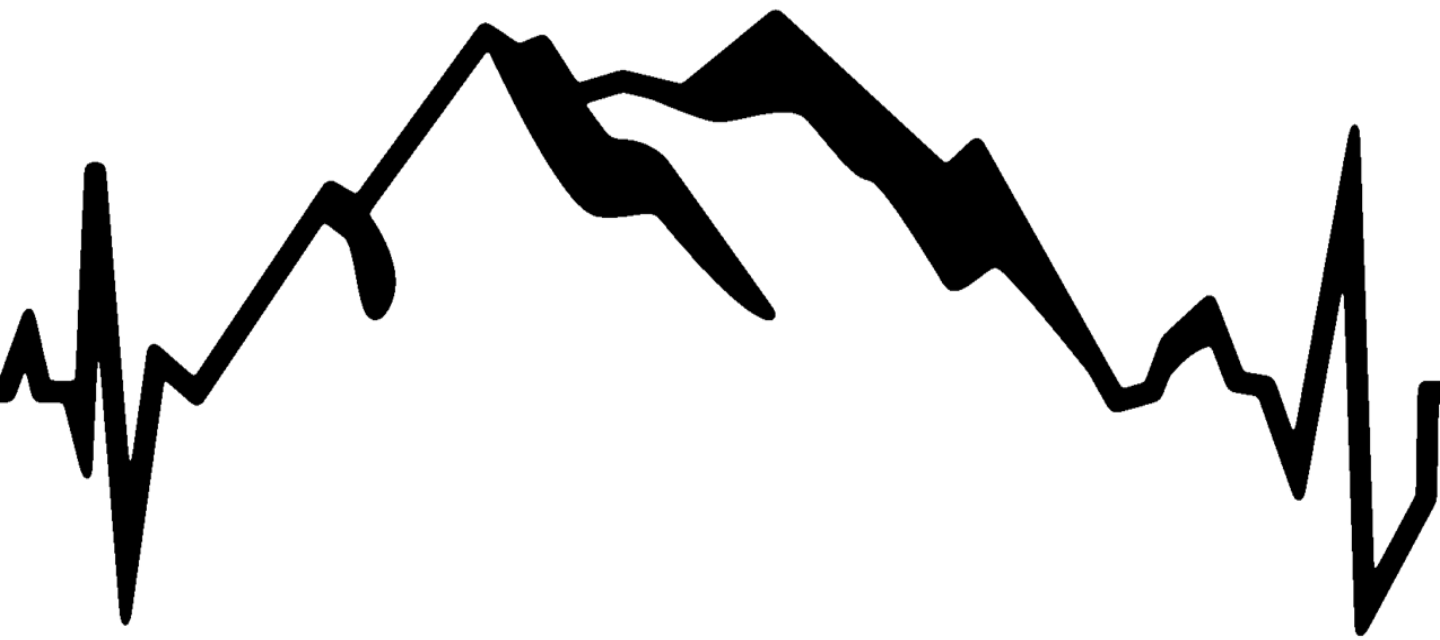
The highs and lows of programmed cardiovascular disease by developmental hypoxia

Valorization



Relevance

Cardiovascular disease kills 1 in 3 people. Every 3 minutes someone in the UK has a heart attack and 30% of these are fatal . Globally, around 17 million people die from cardiovascular disease each year. In 2010, the total costs of cardiovascular disease in the USA was $444 billion and this number is predicted to increase to 800 billion dollars by 2030. In the UK, according to the British Heart Foundation, the annual costs to the nation of premature death, lost productivity, hospital treatment and prescriptions relating to cardiovascular disease is of the order of £19 billion. Therefore, there is no question that cardiovascular dysfunction is a vast problem imposing a significant burden on every country's health and wealth (1).

It is widely accepted that our genes interact with traditional lifestyle factors, such as smoking, obesity and/or a sedentary lifestyle to promote an increased risk of heart disease (2). It has also become established that the gene-environment interaction early in life may be just as, if not more, important in 'programming' heart health and heart disease (3). This is unsurprising because our physiology is much more plastic and malleable during early life and the younger we are, the greater the impact the environment has upon us (3). These concepts have brought attention to adverse pregnancy and whether it can increase the risk of cardiovascular disease in the offspring. Accordingly, we now know that fetal development during suboptimal conditions can indeed trigger a fetal origin of cardiovascular dysfunction and increase the risk of chronic heart disease in the offspring in later life (3-5).

