The Role of Growth in Heart Development

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Abstract

While it is established that the quality of the perinatal environment is critical in sculpting the developing individual, the mechanisms by which this occurs remain poorly defined. The growing fetus is dependent on the nutrients (including oxygen) it receives from the mother via the placenta. When this supply line is compromised, heart growth patterns are altered. In addition, hormones, other circulating factors, and the hemodynamic environment in which the fetus develops are important in determining outcomes for organ structure and function. Numerous studies in sheep have demonstrated that heart development can be modified in a number of ways, and the nature of the change differs between types and gestational timings of insults. Embolization of the placenta leads to the cessation of proliferation and maturation of cardiomyocytes; this may be due in part to changes in circulating insulin-like growth factor-1 levels. Such insults may be the underlying cause of cardiovascular disease in adults. Insults that modify the maturation timeline, final myocyte number, vascularity and endothelial responsiveness in the heart can have effects that persist long after the insult has been ameliorated.

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Introduction

Epidemiological data show that slow growth during fetal life is associated with an increased risk of type 2 diabetes, hypertension, stroke and ischemic heart disease later in life [1]. Retarded growth before birth appears to be caused by depressed transport of nutrients and/or oxygen to the fetus or increased levels of maternal glucocorticoids in the fetal circulation [2], and rarely, genetic abnormalities. All of these causes can occur simultaneously.

Animal models of maternal undernutrition or low oxygenation show that fetal malnutrition leads to permanent structural and physiological changes in
the fetus that increase its susceptibility to adult-onset diseases including hypertension, type 2 diabetes and endothelial dysfunction [3]. The nutritional status of the fetus influences the production rates of circulating growth factors, hormones and cytokines, which are key regulators of organs within the embryonic and fetal body. However, the mechanisms by which these factors cause changes within specific organs are not known.

The sheep fetus is a commonly used model of cardiovascular development. It is an attractive model for studying nutritional effects on the developing heart because the fetal sheep heart grows at a rate similar to that of the human fetal heart and because its growth can be easily monitored throughout gestation. During the first two thirds of gestation, the heart grows by hyperplasia; mononucleated cardiomyocytes proliferate by normal mitosis. The heart then undergoes a maturation period during the last third of gestation when cardiomyocytes gradually undergo binucleation characterized by karyokinesis without cytokinesis. This process is known as terminal differentiation. Thus, the proportion of working myocytes that contain two nuclei can be used as a clear indicator of the progression of maturation. Binucleated cells cannot divide but they can readily enlarge. During late postnatal life most of the growth of the myocardium is driven by the enlargement of binucleated cardiomyocytes. The complexities of the growth and maturation of the myocyte population in sheep has been described [4].

The heart has critical windows of development when it is most vulnerable to specific types of stress. In the pre-implantation rat embryo, nutritional cues affect embryo growth patterns and may increase the vulnerability of the offspring to late life hypertension [5]. In early gestation, nutritional stressors may interrupt the cell proliferation process and reduce the rate at which cardiomyocytes replicate. It is not known whether the loss of heart cell numbers due to low rates of proliferation in the first half of gestation will lead to a permanent reduction in cardiomyocytes for life or whether cell numbers can somehow be replenished when conditions are better. In late gestation, when cardiomyocytes are rapidly becoming binucleated, nutritional stressors may interrupt this maturation process. If the late gestation fetal myocardium is subjected to a period of inadequate nutrition, at least two outcomes are possible. If the binucleation process is impeded the fetus may be born with a heart containing mostly immature mononucleated myocytes that must go through their maturation process at some later time. If cardiomyocyte binucleation rates become augmented, the numbers of mononucleated cardiomyocytes will be relatively fewer before the full complement of cells has been generated. This outcome would reduce the generative capacity of the myocardium as cells are exiting the cell cycle prematurely and would leave the myocardium with a less than optimal number of cardiomyocytes.

The outcome of altered cell numbers could affect the right ventricle differently from the left ventricle. As the postnatal right ventricle becomes the pump for the low pressure pulmonary circuit, right ventricular cell numbers decline.
At birth, the left ventricle must eject its stroke volume into the systemic aorta which becomes a high pressure system upon the loss of the low resistance placenta. To accomplish this task, the left ventricle must already have a sufficiently thick wall, round shape and be metabolically prepared for efficient work. The left ventricle normally has proliferative capacity for a period of time after birth as well [4]. Having too few cells could compromise the function of the ventricle over a lifetime. The ability of the adult heart to make new cardiomyocytes, especially in areas of stress has been clearly demonstrated [6]. However, it is also clear that the regenerative capability of the heart is inadequate to replace cardiomyocyte loss following infarction or failure.

