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Animal Studies of the Effects of Early Nutrition on Long-Term Health

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Abstract

Small size at birth is associated with increased risk of a variety of common chronic diseases in adulthood. Numerous experimental studies in animals have supported the observations in humans, demonstrating that changes in nutrition in early life can lead to altered long-term health. Importantly, these effects can be independent of size at birth, and can depend on the interaction between nutritional events before and after birth. Both macro- and micronutrient intake are important. Furthermore, these effects may vary according to the nature, timing, severity and duration of the nutritional insult. This review provides examples from animal studies of evidence of these long-term effects, and some possible underlying mechanisms whereby nutrition in early life can affect long-term health.

Nutrition and Intrauterine Growth Restriction

Epidemiological studies over the last 20 years have demonstrated that small size at birth is associated with increased risk of a variety of common chronic diseases in adulthood, such as hypertension, coronary heart disease, type 2 diabetes and obesity [1]. This increased risk is not confined to those born with intrauterine growth restriction (IUGR), but appears to be continuous across the birthweight spectrum, including those within the normal birthweight range. The epidemiological evidence from human studies reported by Barker and colleagues, although initially criticized, was rapidly supported by similar observations in animals. For example, the inverse relationship between birth size and adult blood pressure reported in guinea pigs [2] is unlikely to be explained by confounding due to socioeconomic and lifestyle factors.

Previous classic studies in pigs and guinea pigs [3] had demonstrated that undernutrition in early life can result in growth impairment that is permanent, even if nutritional status subsequently improves. Barker thus hypothesized that undernutrition before birth may be a key mechanism leading both to small size at birth and also to the associated long-term outcomes [1].

Animal studies have also demonstrated the important distinction between maternal nutrition, fetal nutrition, and fetal growth. The fetus grows at the end of a long supply line, comprising not only maternal nutrition, but also uterine and umbilical blood flows, placental transport, and placental and fetal metabolism [4]. Therefore, changes in maternal nutrition do not always result in altered fetal nutrition, as there may be sufficient reserve in the fetal supply line. However, as will be discussed below, changes in maternal nutrition can affect long-term health independent of size at birth. Similarly, interference with steps along this fetal supply line in both human and animal pregnancy commonly results in IUGR, since fetal nutrition is the key regulator of fetal growth [4]. These changes also often result in altered long-term health.

The focus of this review is on animal studies that have examined the ways in which nutrition before and after birth can affect long-term health. These effects may vary depending on the nature, timing, severity and duration of the nutritional insult.

Nutrient Balance

Macronutrients

Dietary protein intake varies widely in different communities, and was an early focus for animal studies. Maternal protein restriction during pregnancy in rats resulted in hypertension in the offspring, altered cardiac structure and function, impaired glucose tolerance, and altered fat distribution and food intake [5, 6].

Importantly, postnatal diet, particularly if high in fat, magnified the effects of maternal protein restriction on the offspring. When fed a highly palatable diet from weaning, adult offspring from rat dams exposed to protein undernutrition during gestation and lactation were more insulin resistant, hyperlipidemic, hypertensive, and more obese and sedentary [7]. In utero protein restriction followed by overfeeding during lactation also shortened offspring life span in mice [8].

Other changes in dietary macronutrient balance during pregnancy also alter disease risk in the offspring. Exposure of rat dams to a high-fat diet during gestation resulted in offspring with impaired glucose tolerance, impaired endothelial function, and hypertension [9]. Cardiovascular dysfunction is induced in the offspring by high-fat feeding not only during pregnancy, but

also during lactation. However, if the offspring of dams fed a high-fat diet were also fed a high-fat diet after weaning, the endothelial dysfunction, although not the hypertension, was prevented [10]. In addition, the specific type of dietary fat also modifies the effect of a maternal low-protein diet on offspring blood pressure [11], suggesting that quite subtle changes in the type and balance of dietary macronutrients in early life may have important long-term consequences.

Micronutrients

There are many examples of specific micronutrients in the maternal diet that influence physiology of the offspring. In rats, maternal dietary deficiency of iron, calcium, or zinc during pregnancy is associated with increased blood pressure in the adult offspring [12].

