Cardiac and vascular disease prior to hatching in chick embryos incubated at high altitude

Article · February 2010

DOI: 10.1017/S2040174409990043

11 authors, including:

Carlos E Salinas
Universidad Mayor de San Andres
12 PUBLICATIONS **179** CITATIONS
SEE PROFILE

Emily Camm
University of Cambridge
46 PUBLICATIONS **1,015** CITATIONS
SEE PROFILE

John Tuckett
The Newcastle upon Tyne Hospitals NHS Foundation Trust
6 PUBLICATIONS **46** CITATIONS
SEE PROFILE

Ruwan Weerakkody
Imperial College London
45 PUBLICATIONS **518** CITATIONS
SEE PROFILE

Journal of Developmental Origins of Health and Disease (2010), **1**(1), 60–66.

doi:10.1017/S2040174409990043

ORIGINAL ARTICLE

Cardiac and vascular disease prior to hatching in chick embryos incubated at high altitude

C. E. Salinas[1], C. E. Blanco[2], M. Villena[1], E. J. Camm[3], J. D. Tuckett[3], R. A. Weerakkody[3], A. D. Kane[3], A. M. Shelley[3], F. B. P. Wooding[3], M. Quy[3] and D. A. Giussani[3]*

[1]*Instituto Boliviano de Biología de Altura, Facultad de Medicina, Universidad Mayor de San Andrés, La Paz, Bolivia*
[2]*Department of Pediatrics, Maastricht University, Maastricht, The Netherlands*
[3]*Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, UK*

The partial contributions of reductions in fetal nutrition and oxygenation to slow fetal growth and a developmental origin of cardiovascular disease remain unclear. By combining high altitude with the chick embryo model, we have previously isolated the direct effects of high-altitude hypoxia on growth. This study isolated the direct effects of high-altitude hypoxia on cardiovascular development. Fertilized eggs from sea-level or high-altitude hens were incubated at sea level or high altitude. Fertilized eggs from sea-level hens were also incubated at high altitude with oxygen supplementation. High altitude promoted embryonic growth restriction, cardiomegaly and aortic wall thickening, effects which could be prevented by incubating eggs from high-altitude hens at sea level or by incubating eggs from sea-level hens at high altitude with oxygen supplementation. Embryos from high-altitude hens showed reduced effects of altitude incubation on growth restriction but not on cardiovascular remodeling. The data show that: (1) high-altitude hypoxia promotes embryonic cardiac and vascular disease already evident prior to hatching and that this is associated with growth restriction; (2) the effects can be prevented by increased oxygenation; and (3) the effects are different in embryos from sea-level or high-altitude hens.

Received 28 May 2009; Revised 17 July 2009; Accepted 11 August 2009; First published online 1 October 2009

Key words: cardiovascular disease, chick embryo, high altitude, hypoxia.

Introduction

Despite healthy skepticism,[1,2] evidence derived from human epidemiologic studies linking small size at birth with greater cardiovascular risk has gathered increasing support in recent years.[3,4] This risk of cardiovascular disease not only results from intrauterine growth retardation in complicated pregnancy, but the association also extends across the normal range of birth weight in healthy pregnancy.[1,3] A component of fetal growth is determined by the quality of the intrauterine environment. In turn, the quality of the intrauterine environment is largely determined by the available nutrient and oxygen supply to the growing young. Consequently, there have been many reports investigating the association between reduced fetal growth and increased risk of cardiovascular disease in animal models in which development has been complicated by reductions in fetal nutrition and/or in fetal oxygenation.[5–10]

Under physiologic conditions, in humans, fetal hypoxia occurs most commonly during the hypobaric hypoxia of pregnancy at high altitude.[11] Although several investigators have reported reduced birth weight in human babies with increasing altitude,[12–17] there have been no reports on the association between fetal growth restriction and alterations in cardiovascular development already evident prior to birth at high altitude in any species. Most high-altitude human populations are impoverished, therefore the extent to which any effects on fetal development during pregnancy at high altitude is governed by fetal under-nutrition or fetal under-oxygenation, remains uncertain. By using the chick embryo as an animal model, an earlier study in our laboratory isolated the direct effects of developmental hypoxia owing to high altitude on embryonic growth, independent of changes in maternal nutrition and of the physiology of the mother or the placenta.[18] The data in that study showed that high-altitude incubation of fertilized eggs laid by sea-level hens markedly restricted growth of the chick embryo. Incubation at high altitude of fertilized eggs laid by high-altitude hens also restricted embryonic growth, but to a lesser extent compared to eggs laid by sea-level hens. By contrast, incubation at sea level of fertilized eggs laid by high-altitude hens not only restored, but also enhanced growth relative to sea-level controls. Incubation at high altitude of sea-level eggs with oxygen supplementation completely prevented the high-altitude-induced growth restriction. Thus, the oxygenation of the chick embryo, independent of maternal nutrition, has a predominant role in the control of its growth during development at high altitude. Further, prolonged high-altitude residence confers protection against the deleterious effects of hypoxia on growth.

