Structural failures of the blood–gas barrier and the epithelial–epithelial cell connections in the different vascular regions of the lung of the domestic fowl, *Gallus gallus* variant *domesticus*, at rest and during exercise

John N. Maina[1,*] and Sikiru A. Jimoh[2,‡]

[1]Department of Zoology, University of Johannesburg, Auckland Park 2006, Johannesburg, South Africa
[2]School of Anatomical Sciences, University of the Witwatersrand, Braamfontein 2000, Johannesburg, South Africa

*Author for correspondence (jmaina@uj.ac.za)
‡Present address: Department of Biochemistry, Faculty of Health Sciences, University of Cape Town, Observatory 7935, Cape Town, South Africa

Biology Open 2, 267–276
doi: 10.1242/bio.20133608
Received 12th November 2012
Accepted 11th December 2012

Summary

Structural failure of blood–gas barrier (BGB) and epithelial–epithelial cell connections (EECCs) in different vascular regions of the exchange tissue of the lung was studied in rested and exercised chickens. The number of red blood cells (nRBCs) was counted and protein concentration (PC) measured after lavaging the respiratory system, and blood was sampled to determine the blood lactate levels (BLLs). The numbers of complete BGB breaks (nBGBBs) and those of the EECCs (nEECCBs) were counted in the different vascular territories of the lung. The nRBCs and the PCs increased with increasing exercise intensities but the rate of increase decreased at higher workloads. From rest to the fastest experimental treadmill speed of 2.95 m.sec^{-1}, BLLs increased 4-fold. In all cases, the nEECCBs exceeded those of the BGB, showing that structurally the BGB is relatively weaker than the EECC. The increase in the number of breaks with increasing exercise can be attributed to increase in the pulmonary capillary blood pressure (PCBP) from faster heart rates and higher cardiac outputs, while the leveling out of the measurements made at higher workloads may have arisen from hemodynamic changes that initially ensued from exudation of blood plasma and then flow of blood into the air capillaries on failure of the BGB. The relative differences in the nBGBBs and the nEECCBs in the different vascular regions of the lung were ascribed to diameters of the branches and their points of origin and angles of bifurcation from the pulmonary artery. Presence of RBCs in the air capillaries of the lungs of rested chickens showed that failure of the BGB commonly occurs even in healthy and unstressed birds. Rapid repair and/or defense responses, which were observed, may explain how birds cope with mechanical injuries of the BGB.

Key words: Birds, Exercise, Rest, Chicken, Lung, Blood–gas barrier, Structural failure

Introduction

"Like most machines, components of the body eventually fail. Disease states, mechanical insult or normal wear may initiate the process of tissue degradation." (Li et al., 2010)

The air capillaries and the blood capillaries of the exchange tissue of the avian lung have been reported to behave like 'rigid' tubes (Macklem et al., 1979; Powell et al., 1985; West et al., 2007; Watson et al., 2008). Like mammals, birds are endothermic homeotherms; they operate at relatively higher body temperatures (40–42℃) (Aschoff and Pohl, 1970). Oxygen is acquired by a structurally complex and functionally efficient respiratory system, the lung air sac system (e.g. Powell and Scheid, 1989; Maina, 2005). To support an energetic lifestyle, large hearts (Hartman, 1955) with large cardiac outputs (Grubb, 1983; Bishop, 1997; Seymour and Blaylock, 2000) supply tissues with oxygen. Compared to mammals, the arterial blood pressures in birds are much higher (Spector, 1956; Speckmann and Ringer, 1963; Erickson, 1994; Seymour and Blaylock, 2000; Lichtenberger, 2005). For example, compared to that of 95 mmHg (12.64 kPa) in a mammal like the cynomologus monkey, *Macaca fascularis*, (body mass 4.60 kg), the turkey, *Meleagris gallopavo*, (4.77 kg) has a higher systolic blood pressure of 150 mmHg (19.95 kPa) (Seymour and Blaylock, 2000). Turkeys have exceptionally high systolic blood pressures of as much as 400 mmHg (53.3kPa) (Beller et al., 2004). In ratites (mainly ostrich, emu, and rhea), sudden deaths have been attributed to aortic rupture (Mitchinson and Keymer, 1977) (2008 factsheet 'Aortic ruptures' by Hunter et al.: http://www.agbiosecurity.ca/healthybirds/Factsheets/Disease/AorticRupture.pdf). Termed 'dissecting aneurism' or 'turkey heart attack', fast

growing, healthy turkeys (mostly males of between 7 to 24 weeks of age) die suddenly of bleeding after aortic rupture which is caused by high blood pressure (Collins, 1971; Neumann and Ungar, 1973; Krista et al., 1986; van Veen, 1999). After autopsy of a visibly (externally) healthy cardinal, *Cardinalis cardinalis*, which died after a short territorial spat, a 7-mm hole (probably caused by acute blood pressure surge during the excitement) was reported in the ventricle of the heart (Erickson, 1994). Birds like the fast growing broiler chickens and turkeys frequently experience hypertensive problems that lead to high myocardial stress, resulting in complications such as aortic aneurysm and ascites (Speckmann and Ringer, 1963; Krista et al., 1970; Julian and Wilson, 1986; Currie, 1999; Issac et al., 2010). In hypertensive turkeys, respectively, Simpson, and Krista et al. (Simpson, 1978; Krista et al., 1967) reported mortality rates from aortic rupture of 20% and 43.7%. It is perplexing that with their relatively high systolic and arterial blood pressures, birds possess a blood–gas barrier (BGB) which is ~3× thinner than those of mammals of equivalent body mass (Gehr et al., 1981; Maina and King, 1982; Maina et al., 1989; Maina and West, 2005; Maina, 2005).

This study investigated the effect of exercise on the structural integrity of the BGB and the epithelial–epithelial cell connections (EECCs) in the different regions of the exchange tissue of the lung of the domestic fowl (chicken), *Gallus gallus* variant *domesticus*, which are supplied by the four main branches of the pulmonary artery (PA). Changes of blood lactate levels (BLLs), numbers of red blood cells (nRBCs) and protein concentrations (PCs) in lavaged fluid were assessed and complete blood–gas barrier breaks (BGBBs) and epithelial–epithelial cell connection breaks (EECCBs) were counted. A list of definition of commonly used abbreviations is given in Table 1 for quick reference.

Results

At the slowest treadmill speed of 0.66 m.sec^{-1}, some of the chickens tried to jump off the treadmill belt but were prevented by a transparent perspex sheet, which was placed well above it. At speeds of 2.53 and 2.95 m.sec^{-1}, after 7 minutes of running, the chickens were visibly exhausted; they had to struggle to complete the set exercise time of 10 minutes. The fastest treadmill speed of the experiment (2.95 m.sec^{-1}) appeared to be the maximum exercise intensity that the chickens could tolerate for the set exercise program; they had to be physically nudged to keep running and complete the time.

Table 1. Definition of abbreviations commonly used in the text.

Abbreviation	Definition
BGB	Blood–Gas Barrier
BGBBs	Blood–Gas Barrier Breaks
BLL	Blood Lactate Level
EECCBs	Epithelial–Epithelial Cell Connection Breaks
EECCs	Epithelial–Epithelial Cell Connections
nBGBBs	number of Blood–Gas Barrier Breaks
nEECCBs	number of Epithelial–Epithelial Cell Connection Breaks
nRBCs	number of Red Blood Cells
PA	Pulmonary Artery
PC	Protein Concentration
PCBP	Pulmonary Capillary Blood Pressure
RBCs	Red Blood Cells
$\dot{V}O_2$	Oxygen consumption
$\dot{V}O_{2max.}$	Maximum Oxygen consumption

Blood lactate levels

For the exercised chickens, the pre-exercise BLL values were comparable while the post-exercise ones increased gradually with increasing workload (Fig. 1). In the rested chickens, the difference between the first BLL measurement (1.39±0.01s.d. mmol.L^{-1}) and the second one (1.27±0.09s.d. mmol.L^{-1}), measurements taken 10 minutes apart, was not statistically significant ($0.5>P>0.1$). The small but consistent difference could have ensued from incidental distress of the chickens prior to taking the first measurement. The same can explain the small differences between the resting groups of birds in the different exercise regimens. Between rest and running speed of 0.66 m.sec^{-1}, post-exercise BLL rose from 1.29±0.09s.d. mmol.L^{-1} to 3.18±0.04s.d. mmol.L^{-1}, a 2.5-fold increase; between speeds of 1.97 and 2.95 m.sec.$^{-1}$, the increase of BLL from 4.03±0.18s.d. to 5.14±0.61s.d. mmol.L^{-1} was not significant ($P>0.5$) for a 1.5× increase in the running speed (Fig. 1). This shows that under the exercise regimen, BLL approached or reached the highest physiologically tolerable level and that metabolically, the birds were pushed close to their exercise endurance limit.

