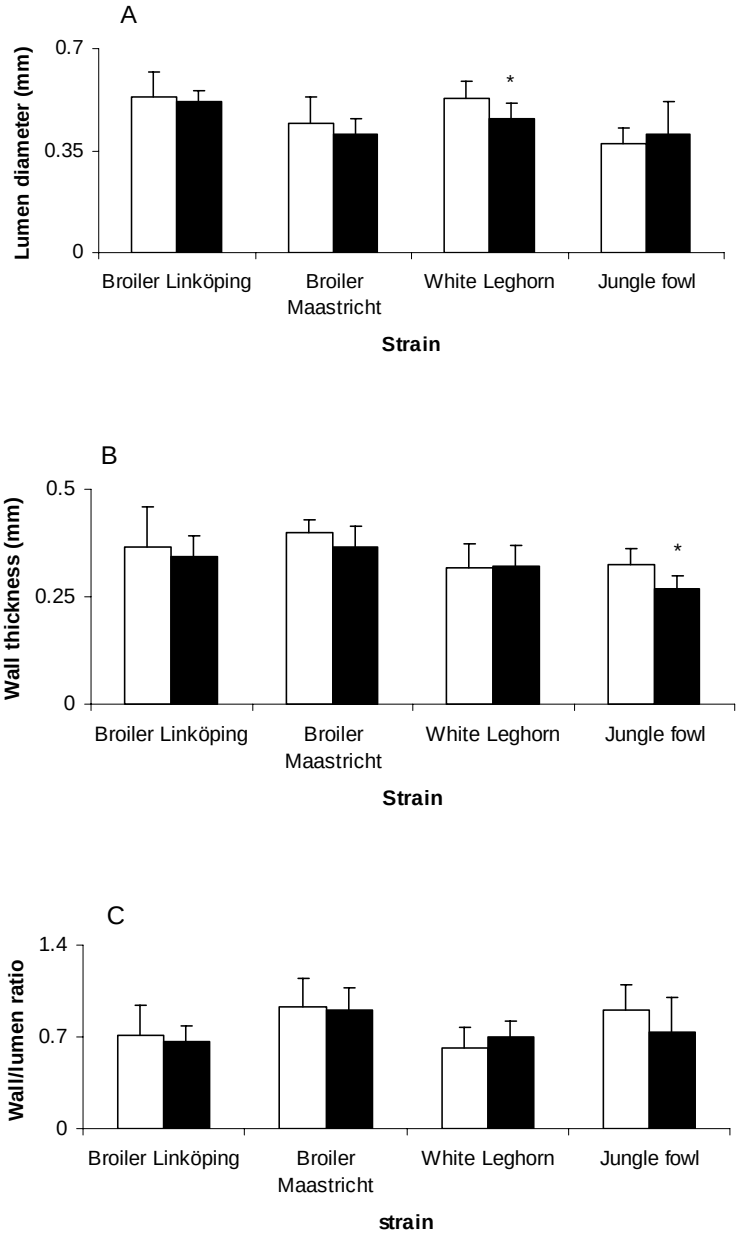

*Figure 2. Effect of hypoxia on vessel morphology. The white columns are control aortas and the black columns are aortas treated with hypoxia. Panel A is the lumen diameter for the different. Panel B shows the wall thickness and Panel C shows the wall/lumen ratio. Values are mean ± SD; *p<0.05.*

4.5 Effects of alterations in relative humidity

The effects of alterations in relative humidity are shown in Table 4. There were no significant differences seen when the broiler Linköping were treated with different degrees of relative humidity low and high (25 % and 70 % relative humidity respectively) when looking at total diameter (fresh and fixed), lumen diameter, wall thickness and wall/lumen ratio.

Table 4. Effects of alterations in relative humidity on vessel morphology. Broiler N is considered as control, broiler 25 % is incubated with 25 % relative humidity and broiler 70 % relative humidity.

