Developmental Origins of the Metabolic Syndrome: Prediction, Plasticity, and Programming

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McMillen, I. Caroline, and Jeffrey S. Robinson. Developmental Origins of the Metabolic Syndrome: Prediction, Plasticity, and Programming. *Physiol Rev* 85: 571–633, 2005; doi:10.1152/physrev.00053.2003.—The “fetal” or “early” origins of adult disease hypothesis was originally put forward by David Barker and colleagues and stated that environmental factors, particularly nutrition, act in early life to program the risks for adverse health outcomes in adult life. This hypothesis has been supported by a worldwide series of epidemiological studies that have provided evidence for the association between the perturbation of the early nutritional environment and the major risk factors (hypertension, insulin resistance, and obesity) for cardiovascular disease, diabetes, and the metabolic syndrome in adult life. It is also clear from experimental studies that a range of molecular, cellular, metabolic, neuroendocrine, and physiological adaptations to changes in the early nutritional environment result in a permanent alteration of the developmental pattern of cellular proliferation and differentiation in key tissue and organ systems that result in pathological consequences in adult life. This review focuses on those experimental studies that have investigated the critical windows during which perturbations of the intrauterine environment have major effects, the nature of the
epigenetic, structural, and functional adaptive responses which result in a permanent programming of cardiovascular and metabolic function, and the role of the interaction between the pre- and postnatal environment in determining final health outcomes.

I. EARLY ORIGINS OF ADULT DISEASE: HYPOTHESES AND CONTROVERSIES

A. Origins of the Hypothesis

The “early” or “fetal” origins of adult disease hypothesis describes a hypothesis that was originally put forward by David Barker and colleagues in Southampton in the United Kingdom which stated that environmental factors, particularly nutrition, act in early life to program the risks for the early onset of cardiovascular and metabolic disease in adult life and premature death. Before the fetal origins hypothesis was articulated, an association between early life events and later cardiovascular disease had been proposed on more than one occasion. In 1934, Kermack et al. (251) demonstrated that death rates from all causes in the United Kingdom and Sweden fell between 1751 and 1930, and they concluded that this was the result of better childhood living conditions during this period. Subsequently, Forsdahl (150) reported that there was a correlation within different geographical regions of Norway between coronary heart disease in 1964–1967 and infant mortality rates, some 70 years earlier. Forsdahl (150) postulated that poverty may act through a nutritional deficit to result in a life-long vulnerability to a more affluent adult life-style. In 1985, Wadsworth et al. (524) in the United Kingdom reported that adult blood pressure was inversely related to birth weight in men and women born in 1946. In 1986, Barker and colleagues were searching for an explanation of the different rates of mortality from stroke and cardiovascular diseases in geographical regions of England and Wales. They noted that the geographical distribution of mortality rates from stroke and cardiovascular diseases in 1968–1978 was closely related to neonatal mortality in 1921–1925, and they concluded that poor health and physique of mothers were important determinants of the risk of stroke in their offspring (21). Soon afterwards, they proposed that environmental influences, which impair growth and development in early life, result in an increased risk for ischemic heart disease (22), and indeed, further studies showed that low birth weight and low weight at 1 yr were associated with the highest rates of cardiovascular death (251, 388). There then followed a worldwide series of epidemiological studies that extended the initial observations on the association between pre- and postnatal growth and cardiovascular disease to include associations between early growth patterns and an increased risk for hypertension, impaired glucose tolerance, non-insulin-dependent or type 2 diabetes, insulin resistance, and obesity in adult life. Insulin resistance (with or without glucose intolerance), raised blood pressure, atherogenic dyslipidemia [elevated triglyceride, small low-density lipoprotein (LDL) particles, low high-density lipoprotein (HDL) cholesterol], central or abdominal obesity, and prothrombotic and proinflammatory states are all major components of the metabolic syndrome (137), and it has been suggested on the basis of these epidemiological data that the metabolic syndrome be renamed “the small baby syndrome” (24). The fetal or developmental origins of adult disease hypothesis therefore extended to include the antecedents of the separate and combined pathologies of the metabolic syndrome. This review focuses specifically on the early origins of hypertension, insulin resistance, glucose intolerance, and obesity as the major components of the metabolic syndrome and as risk factors for cardiovascular disease and type 2 diabetes.

