Animal Models That Elucidate Basic Principles of the Developmental Origins of Adult Diseases

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Abstract

Human epidemiological and animal laboratory studies show that suboptimal environments in the womb and during early neonatal life alter development and predispose the individual to lifelong health problems. The concept of the developmental origins of adult diseases has become well accepted because of the compelling animal studies that have precisely defined the outcomes of specific exposures such as nutrient restriction, overfeeding during pregnancy, maternal stress, and exogenously administered glucocorticoids. This review focuses on the use of animal models to evaluate exposures, mechanisms, and outcomes involved in developmental programming of hypertension, diabetes, obesity, and altered pituitary-adrenal function in offspring in later life. Ten principles of developmental programming are described as fundamental, regardless of the exposure during development and the physiological system involved in the altered outcome. The 10 principles are discussed in the context of the physiological systems involved and the animal model studies that have been conducted to evaluate exposures, mechanisms, and outcomes. For example, the fetus responds to challenges such as hypoxia and nutrient restriction in ways that help to ensure its survival, but this "developmental plasticity" may have long-term consequences that may not be beneficial in adult life. To understand developmental programming, which represents the interaction of nature and nurture, it is necessary to integrate whole animal systems physiology, in vitro cellular biology, and genomic and proteomic approaches, and to use animal models that are carefully characterized and appropriate for the questions under study. Animal models play an important role in this evaluation because they permit combined in vivo and in vitro study at different critical time windows during the exposure and the ensuing developmental responses.

Key Words: animal models; developmental origins of adult disease; exposures; fetus; lactation; neonate; placenta; pregnancy

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Necessity of Using Animal Models

An overwhelming number of human epidemiological and animal laboratory studies confirm that suboptimal environments in the womb, as well as during early neonatal life, alter development and predispose the individual to lifelong health problems. Although the recent rekindling of interest in the developmental origin of adult diseases was initiated by studies in human epidemiology, the epidemiological approach has several important limitations. Environmental conditions that affect study populations in human epidemiological studies are constantly changing. One of the most fascinating and instructive human events that has led to a wealth of human epidemiological information is the Dutch Hunger Winter. Following a failed joint Anglo-American airborne assault on a bridge over the river Rhine at Arnhem in the fall of 1944, the occupying Nazis responded by restricting the supply of food over large areas of Holland from September 1944 to May 1945. That winter was exceedingly cold. Before the imposition of the restrictions on transport of food by the Germans, the average Dutch person's wartime food intake was already reduced to approximately 1500 calories per day. By the end of 1944, as a result of the food restrictions, average intake had fallen to roughly 750 calories per day. At the height of the very harsh winter, some people were eating as few as 450 calories daily. Analysis of outcomes is complicated by the fact that calories available to different segments of the population were very variable. Greater supplies of food were available in the countryside. People who lived in the cities were more seriously undernourished. Thus, there was a very wide divergence in the nutritional challenge. In addition, as the winter became colder, dietary needs increased as did the mental stress of the final months of World War II. All of these adverse conditions —low nutrition levels, exposure to the cold, and mental stress—were imposed on pregnant women as well as other members of the community.

Extensive studies have been carried out on the offspring of pregnant women born during or immediately after the Dutch Hunger Winter—the so-called children of the Dutch Hunger Winter, who reached 60 yr old in 2005 (Barker 1998; Lumey et al. 1993; Roseboom et al. 2001). These studies have demonstrated an increased incidence of several chronic adult conditions such as diabetes and obesity in the children of the Dutch Hunger Winter. However, interpreta-

tion of the data is complicated by the lack of information from contemporaneous control pregnancies of women who were unexposed to these challenges but otherwise experienced a similar environment during pregnancy. In addition, in this type of study it is clearly not possible to control the level of nutrient restriction carefully or to obtain maternal, placental, fetal, and offspring tissues at various stages of exposure and during recovery to evaluate mechanisms and outcomes. Thus, a thorough understanding of the mechanisms, outcomes, and potential interventions related to developmental programming requires controlled animal studies.