*Address for Correspondence: Dr D. A. Giussani, Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, CB2 3EG, UK.
(Email dag26@cam.ac.uk)

The present study tested the hypothesis that development at high altitude is related to a prenatal origin of cardiovascular disease and that hypoxia is the mechanism underlying the relationship. The hypothesis was tested three-fold: (1) by investigating the effects on the cardiovascular development of fertilized eggs laid by sea-level hens when incubated at high altitude; (2) by investigating whether alterations in the embryonic cardiovascular system induced by development at high altitude could be prevented by incubation at sea level of fertilized eggs laid by high-altitude hens; and (3) by investigating whether alterations in the embryonic cardiovascular system induced by development at high altitude could be prevented by incubation at high altitude of sea-level eggs with oxygen supplementation. We were also interested in whether prolonged high-altitude residence conferred any protection against any deleterious effects of hypoxia on cardiovascular development.

Methods

The study was done in Bolivia, in the high-altitude city of La Paz (3600 m, 494 mmHg, approximate ambient dry PO_2 100 mmHg) and the sea-level city of Santa Cruz (420 m, 760 mmHg, approximate ambient dry PO_2 160 mmHg). The incubation procedures have been published earlier in detail.[18] In brief, fertilized eggs were obtained from Black Leghorn chickens that had been reared at the sea-level city of Santa Cruz or at the high-altitude city of La Paz for at least six generations. Fertilized eggs from sea-level hens, laid at sea level, were randomly divided and incubated either at sea level (SLSL, $n = 31$) or high altitude (SLHA, $n = 19$). Eggs from high-altitude hens, laid at high altitude, were randomly divided and incubated either at high altitude (HAHA, $n = 33$) or sea level (HASL, $n = 25$). SLHA embryos were also incubated with oxygen supplementation (SLHA + O_2, $n = 21$) at rates to maintain sea-level oxygen partial pressures according to Dalton's Law.[19]

All incubations (Polyhatch; Brinsea Products Ltd, UK) were carried under conditions to optimize development, with controlled temperature (38°C), humidity (60%) and appropriate egg rotation. On day 20, out of the 21-day incubation period, the egg was weighed, the air cell was exposed and chorioallantoic venous blood was drawn into a 1 ml syringe for analysis of PO_2 (ABL 500; Radiometer, Copenhagen, Denmark), whenever possible in duplicate. Following euthanasia by spinal transection, the embryo was removed from the eggshell and weighed. Head diameter and body length (crown–rump length) were measured with a digital micrometer.

The embryonic heart was dissected and weighed. In a subset of animals, following maximal dilatation using ethylenediaminetetraacetic acid (EDTA; 50 mg/kg), a 5 mm segment of the thoracic aorta was dissected at the level of the apex of the heart, and the heart and aortic segment were fixed in 4% phosphate buffered paraformaldehyde for 24 h and then stored in physiologic buffer. Hearts and vessels were then embedded in paraffin. To account for possible shrinkage because of paraffin processing, the diameter of erythrocytes in heart sections was measured and compared to that obtained by measuring fresh erythrocytes from chick embryos at the same stage of incubation.[20] All measurements were corrected using this factor. Mid-cardiac 4-μm coronal sections and 7-μm transverse aortic sections were stained with van Gieson's solution. Slices were digitally recorded and analyzed by computerized morphometric systems (Quantimet 570; Leica, The Netherlands and Hauppauge Computer Works, UK).

All procedures were approved by the local ethics committee of the Bolivian Institute for High Altitude Biology (Consejo Técnico, IBBA, Universidad Mayor de San Andrés, La Paz, Bolivia). Comparisons between groups were assessed statistically using one-way ANOVA with the Student–Newman–Keuls *post-hoc* test (Sigma-Stat; SPSS Inc., Chicago, IL, USA). The relationships between indices of cardiovascular remodeling and embryonic size or PO_2 were assessed using the Pearson Product-Moment correlation. A comparison between the slopes and intercepts of regression lines was conducted according to Armitage and Berry.[21] For all comparisons, statistical significance was accepted when $P < 0.05$.

Results

Oxygenation and biometry in the chick embryo

Analysis of this subset of animals confirms that incubation at high altitude induced embryonic systemic hypoxia and growth restriction (Table 1). The embryonic growth restriction is disproportionate as the ratio of the head diameter to body length was increased following incubation at high altitude (Table 1). When weight was expressed as a percentage of the initial egg mass, HAHA embryos showed partial protection against the effects of high-altitude incubation on growth. Further, the relative body weight in HASL embryos was greater than any other group (Table 1).

Cardiac measurements in the chick embryo

Relative to SLSL chick embryos, SLHA and HAHA groups showed significant increases of similar magnitude in the relative cardiac weight, and in the relative wall thickness of the left and right ventricles and septum (Fig. 1 and Table 2). In contrast, HASL and SLHA + O_2 embryos had cardiac measurements similar to SLSL embryos (Fig. 1). However, the relative thickness of the walls of the left and right ventricles was significantly reduced when compared to all other groups in SLHA + O_2 embryos (Fig. 1).