B. The Thrifty Phenotype Hypothesis, Programming, and Developmental Plasticity

During the past decade there have been a number of mechanistic frameworks proposed to explain the biological basis of the associations observed between birth weight and health outcomes in the epidemiological studies. In 1992, Hales and Barker (187) coined the term the “thrifty phenotype” hypothesis, derived from the prior “thrifty genotype” hypothesis (362, 363). Neel (362) had proposed that “thrifty” genes were selected during evolution at a time when food resources were scarce and that they resulted in a “fast insulin trigger” and thus an enhanced capacity to store fat, which placed the individual at risk of insulin resistance and type 2 diabetes. The thrifty phenotype hypothesis, however, suggested that when the fetal environment is poor, there is an adaptive response, which optimizes the growth of key body organs to the detriment of others and leads to an altered postnatal metabolism, which is designed to enhance postnatal survival under conditions of intermittent or poor nutrition. It was proposed that these adaptations only became detrimental when nutrition was more abundant in the postnatal environment, than it had been in the prenatal environment (187, 189). The concept that there are embryonic and fetal adaptive responses to a suboptimal intrauterine environment that result in permanent adverse consequences is consistent with the definition of “programming” by Lucas in 1991 (320) as either the induction, deletion, or impaired development of a permanent somatic structure or the “setting” of a physiological system by an early stimulus or insult operating at a “sensi-
tive" period, resulting in long-term consequences for function.

One of the crucial elements of this definition is the concept of a sensitive or "critical" period during which specific nutritional perturbations may operate to cause long-term changes in development and adverse outcomes in later life (26, 144, 506, 539, 540). The existence of critical periods during which nutritional influences can have long-lasting consequences for growth and metabolism had also been highlighted by the early work of McCance and Widdowson (342), who showed that early undernutrition had a permanent effect on the subsequent growth of rats, whereas later undernutrition only had a transient effect. The term metabolic imprinting has also been used to describe the biological phenomena that may underlie the relationship between intrauterine nutritional experiences and subsequent health outcomes (530). This term is intended to encompass adaptive responses of the organism to specific nutritional conditions in early life that are characterized by 1) a susceptibility limited to a critical ontogenic window early in development; 2) a persistent effect lasting through adulthood; 3) a specific and measurable outcome, which may differ quantifiably among individuals; and 4) a dose-response or threshold relation between a specific exposure and outcome (530). The term metabolic imprinting was based on the historical precedent set by Konrad Lorenz who used imprinting to refer to the setting of certain animal behaviors that resulted from early experience. There has been a debate on the appropriateness of both programming (because this term could be misconstrued as referring to events which are reversible, rather than irreversible) and metabolic imprinting (because this term implies early events are exclusively metabolic in origin and because of the possible confusion between metabolic imprinting and "imprinted" genes). It has also been recently stated that whereas developmental physiologists and physicians may use programming to describe the process whereby a stimulus or insult at a sensitive or critical period of development, has lasting effects on the structure or function of the body, that this term has alternative meanings in ethology and other areas of biology, and that therefore programming should no longer be used in the context of the developmental origins of adult disease (23). It has been proposed that the term developmental plasticity rather than programming would be more appropriate. The formal definition of developmental plasticity is the ability of a single genotype to produce more than one alternative form of structure, physiological state, or behavior in response to environmental conditions (23). There has also been a view that the original concept of the thrifty phenotype hypothesis, which applied specifically to conditions of intrauterine deprivation, may be too limited as an overarching hypothesis for the developmental origins of adult disease and that that the adaptive responses of the embryo or fetus to a range of prenatal environments should be viewed in a broader, evolutionary context (28).

I. Evolution, developmental plasticity, and predictive adaptive responses

It is clear from a range of diverse fields including evolutionary ecology and molecular biology that a given genotype can give rise to different phenotypes, depending on environmental conditions. There are many different species where the impact of an environment experienced by one generation determines the development and behavior of the next generation. Female birds are able to alter many aspects of the composition of the egg in response to a range of environmental factors including food availability, levels of sibling competition, and the quality of their mates (311). Such maternal effects can result in the effects of a specific environmental factor persisting across several generations (28, 311). If the effects of the past conditions produce mismatches with current, changed conditions, however, then developmental plasticity may have a detrimental effect on survival and reproductive success (28). Thus Bateson et al. (28) propose that for individuals whose early environment has predicted a high level of nutrition in adult life and who develop a large phenotype, the better the postnatal conditions, the better will be their adult health. For individuals whose conditions in fetal life predicted poor adult nutrition and who develop a small phenotype, the expected outcomes may vary, although they are predicted to be worse off when there is a relative excess of nutrition in postnatal life (28). There has also been a proposal to separate those homeostatic responses that represent fetal adaptations to changes in the intrauterine environment and that may have long-term consequences, from those which need not confer immediate advantage but are induced in the expectation of future adaptive changes; this latter group of responses has been defined as "predictive adaptive" (174). In this model, selection across generations operates to favor protection of those predictive adaptive responses that aid survival to reproductive age (174). The programmed or plastic responses made during development that have immediate adaptive advantage might also act to limit the range of postnatal adaptive responses to a new environment and would be considered to be "inappropriate" predictive adaptive responses (174). This general model is therefore consistent with the original thrifty phenotype hypothesis which stated that fetal adaptations to a poor intrauterine environment may have adverse consequences if there is a relative excess of nutrition available in adult life.