The use of animal models is not subject to the limitations described above. In addition animal models have many advantages over human epidemiological studies. First, it is possible to select and evaluate health in the population of females to be studied even before pregnancy. Features such as diet can also be manipulated before pregnancy. It is now clear that pregnancy is affected in many ways by the prepregnancy environment (especially nutrition) and general health of the mother. To date, very few developmental programming studies in any species have attempted to evaluate the effects of altered maternal status before pregnancy on fetal development and outcomes in the offspring. For example, it is important to know the effect of maternal body mass index and body composition before pregnancy on fetal development. Second, in animal studies it is possible to control food intake and environmental factors rigorously in the different groups under study. Dose response questions can be asked in ways that are very difficult in human studies. Establishment of exposure challenge-response data is critical to a better understanding of mechanism, and ultimately relevance, to human disease. Third, although noninvasive techniques (e.g., ultrasound) can be performed in human pregnancy, it is not possible to repeat many of these evaluations as frequently as might be desired because the burden on the mother (e.g., travel to site of study) is too great. Finally, invasive techniques (e.g., biopsies) are more easily performed in animals, thereby providing multiple point data within the same animal on the profile of development at key times during fetal development and postnatal life.

The mammalian organism passes more biological milestones (e.g., developing adequate organ blood flow) during development than any other stage of life. If these milestones are not passed correctly, the incidence of persistent later life health problems increases greatly. For example, offspring of rats undernourished in pregnancy and lactation have a higher incidence of high blood pressure, diabetes, obesity, and altered sexual function in adult life (Brawley et al. 2003; Dahri et al. 1991; Fernandez-Twinn et al. 2005; Ozaki et al. 2001; Ozanne et al. 1996b; Zambrano et al. 2005a). The purpose of this article is to describe the different animal models that have been studied in an attempt to clarify the principles of developmental programming.

The developing embryo and fetus demonstrate remarkable plasticity in their responses to altered developmental challenges. One good example is the alteration in regional distribution of blood that occurs when the fetus becomes hypoxic (Cohn et al. 1974). In studies conducted in sheep, it has been shown that the fetus responds to decreased oxygen availability by preferentially perfusing the vital organs, brain, heart, and adrenals at the expense of other organs that are not vital to immediate survival (e.g., the carcass and kidneys). Although no firm data exist to show similar redistribution in response to poor nutrition, several investigators have shown in fetal sheep that the undernourished fetus develops a kidney with fewer glomeruli and a smaller liver (Gilbert et al. 2005). Although these fetal compensations may aid short-term survival, suboptimal development of the organs that are deprived to ensure function of the fetal brain, heart, and adrenals can predispose to adult disease. Fewer renal glomeruli increase the offspring's susceptibility to hypertension in later life, and an underperforming pancreas increases the tendency to diabetes if the postnatal diet is not adjusted appropriately. Thus, although the ability of the fetus to respond to challenges is an expression of "developmental plasticity," the long-term consequences may not be beneficial.

Use of Animal Models to Elucidate Ten Principles of Developmental Programming

Ten principles of developmental programming are fundamental, regardless of the exposure during development and the physiological system involved in the altered outcome. These principles are listed in Table 1. Each principle is described below, with the physiological systems involved and the animal models that have been used to conduct studies for evaluating exposures, mechanisms, and outcomes.

Principle 1: During Development, There Are Critical Periods of Vulnerability to Suboptimal Conditions

The first major principle of developmental programming is that critical time windows exist during development when organs are vulnerable to challenges such as decreased oxygenation, nutrient supply, and altered hormone exposure. The period of vulnerability varies from organ to organ. If adverse conditions are experienced in the window of vulnerability, then the trajectory of development of the responding organ may be changed in ways that result in persistent malfunction. It is important to realize that the pathophysiological phenotype resulting from the challenge (e.g., morbid obesity in some rat models [Vickers et al. 2000, 2003]) does not represent a particular human disease entity (e.g., metabolic syndrome in this example). The altered phenotype does, however, illuminate altered function that may be a component of the disease state.