Red blood cells and protein concentrations in lavaged fluid

In both rested and exercised chickens, red blood cells (RBCs) were observed in the recovered fluid (Fig. 2); they were indicative of BGB failure. The nRBCs increased up to the treadmill speed of 1.97 m.sec^{-1} after which it leveled out (Fig. 2). While between resting and the treadmill belt speed of 0.98 m.s^{-1} the nRBCs increased from $3\times10^5\pm4\times10^4$s.d. per cm^3 to $5\times10^5\pm6\times10^3$s.d. per cm^3, a factorial increase of 1.7, between the speeds of 1.97 ($5.9\times10^5\pm2\times10^4$s.d. per cm^3) and that of 2.95 m.s^{-1} ($6.4\times10^5\pm6\times10^3$s.d. per cm^3) the increase was only 1.1. At the treadmill speed of 1.97 m.s^{-1}, the chickens started to struggle to keep running, particularly after 7 minutes on the treadmill. In the fluid recovered from the respiratory system of the chickens, PC increased gradually from rest to different exercise intensities (Fig. 3). Between a lower level of exercise and a higher one, the greatest increase occurred between rest (8.85±0.14s.d. μg.ml^{-1} and the treadmill speed of 1.97 m.sec^{-1} (34.03±0.23s.d. μg.ml^{-1}), a factorial increase of ~4. Showing that exercise was affecting them in the same way, the changes in the nRBCs (Fig. 2) matched those of PC (Fig. 3).

Blood–gas barrier breaks and epithelial–epithelial cell connection breaks in different vascular regions of the lung

In rested and exercised chickens, BGBBs and EECCBs were observed in the different regions of the lung supplied by the four main branches of the PA. In all cases, the nEECCBs exceeded the BGBBs (Fig. 4). The differences between the nBGBBs and the nEECCBs in the different vascular territories of the lung (Figs 5, 6) and the differences between the nEECCBs and those of the BGB in a particular vascular region of the lung (Fig. 7) were expressed as percentages of the total in order to reflect the relative numbers of structural failures in these parts. Fig. 5 shows that in the rested chickens, compared to other regions, the region supplied by the caudomedial branch had most failures; no breaks occurred in the region supplied by the cranial branch; the number of breaks in the areas supplied by the accessory and caudolateral

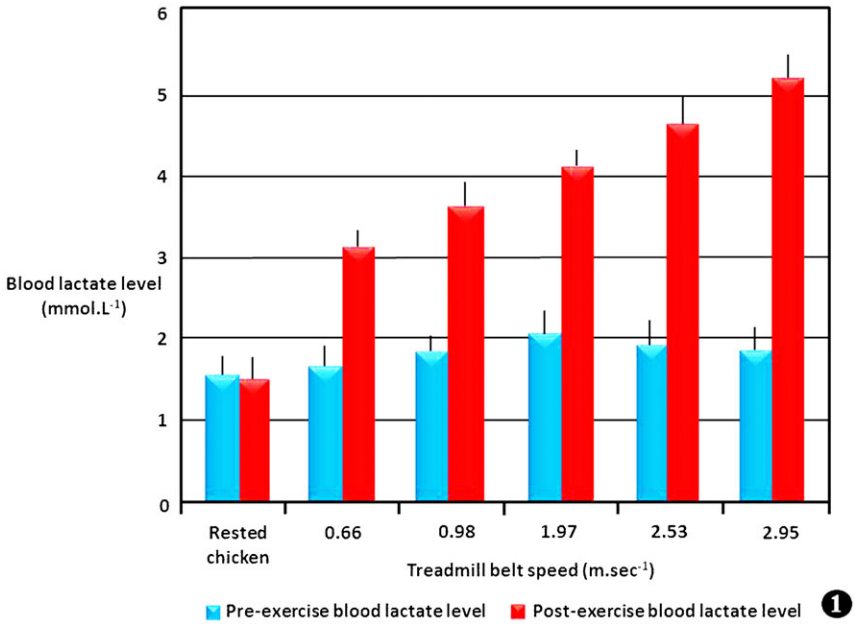

Fig. 1. Blood lactate levels (BLLs) of chickens at rest and after exercise. The post-exercise values increased with increasing exercise intensity but the rate of increase decreased with increasing workload. In rested chickens, the differences between the BLL may be explained by some distress prior to taking the first BLL reading.

branches was comparable. At the treadmill speed of 0.66 m.sec^{-1}, the region supplied by the cranial branch had the least nBGBBs while that supplied by the caudomedial branch had the greatest number; the number of breaks in areas supplied by the accessory and caudolateral branches was comparable. At the treadmill speeds of 2.53 and 2.95 m.sec^{-1}, the nBGBBs did not show much variation in the four regions. Fig. 6 shows that in rested chickens, the greatest nEECCBs occurred in the region supplied by the accessory branch of the PA while the lowest was in the region supplied by the caudolateral one; the values in the regions supplied by the cranial and caudomedial branches were similar. At treadmill speeds of 0.66 and 0.98 m.sec^{-1}, the nEECCBs in the regions supplied by the accessory and caudomedial branches were comparable and more than in the regions supplied by the cranial and caudolateral branches. At the treadmill speed of 1.97 m.sec^{-1}, the nEECCBs showed modest variation. At treadmill speeds of 2.53 and 2.95 m.sec^{-1}, the accessory and caudomedial branches of the PA had the greatest and the cranial and caudolateral branches the lowest numbers of breaks. Fig. 7 shows the relative differences between the nEECCBs and the nBGBBs in the different vascular regions of the lung. It shows the most common type of break and how the numbers of breaks in the four regions of the exchange tissue compare at rest and during different exercise intensities. In the rested chickens, in the area supplied by the cranial branch of the PA, the EECCBs were the only breaks which were observed while the areas supplied by the other branches showed marked differences of the relative numbers of EECCBs to BGBBs; in the area supplied by the caudomedial branch, the EECCBs contributed 55% of the total number of breaks in the vascular region and for those supplied by the accessory and caudolateral branches the values were 77% and 78% respectively. At 0.66 m.sec^{-1} treadmill speed, the percentage differences were lower than those in rested chickens, with the area supplied by the

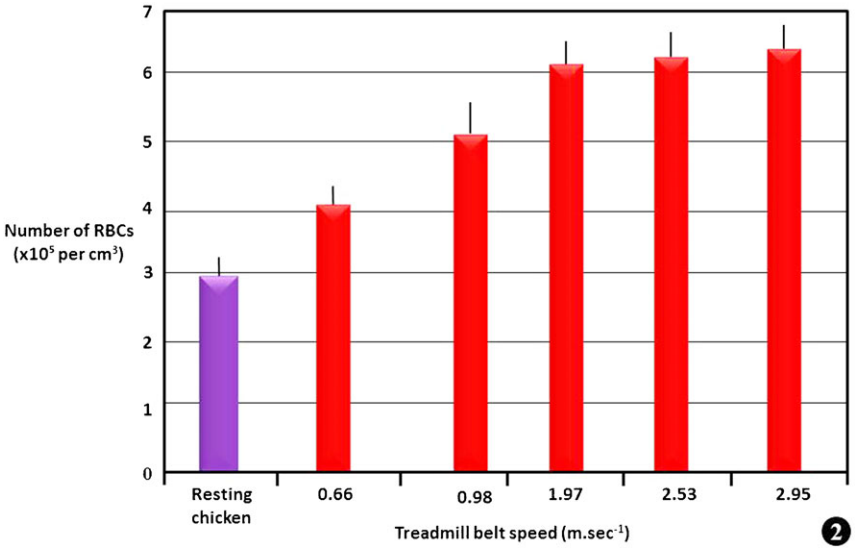

Fig. 2. Changes of the number of red blood cells (nRBCs) in the fluid collected from the respiratory system at rest and after exercise. The greatest increase of the nRBCs occurred between the treadmill exercise speeds of 0.66 and 1.97 m.sec^{-1} while the rate of increase of the nRBCs about leveled off at higher workloads.