Specific amino acids appear to have particularly important roles in fetal development and subsequent disease risk. For example, taurine is critical in pancreatic and neurological development. Fetuses of rat dams exposed to a low-protein diet had smaller pancreatic islets with reduced rates of islet β-cell proliferation and higher rates of apoptosis, but these effects were prevented by maternal supplementation solely with taurine [13]. In sheep, periconceptional undernutrition resulted in elevated maternal and fetal plasma taurine concentrations in late gestation and evidence of accelerated pancreatic maturation [14]; these changes were associated with later impairment of glucose tolerance in the adult offspring [15].

Similarly, glycine is critical for many aspects of fetal development in utero, including synthesis of DNA, heme, collagen and creatine. In rats, maternal glycine supplementation normalizes the high blood pressure induced in offspring by a low-protein diet in pregnancy, perhaps in part by normalizing endothelial function [16].

Timing and Duration of Undernutrition

Nutritional composition in early life has important effects on long-term function, but the timing and duration of nutritional changes is also important. Maternal undernutrition even before conception in rats resulted in increased blood glucose and cholesterol concentrations in adult offspring [17]. Elevated blood pressure in offspring exposed to a maternal low-protein diet was greater when the dietary insult was initiated very early in gestation than when it was initiated in mid-gestation [18]. Indeed, a low-protein diet in rats solely during the blastocyst stage (first 4.5 days of the 21-day pregnancy) led to postnatal hypertension in the offspring [19].

In sheep, periconceptional maternal undernutrition has been shown to alter many aspects of fetal development in late gestation, including altering fetal growth trajectory [20]. Furthermore, the timing of the periconceptional nutri-

tional insult affects fetal growth and metabolic responses to a late gestation stressor, such as an acute maternal fast [20]. Maternal undernutrition either solely before conception or both before and after conception led to decreased fetal growth in response to an acute maternal fast in late gestation compared with fetuses of ewes well nourished throughout. In contrast, fetuses of ewes undernourished only after conception had no reduction in their growth trajectory in response to the maternal fast [20]. Changes in the fetal glucose-insulin axis or the fetal or maternal hypothalamo-pituitary-adrenal (HPA) axis at the time of the fast cannot explain these differences [12]. Therefore, it seems that preconceptional maternal nutrition has an important role in determining the fetal responses to stress in late gestation [12, 20]. Furthermore, maternal undernutrition can affect fetal development without necessarily limiting substrate supply for tissue accretion, since nutrient requirements in early pregnancy are minimal, and also without exposing the fetus to excess glucocorticoids, since maternal glucocorticoid concentrations were actually decreased during undernutrition [21].

Several aspects of endocrine regulation in late-gestation fetal sheep were also altered by periconceptional undernutrition [4, 12]. Maturation of the glucose-insulin and HPA axes were accelerated [22]. Importantly, these changes persisted after birth, with offspring of ewes undernourished in the periconceptional period showing impaired glucose tolerance which worsened with increasing age [15]. Sheep exposed to periconceptional undernutrition also displayed altered behavioral laterality [23] and suppressed behavioral and glucocorticoid responses to 5 min of isolation, a potent psychological stressor in sheep [24]. These data suggest that maternal undernutrition in early gestation has long-term effects on neurological and endocrine function, and that the effects are demonstrable not only by detailed physiological testing, but also during exposure to the kinds of challenges that might be faced in everyday life.

Undernutrition beyond the periconceptional period also has effects on the health of the offspring. Undernutrition in both early and mid-gestation in sheep led to greater adipose tissue deposition [25], and deleterious effects on the ovaries in fetal and adult life [12] such as reduction in the number of large corpora lutea [26]. In rats, maternal undernutrition in the last third of pregnancy resulted in impaired glucose tolerance in the offspring, whereas a similar period of undernutrition earlier in pregnancy did not [27]. Undernutrition in late, but not early, gestation led to similar effects in ovine studies [28]. Also in sheep, a short, acute maternal undernutrition insult in late gestation increased central HPA axis responses to corticotropic stimulation in adult offspring, independent of birthweight [29]. Interestingly, if the undernutrition insult was long enough to reduce birthweight, the effects on HPA axis function were mitigated. Clearly, maternal undernutrition can result in long-term changes in postnatal physiology without necessarily affecting size at birth.

