Effects of pre-natal and early post-natal undernutrition on adult internal thoracic artery function

Omar A. Khan\textsuperscript{a,b}, Christopher Torrens\textsuperscript{b}, David E. Noakes\textsuperscript{c}, Lucilla Poston\textsuperscript{d}, Mark A. Hanson\textsuperscript{b,*}, Lucy R. Green\textsuperscript{b}, Sunil K. Ohri\textsuperscript{a}

\textsuperscript{a}Department of Cardiothoracic Surgery, Southampton General Hospital, UK
\textsuperscript{b}Centre for Developmental Origins of Health and Disease, University of Southampton, Princess Anne Hospital, Southampton SO16 5Y A, UK
\textsuperscript{c}Department of Veterinary Reproduction, Royal Veterinary College, University of London, UK
\textsuperscript{d}Division of Reproductive Health, Endocrinology and Development, Guy's King's St Thomas Hospital, London, UK

Received 23 February 2005; received in revised form 18 August 2005; accepted 31 August 2005; Available online 4 November 2005

Abstract

Objective: Previous studies in humans and animals have suggested that undernutrition in utero and in early post-natal life may lead to altered vascular function in a number of peripheral arteries. We investigated the effect of pre- and post-natal nutrient restriction on the vascular reactivity of the left internal thoracic artery using a sheep model.

Methods: Welsh mountain ewes were mated and assigned to three dietary groups: (1) 100% of total nutritional requirements (control, \(n=6\)); (2) 50% of total nutritional requirements during the first 31 days of gestation (\(n=6\)); and (3) 50% nutritional restriction during the first 31 days of gestation, followed by a restriction in the diet of their offspring 12—25 weeks post-natally, designed to produce a 15% reduction in growth trajectory (\(n=7\)). The male offspring were sacrificed at 130 weeks; the left internal thoracic artery was mounted onto a wire myograph and the reactivity of the vessel to various agonists measured.

Results: The offspring of animals who underwent an early gestation nutrient restriction had a significantly increased basal tone (0.41 /C6 0.25 vs 6.34 /C6 1.35, \(p=0.015\)) and sensitivity to phenylephrine (log EC50: \(-6.23 \pm 0.04 \text{ M} \) vs \(-5.74 \pm 0.17 \text{ M}, \ p=0.036\)) as compared with control animals. However, this phenomenon was not seen in animals that underwent both pre- and post-natal nutrient restriction.

Conclusions: Pre-natal undernutrition increases the basal tone and sensitivity of the left internal thoracic artery to phenylephrine. This effect is significantly attenuated by continued undernutrition in early post-natal life. These experiments suggest that in utero and early post-natal undernutrition may be important determinants of graft function in later life.

\#2005 Elsevier B.V. All rights reserved.

Keywords: Coronary artery bypass conduits; Vascular tone and reactivity

1. Introduction

Over the last decade there has been accumulating evidence that low birth-weight is associated with an increased risk of cardiovascular mortality [1—3]. These findings have led to the development of the ‘fetal origins of adult disease hypothesis’ [4] which states that cardiovascular diseases, and in particular hypertension, originate as a consequence of poor nutrition in utero. However, the mechanisms underlying this link between altered foetal growth and cardiovascular disease remain unclear. In view of this, a number of animal models have been developed in order to better characterise the effect of maternal nutrition on the vascular function of their offspring. One consistent finding from these studies is the observation that undernutrition in utero is associated with altered vascular reactivity in peripheral arteries. This finding has been seen in a number of species and appears to be consistent across a number of vascular beds [5—8]. However, the clinical significance of this finding has yet to be evaluated. In particular, it is unclear whether this vascular dysfunction is seen in arterial vascular conduits utilised in cardiovascular surgery. Over the last 20 years, the left internal thoracic artery (ITA) has become the preferred primary conduit for coronary artery bypass surgery. The purpose of this study was to assess the effect of pre- and peri-natal nutritional challenges on the vascular reactivity of the left internal thoracic artery using a sheep model.

2. Methods

2.1. Animal and study design

All animal procedures carried out in this study were in accordance with the regulations of the British Home Office Animals (Scientific Procedures) Act, 1986 and approved by
the local ethical review committee. The health and welfare of the animals were checked throughout the study and any signs of ill-health or disease were reported to a veterinary surgeon who advised treatment according to standard veterinary practice. Serial anthropometric measurements were taken on all animals at regular intervals throughout the study period.

Welsh mountain ewes of uniform age, weight and body condition were bought from a known supplier (Royal Veterinary College, University of London). Upon enrolment into the study, the ewes were assigned to three dietary groups. The oestrous cycles of the sheep were synchronised using vaginally inserted progesterone-impregnated sponges (Chronogest, Intervet), which were removed 48 h prior to tupping. Rams of the same breed were introduced on Day 1 of the study (Day 0 was assumed to be conception). On Day 16, intravenous blood samples were extracted from the ewes and an ELISA progesterone assay was performed. Animals with low progesterone were deemed not pregnant and removed from the study. On Day 60 of gestation, each ewe was scanned to confirm pregnancy and any animal not pregnant was withdrawn from the study.