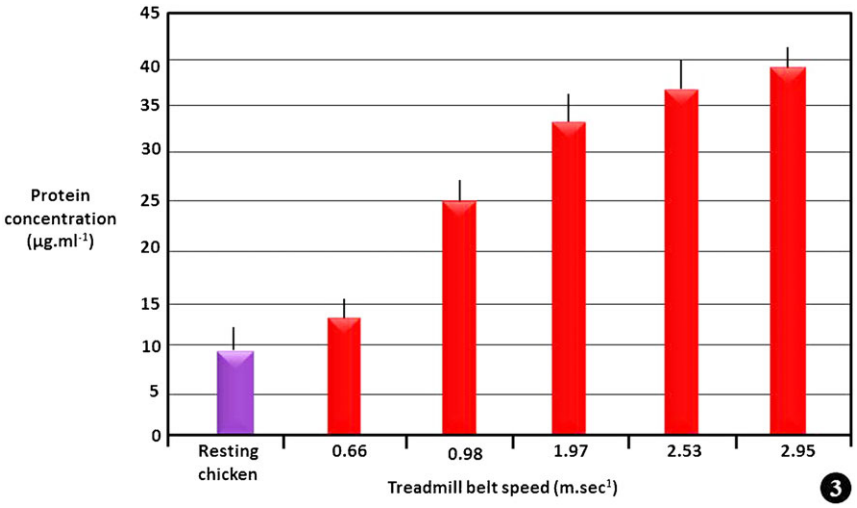

Fig. 3. Changes of protein concentration (PC) in the fluid collected from the respiratory system of the chicken lung at rest and after exercise. The greatest increases of PC occurred between the treadmill exercise speeds of 0.66 and 1.97 m.sec^{-1} while the rate of increase of PC decreased at higher workloads.

cranial branch of the PA showing the greatest relative contribution of the EECCBs and the caudomedial one the lowest. At 0.98 m.sec^{-1}, the values were about equal while at the treadmill speed of 1.97 m.sec^{-1} and higher, the relative proportions between the EECCBs and the BGBBs generally decreased.

Morphologies of the blood–gas barrier breaks and the epithelial–epithelial cell connection breaks

The profuse interdigitation of the air capillaries and the blood capillaries in the exchange tissue of the lung is shown in Fig. 8A,B. The EECCs are for the most part thin cellular extensions that separate the air capillaries while connecting the blood capillaries (Fig. 8A–C). The failure of the EECCs started with small perforations (Fig. 8D) which joined into large slits (Fig. 8E,F), while for the BGB, early injury was heralded by measurable increase of the nRBCs and the PC in the lavage fluid (Figs 2, 3); at lower workloads, linear breaks occurred (Fig. 8G) and at higher ones, the blood capillary wall tore open (Fig. 8H,I).

Discussion

Running on a treadmill is a highly energetic form of exercise that occasions large energy expenditure (Roston et al., 1987; Cabrera et al., 1999). In a horse, Thomas and Fregin observed that at maximum exercise on a treadmill, stroke volume increased by as much 41% above the resting value and cardiac output rose 6-fold (Thomas and Fregin, 1981). Oxygen consumption ($\dot{V}O_2$) of animals running on a treadmill increased linearly as a function of speed (e.g. Gleeson and Baldwin, 1981; Taylor et al., 1981). In the marabou stork, *Leptoptilos crumeniferus*, Bamford and Maloiy reported a direct relationship between $\dot{V}O_2$ and heart rate for birds running on treadmill (Bamford and Maloiy, 1980), while large increases in the respiratory rate and heart rate were reported by Butler et al., Butler, and Peters et al. in birds flying in a wind tunnel (Butler et al., 1977; Butler, 1991; Peters et al., 2005). Eliassen reported that pulse pressure almost doubled from rest to flight in gulls (Eliassen, 1963), while Hart and Roy recorded 3 to 4 times increase in heart rate over the resting level in exercising pigeon (Hart and Roy, 1966). Increase of BLL in blood during exercise has been shown to occur in three phases (Roston et al., 1987; Cabrera and Chizeck, 1996): in the first phase which corresponds to less than 60% of maximum $\dot{V}O_2$ ($\dot{V}O_{2max.}$) and occurs during mild to moderate exercise, the rate of glycolysis increases several times without significant BLL increase; in the second phase which occurs during heavy exercise

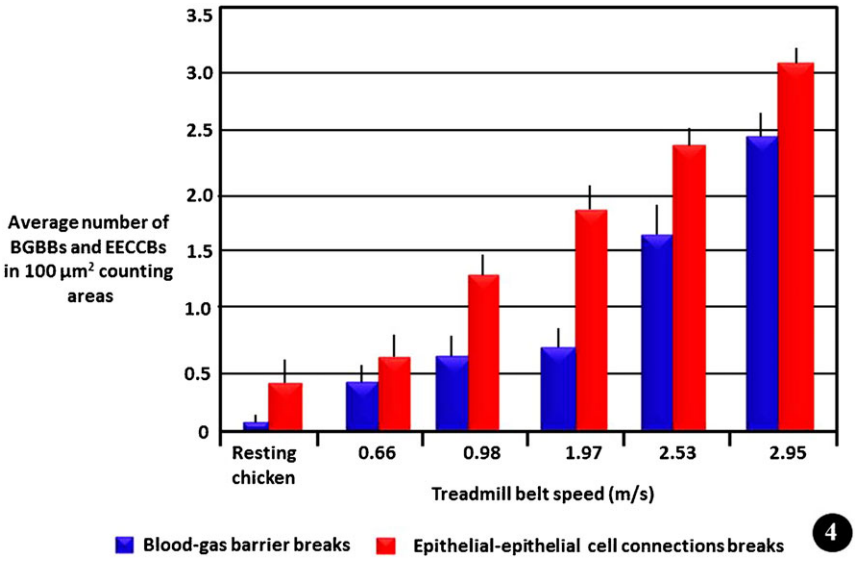

Fig. 4. Changes in the average numbers of blood–gas barrier breaks (nBGBBs) and epithelial–epithelial cell connection breaks (nEECCBs) in lungs of rested and exercised chickens. The breaks increased with increasing exercise intensity, with the differences between the two types of breaks decreasing at higher workload. Both at rest and exercise, the nEECCBs exceeded the nBGBBs.

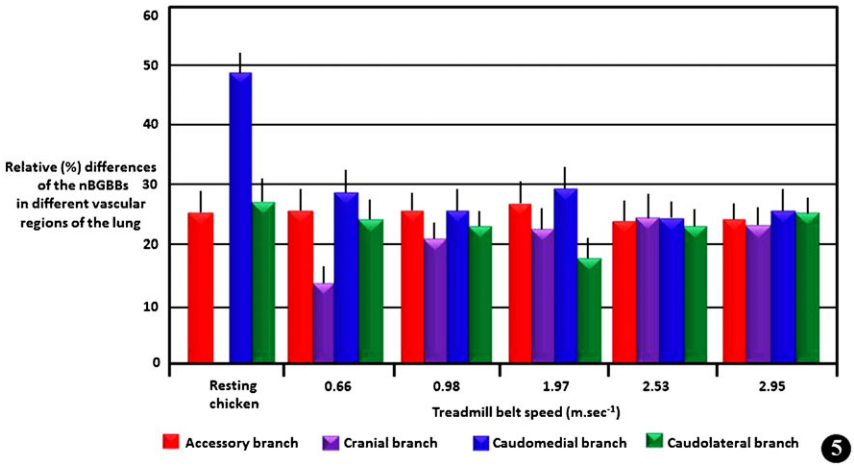

Fig. 5. Relative (%) differences of the numbers of blood–gas barrier breaks (nBGBBs) in the different regions of the lung supplied by the four main branches of the pulmonary artery in rested and exercised chickens. In the rested chickens, the number of breaks in the caudomedial branch was the highest and no breaks occurred in the area supplied by the cranial branch; between the rested chickens and those run at a treadmill speed of 0.66 m.sec^{-1}, the numbers of breaks in the regions supplied by the accessory branch of the PA were comparable. In running chickens, the areas supplied by the caudomedial and accessory branches generally had the highest number of BGB breaks.

equivalent to 60 to 80% of $\dot{V}O_{2max.}$, BLL rises to a higher steady-state level above the resting one, and; during the third phase which occurs when $\dot{V}O_2$ is greater than 80%, anaerobic glycolysis augments the energy derived from aerobic production, causing progressive increase of BLL (Cabrera et al., 1999). Because lactate acid level serves as a diagnostically important oxidizable substrate during exercise, it has been used to access, plan, and predict training and performance endurance programs (Cabrera et al., 1999).

