CHAPTER 18

Hypoxia, Fetal Growth and Developmental Origins of Health and Disease

Dino A. Giussani*

Abstract

The compelling evidence linking small size at birth with later cardiovascular disease, obtained from epidemiological studies of human populations of more than a dozen countries,[1] has clearly renewed and amplified a clinical and scientific interest into the determinants of fetal growth, birth weight and the development of cardiovascular function and dysfunction before and after birth. As early as the 1950s Penrose[2] highlighted that an important determinant of birth weight was the quality of the intrauterine environment, being twice as great a determinant of the rate of fetal growth than the maternal or fetal genotype. Studies of birth weights of relatives[2] together with strong evidence from animal cross-breeding experiments[3,4] have clearly supported this contention. One of the great qualifiers of the fetal environment is the maternal nutritional status during pregnancy. As such, the reciprocal association between low birth weight and increased risk of high blood pressure in adulthood, as first described by Barker,[1] has literally exploded a new field of research investigating the effects of maternofetal nutrition on fetal growth, birth weight and subsequent cardiovascular disease. However, the fetus nourishes itself also with oxygen, and in contrast to the international effort which is assessing the effects of maternofetal under-nutrition on early development, the effects of maternofetal under-oxygenation on fetal growth, birth weight and subsequent increased risk of disease have been little addressed. Here, evidence is presented, which supports the concept that fetal hypoxia alone may provide a candidate prenatal stimulus contributing to fetal growth restriction and the developmental origins of cardiovascular health and disease.

The Fetal Cardiovascular Defence to Short- and Long-Term Hypoxia

Hypoxia is one of the major challenges that the fetus may face during gestation. The immediate fetal defence to hypoxia is largely dependent on its cardiovascular system. During acute hypoxia the fetal strategy is to make best use of the available oxygen delivery. Hence, in response to acute hypoxia, a redistribution of the fetal cardiac output occurs, which shunts blood flow away from peripheral towards essential circulations in order to protect hypoxia-sensitive organs like the fetal brain[5,6] (Fig. 1). Should the duration of the hypoxic challenge become prolonged, the initial homeostatic cardiovascular defence becomes enhanced. In response to chronic hypoxia, there is persistent redistribution of blood flow towards essential circulations, secondary to chronic elevations in peripheral vascular tone.[7-9] Whether this sustained homeostatic vascular response in the fetus becomes vestigial and a maladaptation, akin to the erythrocytotic[10] or pulmonary vasoconstrictor[11] responses to hypoxia in highland neonates and adults, which if transient are beneficial but if persistent lead to pathology, is unclear at present. However, it is

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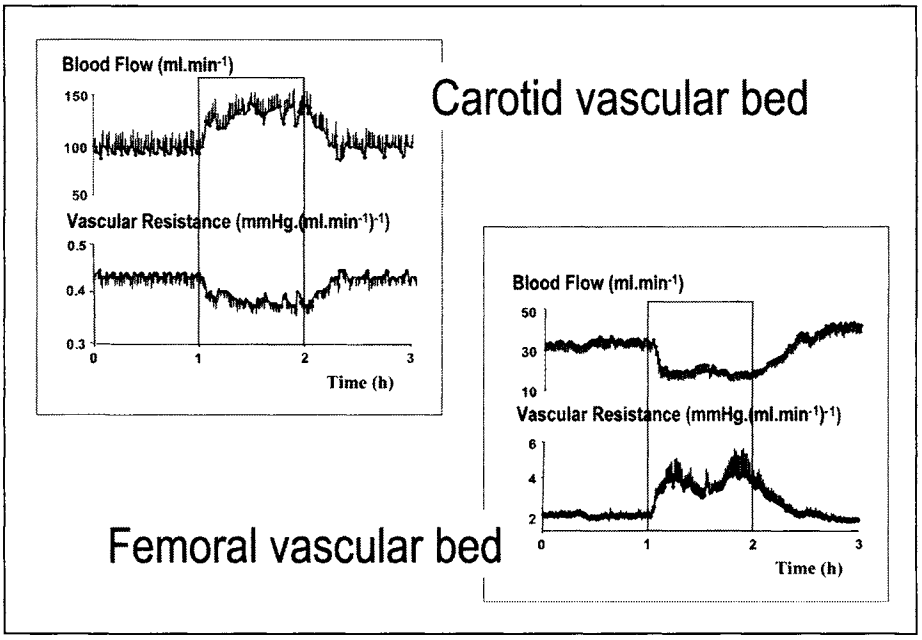