2.2. Dietary manipulation

The animals were individually-housed indoors prior to conception for approximately one week and fed a complete diet ration according to body weight. This diet consisted of barley, wheat, micronised full fat soya, grass meal, molasses, chopped straw, calcium carbonate, dicalcium phosphate salt and sheep vitamin/mineral supplement, which provided 10.8 MJ/kg metabolisable energy, 14.98 g/kg crude protein and 362 g/kg dry matter. Feed was allocated based on the advisory manual prepared by the AFRC Technical Committee on responses to nutrients.

The ewes were then randomly assigned to one of three dietary groups. The nutritional challenges of the groups are summarised in Fig. 1.

Control ewes (CC) were fed 100% of their nutritional requirements throughout gestation (term being approximately 147 days), and their male offspring \( n = 6 \) were fed a pelleted diet with hay following weaning at 12 weeks.

Ewes in the pre-natal restricted group (UC) were fed a complete diet ad libitum until Study Day 0. The animals were then fed 50% of their energy requirement based on body weight until Study Day +31. These animals were then fed 100% of their nutritional requirements throughout the remainder of gestation. Their male offspring \( n = 6 \) were then fed a pelleted diet with hay following weaning at 12 weeks.

Ewes in Group UU underwent an identical pre-natal restriction as those in Group UC. In addition, following weaning, their offspring underwent a further nutrient restriction. This restriction was individually calculated in order to reduce the body weight to 85% of the predicted growth for each lamb based on its 0–12-week growth trajectory. This restricted diet was given between 12 and 25 weeks following birth, after which the male offspring \( n = 7 \) were transferred back onto a pelleted diet with hay.

2.3. Determination of isolated artery function

At 130 weeks of age, the male offspring of the ewes were euthanised using an overdose of pentobarbitone sodium (0.8 ml/kg i.v., 200 mg/ml, Animalcare Ltd., York, UK). Immediately following death, the left internal thoracic was harvested from the 2nd intercostal space to its bifurcation by an investigator who was blinded as to the dietary group of the sheep. Following harvesting, the artery was excised and immersed in ice-cold physiological salt solution (PSS) (consisting of (mM): NaCl, 119; KCl, 4.7; CaCl\(_2\), 2.5; MgSO\(_4\), 1.17; NaHCO\(_3\), 25; KH\(_2\)PO\(_4\), 1.18; EDTA, 0.026; and D-glucose, 5.5) in order to reduce the risk of ischaemic damage to the arteries. The arteries were dissected free of connective tissue and the distal centimetre excised and then divided into four segments as ring preparations. Ring segments were then mounted on a small vessel wire myograph (Multi Myograph Model 610M; J.P. Trading, Aarhus, Denmark). The arterial ring segments were bathed in physiological salt solution at 37 °C gassed with a mixture of 95% O\(_2\) and 5% CO\(_2\) (pH 7.4). After an equilibration period of 30 min, PSS was exchanged with fresh, pre-warmed and pre-oxygenated Ca-free PSS. The rings were then stretched in progressive steps and the tension recorded. The optimal point on their length–tension relationship was then determined by calculating the internal vessel circumference equivalent to a transmural pressure of 100 mmHg (using the Laplace relationship) and setting the arterial diameter equal to 0.9 × IC\(_{100}\) as per previously validated techniques [9].

2.4. Experimental protocol

Following optimisation of the diameter of the rings as described above, all preparations were subsequently challenged with KPSS (125 mM K, equimolar substitution of NaCl with KCl in PSS) for 5 min, to obtain a reference contraction. The rings were then washed out, and a standardised protocol was followed in order to assess the contraction and relaxation properties of the internal thoracic artery of each animal. Contraction data were generated by measuring cumulative dose–response curves to PE (10\(^{-8}\) to 10\(^{-2}\) M) and endothelin (10\(^{-12}\) to 10\(^{-7}\) M). Relaxation was assessed by pre-constricting the ring segments with 10\(^{-7}\) M endothelin and measuring the relaxation to adenosine (10\(^{-11}\) to 10\(^{-4}\) M) and bradykinin (10\(^{-12}\) to 10\(^{-5}\) M). At the end of the experiment, papaverine (10\(^{-5}\) M) was added to the organ bath in order to record the absolute resting passive tension. All drugs and chemicals except endothelin (Bachem, St Helens, UK) were obtained from Sigma Chemical Co. (Poole, UK).
2.5. Data analysis

Basal tone and contraction responses to agonists were calculated as percentage of maximum 125 mM KPSS-induced contraction and expressed as mean ± SEM. The spontaneous or resting basal tone was calculated by comparing the tension prior to KPSS contraction with the absolute resting passive tension using a standardised technique and expressed as a percentage of the amplitude of the reference contraction ± SEM [10]. Relaxant responses to agonists were calculated as percentage relaxation of the endothelin-induced contraction and expressed as mean ± SEM.