In this study, BLLs rose 4-fold between rested chickens and those run at the fastest treadmill speed of 2.95 m.sec^{-1}. The post-exercise BLLs should have been consistent with the exercise intensities since the chickens were not trained to run before the experiments. It cannot, however, be totally ruled out that the chickens may have suffered some degree of stress prior to and during the experiments, even though every care was taken to minimize or eliminate it. The recurrent though insignificant difference between the first and the second BLL measurements in rested birds can be attributed to instinctive stress that may have caused the BLL to remain elevated beyond a 10-minute time interval. The difference between the BLL in the rested chickens and those subjected to the slowest exercise treatment (0.66 m.sec^{-1}) can be explained by delay between lactate acid build-up and release of lactate dehydrogenase.

The initial increase of PC in the recovered fluid may have resulted from increased leakiness of the BGB before failure occurred. A similar process was observed in the dog lungs which were subjected to high transient vascular pressure (Maron et al., 2001). Presence of RBCs on the respiratory surface of normal (healthy) birds has been previously reported, e.g. in rock dove, *Columba livia*, (Maina and Cowley, 1998) and the chicken (Nganpiep and Maina, 2002; Kiama et al., 2008). As confirmed here, moderate inconsequential structural failure of the BGB seems to be a common occurrence even in lungs of resting (unstressed) birds. Compared to mammals, birds have higher arterial blood pressures (Seymour and Blaylock, 2000) and much larger hearts (e.g. Hartman, 1955; Seymour and Blaylock, 2000) which generate large cardiac outputs (e.g. Grubb, 1982). Presence of white blood cells and fibrinous strands within minutes of the experiment showed existence of an efficient cellular defense and/or repair mechanism. It may explain how birds contend with BGBBs that appear to occur commonly in their lungs. The production of the fibrillary structures which may plug BGB breaks may be initiated by activation of small GTPases Rho and Cdc42 that are known to stimulate actin filament synthesis (Wood et al., 2002). In the mammalian lung, repair of the alveolar epithelial cells after pressure was increased to injurious levels and then lowered to non-injurious ones was reported by Vlahakis et al. (Vlahakis et al., 2002). In this study, at higher workloads, the chickens may have struggled to complete the 10-minute exercise program due to build-up of lactic acid in the tissues, especially in the leg muscles and occurrence of a

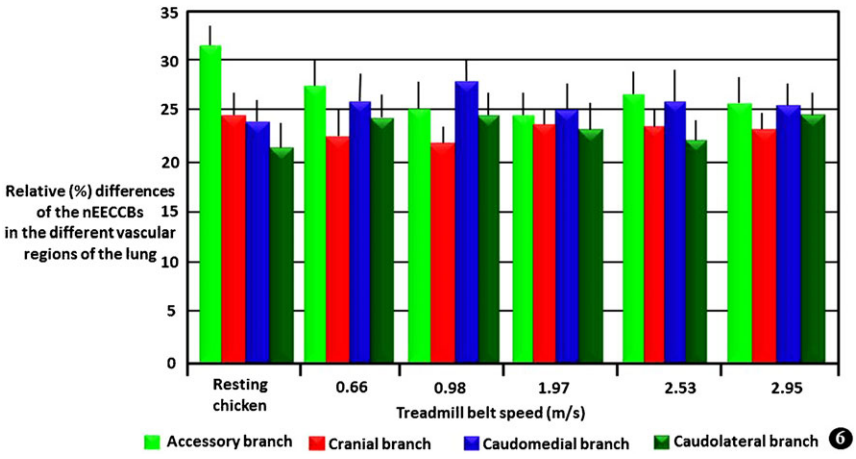

Fig. 6. Relative (%) differences of the numbers of epithelial–epithelial cell connection breaks (nEECCBs) in the different vascular regions of the lung supplied by the four main branches of the pulmonary artery (PA) in rested and exercised chickens. In rested chickens, the regions supplied by the accessory and caudolateral branches of the PA showed the highest and the lowest numbers of breaks respectively while the numbers of breaks were comparable in the regions supplied by the cranial and caudomedial branches. At speeds of 0.66 and 0.98 m.sec^{-1}, the numbers of breaks in the regions supplied by the accessory and caudomedial branches of the PA were comparable and greater than values from the regions supplied by the cranial and caudolateral branches of the PA. Generally, in exercising birds, the areas supplied by the accessory and caudomedial branches had the greatest number of breaks.

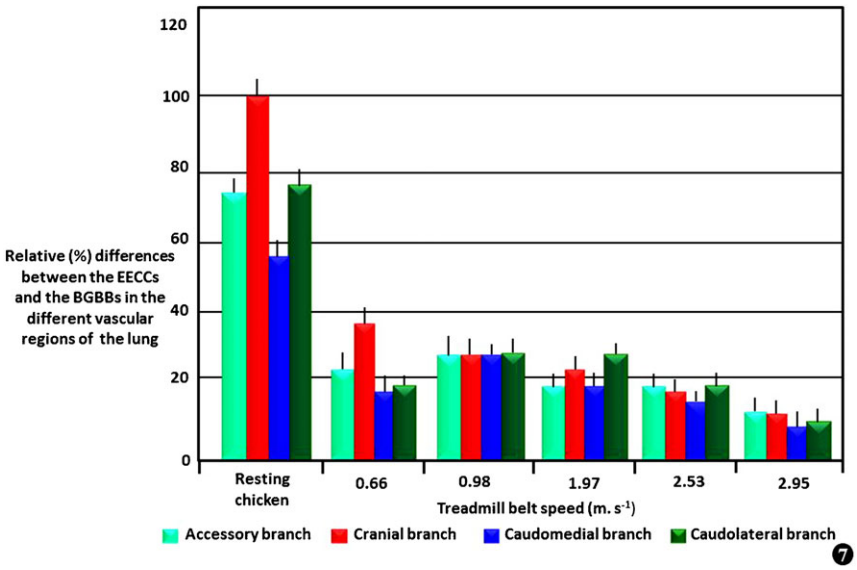

Fig. 7. Relative (%) differences between the numbers of epithelial–epithelial cell connection breaks (nEECCBs) and the numbers of blood–gas barrier breaks (nBGBBs) in different vascular regions of rested and exercised chicken lungs. For the rested chickens, the region supplied by the cranial branch, only EECCBs occurred while at a treadmill speed of 0.98 m.sec^{-1}, the relative proportions of the EECCBs were equivalent. Ostensibly due to hemodynamic changes that occurred after failure of the BGB and flow of blood plasma and then blood into the air capillaries, the relationship between the nEECCBs and the nBGBBs decreased from rested to exercised chickens. In rested chickens, the EECCs were more highly susceptible to failure than the BGB.

'respiratory block' from initial presence of blood plasma and then of blood in the air capillaries. West and Mathieu-Costello observed that stress failure of the BGB is an important but overlooked factor that limits maximum exercise (West and Mathieu-Costello, 1995).

Increase in the pulmonary capillary blood pressure (PCBP) is probably the foremost direct challenge to the mechanical integrity of the blood capillary wall, especially the BGB, and indirectly the EECCs. In racing horses, PCBP rose to 100 mmHg (13.33 kPa) (Jones et al., 1992). While such high pressure may not have been reached in exercised chickens, BGBBs which were physically similar to those reported in the mammalian lung by West et al. (West et al., 1991) were observed. It remains to be determined whether under similar levels of PCBP and states of BGB thickness the avian BGB is stronger than the mammalian one. Bird lungs have BGBs that are ~3 times thinner than those of mammals (Gehr et al., 1981; Maina, 1989; Maina et al., 1989; West, 2009). For that, the avian lung should be more susceptible to pulmonary mechanical injuries and diseases. After comparing the strengths (stress tolerance) of the blood capillaries in the rabbit lungs, the dog lungs, and the horse lungs, Birks et al. observed that stress failure may correlate with the thickness of the BGB (Birks et al., 1994), a property consistent with Laplace's relationship which states that wall stress is proportional to capillary radius but inversely proportional to wall thickness. We have demonstrated collagen fibers in the basement membrane of the BGB and the EECCs of the lung of the chicken (Maina et al., 2010) and immunolocalized type-IV collagen (Jimoh and Maina, 2013), a protein family of triple helical isoforms that form strong two-dimensional planar network of fibers (Timpl, 1989; Hudson et al., 1993; West, 2009), in the exchange tissue of the lung of the domestic fowl. The strength of the BGB of the mammalian lung has been attributed to presence of type-IV collagen in the basement membrane (Crouch et al., 1997; West and Mathieu-Costello, 1999; Maina and West, 2005; West, 2009).

