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Impact and mechanisms of fetal physiological programming

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THE PHYSIOLOGY OF FETAL PROGRAMMING is a quickly maturing science. Whereas initial studies established and expanded our perception of the phenomenon, more recent studies have begun to focus on the molecular and cellular mechanisms underlying the physiological changes considered to be programming. The reader who is unfamiliar with fetal programming is directed to an ever-expanding body of excellent works that review the history and survey a broad spectrum of scientific findings in the area. Aside from the recent references most familiar to the authors (1–3, 9, 12, 16–18, 20, 21, 24, 31, 32, 37, 39, 44–46) are many others, including special issues of Trends in Endocrinology and Metabolism (vol. 13, 2002) and British Medical Bulletin (vol. 60, 2001).

While the concept of physiological programming is now widely accepted, it is fair to say that a precise definition remains a subject of discussion, or at least a definition that is still evolving. Initially, programming was perhaps too simply associated with deprivation during fetal gestation and small weight at birth. This provided us with a working definition of programming that related adjustments made during fetal life in response to adverse changes in the biological environment with permanent consequences that may have been advantageous in fetal life but confer disease after birth. One key example of this is the “thrifty phenotype” hypothesis (22, 23). That is, in order to maintain viable growth and birth, the pattern of expression of genes can alter insulin sensitivity and otherwise impair metabolic regulation when the environment of decreased nutrient availability. These individuals appear to be at risk of developing metabolic syndrome if moderate early nutrient restriction in pregnant guinea pigs, a species that for development arguably resembles humans more than do sheep or rats. When fetuses were examined late in gestation (0.87G), those in food-restricted dams (70% of normal from 4 wk before to 0.5G) were smaller, with disproportionately smaller liver, biceps, thymus and spleen, and relatively larger brain and lungs. Importantly, interscapular and retroperitoneal fat were also increased relative to body weight.

A relatively new perspective on programming is the impact and mechanisms of change to physiological regulatory systems that occur in response to the maternal environment before there is any direct impact on the fetus. In rats, Drake and colleagues (10) have shown that exposure of female fetal rats to excess glucocorticoid can cause physiological changes in their male offspring, a transgenerational effect. Specifically, the investigators found that the offspring of females exposed to dexamethasone as fetuses have diminished birth weight, and male offspring exhibited diminished glucose tolerance and increased hepatic levels of phosphoenolpyruvate carboxykinase (PEPCK). These studies are consistent with a growing body of literature that demonstrates programming across generations (6) and provides evidence for the biochemical mechanisms by which altered metabolic regulation can occur.

Another early time period during which it is becoming increasingly apparent that physiological regulation may be subject to programming is the periconceptional period. For example, maternal undernutrition before implantation in rats results in hypertensive offspring (30). Decreased maternal nutrition in sheep from 61 days before to 30 days after mating (term gestation is ~150 days), a time when the nutritional burden added by the fetus is nil to negligible, is associated with precocious activation of the hypothalamo-pituitary-adrenal (HPA) axis later in gestation (29). The early activation of the HPA axis may not only lead to inappropriate elevation of prostaglandins and early birth but, as noted above, may also be associated with further programming effects due to inappropriate exposure of the fetus to glucocorticoids. Other reports have also noted that HPA activity is altered as a consequence of changes in the periconceptional environment (11, 14, 15). Another recent study in sheep emphasizes differences in early versus late undernutrition and singleton versus twin pregnancies (13). Undernutrition and maternal and fetal hypoglycemia in late gestation significantly increases perirenal fat in twins, suggesting a clear effect on metabolic regulation when the environmental conditions reach certain limits.