The fetal heart and brain are special organs that may be partially protected under conditions of chronic oxygen shortage [7]. For example, under conditions of acute hypoxemia, blood is shunted away from abdominal organs in order to favor blood flow to the fetal heart and brain. This has been shown in a number of experimental circumstances ranging from uterine blood flow reduction to global reduction in maternal oxygen [8, 9]. Thus, babies living in suboptimal environments in the womb may grow asymmetrically with 'spared' head and heart sizes but reduced abdominal girth.

Growth Factor Support of the Heart

During fetal life, insulin-like growth factor-1 (IGF-1) is an important regulator of organ growth and is influenced by fetal nutrient supply [10, 11]. Fetal IGF-1 levels are downregulated under conditions of maternal protein/calorie deprivation or placental reduction in sheep if fetal growth is suppressed. Birthweight has a greater influence than maternal nutritional status on glucose tolerance, blood pressure, and IGF-1 levels in fetal sheep [12, 13]. Offspring born to malnourished mothers are at the low end of the growth spectrum and have decreased heart to bodyweight ratios compared to fetuses that have been exposed to elevated levels of IGF-1 before birth. Mice that are null for IGF-1 or null for the IGF-1 receptor have birthweights that are approximately 50% of normal [14]. When IGF-1 is overexpressed in transgenic mice, the heart to bodyweight ratio and the absolute heart weight are increased by increased cardiomyocyte numbers.

Much of what is known about IGF-1 effects in the fetus comes from experiments in sheep. The IGF-1 analog (IGF-1 LR3) has been used to study fetal growth in sheep [15]. When IGF-1 LR3 was infused into near-term sheep fetuses for 7 days, heart weight increased by 35% over vehicle-infused control hearts and the heart weight to bodyweight ratio was also increased. This observed growth could have been either through the stimulation of cardiomyocyte enlargement, cardiomyocyte proliferation or both. However, it appears that IGF-1 LR3 stimulates an increase in the ratio of mononucleated to binucleated cells in the myocardium as was shown in fetuses exposed for 7 days to
IGF-1 LR3; cardiomyocyte dimensions did not change in these experiments. Thus, IGF-1 is a powerful regulator of cardiac growth in the fetus and it appears that malnourished fetuses have decreased levels of IGF-1 and that cardiomyocyte proliferation rates are thus depressed.

The signaling mechanism by which IGF-1 regulates sheep cardiomyocyte growth has been studied in cardiomyocytes in vitro [15]. It appears that IGF-1 signals through the membrane-bound IGF-1 receptor and stimulates both the extracellular signal-regulated kinase (ERK) and phosphoinositol-3 kinase (PI3K) cascades. IGF-1-induced protein synthesis requires ERK activation and there is evidence that IGF-1-stimulated hypertrophy in neonatal rat cardiomyocytes signals through the ERK cascade. If data from the rat apply to the large mammal (and that has not been proven), then IGF-1 may have roles to play in hypertrophic as well as hyperplastic growth.

**Placental Insufficiency, Nutrient Deprivation and Heart Growth**

The placenta is the organ through which all nutrients must pass to reach the fetus. Poor placentation leads to poor nutrient transport, poor oxygenation and inadequate support for optimal fetal growth [16]. It is now known that subnormal fetal growth is associated with depressed expression of many transport proteins normally found on the microvillous membrane of the placenta, including the transport systems for several amino acids, the sodium hydrogen ion exchanger, sodium potassium ATPase and lipoprotein lipase [17].

One model of fetal malnutrition has been widely used – the placental insufficiency model in sheep [18, 19]. Inert microspheres of some 50 μm can be administered to the placenta via the umbilical arteries. Gradually the exchange area of the placenta is reduced. This model causes global placental insufficiency where the transport of nutrients, including oxygen, is reduced. Typically, fetal bodyweight is reduced compared to controls. Brain growth is mildly protected with an increased brain to liver weight ratio. This mild ‘relative brain-sparing’ pattern of growth has been well described following 20 days of placental embolization [20, 21].