The greater nEECCBs compared to those BGB, points out to the fact that the former sites are structurally weaker than the latter; the EECCs are the areas of the exchange tissue of the avian lung where thin strands of epithelial cells lie back-to-back, separating the air capillaries and connecting the blood capillaries (Fig. 8A–C). The increase in the relative differences between the nEECCBs and the nBGBBs at higher workloads may have been caused by hemodynamic changes which occurred after failure of the BGB, initially leading to exudation of blood plasma and then streaming of blood into the air capillaries. It is plausible that presence of blood in the air capillaries externally stabilized the BGB while accelerating failure of the EECCs. Interestingly, in chickens infused with 2,4 dinitrophenol (DNP), which caused a 4-fold increase in cardiac output, for unclear reasons, while the pulmonary arterial pressure rose steadily (as cardiac output increased), the increase in the arterial pressure was even less than that in a mammal, where recruitment and distension in the pulmonary capillary system occurs (West et al., 2010).

In concurrence with the physics of fluid flow, the point of origin, the angle of bifurcation, and the diameters of the main branches of the PA should profoundly determine the distribution of blood to different regions of the lung. From the pattern of its branching reported by Abdalla (Abdalla, 1989) and the diameters and angles of bifurcation of the four branches (unpublished observations), more blood should flow into the caudomedial branch because it is the widest and the most direct continuation of the PA while the highest resistance should occur in the accessory branch because it is the first and the narrowest branch that originates from the PA. Measurements made on latex casts of the lung of the domestic fowl (unpublished observations) showed that the angles of bifurcation of the accessory, the cranial, the caudomedial, and the caudolateral branches from the PA were respectively 44°, 56°, 17°, and 90° while the diameters were 0.8, 1.5, 2.2, and 1.4 mm. In the chicken lung, no anastomoses occur between the arterial branches of the PA (Abdalla and King, 1976). Therefore, with certainty, the BGBBs and the EECCBs counted in this study can be associated with the individual branches of the PA. The greatest nBGBBs and nEECCBs occurred in the regions of the lung supplied by the caudomedial and accessory branches in accordance with respectively the expected greater blood flow and resistance (pressure) in the two branches. Pulmonary blood flow redistribution was reported in the lung of the panting ostrich, *Struthio camelus*, (Jones, 1982).

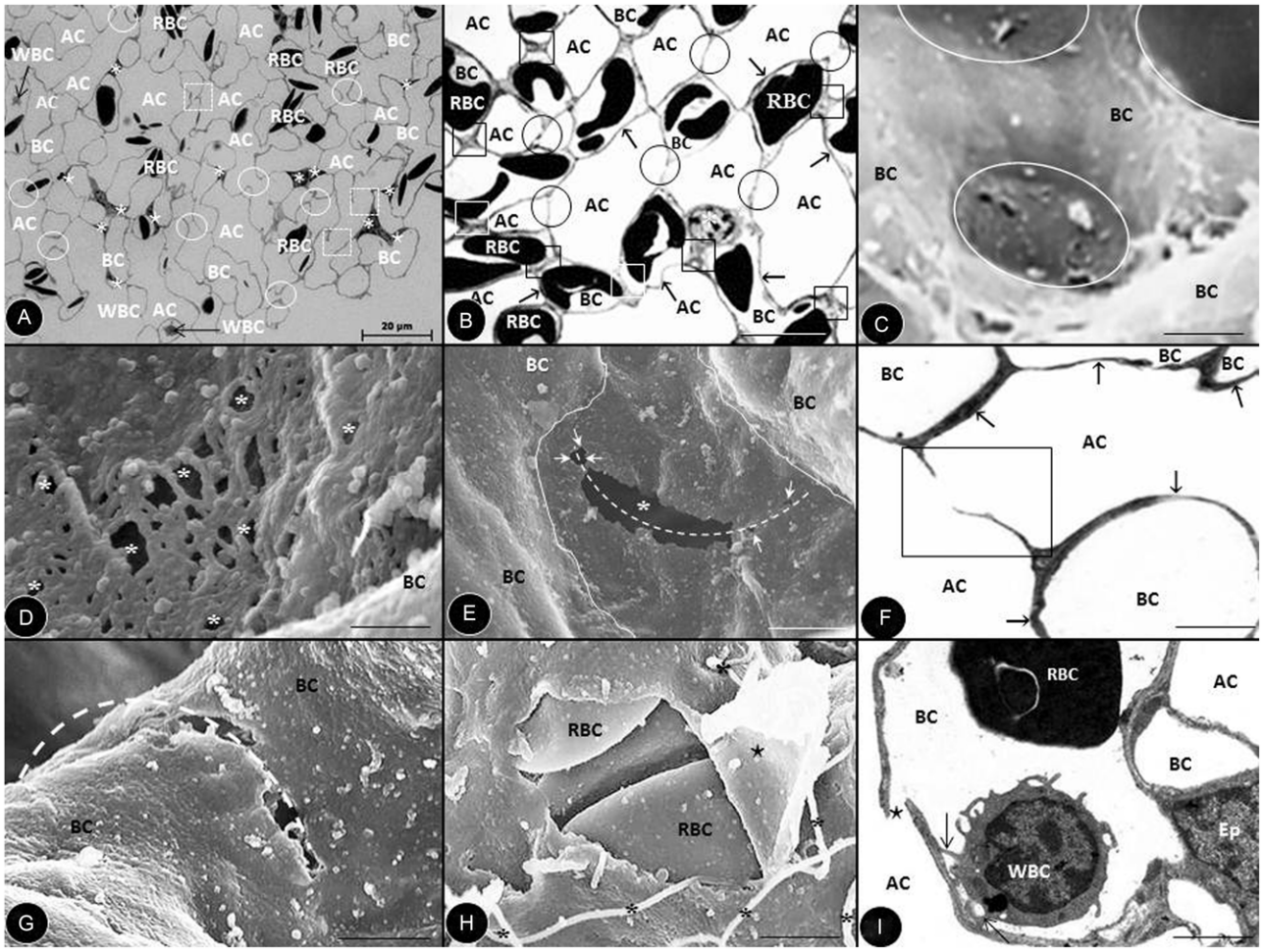

Fig. 8. (**A**) Photomicrograph of the exchange tissue of the chicken lung on which the numbers of complete blood–gas barrier breaks (BGBBs) (dashed squares) and epithelial–epithelial cell connection breaks (EECCBs) (circles) were counted. AC, air capillaries; BC, blood capillaries; asterisks, epithelial cells; stars, endothelial cells; RBC, red blood cells; WBC, white blood cells. Scale bar: 20 µm. (**B**) Transmission electron micrograph showing the components of the exchange tissue: air capillaries (AC), blood capillaries (BC), red blood cells (RBC), epithelial–epithelial cell connections (circles), barriers separating blood capillaries (squares), blood–gas barriers (arrows), and epithelial cell nucleus (asterisk). Scale bar: 20 µm. (**C**) Scanning electron micrograph showing epithelial–epithelial cell connections (encircled areas). BC, blood capillaries. Scale bar: 15 µm. (**D**) Scanning electron micrograph close-up of perforations of an epithelial–epithelial cell connection (asterisks). BC, blood capillary. Scale bar: 25 µm. (**E**) Scanning electron micrograph showing an epithelial–epithelial cell connection (area between continuous lines) with a large failure site (asterisk), smaller holes (arrows) about to merge with it, and the direction of failure shown by the dashed line. BC, blood capillaries. Scale bar: 30 µm. (**F**) Transmission electron micrograph showing an epithelial–epithelial cell connection break (boxed area). AC, air capillary; BC, blood capillary; arrows, blood–gas barrier. Scale bar: 10 µm. (**G**) Scanning electron micrograph showing a transverse linear fracture site (dashed line) of a blood capillary (BC) wall. Scale bar: 25 µm. (**H**) Scanning electron micrograph showing area where the blood–gas barrier has ripped off at high workload (star). RBC, red blood cell; asterisks, fibrinous strands close to and over the failure site. Scale bar: 10 µm. (**I**) Transmission electron micrograph showing a blood–gas barrier rupture site (star). BC, blood capillary; AC, air capillary; RBC, red blood cell; WBC, white blood cell with filopodia (arrows) attaching onto the endothelial cell of the blood capillary; Ep, epithelial cell nucleus. Scale bar: 20 µm.