Possible Mechanisms

Altered Organ Structure

Early nutrient restriction may permanently impair not only overall growth, but growth and development of specific organs that may contribute to long-term disease risk in later life. For example, there is a wide variation in the number of nephrons present in the kidneys at birth, and this number is then fixed for life. Reduced nephron reserve may increase the risk of later hypertension. Maternal nutrient restriction is known to impair fetal nephrogenesis in rats, mice, and sheep [12]. It also reduces angiogenesis and increases peripheral vascular resistance, all of which may contribute to offspring hypertension [30].

Similarly, cardiomyocyte proliferation is essentially confined to the prenatal period, with postnatal cardiac growth occurring by hypertrophy rather than hyperplasia. In rats, a maternal low-protein diet reduced cardiomyocyte numbers in the hearts of newborn offspring [31] and increased cardiomyocyte apoptosis in postnatal life associated with cardiac dysfunction [5]. Chronic protein-calorie undernutrition in rat dams also led to offspring with marked cardiac atrophy [32].

Likewise, most pancreatic β-cells are produced before birth or in the early neonatal period, and there is limited capacity for β-cell neogenesis after this time. Diabetes results when limited capacity to increase insulin production cannot meet the increased demand resulting for example, from insulin resistance, and is, therefore, more likely when pancreatic β-cell number is reduced. IUGR induced by maternal food restriction led to considerable reduction in β-cell mass in neonates and young rats [33]. Maternal food restriction in late gestation in mice also markedly reduced pancreatic β-cell mass at birth, and this relative reduction persisted into adult life [34]. When combined with insulin resistance, this would be expected to result in impaired glucose tolerance in later life.

Altered Placental Function

Maternal undernutrition may alter fetal growth and development by affecting the structure and function of the placenta. This may occur via changes in placental weight, histomorphology, vasculogenesis and angiogenesis, as well as placental nutrient transport capacity [35]. For example, in guinea pigs, maternal undernutrition led to increased placental barrier thickness and a considerable reduction in the surface area of syncytiotrophoblast for exchange, likely reducing the relative placental capacity to deliver substrates to the fetus [36]. In rats, maternal undernutrition led to enhanced apoptosis in the placental junction and labyrinth zones, the site for fetomaternal exchange [35].

Maternal undernutrition may not only affect placental structure, but also placental function. In rats, prolonged maternal malnutrition in late gestation

reduced circulating maternal glucose concentrations and the expression of GLUT3 in the placenta [37]. Protein restriction of rat dams downregulated placental amino acid transport, which appeared to be a possible cause of IUGR in these animals [38].

Altered Metabolic and Endocrine Environment in utero

The preimplantation embryo is particularly sensitive to epigenetic modifications that may have long-term consequences, and, as previously discussed, maternal protein undernutrition during the blastocyst stage of rat pregnancy led to hypertension in the offspring [19]. Kwong et al. [19] suggested that the transient mild hyperglycemia and amino acid deficiency in maternal serum due to dietary restriction may be a key underlying mechanism. However, subsequent embryo transfer experiments indicated that these effects were intrinsic to the blastocyst, rather than the environment in utero [39].

Nonetheless, there is evidence that undernutrition has a number of effects on the maternal physiological and endocrine milieu. In sheep, periconceptional undernutrition resulted in a delayed rise in early gestation progesterone concentrations in the ewe [22], and altered regulation of the maternal insulin/glucose axis that persisted beyond the period of undernutrition. Undernutrition before, but not after, conception also inhibited the normal development of the physiological insulin resistance of pregnancy in mid-gestation, which in turn was directly related to the growth of the fetus in late gestation (fig. 1) [40]. These and many other changes in the maternal physiological environment during pregnancy and lactation may be one mechanism by which relatively brief or specific changes in maternal nutrition can have long-term effects on the developing offspring.

Altered HPA Axis Function

Another mechanism by which early nutrition may have long-term effects on postnatal disease risk is by affecting the exposure of the developing offspring to glucocorticoids. Glucocorticoids affect growth and maturation of multiple tissues and, in particular, can lead to impaired growth, increased blood pressure and impaired glucose tolerance. These effects may occur in utero if maternal glucocorticoid concentrations are elevated, or if the placental barrier that protects the fetus from high concentrations of maternal glucocorticoids, mediated by activity of the 11β-hydroxysteroid dehydrogenase type 2 (11βHSD-2) isozyme, is impaired. Persistence of these changes after birth via permanent alterations in the regulation of the HPA axis in the offspring could explain many of the observed relationships between reduced size at birth and later disease risk.