Treatment	Fresh diameter	Total diameter	Lumen diameter	Wall thickness	wall/lumen ratio	n
Broiler N	1.58 ± 0.12	1.32 ± 0.21	0.53 ± 0.09	0.37 ± 0.09	0.71 ± 0.23	6
Broiler 25%	1.50 ± 0.15	1.37 ± 0.17	0.51 ± 0.13	0.40 ± 0.05	0.81 ± 0.17	10
Broiler 70%	1.47 ± 0.19	1.26 ± 0.19	0.49 ± 0.15	0.36 ± 0.06	0.79 ± 0.20	10

4.6 Pressure-diameter loops

The results of the pressure-diameter loops performed on aortas from broiler Linköping embryos treated with hypoxia and control, are shown in Figure 3. The two lines show no difference in elastic properties between hypoxia and control.

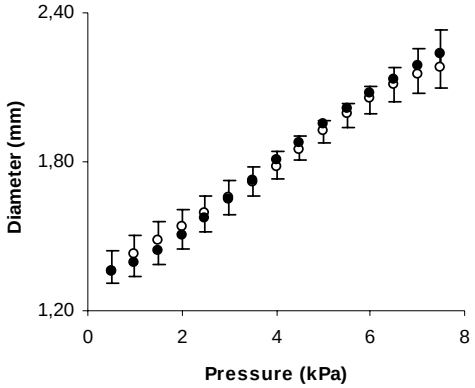

*Figure 3. Pressure-diameter loops. Open circles are 19 days old normoxic broiler Linköping embryos and the closed circles are 19 days old hypoxic broiler Linköping embryos. Values are mean ± SD; *p<0.05.*

5 Discussion

5.1 Effects of fixation

Not unexpectedly, longer fixation time (up to 2 weeks) was associated with a larger shrinking effect. These results are in accordance with previous data by Zenk et al. (1998), that showed a shrinkage factor of 10-17 % for 10 % formalin solution. The effects of shrinking have been studied by others, Enell (2004) showed an average shrinkage of 7 % in the Bouin fixative and Lantendresse et al. (2002) showed that the shrinkage effect of modified Davidson's Fluid fixative was moderate and comparable with that of Bouin.

Controls and hypoxic embryos within each strain were treated the same fixation time so an equal shrinkage effect can therefore be assumed.

5.2 Aortic morphology

Chronic stress during embryonic development leading to a low birth weight is associated with an increased risk to develop coronary heart disease, hypertension, and non-insulin dependent diabetes later in life, a phenomenon widely known as the fetal origins hypothesis (Barker's hypothesis, Barker 1993). There was a pronounced reduction in body weight for embryos incubated in hypoxia compared to the controls in this study (broiler Linköping 38 %, broiler Maastricht 12 %, White leghorn 14 %, and red jungle fowl 21 %). Other studies on the White leghorn show similar results (Jonsson 2005, Ruijtenbeek et al. 2003a, Rouwet et al. 2002). The most common cause of embryonic growth retardation is placental insufficiency, which is a combination of both malnutrition and chronic hypoxia (Ruijtenbeek et al. 2003b). Ruijtenbeek et al. (2003a) studied the effect of both malnutrition and hypoxia separately to see what impact it had on embryonic growth in White leghorns chickens. To test protein malnutrition 10 % of total albumin content was removed before incubation. What they saw was that embryos incubated under hypoxia and incubated under malnutrition both had a 14 % lower body mass compared to the control group. Further they noticed that relative brain weight was not altered in any of the two treatments indicating that growth was disproportional. This indicates that hypoxia can reduce growth rate all by its own as also seen by others (Jonsson 2005, Rouwet et al. 2002).

Ruijtenbeek et al. (2003a) also noted that the embryos incubated in hypoxia had an altered arterial function seen as a reduction in endothelial-dependent relaxation. Alterations to the arterial system caused by hypoxia has also been seen by Rouwet et al. (2002), who saw that hypoxia induced hypertrophic growth in the aortic wall in the White leghorn chicken embryo. This hypertrophy was seen as an increase in aortic wall thickness and a decrease in lumen diameter leading to an increased wall/lumen ratio.