Conclusion

This study corroborates earlier investigations by Maina and Cowley, Nganpiep and Maina, and Kiama et al. (Maina and Cowley, 1998; Nganpiep and Maina, 2002; Kiama et al., 2008) that structural failure of the BGB is common even in resting (unstressed) and normal (healthy) birds. The high arterial blood pressures in birds (e.g. Seymour and Blaylock, 2000) and systolic pressures that range from 108 to 220 mmHg (14.4–29.3 kPa) (Lumeij and Ritchie, 1994), may lead to high PCBPs, explaining the occurrence BGBBs even in inactive/unstressed birds. Presence of white blood cells and prompt deposition of fibrin-like material at the failure sites showed existence of an efficient cellular defense and/or repair mechanism of injured BGB. It may explain how birds cope with modest BGBBs. The occurrence of more EECCBs (compared to the BGBBs) showed that as would be expected from their morphologies (Maina and King, 1982; Maina and West, 2005), the EECCs are weaker than the BGBs. The relative numbers of BGBBs and EECCBs in the different parts of the lung, which are supplied with blood by the four main branches of the PA, correlate with the volumes of blood delivered to these regions. Identifying how and under what conditions BGBBs occur in the avian lung should help in formulation of husbandry practices that lower incidences of deaths from such injuries. For birds bred for human consumption, husbandry methods that minimize stress may help reduce loses and therefore improve commercial productivity.

Materials and Methods

Experimental animals

The University of the Witwatersrand's Animal Ethics Screening Committee granted approval of this study (Clearance Number 2007/53/01). Mature free range mixed breed of domestic chickens which weighed between 1.4 and 2.8 kg were obtained from a reliable supplier. They were kept in an animal holding unit for 3 weeks to acclimatize to the indoor conditions, become accustomed to handling, and for their health to be properly assessed. The temperature in the well-ventilated room in which they were kept was maintained at 22℃, the birds were exposed to 12-hour light and 12-hour darkness cycles, they were fed a commercial food ration and occasional green vegetables, and water was provided *ad libitum*. The experimental animals were divided into two groups; the first group comprised of rested chickens while those in the second group were subjected to five exercise regimens. Eight chickens were used for each test; for the 6 experimental set-ups (1 resting and 5 exercise treatments), a total of 48 birds were used, and; in the four experimental formulations (BLL measurement, counting of RBCs, determination of PC, and counting of BGBBs and EECCBs), 192 chickens were used. The microscopic morphologies of the BGBBs and the EECCBs were studied by transmission electron microscopes and scanning electron microscopes.

Pulmonary lavage

The chickens were killed by injection with 5 ml of thiopentone sodium (200 $mg.cm^{-3}$) (Euthanase®) into the right brachial vein and the trachea exteriorized and cannulated. PBS (pH 7.4) which was warmed to 40℃ was instilled into the respiratory system from a height of 30 cm above the sternum. When it stopped flowing, the trachea was ligated and the fluid left in the respiratory system for 10 minutes before collecting it by gravity, i.e. by holding the bird's head down by its legs.

Counting of the red blood cells

A hemocytometer with Neubauer markings and a special coverslip was used to count the RBCs. Counting was done on five squares at a total magnification of 400×, using a 10× eyepiece and a 40× objective. Double counting was avoided by applying an exclusion rule: cells which overlapped the top and the right boundary of the counting area were included while those that touched the bottom and left boundary were excluded. The nRBCs was calculated from the volume of the fluid under the coverslip in the five squares that were counted.

Determination of protein concentration

The Lowry method of PC determination (Lowry et al., 1951) was used. Triplicates of 50 µl each of serially diluted working solution of BSA were put in 1 mm^3 Eppendorf tubes. The collected lavage fluid was also prepared in triplicate. Two hundred µl of reagent A was added to the contents of the Eppendorf tubes which were left on a mechanical shaker for 10 minutes at room temperature (22℃). Fifty µl of reagent B was added to the content of each tube and shaken for another 30 minutes. Absorbance of each of the solutions in a microwell was read at 690 nm wavelength using Labsystems Multiskan Ascent (Amersham Pharmacia, Buckinghamshire, UK) spectrophotometer. The absorbance values of the BSA standards were plotted against concentrations to construct a standard curve. The PC in the lavage fluid was calculated using the derived mathematical equation relating the absorbance of the BSA standard to the concentration obtained from the graph.

Experiments on rested chickens

To avoid agitating the birds before the start of the experiment, chickens were kept in isolation (in a cage) for 2 hours. Calming the chickens by sedation was considered but not performed due to possibility of introducing experimental 'noise' that would have been difficult to set apart, especially for the exercised birds. After calming down, a chicken was gently removed from the cage and a drop of blood taken by pricking the comb with a lancet. Three measurements of blood lactic acid level (BLL) were made with Lactate Plus® (Nova Biomedical, Waltham, MA, USA) hand-held machine. Next, the chicken was injected with 2.5 cm^3 heparin (1000 IU) through the left brachial vein. Following 10 minutes of its circulation, the bird was killed by injection of 5 ml of thiopentone sodium into the right brachial vein. The respiratory system was then instilled with warm (40℃) PBS from a height of 30 cm above the sternum. The nRBCs were counted and the PCs determined in the fluid recovered from the respiratory system. After flushing the lungs with warm PBS (pH 7.4; temperature 40℃) for 2 to 3 minutes, they were fixed by perfusing them with 2.5% glutaraldehyde (pH of 7.4; osmolarity of 350 $mOsm.L^{-1}$) at an inflow pressure of 12 cmH_2O (8.8 mmHg=1.2 kPa); the outflow tube was placed at the level of the sternum 7 cmH_2O (5.3 mmHg=0.7 kPa) [In a pilot study, it was determined that an inflow pressure of 12 cmH_2O (1.2 kPa=9 mmHg) and an outflow one of 8 cmH_2O (0.8 kPa=6 mmHg) caused minimal or no BGB and EECCs failures]. The progress of intravascular perfusion was assessed from the blanching (whitening) of the lung due to ejection of blood from it. The lungs were removed from the thorax, immersed in the same fixative, and stored at 4℃ before sampling and processing for microscopic analysis and examination.

Experiments on exercised chickens

A Collins treadmill machine® (Upfront Merchants, Boulder, CO, USA) powered by a single electric motor with a variable speed of between 0.5 and 3.0 $m.sec^{-1}$, maximum inclination angle of 5° and total running belt length of 150 cm was used. Prior to the experiments, the chickens were allowed to familiarize themselves with the surroundings and become accustomed to the noise made by the machine; they were placed close to it for 1 hour every morning for 2 weeks. Every other care was taken to reduce stress that could increase heart rate or respiratory rate. Measurements of these parameters were avoided in order not to excite the birds. During the experiment, the chickens were quietly approached and gently placed legs first on the treadmill while the machine was running at the slowest speed and zero inclination. When the chicken started walking, the speed of the belt was slowly increased to the desired exercise level. After 10 minutes of running, a sample of blood was immediately taken and the BLL measured. Pulmonary lavage was performed and the nRBCs and the PC determined in the recovered fluid. In the same exercise protocol, the chickens were first injected with heparin through the left brachial vein and after 10 minutes of circulation killed by injecting them with Euthanase®. The coelomic cavity was opened by a ventral approach, the pericardial sac incised, and the adipose tissue around the root of the pulmonary trunk cleared up to where the blood vessel bifurcated into left and right pulmonary arteries. Incisions were made through the right ventricle and also across the tip of the left ventricle to access their cavities. The inflow tube was inserted through the slit in the right ventricle and pushed up to the pulmonary trunk and the outflow tube was inserted through the opening at the apex of the heart and pushed past the atrioventicular valve into the left atrium. The pulmonary circulation was flushed with warm PBS and the lungs fixed as in the rested birds.