In rats, intrauterine glucocorticoid exposure leads to reduced numbers of glucocorticoid receptors in the hypothalamus, resulting in impaired negative feedback and hence long-term upregulation of the HPA axis after birth [41].

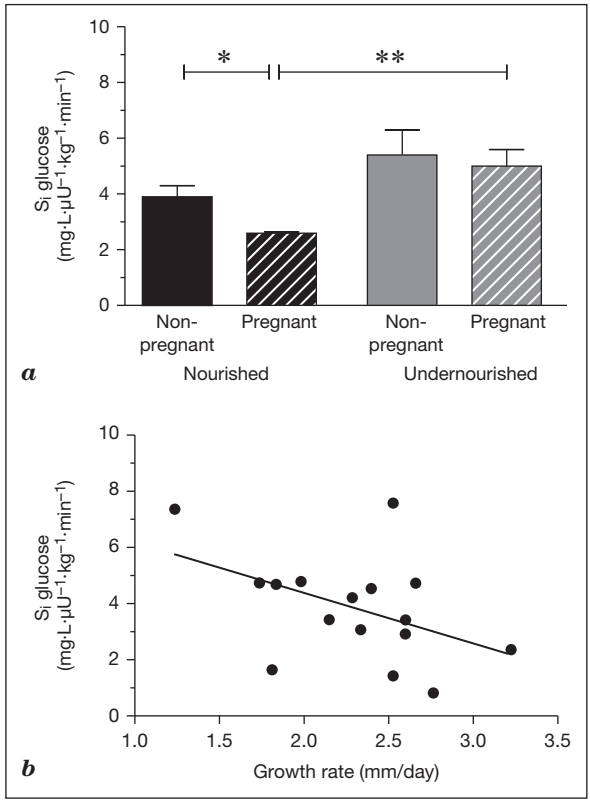

Fig. 1. a Insulin sensitivity (S_i glucose) in mid-pregnancy (65 days' gestation) in normally nourished and periconceptionally undernourished pregnant and nonpregnant ewes (* $p < 0.05$, ** $p < 0.01$). ***b*** Relationship between maternal insulin sensitivity in mid-gestation and fetal growth in late gestation.

This in turn could contribute to increased blood pressure and glucose intolerance in the offspring.

Maternal dietary restriction also increased maternal glucocorticoid secretion in rats, reduced placental 11βHSD-2 activity [42], and altered neonatal HPA axis function. Prevention of the rise in maternal glucocorticoid concentrations by maternal adrenalectomy abolished the effect of a low-protein diet on the outcomes of interest in the offspring [43]. This provides convincing evidence that altering glucocorticoid exposure in early life may be one mechanism by which early nutrition can have long-term consequences for later health.

Maternal undernutrition in sheep also altered HPA axis function in the offspring before and after birth, even when the undernutrition was confined to

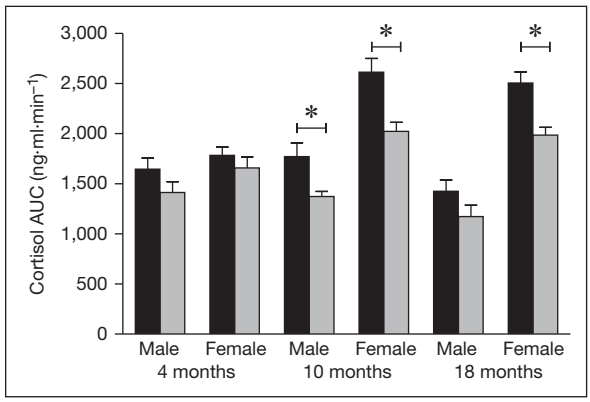

Fig. 2. Cortisol response to an AVP + CRH challenge in male and female sheep at 4, 10 and 18 months of age. Grey bars represent offspring of periconceptionally undernourished ewes, and black bars the offspring of normally nourished ewes. * $p < 0.05$ for nutrition effect. AUC = Area under the curve.

very early in gestation [22]. However, maternal nutrient restriction may not always result in a fetal environment of elevated glucocorticoid concentrations or in increased activity of the HPA axis after birth. Rather, sheep exposed to mild undernutrition for several weeks around the time of conception showed reduced basal and stimulated glucocorticoid concentrations, with altered regulation of the maternal HPA axis [21] and suppressed placental 11βHSD-2 activity beyond the period of undernutrition [44]. Their offspring showed suppression of HPA axis activity that became more marked with age (fig. 2). Therefore, it is possible that the fetus of a chronically undernourished mother develops in a low, rather than high, glucocorticoid intrauterine environment, and that this can also result in long-term disease risk.