Aortic measurements in the chick embryo

SLHA embryos showed significant aortic medial thickening, as indexed by calculation of the aortic wall to lumen area ratio (Fig. 2). Aortae from HAHA embryos had the greatest wall

Table 1. *Oxygenation and biometry in the chick embryo*

	PO_2 (mmHg)	Absolute body weight (g)	Relative body weight (%)	Head diameter : body length
SLSL	56 ± 6 (9)[a]	28 ± 1 (31)[a]	40 ± 1 (31)[a]	0.177 ± 0.001 (31)[a]
SLHA	38 ± 2 (12)[b]	15 ± 1 (19)[b]	22 ± 1 (19)[b]	0.202 ± 0.005 (19)[b]
HAHA	36 ± 2 (10)[b]	15 ± 1 (33)[b]	31 ± 1 (33)[c]	0.201 ± 0.003 (33)[b]
HASL	64 ± 9 (7)[a]	24 ± 1 (25)[c]	55 ± 2 (25)[d]	0.176 ± 0.001 (25)[a]
SLHA + O_2	64 ± 4 (10)[a]	30 ± 1 (21)[a]	45 ± 1 (21)[a]	0.173 ± 0.003 (21)[a]

Values are mean ± SEM for the partial pressure of oxygen in chorio-allantoic venous blood, the embryonic weight expressed as a percentage of the initial egg mass and the ratio of the head diameter to crown–rump length in sea-level chick embryos incubated either at sea level (SLSL) or high altitude (SLHA), high-altitude embryos incubated at high altitude (HAHA) or sea level (HASL), and from sea-level chick embryos incubated at high altitude with oxygen supplementation (SLHA + O_2).

Number (n) of chicks for each variable in parentheses.

[a,b,c,d] Values within columns that have different letters as superscripts are significantly different from each other (one-way ANOVA with Student–Newman–Keuls test; $P < 0.05$).

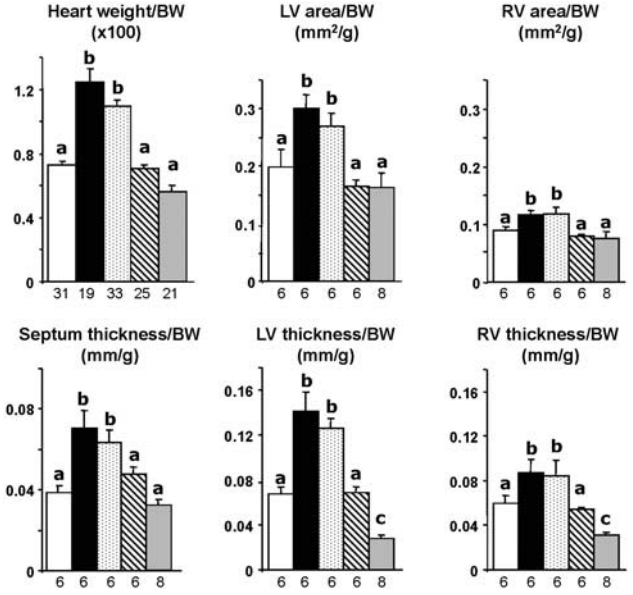

Fig. 1. Bars represent the mean ± SEM for the heart weight, area and thickness of the walls of the left and right ventricles and thickness of the cardiac septum expressed relative to body weight in sea-level chick embryos incubated either at sea level (SLSL, open bar) or high altitude (SLHA, filled bar), high-altitude embryos incubated at high altitude (HAHA, stippled bar) or sea level (HASL, hatched bar), and in sea-level chick embryos incubated at high altitude with oxygen supplementation (SLHA + O_2, gray bar). n of each group for each variable are shown at the bottom of the histograms. Values within columns that have different letters as superscripts are significantly different from each other (one-way ANOVA with Student–Newman–Keuls test; $P < 0.05$).

thickening relative to all other groups, as indexed by significant differences in all aortic measurements and derived calculations (Table 3 and Fig. 2). HASL or SLHA + O_2 prevented the aortic thickening induced by incubation at high altitude (Fig. 2).

Relation between cardiovascular remodeling and embryonic size or PO_2

Correlation analysis revealed that the embryonic body weight and PO_2 were negatively related to the aortic wall to lumen area ratio in all groups independent of treatment (Fig. 3). By contrast, the ratio of the embryonic head diameter to body length was positively related to the aortic wall to lumen area ratio in all groups independent of treatment (Fig. 3). When body weight was related to the cardiac weight across all groups, the association was best described by a reverse exponential ($y = 1.8951e^{-0.036x}$, $r = 0.85$) (Fig. 4*a*). Though SLSL, HASL and SLHA + O_2 embryos were distributed across the right-hand side, SLHA and HAHA groups were distributed across the left-hand side of the association (Fig. 4*a*). The relation between body weight and cardiac weight remained significant even across the normal range for body weight in SLSL embryos (Fig. 4*b*). Though the embryonic body and cardiac weights were obtained in every chick, only organs from smaller subgroups of embryos were prepared for histology. Similarly, chorio-allantoic PO_2 was obtained only from subgroups of embryos. Therefore, the relationship between embryonic body weight and any variable other than cardiac weight (for instance aortic wall to lumen area ratio or PO_2) within any one group could not be investigated.