In one classic early study, female rats exposed to a single dose of male sex steroids in the first 5 days of life failed to show normal reproductive cycles when they

Table 1 Ten principles of developmental programming

1. During development, there are **critical periods of vulnerability** to suboptional conditions. Vulnerable periods occur at different times for different tissues. Cells dividing rapidly are at greater risk. Factors that increases risk include:
   - Too much of a normal chemical (e.g., hormone, critical nutrient, or vitamin);
   - Deficiency of a normal chemical (e.g., hormone, critical nutrient, or vitamin);
   - Abnormal chemicals (e.g., alcohol or nicotine); and
   - Abnormal physical forces (e.g., high blood pressure).
2. Programming has **permanent effects** that alter responses in later life and can modify susceptibility to disease.
3. Fetal development is **activity dependent.** Normal development is dependent on continuing normal activity. Each phase of development provides the required conditions for subsequent development.
4. Programming involves several different **structural changes in important organs:**
   - The absolute number of cells in the organ may increase or decease;
   - The relative proportions and distribution of different types of cell within the organ may be unbalanced;
   - The normal blood supply to the organ may not form; and
   - Too many or too few hormone receptors may form with a resulting resetting of feedback and other control mechanisms.
5. The **placenta** plays a key role in programming.
6. **Fetal compensation carries a price.** In an unfavorable environment, the developing baby makes attempts to compensate for deficiencies. Following compensation, birthweight may be normal or only slightly decreased. However, the compensatory effort carries a price.
7. **Attempts made after birth to reverse the consequences of programming may have their own unwanted consequences.** When postnatal conditions prove to be different from those for which the fetus prepared, problems may arise.
8. **Fetal cellular mechanisms often differ from adult processes.** Fetuses react differently to suboptimal conditions than do newborn babies or adults.
9. The **effects of programming may pass across generations** by mechanisms that do not necessarily involve changes in the genes.
10. Programming often has **different effects in males and females.**

reached puberty. When neonatal female rats were exposed to androgen around 20 days of postnatal life, there was no persistent effect on reproduction (Barraclough and Gorski 1961). Thus, studies aimed at determination of exposures that lead to developmental programming, mechanisms by which programming occurs, and/or outcomes in offspring must pay close attention to the timing of the study in relation to the system under investigation.

Various animal models have been used to study several different exposures that are known to predispose to chronic diseases such as diabetes, obesity, hypertension, altered endocrine function, and mood disorders. The major challenges studied have been poor nutrition, maternal stress, and exposure to various pharmacological agents. This article focuses on in vivo and in vitro tissue studies and approaches in rats, guinea-pigs, sheep, and nonhuman primates.

The vast majority of studies on developmental programming have been conducted using the following variety of different nutritional paradigms as challenges to the developing fetus and neonate: (1) an isocaloric low-protein diet (Ozanne et al. 1996a); (2) global nutrient restriction (Blondeau et al. 1999; Garofano et al. 1999); (3) streptozotocin-induced maternal diabetes (Holemans 1993; Holemans et al. 1991); (4) uterine blood flow restriction (Simmons et al. 2001); and (5) administration of glucocorticoids to expose the fetus to glucocorticoid levels that are inappropriate for the developmental stage at which exposure occurs (Nyirenda et al. 2001). Nutrient restriction models easily enable the investigator to deliver the challenge of interest in a carefully controlled window of development. To explore the concept of critical windows of susceptibility, several studies have used nutritional exposures during short windows of gestation. In other studies that compare outcomes resulting from poor nutrition during fetal life with restriction during early postnatal life, nutrients have been restricted during pregnancy and/or lactation (Ozanne and Hales 2004; Zambrano et al. 2005a,b). Pups may be reared by their biological mother or cross-fostered from a mother maintained on one diet during pregnancy to another mother on the same diet (as a control for cross-fostering) or different diet during lactation.

When offspring are exposed to the consequences of suboptimal nutrition of their biological mother during both pregnancy and lactation, the interpretation of the outcomes is less complicated than when diets are changed during lactation either while leaving the pups with their biological mothers and changing the mother's diet or cross-fostering to a mother on a different diet. When the maternal diet is changed at delivery and pups are left with their mothers, there is a period of time in which the challenge (e.g., restricted diet in a previously well-nourished mother or adequate diet in a previously restricted mother) must establish

itself. Although leaving the pups with their mother and changing the diet may narrow the window of exposure, differences between groups can still be attributed to the period after the change. Cross-fostering to nonbiological mothers does result in immediate exposure to the challenge under study; however, interpretation must take into account considerations of immune and other differences that are not present when pups are reared by their biological mother. In addition, keeping pups with their biological mother is perhaps more relevant to the human situation because potential therapies to reverse the effects of prenatal exposures involve maintaining babies with their biological mother.