Altered glucocorticoid regulation of the anorexigenic hormone pro-opiomelanocortin (POMC) pathways in the ventral hypothalamus may be another mechanism by which fetal responses to maternal undernutrition result in an altered postnatal phenotype. In late gestation, fetuses of ewes undernourished from before conception until day 30 of a 148-day pregnancy displayed hypomethylation and increased histone acetylation of the promoter regions of POMC and the glucocorticoid receptor, 100 days after the undernutrition insult had ceased [45]. These epigenetic changes in the glucocorticoid receptor were reflected in increased gene expression [45]. In the ventral hypothalamus, the glucocorticoid receptor upregulates POMC. Thus, if persistent into postnatal life, these changes could result in disordered appetite regulation and contribute to the reported association between prenatal undernutrition and postnatal obesity.

Conclusions

Animal studies have provided an important contribution to our understanding of the mechanisms underpinning the long-term effects of reduced size at birth that have been observed in human epidemiological studies. Experiments in animals have shown that undernutrition in utero can result in growth impairment that is permanent, even if nutritional status subsequently improves. Observations in rats, mice, sheep, pigs and guinea pigs have also shown that the effects of undernutrition in utero may vary according to the nature, timing, severity and duration of the nutritional insult. Importantly, the long-term effects of early nutritional challenge can occur independently of size at birth, and even when the nutritional challenge is confined to the period before or around the time of conception. Some of the underlying mechanisms include impaired development of individual organs, altered placental function, altered metabolic and endocrine environment in utero, and altered function of the HPA axis and hypothalamic appetite regulatory centers. Clearly, nutritional quality and quantity in early life are critical in determining growth, development and disease risk for life.

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Discussion

Dr. Simmer: I would like to challenge you on one statement, and I think Peter Gluckman also has this theory that if you are well fed in utero you might be better off long-term to stay well fed. I don't think everyone agrees with that, I think there are other researchers in the literature that have challenged that assumption. Would you like to comment on that?

Dr. Harding: I think there is a good deal of confusion in the literature, and that understanding what is 'well fed in utero' is quite problematic. The limited animal data suggest that different outcome measures might give you different answers. In the example I showed you of maternal high-fat diet in rats, maintaining the offspring on a high-fat diet did not improve offspring blood pressure, but did improve endothelial function and oxidative status [1]. We also need to acknowledge that you might not be able to optimize all of the outcomes. It may be that if you are doing well for your brain, you are not doing so well for your heart or the other way round.

Dr. Agarwal: Do you know of any experiments with cereal proteins?

Dr. Harding: There are few data in animals, but I think it would be a very interesting area to investigate in more detail.

Dr. Pereira-da-Silva: I have a question and a comment. Regarding the maturation of the hypothalamic-pituitary-adrenal axis in the fetus, some animal studies suggest overexpression of neuropeptide Y in fetuses subjected to maternal undernutrition [2]. On the other hand, in fetuses growing under diabetic environment overexpression of the orexigenic neuropeptides Y and galanin and underexpression of the anorexigenic neuropeptide cocaine- and amphetamine-regulated transcript, persisting into adult

life, have been recorded [3, 4]. How important are these prenatal disturbances for hypothalamic appetite regulators in programming obesity? My comment is related to the long-term consequences of increasing nutrient intake in preterm infants, including by human milk fortification. The stimulating effect of milk protein on IGF-I secretion in early life, not seen with proteins of other origins [5], may be associated with adipogenic activity, increase in fat mass and programming obesity [6]. Now, you have presented a nice study showing that fortification of mother's milk (increasing the protein intake), increases the future lean mass in sheep. This may be good news for neonatologists who are concerned about providing better somatic and brain nutrition to preterm infants while avoiding programming obesity [7].