The most surprising finding of placental embolization experiments is that heart tissue is often not spared [18, 22]. If the heart were to grow out of proportion to body size, one would expect to find vigorous growth and normal maturation (binucleation) of the cardiomyocytes. However, with embolization heart weight is normal for bodyweight. In essence, the heart stops growing in parallel with the body and the suppression of growth is profound. Both the proliferation of myocytes and the rate of binucleation of myocytes are reduced significantly while cardiomyocyte size is not. The smaller hearts contain fewer working myocytes compared to normal-sized controls and the maturation of the heart is suspended. Thus, the fetal response to placental insufficiency is one of overall fetal organ growth suppression and lack of maturation.
accompanied by little or no special protection of the heart. This condition presents a physiological problem for the small heart, especially at birth when the heart needs to eject blood against a high resistance, high pressure circuit.

Only a few studies have reported the effects of intrauterine growth restriction (IUGR) on cardiomyocyte development in humans. Mayhew et al. [23] suggested that myocyte maturation is delayed in IUGR fetuses, with some ‘catch-up’ later in gestation. In the offspring of rats fed low protein diets during pregnancy, the heart weight/bodyweights of the offspring were not different from controls at birth. Nevertheless, there were fewer cardiomyocyte nuclei in the hearts of offspring born to mothers that were on a low maternal protein diet during pregnancy [24]. These authors conclude that the suboptimal prenatal environment leads to reduced proliferation and fewer cardiomyocytes in their model.

The fetal sheep hypothalamo-pituitary-adrenal axis may be activated when the mother is undernourished, leading to augmented fetal cortisol concentrations and early delivery [25]. In experiments where sub-pressor levels of cortisol were infused into well-nourished sheep fetuses, the cell cycle activity of cardiomyocytes was increased and cardiac mass increased [26]. Thus, one might expect that the increased fetal cortisol levels that usually accompany placental insufficiency would stimulate heart growth [19, 21]. However, this does not happen. In spite of increases in cortisol and renin in fetuses with placental insufficiency [19], both of which may stimulate cardiomyocyte proliferation in well-nourished hearts, heart growth is depressed. These data may indicate that undernourished hearts are not able to grow when nutrients, oxygen and IGF-1 levels are depressed.

**Undernutrition and Fetal Blood Pressure**

In the sheep, blood pressure is maintained at normal levels with placental insufficiency [19, 22]. However, under conditions of acute hypoxemia, sheep fetuses and presumably human fetuses show profound increases in blood pressure. Increases in pressure load to the heart ventricles cause accelerated maturation of cardiomyocytes [27]. This leads to a condition where the fetal myocardium has a decreased proportion of mononucleated cardiomyocytes, a larger proportion of cardiomyocytes that have two nuclei and binucleated cells that are larger than normal (fig. 1). This condition appears to lead to a myocardium with fewer cardiomyocytes and consequently fewer capillaries. This may lead to a long-term vulnerability of the heart for coronary artery disease and heart failure.

The effect of maternal and fetal undernutrition on fetal blood pressure regulation is complicated by the fact that different models lead to different outcomes. Arterial pressure may or may not be altered in fetal sheep being carried by mothers that are undernourished. In the undernourished ewe model, fetal
blood pressure can be decreased [28] or increased [29]. However in the carunclectomy model of fetal undernutrition, blood pressure is maintained at a normal level through adrenergic support [30]. Changes in endothelial function in various organs may predetermine cardiovascular effects in later life [31].

**Low Content of Oxygen in Fetal Blood**

Davis et al. [32] have demonstrated that the coronary tree is highly plastic during the perinatal period. This finding is important because it shows that the coronary tree can respond in a dramatic fashion to changes in the fetal environment. When near-term fetal sheep are made anemic by reducing their circulating red cell mass by about 50% over several days, the cardiovascular system responds by dramatic adaptations. Heart size increases rapidly. Cardiac output goes up. But most importantly, coronary artery conductance doubles. Coronary conductance is the maximal level of blood flow, at any given pressure, that can be supplied to the myocardium when the coronary arteries are fully dilated by adenosine. Thus, at the normal fetal driving pressure across the myocardium (about 40 mm Hg), flow will rise to more than two times higher than normal after a few days of anemia. Figure 2 shows that maximal coronary blood flow increases dramatically over a few days when
Malnutrition versus Hypoxia

While oxygen may be considered a nutrient in one sense, it is reserved for its own special category in this discussion. Li et al. [33] showed that the adult male offspring of maternal rats that were exposed to low oxygen levels during gestation had cardiovascular abnormalities. While the hearts were of normal appearance, they showed defects when studied in an isolated chamber: (1) the hearts had fewer and larger cardiomyocytes upon histological examination, and (2) they had larger infarctions and poorer contractile func-