One recent study demonstrated effects on offspring that are dependent on the period of undernutrition by determining effects of maternal isocaloric protein restriction (50% control of protein intake) during fetal development and/or lactation in rats on the reproductive system of male progeny (Zambrano et al. 2005b). Rats were fed either a control 20% casein diet (C^1) or a restricted diet (R^1) of 10% casein during pregnancy. After delivery, mothers received either C or R diet until weaning to provide four groups: CC, RR, CR, and RC (first letter refers to the diet in pregnancy and the second to the diet in lactation). Maternal protein restriction during pregnancy increased maternal serum corticosterone, estradiol, and testosterone concentrations at 19 days gestation. Pup birth weight was unchanged, but anogenital distance at birth was increased by maternal protein restriction ($p < 0.05$). Testicular descent was delayed 4.4 days in RR, 2.1 days in CR, and 2.2 days in RC and was not related to body weight. Body weight and testis weight were reduced in RR and CR groups at all ages with the exception of CR testis weight at 270 days postnatal age. At 70 days postnatal age, luteinizing hormone and testosterone concentrations were reduced in RR, CR, and RC. Fertility rate was reduced at 270 days postnatal age in RC, and sperm count in RR and RC. Approaches such as these allow isolation of effects of exposures at different times in gestation.

The concept of critical windows of susceptibility applies to challenges other than poor nutrition. Similar studies have shown differential outcomes in response to maternal stress at different stages of development in the rhesus monkey (Clarke and Schneider 1993; Schneider 1992) and in cardiovascular and renal responses to exogenously administered glucocorticoids in pregnant sheep (Derks et al. 1997; Dodic et al. 1998, 2002).

Finally, when extrapolating conclusions based on data from different species, it is important to remember that the trajectory of development of different systems varies between species. The most simple examples are differences between altricial species and precocial species. Thus, the periods of vulnerability of the developing reproductive system that are postnatal in rodents are generally prenatal in sheep and humans.

Principle 2: Programming Has Permanent Effects That Alter Responses in Later Life and Can Modify Susceptibility to Disease

The study by Barraclough and Gorski (1961), in which androgen was delivered to newborn female rats at different postnatal ages, clearly demonstrates the second principle—the persistence of the programmed response. Neonatal androgen exposure was shown to have permanent effects on structures in the brain, particularly the hypothalamus, that regulate female cyclicity.

Detailed evaluation of the long-term outcomes of exposures requires models that are well characterized and in which physiological variables such as blood pressure and heart rate, endocrine secretion, and metabolic responses can be accurately recorded and analyzed in the conscious unrestrained animal. It has been shown that low-protein diets administered to pregnant rats during pregnancy and lactation lead to high blood pressure in adult life (Brawley et al. 2003b; Jackson et al. 2003; Langley-Evans and Jackson, 1995). Offspring of Wistar rats fed ad libitum on 50% global calorie intake of controls have elevated systolic blood pressure when measured by the tail cuff method during adulthood (Franco et al. 2002a,b). Pregnant Wistar rats fed 70% of an ad libitum diet from 0 to 18 days of gestation deliver offspring with elevated mean arterial blood pressure when measured directly via an arterial cannula at 100 days postnatal life (Ozaki et al. 2001). Other rodents including guinea pigs have been studied in similar paradigms. Thus, 30% caloric restriction in guinea pigs results in an 8 mmHg increase in systolic blood pressure at 15 wk postnatal age when measured with an indwelling carotid artery cannula (Kind et al. 2002, 2003).

Different methods have been used to measure blood pressure in the offspring, and the methods used in vivo must be evaluated critically, as in all physiological studies. Concerns have been expressed with the use of the tail cuff methodology to measure blood pressure in offspring. The tail cuff method requires restraint of the rat during the study, and thus any observations may reflect a stressed level of blood pressure (Jamieson et al. 1997). There is evidence that nutrient restriction during development can alter stress-mediated cardiovascular responses. One informative study evaluated the effect of the stress of ammonia exposure in male offspring of mothers fed 9% casein. Using radiotelemetry, the investigators demonstrated that although basal blood pressure did not differ between offspring of control and low-protein diet-fed mothers, offspring of mothers fed the low-protein diet had a greater increase in systolic and diastolic blood pressure than controls in response to the odor stress. This finding suggests that a maternal low-protein diet increases the sensitivity of the sympathetic nervous system to stress stimuli in the offspring. Telemetry or

[1]Abbreviations used in this article: C diet, control 20% casein diet; MAR, maladaptive response; PAR, predictive adaptive response; PEPCK, phosphenolpyruvate carboxykinase; R diet, restricted 10% casein diet.

use of chronically instrumented animals yields firmer data because, unlike the tail cuff method, monitoring by telemetry avoids the need to handle the animals at the time of the study. Remote telemetry methods also allow more detailed analysis of cardiovascular function such as 24-hr rhythms.