Kind and colleagues (28) have examined the effects of moderate early nutrient restriction in pregnant guinea pigs, a species that for development arguably resembles humans more than do sheep or rats. When fetuses were examined late in gestation (0.87G), those in food-restricted dams (70% of normal from 4 wk before to 0.5G) were smaller, with disproportionately smaller liver, biceps, thymus and spleen, and relatively larger brain and lungs. Importantly, interscapular and retroperitoneal fat were also increased relative to body weight. These findings are significant because they resemble the characteristics at birth of humans born after developing in an environment of decreased nutrient availability. These individuals appear to be at risk of developing metabolic syndrome if
postnatal life is spent in more affluent conditions (51, 52). Another study has utilized moderate early food restriction in pregnant sheep to further examine the programming of cardiovascular changes. Symonds and associates (19) fed sheep 50% recommended caloric intake until 0.65G, followed by 100%. At 3 yr of age, the offspring had higher resting blood pressure and heart rate than controls and a decreased heart rate response to elevation of blood pressure by norepinephrine.

**GENOMIC, GENETIC, AND EPIGENETIC CHANGES**

The transgenerational effects demonstrated in the studies with glucocorticoids in rats suggest the potential involvement of epigenetic changes (10). In another recent study, genomic mechanisms are also thought to play a pivotal role (47). In this model, there is nutritional abundance during the programming period, a high-fat diet during gestation and suckling in rats. This model is also associated with impaired metabolic regulation. At 1 yr of age, the offspring rats have higher insulin resistance, and at 9 mo, their pancreatic β-cells release less insulin in response to glucose. Furthermore, the potential genomic mechanisms are apparent in the observations that at 3 mo, these rats have relatively less mitochondrial DNA in their kidneys, and at 6 mo, they have hyperinsulinemia and decreased expression of the mitochondrial genome in aortic cells, as well as increased expression of the apoptotic voltage-dependent ion channel VDAC1.

**UTERINE BLOOD FLOW**

An important component of maintaining the fetal environment is control of uterine blood flow. Uteroplacental insufficiency is a cause of intrauterine growth retardation and fetal programming. In numerous animal models, researchers use compromised uterine blood flow to demonstrate fetal programming (25). Wood and co-workers (26) have been using an adrenalectomized pregnant sheep model to delineate the role of maternal glucocorticoids in regulating uterine blood flow. They report that with minimal replacement of cortisol, the usual increase in uterine blood flow over days 120–130 of gestation does not occur, nor is there an increase in fetal blood pressure. Interestingly, the group also reports that both low and high levels of cortisol replacement result in decreased rates of fetal growth, and high and low cortisol levels were associated with altered cardiac and renal growth, findings that further emphasize the role of appropriate exposure to glucocorticoids, on the maternal, as well as fetal side of the placenta. Bird and associates (53) have examined aspects of the control of uterine blood flow at the cellular level to provide more mechanistic information on regulation of uterine hemodynamics in pregnancy, a line of work that opens potential new avenues for therapeutic intervention. Measuring nitric oxide production directly for the first time in endothelial cells of the uterine artery, these researchers found that the uterine endothelial cells of pregnant sheep produce more nitric oxide by both calcium-dependent and calcium-independent mechanisms than cells from nonpregnant sheep. Therefore, the ability of the uterine artery to dilate during pregnancy may be specifically related to upregulation of multiple pathways for production of nitric oxide.

**MOLECULAR CHANGES**

The cellular and tissue mechanisms associated with programming are becoming increasingly linked to the expression and elaboration of specific enzymes and genes. As noted above, for example, the transgenerational effect of excessive fetal exposure to glucocorticoids is associated with increased hepatic PEPCK activity (10), and the effect of a high-fat diet during gestation and suckling is associated with decreased mitochondrial DNA (47). In another recent study with a maternal high-protein and high-fat diet in mice during pregnancy and suckling, the early environmental change is associated with markedly decreased hepatic triglycerides in adult female offspring (55). This is, in turn, linked with increased protein levels of the long-chain fatty acid transporter CD36, carnitine palmitoyltransferase-1, and PPAR-α. Similarly, a maternal diet deficient in iron is associated with decreased plasma triglycerides in adult offspring, and this has been linked in the near-term fetus to decreased hepatic triglycerides and decreased hepatic mRNA for cholesterol 7α hydroxylase, liver X receptor-α, and sterol response element binding protein-1c (54).