Fig. 2. Maximal coronary blood flow at normal arterial pressures for the fetus and the adult sheep. Maximal flow was obtained during continuous adenosine infusion. For the fetus, flow data are shown in the same near-term fetus at two hematocrits (Hct): normal 32%, and following 6 days of anemia with Hct at 16%. Both fetal flows were measured when arterial pressure was 40 mm Hg and the coronary vessels were maximally dilated. For the adult, flow data from a 6-month-old adult sheep after having been made anemic as a fetus for only 6 days (expt; pre-partum blood transfusion returned Hct to normal levels) compared to its normal twin that was never anemic. Arterial pressure was 100 mm Hg and the coronary vessels were maximally dilated.
tion following ischemia reperfusion injury. The authors of this study surmised that the abnormal histology and function were an outcome of fetal hypoxemia. However, it has been shown that when maternal rats are placed in an environment where ambient oxygen levels are decreased, their food intake is also decreased. Thus the studies of Li et al. [33] are showing the combined effect of hypoxia and undernutrition.

Experiments from the laboratory of Xu et al. [34] have shed some light on the oxygen versus nutrition issue. Rats that developed in utero under conditions of (1) undernutrition or (2) with depressed oxygen levels, and its accompanying depressed maternal nutrition, were found to grow more slowly than normal before birth. Xu et al. [34] showed increases in the expression of several genes that are known to be associated with cardiac pathology. The hearts from the two protocols may have followed different pathways to reach a similar pathological outcome. The offspring that were once hypoxic had cardiac hypertrophy at birth whereas the nutritionally deprived offspring did not. However, the growth and functional deficits that accompany slow intrauterine growth were apparent in both groups by 7 months of age. Thus parallel but different pathways seem to lead to the same pathophysiology.

These rodent findings may not apply perfectly to large mammals. Fetal sheep that are growth restricted due to maternal undernutrition have enlarged hearts [35]. However, as mentioned above, in sheep that are both nutritionally deprived and are hypoxic, heart weight is normal for bodyweight at birth. Thus, it is not possible to predict the level of vulnerability for disease that is contained within the myocardium by observing the newborn heart. Complex gene expression patterns may set into motion a series of pathological processes that arise within the process of aging.

**Mechanisms of Nutrient Action**

To the biologist the relationships between malnutrition, growth in early life and late life cardiovascular disease beg for explanation. Animal experiments have been highly successful in demonstrating potential links between early life adversity and late life chronic disease. Yet, an integrated and thorough explanation of the mechanisms that underlie the early life origins of disease awaits further study. It is clear that fetuses whose growth is reduced because of maternal and/or fetal undernutrition face some common anatomic and physiological deficits. These include low nephron numbers, reduced numbers of β cells in the endocrine pancreas, enlarged hearts, and resetting of the hypothalamic pituitary adrenal axis. However, the effects of undernutrition may be at once subtle and powerful. The anatomic changes listed may not occur with milder forms of nutrient deprivation. Nevertheless, the vulnerability for disease may be significant. Epigenetic regulation of the genome through methylation of DNA or methylation or acetylation of histones may set
the course for vulnerability for the immediate offspring as well as for future generations. The roles of epigenetic mechanisms are only now coming into view but it is certain that fetal nutritional status affects the epigenetic regulation of genes that have life-long consequences. Because it is now clear that the health of future generations depends upon a deeper understanding of gene regulation and diet in the mother and fetus, it is unlikely that other areas of research will have a greater impact on the health of humankind.

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Discussion

Dr. Bier: It seems to me we have an ongoing nutritional experiment everyday in the US and other countries dealing with potentially altering cardiac developmental programming. After birth very low birthweight infants, who were born at 60% of normal gestation, get parenteral nutrition. If they had stayed in the womb their cardiac myocytes would have been working on a glucose fuel. They are born and right away they are presented with a fatty acid fuel, and it seems to me that this is an extraordinary change in potential programming. Do we know anything about this and what the long-term consequences are?

Dr. Thornburg: Thank you for raising that issue; I didn't have time to talk much about the metabolic effects in the heart. Some of you may not know that at the time of birth the heart switches from using carbohydrate fuel to free fatty acid fuel; mature hearts prefer free fatty acids as a primary fuel. However, when babies are born prematurely the enzymes that are present are not able to use free fatty acids properly. Nevertheless, when we treat these small babies we continue to give them high levels of
lipid. Those of us in the fetal science world are worried about this approach. In other words, if you don’t have the right fuel mix for your car, it doesn’t run right. We think that the baby’s heart also needs the proper fuel mixture. Unfortunately, no one has yet performed the appropriate biochemical studies to observe heart function in premature babies receiving different fuel mixtures of lipid and carbohydrate to determine the degree to which we should change the lipids in commercial nutrient solutions according to the level of maturity of the heart.