The sheep has been studied extensively to evaluate the postnatal effects of maternal nutrient restriction during pregnancy (Gilbert et al. 2005; Vonnahme et al. 2003). The sheep has many advantages for these studies. For example, normal nutrient requirements are well documented in the nonpregnant and pregnant state; there is an enormous database of information on the regulation of fetal growth in sheep; methods exist for instrumenting the fetus in utero to observe the effects of challenges such as hypoxia directly and thus provide information on the immediate effects of exposures on maternal, placental, and fetal function. Maternal nutrient restriction by 50% of global caloric intake results in high blood pressure in the offspring at 9 mo of age (Gilbert et al. 2005). This finding contrasts with the effects of late gestational placental insufficiency and growth restriction induced in sheep by umbilico-placental embolization, after which offspring remained small and had lower arterial pressure than controls in the first 2 yr of life (Louey et al. 2003). The differences in these two studies indicate that similar—but not identical—challenges that include nutrient deprivation (but are not restricted to nutrient supply) may have very different outcomes.

Dexamethasone administered to pregnant rats in the last week of pregnancy elevates blood pressure measured by the tail cuff method in the adult offspring (McMullen and Langley-Evans 2005). In contrast, when measured with an indwelling arterial catheter, McDonald and coworkers (2003) found no increase in blood pressure in adult offspring following betamethasone exposure during fetal life. They attributed the lack of persistent hypertension to three potential reasons: (1) differences in response to prenatal glucocorticoids in the strains of rat utilized; (2) different responses to the administration of betamethasone versus dexamethasone; and/or (3) measurement of blood pressure directly with indwelling catheters, whereas the previous studies measured blood pressure indirectly over a short period of 10 min using tail cuffs. In the study that found no increase in blood pressure, the investigators recorded and averaged blood pressures over an entire 24-hr period.

When interpreting results obtained during different procedures in various studies, it is important to note that an environmental alteration as simple as changing a rat to a clean cage may act as a stressor with marked short-term cardiovascular effects (Duke et al. 2001). Thus, handling the rats or moving them to a novel place to perform recordings may well elevate blood pressure by itself. It could be argued that the presence of controls will remove this concern, but that argument is not theoretically or practically accurate. If the experimental group shows higher blood pressure in a stressed situation compared with the control group, that result does not necessarily indicate that the original challenge during development altered blood pressure. An alternative outcome may be that the developmental challenge has altered the cardiovascular responses to stress—as described above with the odor response—which, although interesting, is a very different finding.

Principle 3: Fetal Development Is Activity Dependent

A fundamental premise of fetal development is that the normal activity of developing organs is vital to normal development. For example, fetal breathing movements are essential to normal lung development. Much experimental work has been performed on this concept in relation to fetal lung development using the chronically instrumented fetal sheep. In this preparation, electrodes can be placed on the fetal chest wall musculature and the diaphragm to record the intermittent fetal breathing movements. A pressure catheter can also be placed in the trachea within the chest. In addition, electrodes can be placed on the skull and across the eyes to record rapid eye movement sleep patterns and fetal electrocorticogram. In various situations such as hypoxemia, fetal breathing movements are depressed (Harding et al. 2000). A decrease in fetal breathing activity results in retardation of fetal lung maturation (Harding et al. 1993).

Activity dependence is a major feature of development, and it corresponds to the concept in the adult of "use it or lose it." For example, as is well known, chronically immobilized muscles waste. In terms of the overall growth and development, studies such as those with fetal breathing show that each phase of development provides the required conditions for subsequent development. The fetal sheep preparation is a powerful model for obtaining a description of the trajectory of structural and functional development.