Concentration—response curves to agonists were fitted to a sigmoidal curve with a four-parameter logistic equation using non-linear regression (Prism 3.0; GraphPad Software Inc, San Diego, CA, USA). It should be noted that all agonists did produce curves which could be fitted to a sigmoidal curve.

Maximum responses and pEC50 values were obtained from the fitted data, where the EC 50 was the concentration (molar) of the agonist that produced 50% of its maximum response. Statistical analyses were performed using one-way ANOVA and a Bonferroni post hoc test to compare the different groups. Significance was assumed if \( p < 0.05 \).

3. Results

3.1. Biometric data

No significant differences in birth weight were seen between the control and restricted groups (3.46 ± 0.19 kg vs 3.86 ± 0.23 kg; \( p = 0.18 \)). Similarly, at post-mortem, there were no differences in the biometric data of the three groups (see Table 1).

3.2. Resting vessel measurements

The optimal mean internal diameter was similar in all three groups as was the maximal response to KPSS (see Table 2). As shown in Table 2, the basal tone of the ITAs in Group UC was significantly increased as compared to the control animals (\( p = 0.015 \)).

3.3. Contractile responses

The results of the contractile responses are summarised in Table 2.

Animals in Group UC showed statistically significant increase in sensitivity to phenylephrine (PE) as compared with Group CC (\( \log \text{EC}_{50} \): 6.23 ± 0.04 M vs 5.74 ± 0.17 M, \( p = 0.036 \)), as shown in Table 2. The overall maximal response to PE was, however, similar in all three groups, and there was no significant difference in the PE dose—response between Groups CC and UU, as illustrated in Fig. 2.

Endothelin produced a sigmoidal CRC in all cases. Both the maximal contractile response and the EC50 values were comparable between the three groups.

3.4. Relaxant responses

Endothelin pre-contraction was similar in all three groups (results not shown). Bradykinin and adenosine produced sigmoidal concentration-dependent relaxation of endothelin

Table 2

| Summary of the contractile vascular function in the three nutritional groups |
|-----------------|--------|--------|--------|
|                  | Group CC | Group UC | Group UU |
| Vessel diameter (mm) | 340.0 ± 19.8 | 354.6 ± 15.6 | 336.7 ± 10.5 |
| KPSS (mM/mm)        | 13.0 ± 2.2  | 12.9 ± 2.1  | 12.1 ± 2.65 |
| Basal tone           | 0.41 ± 0.25 | 6.34 ± 1.35 | 4.08 ± 1.81 |
| Maximal contraction  | Phenylephrine | 145.8 ± 21.0 | 147.6 ± 20.3 | 145.0 ± 11.74 |
| Endothelin           | 123.0 ± 19.3 | 116.4 ± 13.1 | 102.8 ± 16.5 |
| pEC50 (–log M)       | Phenylephrine | 5.74 ± 0.17 | 6.23 ± 0.04 | 6.06 ± 0.03 |
| Endothelin           | 8.76 ± 0.19  | 8.83 ± 0.16  | 8.82 ± 0.20  |

Fig. 2. Responses of three groups to the contractile agonist phenylephrine. (○) Group CC, (■) Group UU, and (●) Group UC.

Table 3

| Summary of the relaxant vascular function in the three nutritional groups |
|-----------------|--------|--------|--------|
|                  | Group CC | Group UC | Group UU |
| Maximal relaxation | Bradykinin | 55.3 ± 11.8 | 29.7 ± 8.1 | 40.5 ± 7.0 |
|                  | Adenosine  | 59.4 ± 3.8  | 51.2 ± 8.4  | 48.0 ± 9.0  |
| pEC50 (–log M)   | Bradykinin | 8.55 ± 0.25 | 8.78 ± 0.25 | 8.34 ± 0.30 |
|                  | Adenosine  | 8.00 ± 0.40  | 8.03 ± 0.28  | 7.91 ± 0.45  |

Maximal relaxation data are expressed as percentage relaxation of endothelin-induced contraction.
pre-contracted arteries but there were no differences in the maximal response or EC$_{50}$ values between the groups (Table 3).