Discussion

Several experimental techniques, employed primarily in pregnant sheep, rats and guinea pigs, have been used to induce sustained fetal hypoxemia, including reductions in uterine and umbilical blood flow,[22–25] placental embolization,[26] pre-conceptual removal of endometrial caruncles[27,28] and maternal chronic hypoxia.[29–31] All these elegant studies have reported marked effects on the developing cardiovascular system. More recently, attention has focused on whether sustained prenatal hypoxia may have adverse consequences for

Table 2. *Cardiac measurements in the chick embryo*

	Heart weight (mg)	LV area (mm^2)	RV area (mm^2)	Septum thickness (mm)	LV wall thickness (mm)	RV wall thickness (mm)
SLSL	196 ± 4 (31)	5.5 ± 0.8 (6)	2.2 ± 0.2 (6)	1.11 ± 0.05 (6)	1.87 ± 0.16 (6)	1.59 ± 0.17 (6)
SLHA	185 ± 7 (19)	4.6 ± 0.2 (6)	1.8 ± 0.2 (6)	1.10 ± 0.05 (6)	2.13 ± 0.07 (6)	1.32 ± 0.07 (6)
HAHA	175 ± 5 (33)	4.2 ± 0.5 (6)	1.8 ± 0.3 (6)	1.02 ± 0.05 (6)	2.08 ± 0.09 (6)	1.31 ± 0.07 (6)
HASL	188 ± 6 (25)	4.0 ± 0.3 (6)	1.8 ± 0.1 (6)	1.00 ± 0.06 (6)	1.74 ± 0.07 (6)	1.47 ± 0.07 (6)
SLHA + O_2	194 ± 9 (21)	5.0 ± 0.6 (8)	2.3 ± 0.3 (8)	1.01 ± 0.09 (8)	0.93 ± 0.15 (8)	1.08 ± 0.07 (8)

Values are mean ± SEM for absolute cardiac measurements in sea-level chick embryos incubated either at sea level (SLSL) or high altitude (SLHA), high-altitude embryos incubated at high altitude (HAHA) or sea level (HASL), and from sea-level chick embryos incubated at high altitude with oxygen supplementation (SLHA + O_2).

Number (*n*) of chicks for each variable in parentheses.

*$P < 0.05$ *v.* SLSL, ANOVA + Student–Newman–Keuls test.

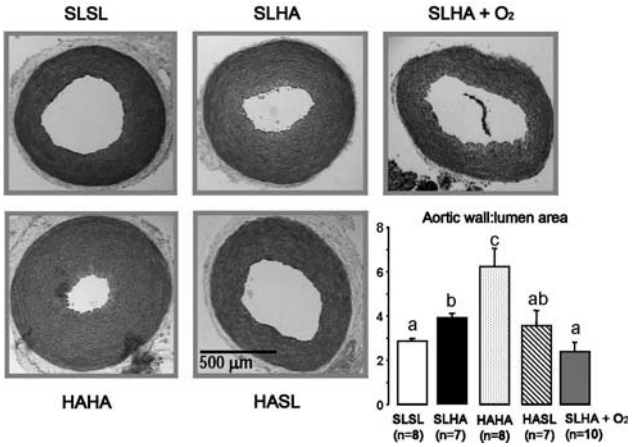

Fig. 2. Photomicrographs of representative examples of aortic sections and the mean ± SEM of the aortic wall to lumen area ratio for sea-level chick embryos incubated either at sea level (SLSL, open bar) or high altitude (SLHA, filled bar), high-altitude embryos incubated at high altitude (HAHA, stippled bar) or sea level (HASL, hatched bar), and in sea-level chick embryos incubated at high altitude with oxygen supplementation (SLHA + O_2, gray bar). Values within columns that have different letters as superscripts are significantly different from each other (one-way ANOVA with Student–Newman–Keuls test; $P < 0.05$).

the function of the cardiovascular system after birth and in later life. For instance, the groups of Zhang,[8] McMillen and Davidge[32–34] have reported that pregnant dams exposed to chronic hypoxia produce offspring with unequivocal cardiac and vascular dysfunction. However, because placental insufficiency decreases the delivery of nutrients as well as oxygen to the fetus and because chronic maternal hypoxia decreases maternal food intake,[32–35] the extent to which the effects on the developing cardiovascular system of all the above interventions are because of fetal under-nutrition or under-oxygenation remains uncertain. Employing the chick embryo as an animal model, a few studies have been able to isolate the effects on the developing cardiovascular system of chronic hypoxia, independent of changes in maternal nutrition and of the physiology of the mother and the placenta. Studies by Blanco and colleagues[36] and the group of le Noble[37] have confirmed that oxygen deprivation can act alone to remodel the developing cardiovascular system. Incubation of chick embryos with isobaric hypoxia induced embryonic aortic hypertrophic growth, left ventricular dysfunction and sympathetic hyperinnervation of peripheral arteries.[36–39] The present study combined the use of the chick embryo model with incubation at high altitude to determine for the first time: (1) whether chronic hypoxia during development at high altitude is the mechanism underlying the relationship between growth restriction and cardiovascular disease already evident prior to hatching; (2) whether such effects are different in embryos from sea-level or high-altitude hens; and (3) whether the effects could be prevented by incubation of fertilized eggs from sea-level hens at high altitude with oxygen supplementation, or by incubation of fertilized eggs from high-altitude hens at sea level.