Dr. Ogra: These are very elegant observations. I would like to pursue two areas of your presentation; one relates to the hematocrit-related database. How does hematocrit impact on the oxygen-carrying capacity for red cells in these experiments? As a corollary, have you looked at situations of polycythemia, for example in people living in high altitudes; what happens under those circumstances in terms of tissue architecture?

Dr. Thornburg: These studies are begging to be done. No one has looked at polycythemia during this period; the data we are showing you are about the only data available in terms of modifying the architecture in the coronary tree. What we do know is that the heart is very hungry for oxygen; it is able to remodel its architecture and it can dramatically increase blood flow so that oxygen delivery can be approximately maintained. This is one way the heart protects itself. It is known that babies who are born small from hypoxemia have protected hearts, larger hearts and larger coronary trees than babies who are severely malnourished.

Dr. Ogra: Is the incidence of heart disease lower in Scottish Highlanders?

Dr. Thornburg: I don’t know of any data where this has been followed. There is a cohort in New Zealand where the babies had low hematocrits and our colleagues are looking at these individuals who are now young adults to see whether their coronary trees are altered for life.

Dr. Ogra: The second question is related to endothelial dysfunction. Has anyone looked at the endothelial architecture in terms of integrin expression which is very critical for function? Such functional aspects may be related to disease expressions later on.

Dr. Thornburg: That’s a good question. I didn’t have the time to show you that undernourishment, for any significant window of time, will cause endothelial dysfunction in virtually every organ of the body. This appears to be related to the generation of more reactive oxygen species. Antioxidants can in large part reverse that endothelial dysfunction. We believe that’s also true in humans who carry with them endothelial dysfunction based on a low birthweight. But that in itself has not been studied well in humans; we know a lot more about it in animals.

Dr. Prentice: I am still struggling to understand the likely direction of later effects that we would anticipate from very profound early effects you showed by the induction of anemia. Can you help me to resolve this?

Dr. Thornburg: Yes, we have studied this quite a bit. What happens is that a fetus makes what we call a super-coronary tree in response to anemia; it will have larger coronary vessels and early in adult life, in what we would call up through adolescence in sheep life, these hearts seem to be very capable; if you reduce oxygen levels they are able to maintain their contractile force even though their oxygen levels go down. At first we thought this was really good and all babies should be anemic just for a while so they will have a tree that is super-functional. But it is likely that these hearts will actually be more vulnerable to infarction, and when they have an infarction they will have an infarction that is actually larger than normal. We are now worried that these hearts might be more vulnerable than we thought, and that the message might be that these coronary trees, although you have more of them, may not behave normally under conditions of ultimate stress, which is when they are deprived of oxygen or are ischemic later in life.
**Dr. Giovannini:** Could you speculate whether the lower hematocrit levels commonly found in breastfed compared to formula-fed babies could be protective from cardiovascular disease in later life?

**Dr. Thornburg:** I wish that I could comment on that because your question is very important. We have also been wondering about this and we have looked for clues in the literature without success. We hope that someone will now take this up because how hematocrit affects the heart and cardiovascular function could be important.

**Dr. Makrides:** I want to ask a question from the opposite view: have you any information about the hemoglobin concentration in pregnancy and what impact that may have on the fetal heart?

**Dr. Thornburg:** First of all we know that when vascular resistance increases in the fetus, for any reason, the heart has to generate pressure against the higher load. We know that this cardiac load stimulates heart growth, hypertrophy and premature maturation of the myocyte. Pressure can be increased by two different mechanisms, either by increasing placental vascular resistance or just by increasing the load by a mechanical method (vascular occluder). With hemoconcentration there is an increase in blood pressure partly because of viscosity and partly because of vasoconstriction, and the same kind of hemodynamic load that occurs with a very small placenta (autoregulation). Hemodynamic load then brings about changes in the growth of the coronary tree and the heart muscle.

**Dr. Makrides:** So with that could you speculate on what the optimal hemoglobin range in the late pregnancy might be and if a pregnant woman's hemoglobin was too high whether that would be a negative thing in terms of over-supplementation with iron?