There was no evidence of aortic hypertrophy found in the aortic morphology in any of the four strains used in this study. Neither of the two broiler strains (Linköping and Maastricht) showed any differences to the control groups in regards to wall thickness, lumen diameter or wall/lumen ratio. In fact the hypoxic broilers used tended to have a thinner aortic wall though not significant from the control group. This is in contradiction to the findings by Rouwet et al. (2002). To see if an increase in hypoxia would yield any different results in the broiler chicken embryos an attempt were made with 12 % O_2, which resulted in 100 % mortality. The White leghorns used had indeed a significantly smaller inner lumen but not a thicker aortic wall so the diameter of the lumen might be due the smaller size of the total aorta. This is in agreement with other studies conducted by Jonsson (2005) who saw no changes in lumen diameter, wall thickness and wall/lumen ratio in 19 days old White Leghorn chickens. There are no traces of aortic hypertrophy in the jungle fowl embryos. Rather the opposite when the hypoxic embryos had a larger lumen and a thinner

aortic wall. This is the complete opposite reaction to the ones presented by Rouwet et al. (2002).

The cause of hypertrophy in the aorta is thought to be a consequence of hypertension. The viscoelastic properties of mature arteries are determined largely by the relative proportions of elastin, collagen, and vascular smooth muscle cells and each of the main components of the arterial wall contributes uniquely to the overall mechanical properties of the tissue (Wells 1999, Speckmann and Ringer 1966).

The oscillations in blood pressure and flow generated by contractions of the heart are dampened in the arterial system by the elasticity of the arterial walls. As blood is injected into the arterial system, pressure rises and the vessel expands. As the heart relaxes, blood flow to the periphery is maintained by the elastic recoil of the vessel walls, resulting in a reduction in arterial volume. The transfer of stress from elastin to collagen with increasing strain (stress) has been attributed to the progressive engagement of collagen fibers that are crimped at low strains. Thus low-modulus elastin dominates wall mechanics at low stress, whereas high-modulus collagen dominates at high stress (Braun et al. 2003, Wells 1999). So therefore if the aortic wall was hypertrophic the consequence would result in a much stiffer aorta. A stiffer aorta would be, as a consequence, unable to compensate for the oscillations in blood pressure and also would affect the function of the baroreceptors located in the aorta in chickens. All this could in the long run lead to circulatory problems and heart disease.

I tested the effect of hypoxia on the elastic properties of the broiler chicken embryo. No difference could be found between the hypoxic embryos and the control group. This is in accordance with the study done by Jonsson (2005) who tested the elastic properties of the aortic wall in 19 days old White leghorn chicken embryos that had been incubated in hypoxia. He found no difference in elasticity between hypoxic and control animals. The fact that I found no change in elasticity in study supports the lack of hypertrophy found in all four of my chicken strains used.

Another factor that could have a stressful effect on the chicken embryos during incubation is the relative humidity inside the incubator. To be sure that relative humidity did not play a major role in my findings and to test the impact of altered relative humidity two experiments were made: one with low relative humidity (25 %) and one with high relative humidity (70 %) both using normal oxygen levels (21 % O_2). There was no difference in lumen diameter, wall thickness or wall/lumen ratio and no difference in embryonic mass. What could be seen though was a significant difference in amount of water lost during incubation between the control and the 70 % relative humidity as would be expected. This is also in accordance with the data presented by Buhr (1995) showing a 6 % water loss for White Leghorn embryos incubated at 70 % relative humidity. On the other hand, the low relative humidity was not significantly different from the control which might indicate that 25 % relative humidity in

fact is not so low that it would cause any dehydration and stress to the embryo. Further investigations in even lower relative humidity might be of interest to see the effects on the circulatory system.

5.3 Conclusion

In conclusion, no evidence of aortic hypertrophy was found, but differences in responses to hypoxia could be seen in the different strains used. The differences between the broilers and the White Leghorn found in this study might be because of the difference in selection pressure for different traits done by breeding (Currie 1999, Dewil et al. 1996). Therefore even though no aortic hypertrophy was found in this study, it cannot exclude alterations in the composition of the aortic wall. Looking at the specific layers in the aortic wall would be the good subject of further studies and investigations in the alterations in the wall composition would be of interest.

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