Analysis of this subset of animals confirms that incubation at high altitude induced embryonic growth restriction and that this effect was diminished in embryos from high-altitude hens. These findings support the observations of other studies reporting that in human populations prolonged high-altitude residence ancestry can confer protection against the effects of high altitude on fetal growth.[11,14,16] The cardiovascular data in the present study show that incubation at high altitude leads to cardiac and aortic wall thickening in the chick embryo, independent of highland ancestry. Such cardiac and vascular remodeling could be prevented by incubation at sea level of fertilized eggs laid by high-altitude hens, or by incubation at high altitude of sea-level eggs with oxygen supplementation. Significant negative relationships were obtained between embryonic body weight or chorio-allantoic venous PO_2 (equivalent to umbilical venous PO_2 in mammalian pregnancy) with aortic wall thickening, and a significant positive relationship occurred between the ratio of

Table 3. *Aortic measurements in the chick embryo*

	Outer diameter (μm)	Lumen diameter (μm)	Wall thickness (μm)	Wall thickness/ lumen radius ratio	Wall area (mm^2)	Lumen area (mm^2)
SLSL	995 ± 63	508 ± 30	244 ± 17	0.96 ± 0.03	593 ± 78	207 ± 24
SLHA	1085 ± 39	488 ± 18	288 ± 14	1.14 ± 0.05	727 ± 57	184 ± 14
HAHA	1036 ± 39	396 ± 24*	320 ±16*	1.66 ± 0.14*	794 ± 58	126 ± 15*
HASL	996 ± 82	464 ± 36	266 ± 29	1.16 ± 0.12	637 ± 108	175 ± 27
SLHA + O_2	939 ± 68	515 ± 38	212 ± 28	0.85 ± 0.13	500 ± 85	216 ± 32

Values are mean ± SEM for aortic measurements in sea-level chick embryos incubated either at sea level (SLSL, $n = 8$) or high altitude (SLHA, $n = 7$), high-altitude embryos incubated at high altitude (HAHA, $n = 8$) or sea level (HASL, $n = 7$), and from sea-level chick embryos incubated at high altitude with oxygen supplementation (SLHA + O_2, $n = 10$).

*$P < 0.05$ *v.* SLSL, ANOVA + Student–Newman–Keuls test.

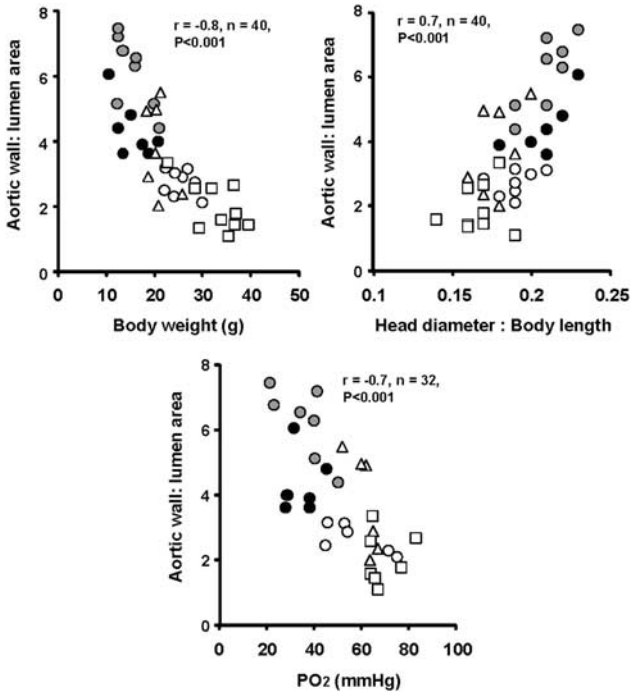

Fig. 3. Relationship between cardiovascular remodeling and embryonic size or PO_2. Body weight, the head diameter to body length ratio and chorio-allantoic venous PO_2 at the end of the incubation period were related to the aortic wall to lumen area ratio in all embryos independent of treatment. *r*, Pearson Product-Moment correlation coefficient; *n*, number of observations. SLSL (○); SLHA (●); HAHA (gray circles); HASL (△) and SLHA+O_2 (□).

the embryonic head diameter to body weight (an index of growth symmetry) and the vascular changes. Combined, therefore, the data presented strongly implicate that hypoxia owing to high altitude is an important mechanism, retarding embryonic growth as well as triggering a developmental origin of cardiovascular disease, already evident prior to hatching/birth. Interestingly, when body weight was related to cardiac weight, data in the present study also show that: (1) a significant negative relationship occurs across the normal range of weights; (2) that this relationship is shifted to the left and upwards by developmental high-altitude hypoxia; and (3) that the shift of the relationship could be restored by incubation at sea level of eggs from high-altitude hens, or by incubation at high altitude of sea-level eggs with oxygen supplementation. These observations have many commonalities with the original findings of Barker and colleagues,[1,3] who related birth weight with increased rates of cardiovascular disease in human populations. They also reported a phenotypic association between asymmetric fetal growth restriction and cardiovascular risk factors, and that this relationship extended across the normal range of birth weights.[1,3]