Dr. Harding: There has been a huge amount of work on how early nutrition changes appetite. There are major changes not only in neuropeptide Y, but in many of the hypothalamic appetite regulatory centers and signaling pathways [8, 9], and many of these changes have been shown to be epigenetically regulated [10]. How readily reversible those are is currently of great interest. In rats, for example, administering leptin in early postnatal life reverses most of the postnatal effects of maternal undernutrition [11]. Leptin is an important regulator of the neural connections in the hypothalamus in the early postnatal period in rats [12]. We don't know yet when that critical period is in humans, but this does point to the potential to reverse some of these effects.

Dr. Gottrand: I have a general question about the animal models of intrauterine growth retardation. Rats and mice have a very rapid growth during the first days after delivery, and some researchers consider that they could be a good model for studying late intrauterine growth retardation in humans for programming issues and so on. My question is, do you think that early undernutrition in the rat model or the mice model could be extrapolated to late intrauterine growth retardation in human and be used as a good model to test for programming or other issues about the impact of late nutritional defect?

Dr. Harding: One of the things that I learned as a PhD student from Geoffrey Dawes, who was the Doyen of Fetal Physiology, is that one should be careful in using the term 'model'. Animal studies tell you about animals, they don't model anything else. But, I do think you can learn what happens by looking for consistency across several species, and I have tried to show you that across a range of species similar things happen. So, having made that point, I am sure that early postnatal events in rodents are very similar developmentally and have similar long-term consequences to late gestational events in more mature species like sheep and humans, and I am sure that we can learn a great deal from influencing nutrition at that time. There is a large literature on the effects of changing nutrition during lactation in rodents, which is similar to changing nutrition in late gestation in more mature species. Similarly, many studies done in prenatal animals are very relevant to preterm infants because we are looking at similar developmental windows.

Dr. Lack: You showed on a number of graphs the separation between male and female animals, but didn't really comment on what the interactions might be between nutritional insults and sex.

Dr. Harding: Nearly all of the animal studies show sexually dimorphic effects, but they are not particularly consistent. The early and probably simplistic explanation was that the more rapidly growing animals, who are usually males, are more vulnerable to additional insults, be they pre- or postnatal, and therefore we saw greater effects in males than females [13]. I don't think that has held up with subsequent experiments, and we don't yet understand this inconsistency. However, this does suggest that in human studies we need to look carefully at different postnatal ages and in both sexes, when we are looking for long-term consequences.

Dr. van Goudoever: I was intrigued by the glycine aspect because I didn't hear anything about that before. What we did in studies in the preterm infants, especially in the growth-retarded preterm infants, we found that they were more likely to be glycine deficient than AGA infants. Can you relate that to any of your work which you have just been presenting? Is there a reason why especially SGA infants need more glycine than AGA infants?

Dr. Harding: Glycine is required for synthesis of things like heme and DNA and collagen, which are glycine rich and essentially end products in that the glycine cannot be recycled from these materials [14]. I could speculate that growth-restricted infants have a greater proportionate demand because they haven't made enough of these materials before birth. Whether this is causal or consequential, I cannot tell you.

Dr. van Goudoever: The other question I have is: how does high-protein diet affect blood pressure? How does it work?

Dr. Harding: There are numbers of possible mechanisms. Dietary protein can affect growth of nephrons and hence filtration load of individual nephrons [15]. It also can affect regulation of the renin-angiotensin system [16], impair endothelial function [17], and alter capillary density [18].

Dr. Rings: May I also add a question with respect to the blood pressure? I am so surprised that you see the effects of undernutrition so early in life, and my question is: would you advocate starting to lower blood pressure of these children as early as can be, like after birth? Would there be any reason to do that? And can you tell from your animal studies if interventions will be helpful to reduce the risk of cardiovascular diseases?

Dr. Harding: That is a really interesting question. The effects I showed you tend to be correlated with age, so the effects in childhood and early adulthood will tend to be small. They are, for example, much smaller than the effects of preterm birth. In our study, being born mildly preterm (median of 35 weeks) doubled the risk of hypertension by the age of 30 [19], whereas the effect of size at birth is in the order of 2 mm Hg/kg birthweight [20]. However, there is some evidence from the animal literature that early interventions may prevent the long-term changes. In rats, if the offspring of dams fed a low-protein diet are treated with an ACE inhibitor (for 3 weeks) in the neonatal period, they do not develop later hypertension [21]. But this is less a treatment effect than intervention during a critical window to reverse an earlier programming effect. It is like the effects of early leptin administration that we discussed earlier [11]. It suggests that if we understood the mechanisms and could identify the critical windows, we might be able to prevent some of the long-term effects.