Sampling and processing of lung tissues for light microscopy, transmission electron microscopy and scanning electron microscopy

Transverse incisions of the fixed lung were made along the costal sulci and the acquired slices cut in halves. An acetate paper with a quadratic square lattice grid of a total area of 6 mm×6 mm divided into 1 mm×1 mm numbered squares was placed on the surface of the cranial aspect of the slice. Four random numbers were generated using a random number generator (free software) and samples taken from areas corresponding to the numbers. Tissue samples were taken from the regions perfused by the four main branches of the pulmonary artery (PA), namely the cranial, the accessory, the caudomedial and the caudolateral branches. The samples taken were processed for light microscopy (LM), transmission electron microscopy (TEM) and scanning electron microscopy (SEM) by the standard laboratory techniques. Briefly, for LM and TEM, the samples were embedded in epoxy resin, semithin sections contrasted with toluidine blue, and for TEM ultrathin sections were contrasted with lead nitrate and uranyl acetate and viewed on a JEOL100S microscope. Samples for SEM were dehydrated, sputter-coated with gold–palladium complex, and viewed on a JEOL 840 microscope.

Determining numbers of blood–gas barrier breaks (BGBBs) and epithelial–epithelial cell connection breaks (EECCBs)

The BGBBs and the EECCBs in the vascular regions supplied by the PA were counted on semithin toluidine-blue stained sections at a final magnification of ×1000, using a 10× eyepiece and a 100× oil objective (Fig. 8A). The analysis was made on eight randomly selected microscopic fields per section. The sections were randomly selected from the different vascular regions of the lung. The average nBGBBs was calculated by adding the total number of breaks in the vascular regions of the lung and dividing it by the number of 100 μm^2 counting fields; the same was done for the EECCBs. The relative (%) proportions of the BGBBs and the EECCBs in the vascular regions of lung were calculated for comparisons.

Statistical evaluation

A nested analysis of covariance (ANCOVA) was used to test the differences between some of the parameters (Littell et al., 1991), with the statistical level of significance set at $P<0.05$.

Acknowledgements

This work was funded by the National Research Foundation (NRF) of South Africa. We are grateful to the technical staff of the School of Anatomical Sciences of the University of Witwatersrand for their immense assistance; to Mr C. van der Merwe of University of Pretoria for assistance with scanning electron microscopy; Dr V. Meskiani for training (S.A.J.) in techniques of immunoelectron microscopy; staff of the Central Animal Service (CAS) Unit of the

University of the Witwatersrand for their help with care of the animals.

Competing Interests

The authors have no competing interests to declare.

References

Abdalla, M. A. (1989). The blood supply to the lung. In *Form And Function In Birds*, Vol. 4 (ed. A. S. King and J. McLelland), pp. 281-306. London: Academic Press.

Abdalla, M. A. and King, A. S. (1976). Pulmonary arteriovenous anastomoses in the avian lung: do they exist? *Respir. Physiol.* **27**, 187-191.

Aschoff, J. and Pohl, H. (1970). Rhythmic variations in energy metabolism. *Fed. Proc.* **29**, 1541-1552.

Bamford, O. S. and Maloiy, G. M. O. (1980). Energy metabolism and heart rate during treadmill exercise in the Marabou stork. *J. Appl. Physiol.* **49**, 491-496.

Beller, C. J., Labrosse, M. R., Thubrikar, M. J. and Robicsek, F. (2004). Role of aortic root motion in the pathogenesis of aortic dissection. *Circulation* **109**, 763-769.

Birks, E. K., Mathieu-Costello, O., Fu, Z., Tyler, W. S. and West, J. B. (1994). Comparative aspects of the strength of pulmonary capillaries in rabbit, dog, and horse. *Respir. Physiol.* **97**, 235-246.

Bishop, C. M. (1997). Heart mass and the maximum cardiac output of birds and mammals: implications for estimating the maximum aerobic power input of flying animals. *Philos. Trans. R. Soc. B* **352**, 447-456.

Butler, P. J. (1991). Exercise in birds. *J. Exp. Biol.* **160**, 233-262.

Butler, P. J., West, N. H. and Jones, D. R. (1977). Respiratory and cardiovascular responses of the pigeon to sustained, level flight in a wind-tunnel. *J. Exp. Biol.* **71**, 7-26.

Cabrera, M. E. and Chizeck, H. J. (1996). On the existence of a lactate threshold during incremental exercise: a systems analysis. *J. Appl. Physiol.* **80**, 1819-1828.

Cabrera, M. E., Saidel, G. M. and Kalhan, S. C. (1999). Lactate metabolism during exercise: analysis by an integrative systems model. *Am. J. Physiol.* **277**, R1522-R1536.

Collins, J. J., Jr. (1971). Dissecting aneurysms in turkeys and man. *AMA Arch. Surg.* **102**, 159-160.

Crouch, E. C., Martin, G. R., Jerome, B. S. and Laurie, G. W. (1997). Basement membranes. In *The Lung: Scientific Foundations*, Vol. 1 (ed. R. G. Crystal, J. B. West, E. R Weibel and P. J. Barnes), pp. 769-791. Philadelphia (PA): Lippincott-Raven.

Currie, R. J. W. (1999). Ascites in poultry: recent investigations. *Avian Pathol.* **28**, 313-326.

Eliassen, E. (1963). Preliminary results from new methods of investigating the physiology of birds during flight. *Ibis* **105**, 234-237.

Erickson, L. L. (1994). *For The Birds: An Uncommon Guide*. Duluth (MN): Pfeifer-Hamilton.

Gehr, P., Mwangi, D. K., Ammann, A., Maloiy, G. M. O., Taylor, C. R. and Weibel, E. R. (1981). Design of the mammalian respiratory system. V. Scaling morphometric pulmonary diffusing capacity to body mass: wild and domestic mammals. *Respir. Physiol.* **44**, 61-86.

Gleeson, T. T. and Baldwin, K. M. (1981). Cardiovascular response to treadmill exercise in untrained rats. *J. Appl. Physiol.* **50**, 1206-1211.

Grubb, B. R. (1982). Cardiac output and stroke volume in exercising ducks and pigeons. *J. Appl. Physiol.* **53**, 207-211.

Grubb, B. R. (1983). Allometric relations of cardiovascular function in birds. *Am. J. Physiol.* **245**, H567-H572.

Hart, J. S. and Roy, O. Z. (1966). Respiratory and cardiac responses to flight in pigeons. *Physiol. Zool.* **39**, 291-306.

Hartman, F. A. (1955). Heart weight in birds. *Condor* **57**, 221-238.

Hudson, B. G., Reeders, S. T. and Tryggvason, K. (1993). Type IV collagen: structure, gene organization, and role in human diseases. Molecular basis of Goodpasture and Alport syndromes and diffuse leiomyomatosis. *J. Biol. Chem.* **268**, 26033-26036.

Issac, Y. M., Abraham, J., Sreeparvathy, George, and Balusami, J. and C. (2010). Managemental practices to control ascitis in a flock. *Vet. World* **3**, 250-252.

Jimoh, S. A. and Maina, J. N. (2013). Immuno-localization of type-IV collagen in the blood-gas barrier and the epithelial-epithelial cell connections of the avian lung. *Biol. Lett.* **9**, 20120951.

Jones, J. H. (1982). Pulmonary blood flow distribution in panting ostriches. *J. Appl. Physiol.* **53**, 1411-1417.

Jones, J. H., Smith, B. L., Birks, E. K., Pascoe, J. R. and Hughes, T. R. (1992). Left atrial and pulmonary arterial pressures in exercising horses. *FASEB J.* **6**, A2020.

Julian, R. J. and Wilson, J. B. (1986). Right ventricular failure as a cause of ascites in broiler and rooster chickens. In *Abstracts: IVth International Symposium Of Veterinary Laboratory Diagnosticians: June 2-6, 1986, Internationaal Congrescentrum RAI, Amsterdam, The Netherlands*, pp. 608-611. Utrecht: Koninklijke Nederlandse Maatschappij voor Diergeneeskunde.

Kiama, S. G., Adekunle, J. S. and Maina, J. N. (2008). Comparative *in vitro* study of interactions between particles and respiratory surface macrophages, erythrocytes, and epithelial cells of the chicken and the rat. *J. Anat.* **213**, 452-463.

Krista, L. M., Waibel, P. E., Shoffner, R. N. and Sautter, J. H. (1967). Natural dissecting aneurysm (aortic rupture) and blood pressure in the turkey. *Nature* **214**, 1162-1163.