There is general agreement that cardiovascular remodeling of this type results from an increase in peripheral resistance.[26,29,37] The aortic thickening may be a response to restore wall stress, as is typical of an increase in load, and the ventricular wall thickening occurs in response to the increased cardiac afterload.[35,36] The hemodynamic overload may increase protein synthesis via a plethora of cellular and molecular pathways, including activation of stretch receptors, proto-oncogenes and vascular growth trophic factors.[40,41] Hypoxia may also affect hypoxia-sensitive growth factors, such as VEGF (vascular endothelial growth factor).[39,42] Consistent with the idea that this cardiovascular remodeling results from an increase in peripheral resistance, it has been reported that chronic hypoxia in the chick embryo promotes sympathetic hyper-innervation and enhanced norepinephrine release from perivascular sympathetic nerves;[36,37] that it decreases NO-dependent relaxation; and that it increases constrictor reactivity in the peripheral vasculature.[36]

The data presented using the chick embryo model are of important human relevance. Three separate clinical studies[43–45] have reported that babies born from pregnancies complicated by placental insufficiency show aortic thickening with increased vascular stiffness and reduced distensibility. A component of aortic thickening in the human fetus in pregnancies complicated by placental insufficiency may therefore be triggered by developmental hypoxia alone.

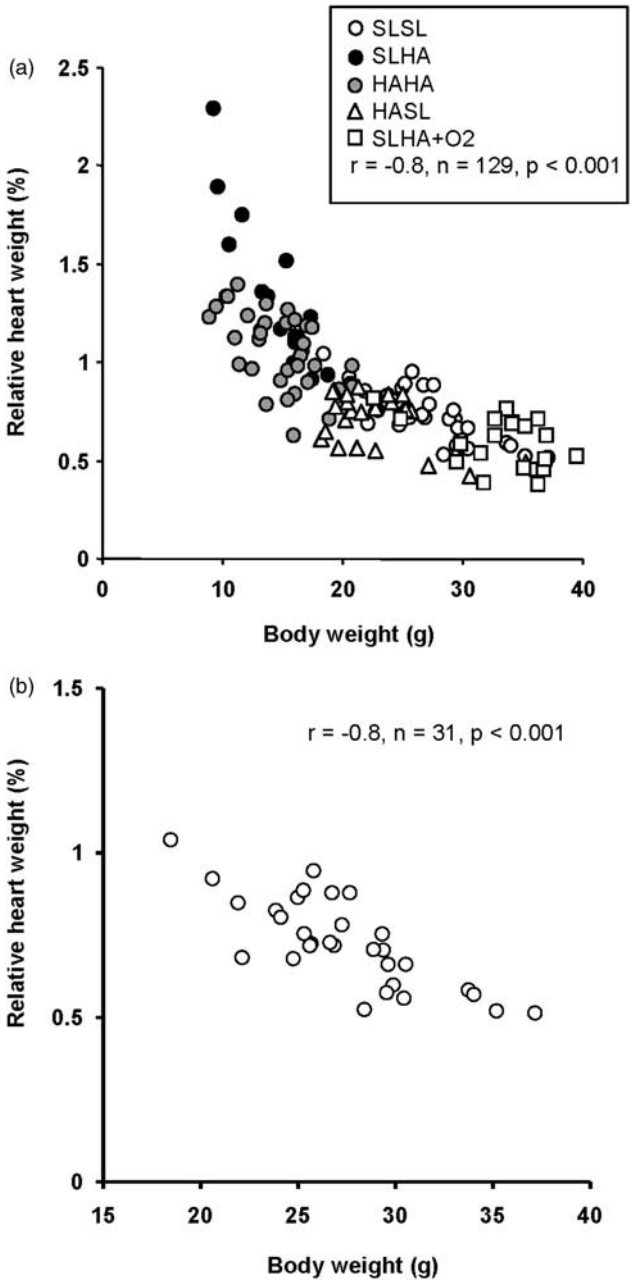

Fig. 4. Relationship between the cardiac weight and body weight in embryos following incubations at sea level and at high altitude (*r*, Pearson Product-Moment correlation coefficient; *n*, number of observations): (*a*) shows that a significant negative relationship occurs across all groups independent of treatment ($P < 0.001$); and (*b*) shows that a significant negative relationship occurs across the normal range of weights in SLSL embryos ($P < 0.001$).

In conclusion, the data show that hypoxia owing to high altitude induces pronounced cardiovascular changes associated with disease in the chick embryo, which are already evident by the end of the incubation period, and that these cardiovascular alterations are associated with disproportionate growth restriction. The effects can be prevented by incubation at sea level of fertilized eggs laid by high-altitude hens, or by incubation at high altitude of sea-level eggs with oxygen supplementation. Prolonged high-altitude residence ancestry confers partial protection against the effects of high-altitude incubation on growth but not on cardiovascular remodeling. It is of obvious interest whether these cardiovascular changes *in ovo* persist, resolve or amplify in later life.