Figure 1. The fetal vascular defence to acute hypoxia. Values represent the minute mean ± S.E.M for blood flow and vascular resistance in the carotid and femoral circulations in 8 fetal sheep between 125-130 days of gestation. Note that the carotid vascular bed undergoes vasodilatation during acute hypoxaemia (box) as indexed by a fall in carotid vascular resistance and an increase in carotid blood flow. In marked contrast, the femoral vascular bed undergoes vasoconstriction, aiding the redistribution of blood flow away from peripheral circulations.

suggested that plausible biological trade-offs of fetal persistent peripheral vasoconstriction are asymmetric growth retardation and increased cardiac afterload, proposing that the classical phenotypic association between intrauterine growth retardation with cardiovascular dysfunction in adult life may originate from the same developmental stimulus—fetal hypoxia.

Hypoxia and Fetal Growth Retardation

Although several studies in animals have shown that chronic hypoxia during pregnancy can lead to slow, disproportionate fetal growth,[12,13] whether the effects are due to sustained under-oxygenation or partial under-nutrition is uncertain as chronic hypoxia also reduces maternal food intake.[13] In human populations, materno-fetal hypoxia occurs most commonly during the hypobaric hypoxia of pregnancy at high altitude. In support of data gathered from animal experiments, several investigators have also reported reduced birth weight and asymmetric growth retardation in human babies with increasing altitude.[14-16] However, because most high altitude populations are also impoverished, the extent to which this reduction in fetal growth is governed by maternal nutritional status or the hypoxia of high altitude, again, remains uncertain. To assess the partial contributions of fetal under-oxygenation and under-nutrition in the control of fetal growth, we have recently adopted a two-prong approach addressing questions in a specific human population and in a specific experimental animal model.

Epidemiological studies of human populations were carried out in Bolivia as this country is geographically and socio-economically unique. Bolivia lies in the heart of South America and it is split by the Andean cordillera into areas of very high altitude to the west of the country (4000 m) and sea level areas as the east of the country spans into the Brazilian Amazon. Facilitating

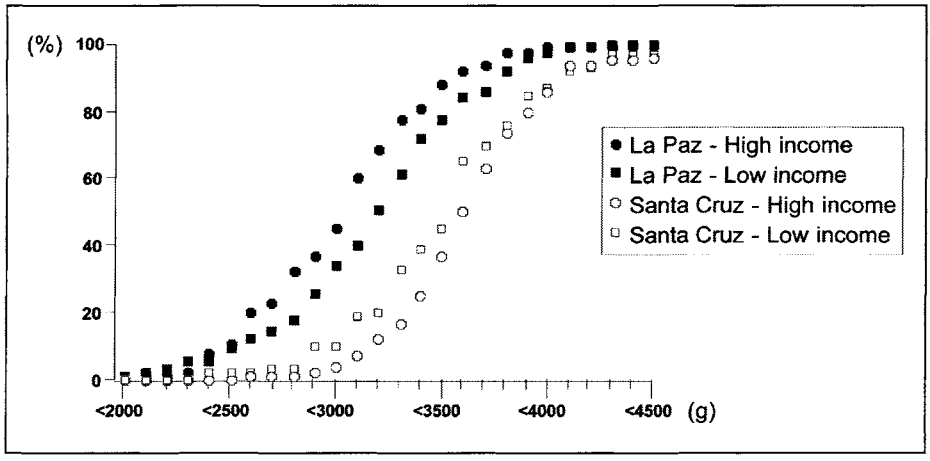

Figure 2. High altitude versus socio-economic stastus on birth weight. The curves represent the cumulative frequency distribution across all birth weights in term babies born from mothers of opposing economic status in La Paz (4000 m) and in Santa Cruz (sea level), in Bolivia. Modified from Giussani et al. Pediatric Research 2001; 49:490-494.[16]