Krista, L. M., Waibel, P. E., Shoffner, R. N. and Sautter, J. H. (1970). A study of aortic rupture and performance as influenced by selection for hypertension and hypotension in the turkey. *Poult. Sci.* **49**, 405-411.

Krista, L. M., Beckett, S. D., McDaniel, G. R., Patterson, R. M. and Mora, E. C. (1986). Rupture pressure of elastic and muscular aortic segments from hypertensive and hypotensive turkeys. *Br. Poult. Sci.* **27**, 207-213.

Li, J., Connell, S. and Shi, R. (2010). Biomimetic architectures for tissue engineering. In *Biomimetics, Learning From Nature* (ed. A. Mukherjee), pp. 488-505. Rijeka: InTech.

Lichtenberger, M. (2005). Determination of indirect blood pressure in the companion bird. *Seminars in Avian and Exotic Pet Medicine* **14**, 149-152.

Littell, R. C., Freund, R. J. and Spector, P. C. (1991). *SAS' System For Linear Models (SAS' Series In Statistical Applications)*, 3rd edition. Cary (NC): SAS Institute.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265-275.

Lumeij, J. T. and Ritchie, B. W. (1994). Cardiology. In *Avian Medicine: Principles And Application* (ed. B. W. Ritchie, G. J. Harrison and L. R. Harrison), pp. 695-722. Lake Worth (FL): Wingers Publishing.

Macklem, P. T., Bouverot, P. and Scheid, P. (1979). Measurement of the distensibility of the parabronchi in duck lungs. *Respir. Physiol.* **38**, 23-35.

Maina, J. N. (1989). The morphometry of the avian lung. In *Form And Function In Birds*, Vol. 4 (ed. A. S. King and J. McLelland), pp. 307-368. London: Academic Press.

Maina, J. N. (2005). *The Lung-Air Sac System Of Birds: Development, Structure, And Function*. Berlin; Heidelberg: Springer-Verlag.

Maina, J. N. and Cowley, H. M. (1998). Ultrastructural characterization of the pulmonary cellular defences in the lung of a bird, the rock dove, Columba livia. *Proc. Biol. Sci.* **265**, 1567-1572.

Maina, J. N. and King, A. S. (1982). The thickness of avian blood-gas barrier: qualitative and quantitative observations. *J. Anat.* **134**, 553-562.

Maina, J. N. and West, J. B. (2005). Thin and strong! The bioengineering dilemma in the structural and functional design of the blood-gas barrier. *Physiol. Rev.* **85**, 811-844.

Maina, J. N., King, A. S. and Settle, G. (1989). An allometric study of pulmonary morphometric parameters in birds, with mammalian comparisons. *Philos. Trans. R. Soc. B* **326**, 1-57.

Maina, J. N., Jimoh, S. A. and Hosie, M. (2010). Implicit mechanistic role of the collagen, smooth muscle, and elastic tissue components in strengthening the air and blood capillaries of the avian lung. *J. Anat.* **217**, 597-608.

Maron, M. B., Fu, Z., Mathieu-Costello, O. and West, J. B. (2001). Effect of high transcapillary pressures on capillary ultrastructure and permeability coefficients in dog lung. *J. Appl. Physiol.* **90**, 638-648.

Mitchinson, M. J. and Keymer, I. F. (1977). Aortic rupture in ostriches (*Struthio camelus*)—a comparative study. *J. Comp. Pathol.* **87**, 27-33.

Neumann, F. and Ungar, H. (1973). Spontaneous aortic rupture in turkeys and the vascularization of the aortic wall. *Can. Vet. J.* **14**, 136-138.

Nganpiep, L. N. and Maina, J. N. (2002). Composite cellular defence stratagem in the avian respiratory system: functional morphology of the free (surface) macrophages and specialized pulmonary epithelia. *J. Anat.* **200**, 499-516.

Peters, G. W., Steiner, D. A., Rigoni, J. A., Mascilli, A. D., Schnepp, R. W. and Thomas, S. P. (2005). Cardiorespiratory adjustments of homing pigeons to steady wind tunnel flight. *J. Exp. Biol.* **208**, 3109-3120.

Powell, F. L. and Scheid, P. (1989). Physiology of gas exchange in the avian respiratory system. In *Form And Function In Birds*, Vol. 4 (ed. A. S. King and J. McLelland), pp. 393-437. London: Academic Press.

Powell, F. L., Hastings, R. H. and Mazzone, R. W. (1985). Pulmonary vascular resistance during unilateral pulmonary arterial occlusion in ducks. *Am. J. Physiol.* **249**, R39-R43.

Roston, W. L., Whipp, B. J., Davis, J. A., Cunningham, D. A., Effros, R. M. and Wasserman, K. (1987). Oxygen uptake kinetics and lactate concentration during exercise in humans. *Am. Rev. Respir. Dis.* **135**, 1080-1084.

Seymour, R. S. and Blaylock, A. J. (2000). The principle of Laplace and scaling of ventricular wall stress and blood pressure in mammals and birds. *Physiol. Biochem. Zool.* **73**, 389-405.

Simpson, C. F. (1978). Relation of hemodynamics to the incidence of diethylstilbestrol-induced aortic ruptures in hypertensive and hypotensive lines of turkeys. *Atherosclerosis* **30**, 249-254.

Speckmann, E. W. and Ringer, R. K. (1963). The cardiac output and carotid and tibial blood pressure of the turkey. *Can. J. Biochem. Physiol.* **41**, 2337-2341.

Spector, W. S. (1956). *Handbook Of Biological Data*. Philadelphia (PA): W. B. Saunders.

Taylor, C. R., Maloiy, G. M. O., Weibel, E. R., Langman, V. A., Kamau, J. M. Z., Seeherman, H. J. and Heglund, N. C. (1981). Design of the mammalian respiratory system. III Scaling maximum aerobic capacity to body mass: wild and domestic mammals. *Respir. Physiol.* **44**, 25-37.

Thomas, D. P. and Fregin, G. F. (1981). Cardiorespiratory and metabolic responses to treadmill exercise in the horse. *J. Appl. Physiol.* **50**, 864-868.

Timpl, R. (1989). Structure and biological activity of basement membrane proteins. *Eur. J. Biochem.* **180**, 487-502.

van Veen, L. (1999). [Aortic rupture in poultry: a review]. *Tijdschr. Diergeneeskd.* **124**, 244-247.

Vlahakis, N. E., Schroeder, M. A., Pagano, R. E. and Hubmayr, R. D. (2002). Role of deformation-induced lipid trafficking in the prevention of plasma membrane stress failure. *Am. J. Respir. Crit. Care Med.* **166**, 1282-1289.

Watson, R. R., Fu, Z. and West, J. B. (2008). Minimal distensibility of pulmonary capillaries in avian lungs compared with mammalian lungs. *Respir. Physiol. Neurobiol.* **160**, 208-214.

West, J. B. (2009). Comparative physiology of the pulmonary blood-gas barrier: the unique avian solution. *Am. J. Physiol.* **297**, R1625-R1634.

West, J. B. and Mathieu-Costello, O. (1995). Stress failure of pulmonary capillaries as a limiting factor for maximal exercise. *Eur. J. Appl. Physiol. Occup. Physiol.* **70**, 99-108.

West, J. B. and Mathieu-Costello, O. (1999). Structure, strength, failure, and remodeling of the pulmonary blood-gas barrier. *Annu. Rev. Physiol.* **61**, 543-572.

West, J. B., Tsukimoto, K., Mathieu-Costello, O. and Prediletto, R. (1991). Stress failure in pulmonary capillaries. *J. Appl. Physiol.* **70**, 1731-1742.

West, J. B., Watson, R. R. and Fu, Z. (2007). Major differences in the pulmonary circulation between birds and mammals. *Respir. Physiol. Neurobiol.* **157**, 382-390.

West, J. B., Fu, Z., Gu, Y., Wagner, H. E., Carr, J. A. and Peterson, K. L. (2010). Pulmonary artery pressure responses to increased cardiac output in chickens with raised metabolic rate. *Comp. Biochem. Physiol.* **156A**, 430-435.

Wood, W., Jacinto, A., Grose, R., Woolner, S., Gale, J., Wilson, C. and Martin, P. (2002). Wound healing recapitulates morphogenesis in *Drosophila* embryos. *Nat. Cell Biol.* **4**, 907-912.