Principle 4: Programming Involves Several Types of Structural Change in Important Organs

When an organism develops in a suboptimal environment, organ growth is altered. The absolute numbers of cells in the organ may increase or decrease as a result of nutrient deficiency and impairment of cell growth and division. Organ growth restriction has been studied in models of maternal nutrient restriction (Clarke et al. 2001; Vonnahme et al. 2003), a decrease in uterine blood supply as a result of uterine artery ligation (Singh et al. 1995; Wlodek et al. 2005), and uterine or umbilical artery embolization (Block et al. 1989; Gagnon et al. 1994). In each of these cases, the fetal supply line is decreased.

Organ growth restriction may manifest itself as a differential restriction of certain components. Thus, maternal low-protein diets given to rats in pregnancy result in a smaller number of blood vessels per unit area in the fetal rat

pancreas (Snoeck et al. 1990). This decrease in potential vascular perfusion of the pancreas is likely to limit pancreatic islet function and be one of the major factors in the predisposition of offspring of nutrient-restricted mothers to develop diabetes.

A decrease in receptor populations further refines the concept of lack of structure in organs that have developed in a suboptimal environment. In one study, pregnant rats were submitted to daily restraint stress for short periods in the last week of pregnancy (Barbazanges et al. 1996). Offspring demonstrated an exaggerated pituitary adrenal response to stress that was shown to accompany a decrease in the number of glucocorticoid receptors in the hippocampus, which in turn results in a decrease in the sensitivity of the negative feedback of corticosterone.

Principle 5: The Placenta Plays a Key Role in Programming

The importance of paying close attention to the effects of different exposures on the placenta is evident in a very interesting study by Langley-Evans and Nwagwu (1998). Pregnant rats were fed either a control diet or a low-protein diet, and fetal and placental weights were measured at day 14 of gestation. Fetal and placental weights were, somewhat surprisingly, actually increased by protein restriction in the gestational window of conception to 7 days of gestation, and fetal weights increased when protein restriction occurred at 8 to 14 or 0 to14 days gestation. These findings emphasize the need to evaluate the mechanisms that regulate placental growth. It is imperative to remember that weight alone is a poor marker for function and that studies on placental efficiency and fetal body composition are needed to understand these different changes.

Another important study relating to the importance of growth of the placenta investigated the effects of feeding a low-protein diet to pregnant rats solely during the preimplantation period: 0 to 4.25 days of pregnancy with the control diet restored for the rest of gestation (Kwong et al. 2001). Offspring were hypertensive. Embryos examined after 0 to 4.25 days of exposure to the maternal low-protein diet had reduced cell numbers, initially in the inner cell mass and subsequently in both the inner cell mass and trophectoderm lineages. The effects appeared to be due more to reduced cellular proliferation than to increased apoptosis. This altered distribution of embryo cell types was likely due to the exposure of the developing embryo to the reduced insulin and essential amino acid levels and increased glucose levels in maternal blood produced by the low-protein diet.

Principle 6: Fetal Compensation Carries a Price

Although the fetus is able to compensate, the mechanisms by which the fetus changes the trajectory of development may affect postnatal function and have positive or negative outcomes depending on the postnatal environment. Studies in chronically instrumented fetal sheep indicate that in the face of various challenges such as hypoxemia, the fetus can redistribute its blood flow in an attempt to protect its vital organs, brain, heart, and adrenals, which are "spared" the full consequences of the shortage in oxygen. This regional redistribution of blood is the cause of the asymmetric growth retardation observed in babies with placental insufficiency. It should be noted that the protection should be called "relative sparing," rather than "sparing." Although the fetus can redistribute its blood in an attempt to protect vital organs, the redistributed blood remains deficient in oxygen and nutrients. Therefore even "spared" organs—the brain, heart, and adrenals—may not be adequately supplied to maintain normal growth and function. However, the organs that are relatively deprived of blood fare even worse. For example, the impaired growth of the fetal liver results in a smaller abdominal circumference and the observed increase in head to abdominal circumference ratio observed in growth-restricted neonates.

Following compensation, birth weight may be within the normal range or only slightly decreased. The difficulty in determining the effect of a poor intrauterine environment solely from birth weight is the inability to know the size that fetus would have attained under optimal conditions. In addition, as mentioned above, weight is a poor measure of normal function because composition, receptor numbers, and organ structure (e.g., vascularity) may be changed in the presence of a relatively normal weight.