Dr. Kleinman: That was an extraordinary review of a very complicated area, and I congratulate you on it. There are so many principles and mechanisms that this work reveals. At the same time, these findings suggest that pregnancy is an exceptionally sensitive and vulnerable period for the developing fetus. Can you comment on the threshold of dietary insufficiency here that leads to potentially harmful chronic health outcomes, because it doesn't seem like human biological systems would have evolved in such a vulnerable way to be so sensitive to the environment at this early stage of development.

Dr. Harding: I don't have an answer, but I have a couple of comments. One is the concept of the predictive adaptive response [22]. This proposes that the effects we are discussing are not evolutionary mechanisms, but rather adaptive mechanisms. During pregnancy, the fetus receives signals from the mother about the nutritional environment and makes the adaptations appropriate for that environment, so that the fetus is prepared as best it can be for the forthcoming extrauterine nutritional environment. Where the pre- and postnatal nutritional environments match, there is no particular health problem. It is only when you get a mismatch, for example when the offspring of

a small mother who is relatively undernourished grow up in a much better nutritional environment, that you start to see the kind of epidemics of diabetes and heart disease that we are seeing in some developing countries.

My second comment is that evolutionary explanations are unlikely to be helpful, because almost all of the relevant long-term diseases (hypertension, diabetes and so on) don't cause major morbidity until after childbearing, so that evolution does not act on those effects.

Dr. Giraldo: What is the impact of early nutrition on behavioral and neurological development?

Dr. Harding: Much of the animal work has been focused on changes in the HPA axis and related stress and anxiety responses. I don't think many of the animal studies are particularly helpful for understanding more subtle behavioral effects because of the relatively unique size, structure and timing of brain development in humans. We can show behavioral changes in sheep after periconceptional undernutrition [23], and others have undertaken behavioral studies in rats [24] and guinea pigs [25], but I don't know how relevant they are to human behavior.

Dr. Lack: The nutritional damage that is done to the offspring of the compromised mother, is that long-lasting into the next generation or is it done by the next generation?

Dr. Harding: There are many multigeneration effects. Altered maternal glucose tolerance in one generation affects pancreatic function in 2 or 3 generations, at least in rats [26]. Some of these effects can be passed through the paternal as well as the maternal line [27], suggesting that not all of the effect is via changes in the intrauterine environment. Rather, there are epigenetic changes in the early embryo.

Dr. Mohanty: I come from the part of India where infant mortality is the highest in the country, and 62% of that mortality is related to the neonatal period. People in India, especially the pregnant women, have a very low intake of food in terms of calorie, protein and fat. But we have a system that when there is a pregnant woman in the neighborhood, the relatives and friends invite her for dinner or lunch once or twice a month, and this goes on until delivery. What we find is that one third of deliveries belong to the low birthweight category, whereas two thirds are still appropriate-for-date babies. So the theory that the fetus is an obligatory parasite is true because the requirement of the fetus is quantitatively very small. So, I have two questions. Does the extra feeding that these ladies get during their pregnancy help them to recover and deliver appropriate-for-date babies, not very low-birthweight babies? Question two: is the theory of the fetus being a parasite irrespective of the protein and carbohydrate intake of the mother relevant today, since the food quantity required by the fetus is very small?

Dr. Harding: I don't know whether the extra feeding of the mother, even if it helps prevent growth restriction, is able to prevent the long-term consequences. The analogy in our sheep studies is of periconceptional undernutrition, followed by good nutrition for the remainder of pregnancy. We find that there is no effect on birthweight [28], but there are substantial changes in adult physiology [29]. Your second comment about the fetus being a wonderful parasite is an interesting one because you are right, the fetal nutrient demands, in absolute terms, are minute, particularly in early pregnancy. Nevertheless, those early pregnancy effects have long-term consequences. So, this story is not about deficiency of the nutrients required to build a fetus, and the fetus as an efficient parasite. Rather, it is about the way the developing embryo adapts to those nutritional signals.

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