Acknowledgements

We thank Dr Diva Bellido, Dr Wilma Tellez, Mr Armando Rodriguez, Mrs Martha Aguilar, Mrs Loyola Riveros, Mr Wilmar Velasquez and Mr Didi Maquera at IBBA, La Paz; Dr Ginella, Dr Nioshi and Dr Roca at CENETROP, Santa Cruz; and Ms Lilian Kessels at Maastricht University, for their help with these studies. The present study was funded by The British Heart Foundation and The Lister Institute for Preventive Medicine. D.A.G. is a Royal Society-Wolfson Foundation Research Merit Award Holder.

Statement of Interest

There are no conflicts of interest.

References

1. Huxley RR, Shiell AW, Law CM. The role of size at birth and postnatal catch-up growth in determining systolic blood pressure: a systematic review of the literature. *J Hypertens*. 2000; 18, 815–831.
2. Lenfant C. Low birth weight and blood pressure. *Metab Clin Exp*. 2008; 57, S32–S35.
3. Godfrey KM, Barker DJ. Fetal programming and adult health. *Public Health Nutr*. 2001; 4, 611–624.
4. Gluckman PD, Hanson MA, Cooper C, Thornburg KL. Effect of *in utero* and early-life conditions on adult health and disease. *N Engl J Med*. 2008; 359, 61–73.
5. McMillen IC, Robinson JS. Developmental origins of the metabolic syndrome: prediction, plasticity, and programming. *Physiol Rev*. 2005; 85, 571–633.
6. Armitage JA, Taylor PD, Poston L. Experimental models of developmental programming: consequences of exposure to an energy rich diet during development. *J Physiol*. 2005; 565(Pt 1), 3–8.
7. Langley-Evans SC, Bellinger L, McMullen S. Animal models of programming: early life influences on appetite and feeding behaviour. *Matern Child Nutr*. 2005; 1, 142–148.
8. Zhang L. Prenatal hypoxia and cardiac programming. *J Soc Gynecol Investig*. 2005; 12, 2–13.
9. Fowden AL, Giussani DA, Forhead AJ. Intrauterine programming of physiological systems: causes and consequences. *Physiology (Bethesda)*. 2006; 21, 29–37.
10. Morrison JL. Sheep models of intrauterine growth restriction: fetal adaptations and consequences. *Clin Exp Pharmacol Physiol*. 2008; 35, 730–743.
11. Moore LG. Maternal O_2 transport and fetal growth in Colorado, Peru and Tibet high-altitude residents. *Am J Hum Biol*. 1990; 2, 627–637.