Nutrient restriction results in a decrease in the number of renal glomeruli, thereby increasing the likelihood of renal problems and associated hypertension in later life. A more subtle change is the type of change in function that is seen in some tissues and has been called a predictive adaptive response (PAR[1]). When fetal rats are undernourished in utero, their livers are dramatically altered in function and structure (Hales et al. 1996). The individual lobules have more cells with phosphenolpyruvate carboxykinase (PEPCK[1]) activity than the livers of fetal rats receiving adequate nutrition. PEPCK is the key gluconeogenic enzyme, and this nutrionally induced change indicates the fetal need to increase gluconeogenesis in the face of decreased glucose availability. Using the chronically instrumented fetal sheep preparation, Fowden and colleagues have shown that an increase in PEPCK is one of the many changes the fetus normally makes in late development to prepare to perform gluconeogenesis in neonatal life (Forhead et al. 2003). Hales and colleagues (1996) have argued that if the neonate has an imbalance in liver glucose metabolism tending toward increased glucose production, then adaptation will be of value when food shortage is experienced postnatally. They thus coined the term "thrifty phenotype" to highlight the way in which this preparation for extra uterine life may be of value if food supply is short postnatally. This change in functional capacity has been called a PAR to denote its potential value in helping the offspring survive (Gluckman and Hanson 2004a,b). However, if the postnatal

dietary regimen is adequate—or even overabundant, as in our overconsumption-orientated society—the PAR is maladaptive and may predispose to obesity and other related complications.

The concept of matching prenatal development with the postnatal environment has very important implications for the management of growth-restricted human neonates—especially premature babies. The use of animal models has a major role because it is possible to conduct studies on the effects of altering the diet at different times of development in one direction and then subsequently in another direction. Some of the outcomes of developmental programming can be considered as PARs (e.g., increased tendency to gluconeogenesis, which will be beneficial to survival under certain circumstances). Others, however, are distinctly maladaptive responses (MARs[1]). Outcomes that could be considered MARs, with no apparent value to survival, are the decreased muscle mass or numbers of glomeruli that result from maternal nutrient deficiencies and that predispose directly and inevitably to suboptimal health in later life.

Principle 7: Attempts Made After Birth to Reverse the Consequences of Programming May Have Their Own Unwanted Consequences

When postnatal conditions are different from those for which the fetus prepared, problems may arise. Several studies have been conducted in the rat and mouse in which neonates that are growth restricted at birth have been fed postnatally to induce catch-up growth (Ozanne and Hales 2004; Zambrano et al. 2005a). As mentioned above in relation to PARs, if the postnatal environment is different from the one in which the fetus developed, the mismatch may lead to health problems in later life. To investigate this possibility, Ozanne and Hales (2004) fed pregnant mice either a 20% protein or an 8% low-protein diet to restrict fetal growth. Pups were cross-fostered at birth so that the offspring of mothers fed on a low-protein diet during pregnancy were reared by normally fed dams. These offspring were designated the catch-up group; offspring of mothers fed the normal protein diet during pregnancy and reared by mothers fed on the low-protein diet were designated the postnatal low-protein group. Mice in the catch-up group showed rapid catch-up growth and died at a younger age than controls. Notably, mice that grew normally before birth but were fed by mothers on low-protein diets lived 57% longer than the catch-up group. The authors indicate that this difference is equivalent to a human living 75 rather than 50 yr.

Principle 8: Fetal Cellular Mechanisms Often Differ from Adult Processes

Identifying the underlying mechanisms of developmental programming requires detailed study of the fetus as well as the neonate. The fetus develops in a very different oxygen, nutrient, and hormone environment from a postnatal animal. The model that has provided the most information regarding the physiological function of the fetus is the chronically instrumented fetal sheep. Because of its long gestation (approximately 150 days) and the size of the fetus at birth (5-8 kg), the pregnant sheep allows instrumentation of the fetus under general anesthesia in the second half of gestation with subsequent recovery. An extensive database has been compiled from studies of in vivo function of the fetal sheep (Harding et al. 1989; Nathanielsz et al. 1987). These studies show that fetal responses are often very different from those seen in the adult mammal. The paradoxical response of the fetus to hypoxia and the effects of glucocorticoids in increasing activity of the thyroid axis are but two examples.