One of the most common adverse intrauterine conditions in complicated pregnancy is chronic fetal hypoxia (4-6). However, the contribution of chronic fetal hypoxia in promoting intrauterine growth restriction (IUGR) and programmed cardiovascular risk has been difficult to isolate for a number of reasons. For instance, it is established that high altitude pregnancy leads to IUGR (5). However, most high altitude populations are impoverished with significant maternal malnutrition (5). Therefore, the contribution of chronic fetal hypoxia versus chronic fetal undernutrition in slowing fetal growth and in setting future cardiovascular risk under these conditions is uncertain. The same applies to sea level pregnancy complicated by preeclampsia, placental insufficiency, gestational diabetes and even maternal obesity. All these conditions are associated with an increase in placental

vascular resistance (see 6), which will decrease oxygen as well as nutrient delivery to the growing fetus. This makes it impossible to disentangle the effects of chronic hypoxia versus chronic fetal undernutrition in promoting IUGR and programming of cardiovascular disease in human complicated pregnancy at sea level. Similarly, several experimantal studies including our own in mammalian animal models have shown that maternal chronic hypoxia during pregnancy can lead to IUGR and programme increased cardiovascular risk in the offspring (5,7). However, because experimental induction of chronic hypoxia in rodents can reduce maternal food intake and/or alter the quality of the maternal milk (5,7), the contribution of chronic fetal hypoxia versus chronic fetal and/or neonatal under-nutrition under these conditions, again, remains uncertain.

Innovation

By combining the chick embryo model with incubation at high altitude, this PhD thesis has been able to isolate the direct effects of chronic hypoxia due to high altitude on fetal growth, cardiovascular development, effects on the fetal stress axes and on the developmental programming of systemic and pulmonary vascular disease in the offspring. This is because in contrast to mammals, with the exception of monotremes, in the chicken the effects of changes in oxygenation on the embryo can be isolated and determined directly, independent of effects of chronic hypoxia on maternal food intake or the qulity of the maternal milk for lactation. In addition, chickens have a short incubation period (21 days), meaning embryonic studies can be conducted quickly. Maternal lives do not have to be taken in order to study the fetus and so for a fetal study the use of chickens could be described as more ethical, reducing the number of animal use, thereby complying with the 3R principle enshrined by the Home Office. The relatively large size of the chicken embryo at term (> 25g on day 19, term is 21 days) compared to the rodent fetus (< 4 g on day 20, term is 21 days) means that the chicken embryo model has the added advantage of facilitating study of cardiovascular function *in ovo* or in isolated organs, for instance by using the Langendorff preparation or the myograph (8). Interestingly, and perhaps surprisingly, the chronology of cardiac development in the chicken is much more comparable to the human than is the rodent, in which cardiac development continues into the postnatal period (8). Rodents are also polytocous, and the physiology of multiple pregnancies can be quite different to singletons, with different adaptations in place to support more

than one fetus (8). Combined, these advantages make the chicken embryo an animal model to isolate the effects of chronic hypoxia on cardiovascular development and programming of cardiovascular dysfunction.

The data provided by the chicken studies performed in this thesis adds considerably to the existing body of literature to demonstrate that chronic fetal hypoxia can programme a developmental origin of cardiovascular disease independent of any effects of undernutrition. This is important because of the similarities between the patterns of cardiovascular disease in hypoxic animals and the cardiovascular disease found in human offspring of complicated pregnancy. For example, IUGR human fetuses and neonates show decreased cardiac ejection force, diastolic dysfunction, decreased cardiomyocyte numbers, hypertrophic hearts and increased aortic stiffness (8-10). Some factors, including aortic stiffness and endothelial dysfunction have also been reported to be more prevalent in adults who were growth restricted as fetuses (11). Whilst IUGR is not necessarily caused by chronic fetal hypoxia alone, the fact that animal models which isolate the effects of chronic hypoxia produce similar phenotypes confirms that chronic fetal hypoxia is likely to be an important factor in the correlations between complicated human pregnancy, IUGR and an increased risk of cardiovascular disease later in life.

Target groups

This sound knowledge base generated in this PhD study has the potential to significantly facilitate the national and international development of the field, providing a significant academic advance within the field and between related disciplines. The PhD candidate and supervisors attend national and international scientific meetings routinely and are deeply involved in undergraduate and graduate teaching. Therefore, we will refer to this PhD project at least at three levels: 1) at international and national scientific meetings; 2) in laboratory meetings to postgraduate and graduate students and technicians, and 3) in lectures to undergraduate students, especially those in their final year who may be contemplating potential PhD research projects. It is important that investment is made to train new researchers, particularly in the vulnerable skills of fetal surgery and studying fetal cardiovascular function. To deliver maximum benefit in development of skills, capacity and capability, this PhD work will also serve as the basis to: 1) Provide cross-disciplinary training for emerging scientists in all

components of the thesis, spanning in vivo cardiovascular experimentation and stereological and histological analyses of the cardiovascular system; 2) Ensure that other scientists worldwide are exposed to the expertise available through international communications at scientific meetings; 3) Ensure that other scientists at the University of Cambridge, University of Maatricht and The Bolivian Institute for High Altitude Biology (IBBA) are exposed to the expertise available through maintained international collaboration. Thereore, this PhD also serves in outreach programmes.