A somewhat more complex mechanism may operate to produce the hypertension observed in the offspring of rats fed a low-protein diet. The maternal low-protein diet is associated with many changes, including hypertension and impaired nephrogenesis in the offspring (33, 48). A recent study by McMullen and Langley-Evans (36) was aimed at determining the extent to which cardiovascular changes in response to low protein are dependent on glucocorticoids. At 4 wk of age, offspring of rats fed a low-protein diet throughout pregnancy had increased systolic blood pressure, which required the presence of glucocorticoids on days 1–14 of gestation in male offspring only. Further research into specific mechanisms indicated that renal expression of ANG II type 2 receptor mRNA was altered in females only, where it was reduced and the reduction was independent of the presence of glucocorticoids on days 1–14.

**VASCULAR CHANGES IN THE OFFSPRING**

Other recent studies have focused on changes in the vasculature to explain the mechanical aspects of cardiovascular programming. In a comprehensive study by Khan et al. (27), the authors compared changes among the offspring of rats fed a high-fat or normal diet during pregnancy and or suckling. Female offspring of dams on a high-fat diet had elevated systolic blood pressure, regardless of whether the exposure to high fat was during pregnancy or suckling. At the level of vascular mechanisms, the authors found a decreased endothelium-dependent relaxation of mesenteric small arteries in response to acetylcholine in males and females that had been exposed to high fat at either stage of development. Interestingly, arteries of rats exposed to high fat only during suckling had a decreased dilatory response relative to those from rats exposed to high fat during both pregnancy and suckling, suggesting a protective effect of the earlier exposure to high fat. This is not dissimilar to the concept that earlier environmental changes may set a trajectory for later development; and if the same conditions do not occur until later (undernutrition, for example), the programming effects are more pronounced, because the fetus has not accommodated for them.
Roghair et al. (40) have been performing intriguing studies of changes in postnatal arteries from sheep that had been exposed early in gestation to excess glucocorticoids. These studies follow a model that was developed by Wintour and colleagues, who found that exposure to increased levels of glucocorticoids around day 27–29 of gestation in sheep produces hypertension and other manifestations of impaired cardiovascular regulation during postnatal life (7, 8, 50). Roghair et al. (40) observed increased blood pressure at 4 mo of age in lambs exposed to the synthetic glucocorticoid dexamethasone at gestational days 27–28. Exploring mechanisms for the hypertension, they found increased constriction in response to ANG II in the coronary arteries from these lambs but decreased constriction of mesenteric arteries in response to ANG II, phenylephrine, or the thromboxane mimetic U-46619, suggesting a very complex set of vascular changes accompanying the exposure to dexamethasone.

Two recent papers from the Center for Perinatal Biology at Loma Linda University further illustrate the complex changes that occur in blood vessels with ordinary postnatal development or in response to alterations in the fetal environment. The review by Longo and Pearce (34) describes in exquisite detail functional changes in cerebral blood vessels of fetal sheep acclimatized to long-term hypoxia. Among the more interesting findings, in general, is the simultaneous decrease in function related to calcium-dependent contractile mechanisms and an increase in calcium-independent cellular pathways in vessels from fetal lambs subjected to hypoxic conditions. Williams et al. (49) interrogated normal postnatal vascular development and found that dilator responses of carotid and cerebral arteries increase with age in sheep. This effect is clearly apparent in the maximum dilatory responses of carotid and cerebral arteries increase with age in sheep. This effect is clearly apparent in the maximum dilatory effect and sensitivity of vessels to the calcium ionophore A2317. These responses are dependent on nitric oxide because they are absent in endothelium-denuded preparations and blocked by inhibitors of nitric oxide synthase. If these vessels are normally programmed to dilate with the increased metabolic demand of the growing sheep brain, it is not unreasonable to suggest that precious activation of this mechanism during development, as a means of maintaining the delivery rate of substrates at times of deprivation, could permanently alter the ongoing process. Herein may lie clues to other mechanisms involved in adverse programming.