When the fetal sheep is submitted to moderate hypoxemia, fetal breathing movements are suppressed (Harding et al. 2000). This hypoxia-induced decrease in fetal breathing movements is in stark contrast to the adult animal, in which hypoxemia stimulates breathing. Administraton of glucocorticoids to the fetal sheep increases the deiodination of thyroxine in the outer benzene ring, thereby generating more of the active form of the hormone triiodothyronine in preparation for extrauterine life. In the adult mammal, in contrast, glucocorticoids decrease thyroid activity (Thomas et al. 1978).

Principle 9: The Effects of Programming May Pass Across Generations by Mechanisms That Do Not Necessarily Involve Changes in the Genes

Several studies have demonstrated that the consequences of various challenges to which the developing organism is exposed can be passed transgenerationally from female offspring challenged during their own development to their own offspring. In early studies that to date have lacked recognition, two independent groups of investigators demonstrated that the F_1 female diabetic offspring of F_0 rats treated with streptozotocin during pregnancy themselves have F_2 offspring with altered glucose and carbohydrate metabolism (Aerts et al. 1990; Oh et al. 1991).

Drake and colleagues observed transgenerational passage of effects resulting from treatment of pregnant rats with dexamethasone from day 15 to 21 of pregnancy. First generation male and female offspring were not treated in any way. Male offspring of the daughters of treated mothers had lower birth weights, glucose intolerance, and elevated hepatic PEPCK activity. These changes did not continue to the third next generation. Remarkably, female offspring of male rats exposed prenatally to dexamethasone and mated with control females exhibited similar changes (Drake et al. 2005).

A maternal low-protein diet (Reusens and Remacle 2001), global caloric restriction (Garofano et al. 1997), or

bilateral uterine artery ligation in late pregnancy (Simmons et al. 2001) can result in altered carbohydrate metabolism in first generation offspring as well as the second generation derived from females in the first generation. A recent study by Zambrano and colleagues (2005b) investigated whether the female pups (the F_1 daughters) of F_0 female rats exposed to protein restriction during pregnancy and/or lactation deliver offspring (the F_2, or granddaughters and grandsons) with in vivo evidence of altered glucose and insulin metabolism. The original F_0 rats were fed a normal control 20% casein diet (C) or a restricted diet (R) of 10% casein during pregnancy. As in the study reported in Principle 1 above, after delivery, the mothers received either C or R diet during lactation to provide four sets of offspring—groups CC, RR, CR, and RC. All of the female offspring were fed ad libitum with C diet after weaning and during their first pregnancy and lactation. As they grew, the female offspring of RR and CR mothers had low body weight and food intake with increased sensitivity to insulin during a glucose tolerance test at 110 days of postnatal life. Grandsons of the CR mothers showed evidence of insulin resistance. In contrast, granddaughters of the RC mothers showed evidence of insulin resistance. Thus, gender differences showed stage of development time window-specific differences. Gender differences were also observed in the second generation in resting glucose and insulin and insulin:glucose ratios. This study demonstrates that maternal protein restriction adversely affects glucose and insulin metabolism of male and female second generation offspring in a gender-specific and developmental time window-specific manner.

Principle 10: Programming Often Has Different Effects in Males and Females

Responses to exposures differ according to the sex of the fetus as shown in the transgenerational study cited above. Programming of cardiovascular responses is another area in which fetal gender differences have been well documented. One study using radiotelemetric recording methods in conscious rats demonstrated elevated blood pressure in female but not male offspring of dams fed a diet rich in animal lard (Khan et al. 2004).

Conclusions

Developmental programming is the discipline in which studies of nature and nurture come together. It is a key area of research for the health of the national population. Understanding developmental programming will enable provision of better antenatal advice and care to improve the health of *tomorrow's children*. Knowledge of the mechanisms of developmental programming will allow physicians to develop treatments that are best suited to each newborn and adult patient. Making treatment specific to the pregnancy and/or the phenotype of the newborn child will lead to better management and prevention of the complications of diabetes, obesity, heart disease, and other chronic, debilitating, and economically important diseases. In addition to human epidemiology, the study of animal models is needed to answer the key questions relating to exposures, mechanisms, and outcomes of developmental programming.

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