the study design, the two largest cities, and therefore the most populated with approximately 2 million inhabitants each, are La Paz (4000 m) and Santa Cruz (400 m). Bolivia is also socio-economically unique as both La Paz and Santa Cruz are made up of striking economically-divergent populations.[17] In third world countries, and especially in Bolivia, there is an unsurprising strong relationship between socio-economic status and nutritional status.[18] Joining strengths with Barker, a recent study investigated whether the intrauterine growth retardation observed in the high altitude regions of Bolivia was primarily due to intrauterine hypoxia or due to the maternal socio-economic nutritional status.[16] Birth weight records were obtained from term pregnancies in La Paz and Santa Cruz, especially from obstetric hospitals selectively attended by wealthy or impoverished mothers. Plots of the cumulative frequency distribution across all birth weights gathered revealed a pronounced shift to the left in the curve of babies from high altitude than from low altitude, despite similarly high maternal economic status (Fig. 2). Interestingly, when lowland babies born from mothers with high or low economic status were compared, a shift to the left in birth weight occurred in low versus high income groups, however this shift was not as pronounced as the effect on birth weight of high altitude hypoxia alone. Additional data also showed that highland babies from poor families did not have the greatest leftward shift of the relationship as one would have expected. Rather, counter-intuitively, these babies were actually heavier than highland babies born from families with a high socio-economic status. The apparent conundrum is easily explained by assessing the ancestry of the families. In our study, the low socio-economic group of La Paz contained a high percentage (92%) of women from Amerindian origin with Aymara indian paternal and maternal surnames.[16] In contrast, the high socio-economic group of La Paz contained a high European admixture. These findings are reminiscent of the observations of Hass et al[19] and Moore[20] who suggested that fetal growth retardation at altitude is correlated to the duration of high altitude residence, independent of maternal nutrition: the longest resident population experiencing the least decline and the shortest residence groups demonstrating the most reduction in birth weight. Accordingly, reductions in birth weight at elevations greater than 3000m above sea level are greatest in Colorado, intermediate in Andeans and least in Tibetans.[20]

The second prong of our approach exploited the chick embryo as an animal model. In contrast to all mammals, in avian species the effects of hypoxia on the fetus can be assessed

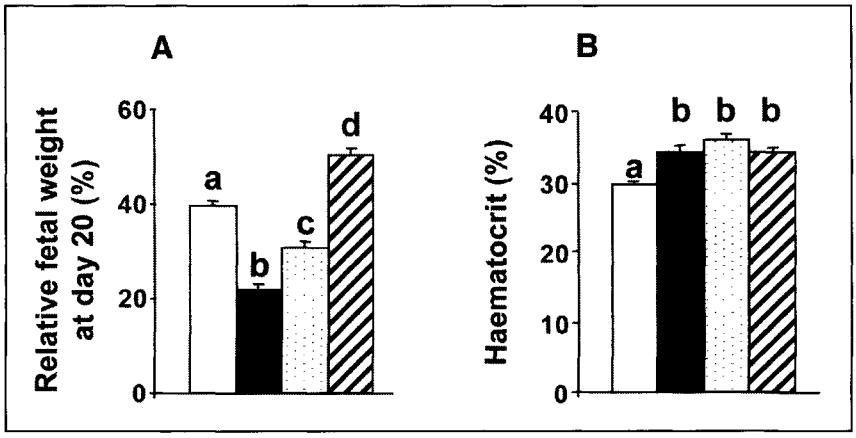

Figure 3. The role of oxygen in the growth of the chick embryo. Values are mean ± S.E.M. for the fetal weight at the end of the incubation period expressed as a percentage of the initial egg mass (A) and the fetal haematocrit at the end of the incubation period (B). Groups are sea level chick embryos incubated either at sea level (SLSL, open bar, n = 31) or high altitude (SLHA, closed bar, n = 19) and high altitude embryos incubated at high altitude (HAHA, stippled bar, n = 33) or sea level (HASL, hatched bar, n = 30). Different letters are significantly different by one way ANOVA + Student Newman Keuls Test ($P < 0.05$).

directly, without additional effects of hypoxia on the mother and the placenta, and without confounding problems associated with reductions in maternal food intake. The study reasoned that if oxygen alone has a real role in the direct control of fetal growth, then fertilised eggs from hens native to sea level should show growth restriction when incubated at high altitude, and fertilised eggs from hens native to high altitude, which usually show growth restriction, should at least recover their growth when incubated at sea level.[21] The data of the study showed that incubation of sea level embryos at high altitude led to a 45% growth restriction, but incubation at high altitude of embryos from hens native to high altitude only led to 22% growth restriction (Fig. 3). The embryonic growth restriction of incubations at high altitude was asymmetric as brain weight was preserved at the expense of body length. Another component of this study showed that when fertilised eggs laid by hens native to high altitude were incubated at sea level, the resulting embryos not only recovered their growth, but they grew heavier than sea level controls. The haematocrit data reveal that this group of embryos retained an increased oxygen carrying capacity despite incubation at sea level (Fig. 3). This suggests that embryos from hens native to high altitude incubated at sea level had a greater oxygen content than sea level controls, further supporting a role for oxygen in the control of fetal growth. The mechanism via which elevated haematocrit levels are maintained in the absence of a hypoxic stimulus is unknown, but the data may reflect an adaptive response, transmitted by the mother to the oocyte prior to egg laying, predictive of fetal development in a hypoxic environment. Another example of a predictive adaptive response[22] is that of the meadow vole, in which the photoperiodic history of the dam prior to conception, rather than the perinatal thermal environment, can better determine the offspring's coat thickness at birth.[23] Alternatively, the maintained elevated haematocrit in HASL embryos in the present study may highlight that genetic control of factors determining oxygen carrying capacity is regulated very early on in the developmental process of the oocyte by the available oxygen concentration at that time.