**Dr. Thornburg:** That's a very good question, and what I have been referring to of course is fetal hemoglobin levels. It turns out that in spontaneously polycythemic animals when hematocrit gets to about 55% we see arterial pressure going up and cardiac load going up. This will, undoubtedly be true for pregnant women as well.

**Dr. R. Bergmann:** I wonder what happens if a nonanemic pregnant woman receives iron or an anemic mother receives too much iron. Would the oxidative stress of pregnancy be aggravated?

**Dr. Thornburg:** You raise a good point. If a woman is iron-deficient, then giving iron is important for trying to reverse the anemia to maintain hematocrit. However iron goes across the placenta via transferring and ferritin, and high iron levels in the fetus are harmful.

**Dr. R. Bergmann:** Perhaps the best thing would be to start iron supplements before becoming pregnant [1].

**Dr. Thornburg:** Absolutely, the take-home point in this is that pre-pregnancy nutrition and good nutrition over the life time will promote good health for both the mother and the fetus.

**Dr. Batubara:** You said that a critical period in heart development is near term. What if the insult occurs in early pregnancy; in the first 3 months of pregnancy?

**Dr. Thornburg:** If you remember from embryology, the heart goes through a tube stage and then it loops, and when it goes through this looping state it is very vulnerable to blood flow abnormalities determined by the establishment of the placenta. Therefore, if the placenta in the very early stages is too small and the blood flow going through the heart is too low, the embryo will have a different shaped heart. We believe that many heart defects derive from these early stage blood flow deficiencies. If venous blood flow patterns going into the embryo heart are changed, the blood flow pattern going through the heart will be changed and that blood flow pattern will then change the way the four chambers of the heart are formed; the heart may have atrial septal and ventricular septal defects.
Dr. Hanson: When the placenta is formed, its trophoblasts grow into the mucosa of the uterus. Thus the cells come from different individuals to some extent so that there is an immune reaction, and it seems as if this difference enhances the formation of the placenta and the growth. In some areas of the world where marriages between cousins are common, in some cases possibly between cousins for many generations, would the placenta form in a different way; would it be less efficient or the fetus grow less?

Dr. Thornburg: I can't give you an answer. But I can give you some reassurance that scientists are looking at the interaction between immune cells and the establishment of trophoblast invasion. It has been shown in particular populations where there tends to be more inbreeding that the genetic makeup of the partners has a great influence on how many immune cells actually migrate to the site of implantation. So your point is well taken.

Dr. Guinto: As obstetricians, we do Doppler studies in growth-restricted babies. We use the uttering arteries to predict intrauterine growth restriction and the umbilical arteries to monitor patients whose babies already have growth restriction. We use abnormalities in the fetal venous Doppler studies and the demonstration of fetal coronary arteries through color flow mapping to guide us as to when the babies should be delivered. So perhaps this is similar to what you have been telling us, that there is an increase in the coronary artery flow, and thus a demonstration of the coronary arteries on Doppler ultrasound, in growth-restricted fetuses in the end stages of compensation.

Dr. Thornburg: Thank you for that comment. One of the great things that we as a scientific community can look forward to is increasingly expert individuals who will be able to image the heart using MRI and ultrasound to obtain images of the fetal coronary vessels. We have been imaging fetal hearts using MRI and ultrasound. We believe that with time we will become much better at predicting those hearts that are likely to have a very poor outcome in terms of their vulnerability for disease. I am very pleased to hear that you are using all these tests to make clinical decisions.

Dr. Walker: Can you reverse the damage that you described by injecting insulin-like growth factor or blocking renin or corticosteroids? In other words, is it a direct effect or is it just a marker of the effect?

Dr. Thornburg: In New Zealand, Jane Harding has infused IGF-1 into fetal sheep. Her group has shown that some of the negative effects accompanying slow fetal growth can be reversed. The real question is whether or not IGF-1 treatment can cause a fetus to outgrow its nutrient supply and you may actually lose more than you gain.

Dr. Smith: Lovely work on the cardiac myocytes. I wonder whether the fetal endothelium loses some of its plasticity as well, and if you are looking at markers loaded on the cardiac endothelium, for example, does the endothelium go up and stay up? Is the dipeptopetidase, which might be important in heading a glucose load after a feed, changed with early load?

Dr. Thornburg: We have done a little bit and we know that in anemic animals endothelin-1 is not actually increased. However, coronary arteries from fetuses that have been malnourished show endothelial defects; they don't dilate very well in the presence of normal vasodilators, they don't generate nitric oxide very well, and their function is improved a great deal by antioxidants.

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