12. Lichty JA, Ting RY, Bruns P, Dyer E. Studies of babies born at high altitude. 1. Relation of altitude to birth weight. *Am J Dis Child*. 1957; 93, 666–669.
13. McClung J. *Effects of High Altitude on Human Birth*, 1969. Harvard University Press, Cambridge.
14. Haas JD, Frongillo EF, Stepcik C, Beard J, Hurtado L. Altitude, ethnic and sex differences in birthweight and length in Bolivia. *Hum Biol*. 1980; 52, 459–477.
15. Moore LG, Niermeyer S, Zamudio S. Human adaptation to high altitude: regional and life-cycle perspectives. *Am J Phys Anthropol Suppl*. 1998; 27, 25–64.
16. Giussani DA, Phillips PS, Anstee S, Barker DJ. Effects of altitude vs. economic status on birth weight and body shape at birth. *Ped Res*. 2001; 49, 490–494.
17. Zamudio S, Postigo L, Illsley NP, *et al*. Maternal oxygen delivery is not related to altitude- and ancestry-associated differences in human fetal growth. *J Physiol*. 2007; 582(Pt 2), 883–895.
18. Giussani DA, Salinas CE, Villena M, Blanco CE. The role of oxygen in prenatal growth: studies in the chick embryo. *J Physiol*. 2007; 585(Pt 3), 911–917.
19. West JB. *Respiratory Physiology. The Essentials*, 7th edn, 2004. Lippincott Williams & Wilkins, Baltimore.
20. Burton GJ, Palmer ME. Eradicating fetomaternal fluid shift during perfusion fixation of the human placenta. *Placenta*. 1998; 9, 327–332.
21. Armitage P, Berry G. Further analyses of straight-line data. In *Statistical Methods in Medical Research* (eds. Armitage P, Berry G, Matthews JNS), 1994; 292–305. Blackwell, Oxford.
22. Clark KE, Durnwald M, Austin JE. A model for studying chronic reduction in uterine blood flow in pregnant sheep. *Am J Physiol*. 1982; 242, H297–H301.
23. Jensen A, Roman C, Rudolph AM. Effects of reducing uterine blood flow on fetal blood flow distribution and oxygen delivery. *J Dev Physiol*. 1991; 15, 309–323.
24. Richardson BS, Bocking AD. Metabolic and circulatory adaptations to chronic hypoxia in the fetus. *Comp Biochem Physiol A Mol Integr Physiol*. 1998; 119, 717–723.
25. Gardner DS, Fletcher AJ, Fowden AL, Giussani DA. A novel method for controlled and reversible long term compression of the umbilical cord in fetal sheep. *J Physiol*. 2001; 535(Pt 1), 217–229.
26. Murotsuki J, Challis JR, Han VK, Fraher LJ, Gagnon R. Chronic fetal placental embolization and hypoxemia cause hypertension and myocardial hypertrophy in fetal sheep. *Am J Physiol*. 1997; 272(1 Pt 2), R201–R207.
27. Walker AM, de Preu ND, Horne RS, Berger PJ. Autonomic control of heart rate differs with electrocortical activity and chronic hypoxaemia in fetal lambs. *J Dev Physiol*. 1990; 14, 43–48.
28. Robinson JS, Kingston EJ, Jones CT, Thorburn GD. Studies on experimental growth retardation in sheep. The effect of removal of endometrial caruncles on fetal size and metabolism. *J Dev Physiol*. 1979; 1, 379–398.
29. Kitanaka T, Alonso JG, Gilbert RD, Siu BL, Clemons GK, Longo LD. Fetal responses to long-term hypoxemia in sheep. *Am J Physiol*. 1989; 256, R1348–R1354.
30. Kamitomo M, Longo LD, Gilbert RD. Right and left ventricular function in fetal sheep exposed to long-term high-altitude hypoxemia. *Am J Physiol*. 1992; 262(2 Pt 2), H399–H405.
31. Thompson LP, Dong Y. Chronic hypoxia decreases endothelial nitric oxide synthase protein expression in fetal guinea pig hearts. *J Soc Gynecol Investig*. 2005; 12, 388–395.
32. Williams SJ, Hemmings DG, Mitchell JM, McMillen IC, Davidge ST. Effects of maternal hypoxia or nutrient restriction during pregnancy on endothelial function in adult male rat offspring. *J Physiol*. 2005; 565, 125–135.
33. Xu Y, Williams SJ, O'Brien D, Davidge ST. Hypoxia or nutrient restriction during pregnancy in rats leads to progressive cardiac remodeling and impairs postischemic recovery in adult male offspring. *FASEB J*. 2006; 20, 1251–1253.
34. Rueda-Clausen CF, Morton JS, Davidge ST. Effects of hypoxia-induced intrauterine growth restriction on cardiopulmonary structure and function during adulthood. *Cardiovasc Res*. 2009; 81, 713–722.
35. De Grauw TJ, Myers R, Scott WJ. Fetal growth in rats from different levels of hypoxia. *Biol Neonate*. 1986; 49, 85–89.
36. Ruijtenbeek K, le Noble FAC, Janssen GMJ, *et al*. Chronic hypoxia stimulates periarterial sympathetic nerve development in chicken embryo. *Circulation*. 2000; 102, 2892–2897.
37. Rouwet EV, Tintu AN, Schellings MW, *et al*. Hypoxia induces aortic hypertrophic growth, left ventricular dysfunction, and sympathetic hyperinnervation of peripheral arteries in the chick embryo. *Circulation*. 2002; 105, 2791–2796.
38. Villamor E, Kessels CG, Ruijtenbeek K, *et al*. Chronic *in ovo* hypoxia decreases pulmonary arterial contractile reactivity and induces biventricular cardiac enlargement in the chicken embryo. *Am J Physiol Regul Integr Comp Physiol*. 2004; 287, R642–R651.
39. Tintu A, Rouwet E, Verlohren S, *et al*. Hypoxia induces dilated cardiomyopathy in the chick embryo: mechanism, intervention, and long-term consequences. *PLoS ONE*. 2009; 4, e5155.
40. Anderson PA, Kleinman CS, Lister G, Talner NS. Cardiovascular function during normal fetal and neonatal development and with hypoxic stress. In *Fetal and Neonatal Physiology* (eds. Polin AR, Fox WW), 1998; pp. 876–889. W.B. Saunders, Philadelphia.
41. Kempf H, Linares C, Corvol P, Gasc JM. Pharmacological inactivation of the endothelin type A receptor in the early chick embryo: a model of mispatterning of the branchial arch derivatives. *Development*. 1998; 125, 4931–4941.
42. Byrne AM, Bouchier-Hayes DJ, Harmey JH. Angiogenic and cell survival functions of vascular endothelial growth factor (VEGF). *J Cell Mol Med*. 2005; 9, 777–794.
43. Skilton MR, Evans N, Griffiths KA, Harmer JA, Celermajer DS. Aortic wall thickness in newborns with intrauterine growth restriction. *Lancet*. 2005; 365, 1484–1486.
44. Koklu E, Kurtoglu S, Akcakus M, *et al*. Increased aortic intima-media thickness is related to lipid profile in newborns with intrauterine growth restriction. *Horm Res*. 2006; 65, 269–275.
45. Akira M, Yoshiyuki S. Placental circulation, fetal growth, and stiffness of the abdominal aorta in newborn infants. *J Pediatr*. 2006; 148, 49–53.