4. Discussion

The ITA is now universally accepted as the preferred primary conduit for coronary artery bypass surgery. Although this graft has been shown to have a superior long-term patency rate as compared with saphenous veins [11, 12], the small diameter and large elastic lamina of this artery makes it vulnerable to spasm [13]. As a consequence of this, there has been considerable interest in the effects of pharmacological properties of this vessel [14—16]. Previous studies have shown that a number of conditions such as hypercholesterolaemia, heart failure and diabetes are associated with impaired ITA dilator function [16]. This study highlights the role of pre-natal nutrition as a determinant of adult ITA function. Our findings show that the ITAs of sheep who underwent pre-natal nutrient restriction showed increased basal tone and an increased sensitivity to phenylephrine. Although this is the first study to examine ITA function in the context of in utero nutrient restriction, previous groups have conducted comparable studies examining other vascular beds. For example, Ozaki et al. [6] have demonstrated that in rats, global undernutrition during gestation caused enhanced thromboxane-induced contraction in the femoral arteries of the male offspring. Similarly, Brawley et al. [5] have shown that dietary protein restriction in pregnancy leads to impaired endothelium-dependent and endothelium-independent relaxation in male offspring. Using a sheep model, Nishina et al. [8] have also shown that global nutrient restriction in early gestation produces foetuses with impaired endothelium-dependent and endothelium-independent femoral artery dilation. With regard to human studies, Martin et al. [17] have shown that low birth-weight neonates show impaired acetylcholine-induced vasodilation in skin arterioles. Additional studies have also demonstrated that low birth-weight is associated with reduced flow-mediated dilatation of the brachial artery and that this dysfunction persists into adult life [18, 19]. In the context of these findings, our ITA results appear to be consistent with previous reports.

Although this study did show that pre-natal undernutrition was associated with vascular dysfunction, it should be noted that there were no differences in birth weight between the three groups. This finding correlates with previous studies [20] in illustrating that following undernutrition in utero, reduction in birth weight is not a necessary pre-requisite for perturbed cardiovascular function in adulthood. Similarly at post-mortem, we found no differences in body weight, crown—rump length, BMI or ponderal index between the three groups. Although the effect of foetal undernutrition on adult growth parameters is beyond the scope of this report, these findings do suggest that birth weight and biometric measures of post-natal growth are relatively poor proxies for foetal nutrition.

The new major finding in this study is the observation that the effects of a pre-natal nutritional challenge are attenuated by continuing this restriction after birth. The issue of interactions between the pre- and early post-natal environment has recently been discussed by Gluckman and Hanson [21] in terms of a so-called 'predictive-adaptive response'. In their model, the foetus constantly interprets the environment created by the maternal milieu and placental function and uses this information to predict the likely post-natal environment. For example, if the foetus has a poor nutritional diet in utero, it will then predict a nutritionally poor post-natal environment and then chooses a developmental path which will optimise its survival in such an environment. If however, the post-natal environment fails to confirm with these expectations, then the offspring will have made inappropriate metabolic adaptations, which instead of aiding its survival, may lead to disease in adulthood. Our findings do appear to support this hypothesis by demonstrating that mismatches between foetal and post-natal environment are the strongest determinants of vascular dysfunction. What remain unclear are the cellular mechanisms underlying these vascular changes. One possibility may lie in epigenetic changes—it is possible that changes in the post-natal environment may lead to changes in DNA methylation [21], which may in turn lead to alterations in the gene expression of vasoactive substances such as vascular endothelial growth factor [22]. Whilst highly speculative, this explanation does at least illustrate a potential mechanism for linking perinatal nutritional with vascular function.

The findings in this study raise the obvious question as to the relevance of this work in humans. Although it would be preferable to directly analyse the effect of pre-natal factors on human ITA function in humans, a prospective study on vascular function would clearly be ethically inconceivable. Our rationale for using sheep in this study is the fact that they share a number of developmental features with humans; and unlike rodents they typically produce singleton offspring. In addition, the sheep ITA has been extensively utilised as a model for studying graft function following coronary artery bypass surgery [23, 24]. In view of this, we believe it is reasonable to consider extrapolating the findings of our study to humans. If so, this in turn raises questions of the potential clinical importance of these findings. Although our animals were studied at the age of only 130 weeks (which in human terms equates to a post-adolescent male), evidence from epidemiological studies suggests that the vascular dysfunction seen as a consequence of foetal undernutrition persists into old age [25]. Given this finding, and the fact that many of the inotropic drugs used in the peri-operative period following cardiac surgery have alpha and beta-mimetic activity, alterations in the vascular reactivity of the type seen in this study may indeed have important clinical consequences in patients undergoing coronary artery bypass surgery.

In summary, we have shown that foetal and early post-natal undernutrition leads to altered internal thoracic artery vasoreactivity in sheep. These findings suggest that under-nutrition in foetal and early life may be an important determinant of graft function in later life.

Acknowledgments

The authors gratefully acknowledge the contribution of Jane Cleal, Piet Boels, Kirsten Poore and Jim Newman in the design of these experiments. This work was supported by the British Heart Foundation and HOPE.
References