Manning and Vehaskari (35) recently reported that the hypertension, which develops in offspring of rats fed a low-protein diet during the last half of pregnancy, can be blocked if the offspring are placed on a low-sodium diet or treated with the angiotensin-converting enzyme inhibitor enalapril for 3 wk after weaning. This suggests a potential interaction with volume expansion or sensitivity of vessels to the calcium ionophore A2317. These responses are dependent on nitric oxide because they are absent in endothelium-denuded preparations and blocked by inhibitors of nitric oxide synthase. If these vessels are normally programmed to dilate with the increased metabolic demand of the growing sheep brain, it is not unreasonable to suggest that precious activation of this mechanism during development, as a means of maintaining the delivery rate of substrates at times of deprivation, could permanently alter the ongoing process. Herein may lie clues to other mechanisms involved in adverse programming.

MECHANISMS STILL TO BE ELUCIDATED

Other models used to study programming have been more parsimonious when it comes to providing insights into its underlying mechanisms of programming. In another study using the model of early exposure (26–28 days of gestation) of fetal sheep to elevated levels of glucocorticoids, the increases in renal mRNA for glucocorticoid and mineralocorticoid receptors was hypothesized to contribute to postnatal hypertension. In a carefully designed study to test whether the changes in receptors produced functional physiological changes in the fetal kidney at 115–122 days gestation in vivo, Moritz and colleagues (38) found no differences in the renal responses to cortisol and, although there was a difference in the glomerular filtration rate in response to aldosterone, renal excretory function was similar to that in control fetuses.

The set point at which postnatal plasma osmolality is maintained in sheep can be elevated by increasing osmolality during fetal life (42). As Ross and colleagues (43) have reported, however, this change cannot be accounted for by changes in plasma concentrations of vasopressin or the rates of glomerular filtration, osmolar clearance, or renal sodium excretion. Ross has also made some key observations on the programming effects of nutritional restriction and along with colleagues has studied the mechanisms for the development for impaired cardiovascular and metabolic regulation (42).

A pivotal element associated with small weight at birth and programming is postnatal catch-up growth (42). In a recent study, Desai and associates have determined that if catch-up growth is controlled, by continuing maternal food restriction during the period of suckling, then individuals born with low birth weights are no different from controls in adulthood, with respect to body weight, fat, or leptin (6a). The mechanisms by which the catch-up growth is linked to programming, however, remains elusive and the subject of further research.

The maturation of research into physiological programming, alluded to in the opening paragraph of this essay, has resulted in varied new lines of research. Some of the studies surveyed here may also provide a curious new direction for this field to take. It can reasonably be said that the concept of fetal programming grew out of human epidemiological studies that linked measurements at birth, usually smallness and thinness, with increased rates of disease later in life (4). These initial studies prompted other retrospective and prospective epidemiological studies on the rates of disease to emerge in human populations known to have been subject to adverse environments, such as starvation, in fetal life (41). Animal experimentation dovetailed into these modes of research: at first largely descriptive in nature and involved to a large degree with the development of suitable models in which to study programming, and more recently with more mechanistic studies. Clearly, there have been fascinating findings, and these will therefore prompt even more extensive animal studies. One wonders whether there may also be a cycle of research forming. If animal studies of mechanisms of programming can demonstrate that the phenomenon can occur even in individuals one generation removed from the fetuses subjected to an altered environment, will that now prompt a “return” of epidemiological studies to the offspring of individuals who were subject to a programming phenomenon as fetuses? Perhaps herein lies a definition of “transgenerational programming.”

REFERENCES