Hypoxia and Developmental Origins of Cardiovascular Disease

An increasing number of experimental studies are beginning to support the argument that developmental hypoxia can give rise to cardiovascular dysfunction before and after birth.

Studies in fetal sheep have shown that chronic hypoxia, secondary to pregnancy at high altitude, sustained anaemia or chronic fetal placental embolization, led to suppressed cardiac function and contractility, and resulted in cardiac hypertrophy (see ref. 24). Studies in chick and rat embryos have shown that the exposure to sustained hypoxia during development, which leads to asymmetric growth retardation, is accompanied by ventricular and aortic hypertrophy.[24,25] Three elegant studies have now shown that fetal hypoxia alone may have persisting consequences for cardiac and vascular health in the offspring at adulthood. Li et al[26] reported that exposure of pregnant rats to hypoxia from day 15 to 21 of gestation produced offspring with increased cardiac susceptibility to ischaemic-reperfusion injury at 6 months of age. Williams et al[27] reported that exposure of pregnant rats to hypoxia from day 15 to 21 of gestation produced offspring with impaired NO-dependent vasodilatation in the mesenteric circulation at 4 months of age. Ruijtenbeek et al[28] reported that exposure of chick embryos to hypoxia from day 6 to 19 of the incubation period produced offspring with exaggerated responses to peri-arterial sympathetic nerve stimulation and down-regulated NO-dependent dilator function in the femoral vascular endothelium at 15-16 weeks of adulthood.

In humans, it remains to be elucidated whether hypoxia-induced reductions in birth weight, as a result of placental insufficiency or of pregnancy at high altitude are associated with increased health risks after birth. Clearly, it is close to impossible to isolate the partial effects of fetal hypoxia and fetal undernutrition in promoting intrauterine growth retardation in human pregnancies complicated with placental insufficiency or preeclampsia. In contrast, investigation of the contribution of fetal hypoxia alone in promoting effects on fetal growth and programming health risks before and after birth can be achieved in human pregnancy at high altitude, carefully controlled for maternal nutritional status, making this a powerful model. Preliminary data[29] suggest that the rates of infant mortality, within the first year of newborn life, are positively correlated with altitude, increasing at a rate of *ca.* 8 deaths per 1000 m increase, and that this relationship is independent of the maternal socio-economic and nutritional status. However, the negligible number of studies in which basal arterial blood pressure was measured in adult residents rather than climbers at high altitude, report conflicting results suggesting either a higher incidence of hypertension in the inhabitants of high altitude regions of Saudi Arabia[30] or lower resting blood pressure in Peruvian highlanders.[31] These inconsistencies may be related to the high altitude residence ancestry of the individuals being studied. Thus, while hypoxia during development may increase the risk of hyertension at adulthood in people who originated from sea level regions, highland natives may have developed a protection, masking the effect. This point reemphasises the clear need for future epidemiological studies of human populations at altitude to relate the effects of prenatal hypoxia with postnatal cardiovascular function, separately, in lowland and highland natives. It is also likely that the deleterious programming effects of prenatal hypoxia on cardiovascular function in later life may express themselves, not during basal conditions, but only once the cardiovascular system is stressed. In this context, it is extremely interesting to highlight one study in India reporting a much higher incidence of ischaemic stroke in 20-50 year old men resident at high altitude.[32]

In summary, our observations in human babies and experiments in the chick embryo at high altitude strongly support the hypothesis that fetal oxygen, independent of genetic and maternal nutritional factors, is an important regulator of fetal growth. Accumulating evidence suggests that prenatal hypoxia alone can also be a potent stimulus triggering a developmental origin of cardiovascular dysfunction in the offspring. In both human and avian species, prolonged high altitude residence ancestry can develop a protection against unwanted biological trade-offs, such as the effects of hypoxia on fetal growth. Whether this protection spans into the postnatal and adult periods, minimising the risk of developing cardiovascular disease, remains to be elucidated. The mechanism of this protection is clearly of paramount scientific interest and important clinical application, not only in pregnancy at high altitude but in sea level pregnancies complicated with reduced oxygen delivery to the fetus, such as during placental insufficiency and preeclampsia.

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