The sound knowledge base generated in this PhD has also been published in high impact journals as original research articles. Combinations of original articles have been further disseminated in Topical Reviews and letters to the Editor of high impact journals. To further achieve excellence with impact, the University Communications Office at Cambridge has been alerted of the potential influence for human health of the scientific findings. Therefore, the work has had an immediate impact on clinicians, basic scientists, healthcare professionals, expectant mothers and their families in terms of providing them with information on the effects on the health of the offspring of high altitude pregnancy or reductions in fetal oxygenation in sea level complicated pregnancy. In addition, the data will benefit the design of therapeutic strategies to protect pregnancy complicated by chronic fetal hypoxia and/or improve fetal growth and development in adverse pregnancy with drugs that limit the adverse effects of fetal hypoxia, such as with spcific antioxidants. The proposed research is therefore likely to be of significant interest and benefit not only to researchers carrying out similar or related research in the field, but also to national and international researchers in other disciplines, such as experts in the metabolic syndrome, diabetes and gestational diabetes, scientists in biochemistry, pharmacology and nanotechnology, as well as cross-disciplinary teams in the pharmaceutical industry. Therefore, the data generated directly and indirectly has the potential to reduce the burden of cardiovascular disease throughout the life course, thereby having a major clinical, economic and societal impact on health.

Conclusion

It is now established that adverse conditions during pregnancy can trigger a fetal origin of cardiovascular dysfunction and/or increase the risk of heart disease in later life. Sub-optimal environmental conditions during early life that may promote

the development of cardiovascular dysfunction in the offspring include alterations in fetal oxygenation and nutrition as well as fetal exposure to stress hormones, such as glucocorticoids. There has been growing interest in identifying the partial contributions of each of these stressors to programming of cardiovascular dysfunction. However, in humans and in many animal models this is difficult, as the challenges cannot be disentangled. By using the chicken embryo as an animal model and intertwining this with high altitude incubation, this PhD has been able to circumvent a number of problems. The work has isolated an important direct contribution of chronic fetal hypoxia in regulating fetal growth, cardiovascular and endocrine development as well as in the programming of systemic and pulmonray vascular disease in the adult offspring.

References

1. https://www.bhf.org.uk/research/heart-statistics
2. Nabel EG. Cardiovascular disease. N Engl J Med 2003; 349:60-72.
3. 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(1):61-73.
4. Giussani DA, Niu Y, Herrera EA, Richter HG, Camm EJ, Thakor AS, Kane AD, Hansell JA, Brain KL, Skeffington KL, Itani N, Wooding FB, Cross CM, Allison BJ. Heart disease link to fetal hypoxia and oxidative stress. Adv Exp Med Biol. 2014; 814:77-87. Review.
5. Giussani DA, Davidge ST. Developmental programming of cardiovascular disease by prenatal hypoxia. J Dev Orig Health Dis 2013; 4(5), 328-37. Review.
6. Giussani DA. The fetal brain sparing response to hypoxia: physiological mechanisms. J Physiol. 2016; 594(5):1215-30. Review.
7. Giussani DA, Camm EJ, Niu Y, Richter HG, Blanco CE, Gottschalk R, Blake EZ, Horder KA, Thakor AS, Hansell JA, Kane AD, Wooding FB, Cross CM, Herrera EA. Developmental programming of cardiovascular dysfunction by prenatal hypoxia and oxidative stress. PLoS One 2012; 7(2):e31017.
8. Itani N, Salinas CE, Villena M, Skeffington KL, Beck C, Villamor E, Blanco CE, Giussani DA. The highs and lows of programmed cardiovascular disease by developmental hypoxia: studies in the chicken embryo. J Physiol. 2017 Oct 5. doi: 10.1113/JP274111.

9. Rizzo G, Capponi A, Rinaldo D, Arduini D & Romanini C. Ventricular ejection force in growth-retarded fetuses. Ultrasound Obstet Gynecol 1995; 5, 247-255.
10. Veille JC, Hanson R, Sivakoff M, Hoen H & Ben-Ami M. Fetal cardiac size in normal, intrauterine growth retarded, and diabetic pregnancies. Am J Perinatol 1993; 10, 275-279.
11. Leeson CP, Kattenhorn M, Morley R, Lucas A & Deanfield JE. Impact of low birth weight and cardiovascular risk factors on endothelial function in early adult life. Circulation 2001; 103, 1264-1268.