Although the evolutionary view provides a clear biological framework to understand the importance of the prenatal environment for the continued reproductive success and health of subsequent adult populations, it is
recognized that not all responses to the current prenatal environment may be potentially predictive of the postnatal environment. As summarized in the remainder of this review, in conditions of severe intrauterine deprivation, there is the capacity to lose structural units such as nephrons, cardiomyocytes, or pancreatic β-cells within developing organ systems. Such decreases in structural and hence the life-long functional capacity of an organ system may be an inadvertent consequence of a decrease in energy supply across the placenta or a selective trade off to maintain the development of more important tissues, such as the brain. At this stage it is not clear that such responses are either adaptive or predictive, although it is clear that they will result in the programming of a reduced functional capacity for life. For this reason we will retain programming as an overarching term that usefully allows the inclusion of such developmental deficits and their consequences in this review of the developmental origins of adult disease (Fig. 1).

Furthermore, there are still uncertainties regarding which of the array of the embryonic or fetal adaptations to environmental changes are examples of developmental plasticity that confer an immediate adaptive benefit and have no functional consequences in the postnatal period beyond fetal survival, or confer an immediate adaptive benefit and act to limit the range of adaptive responses to a mismatched postnatal environment, or confer no immediate adaptive benefit but are induced in expectation of future adaptive advantage. Finally, although the main focus of this field of research to date has been on the impact of poor fetal nutrition, the issue of maternal and hence fetal overnutrition is of particular importance in the context of the current global obesity epidemic. In evolutionary terms, the period of history during which populations have been exposed to an excess of nutrition has been relatively brief, and it is not clear whether the fetal adaptations to periods of relatively high nutrition confer any immediate or subsequent benefit. As summarized in this review, exposure to relative overnutrition during prenatal life may have adverse consequences for individuals, independently of whether the relatively high nutrient intake is or is not a feature of postnatal life (Fig. 1).

The mechanisms through which specific tissues could be affected permanently by nutritional perturbations in early life have been the focus of a range of commentaries (320, 530) and include epigenetic changes in gene regulation, variations in organ structure, alterations in cell number, hepatocyte polyploidization or cardiomyocyte multicleation, clonal selection of specific populations of cells, apoptotic remodeling, and metabolic differentiation (530, 531). Similarly a range of endocrine signals have been implicated as key mediators of the impact of prenatal nutrition on subsequent development, consistent with the concept of “hormonal imprinting” whereby the concentrations of hormones and hormone analogs present during critical windows of development can permanently alter the hormonal responses to specific stimuli and/or tissue sensitivity to specific hormones (85). This review considers the molecular, cellular, metabolic, neuroendocrine, and physiological responses that occur when there is an alteration in fetal substrate supply and highlight those specific responses that appear to have immediate adaptive benefit but which result in a permanent alteration of the developmental pattern of cellular proliferation and differentiation in key tissue and organ systems to result in pathological consequences in adult life (Fig. 2).

2. Fetal growth and birth weight

One important issue that has been flagged in discussions of the importance of the fetal origins of adult disease is the contribution of low birth weight to the burden of adult disease and the extent to which birth weight is a useful measure or surrogate of fetal growth (440). Term is defined as a pregnancy which lasts 37–41 wk, and ~2% of term babies are of low birth weight, i.e., weigh <2,500 g. Infants can also be characterized as small or large for their gestational age in the context of a larger reference population (453). Being small for gestational age is defined as having a birth weight and/or length at least two standard deviations below the mean for gestational age in the reference population (294). While these definitions identify infants that are relatively smaller at birth, they do not distinguish the different growth patterns by which infants may have arrived at their weight at birth. Importantly, some small babies have achieved their genetic growth potential and are simply in the lower tail of the birth weight distribution, whereas other babies may have experienced a prenatal nutritional deficit which slowed their growth but still have a birth weight that falls within the “normal” portion of the birth weight distribution. Currently there are no clinical measures routinely available to enable the distinction to be made between babies with the same birth weight which have experienced different patterns of growth. A second and related area of debate is to what extent maternal nutrition is implicated in the fetal origins of adult disease. It has been argued that it is likely that maternal nutrition has been adequate in the majority of the populations in which the fetal origins hypothesis has been tested (205) and that long-term surveys of maternal nutrition indicate that variation in food supply produce a relatively minor effect on birth weight (437). It is well established, however, that fetal growth in late gestation is normally limited by maternal size and her capacity to supply nutrients to her fetus, a phenomenon known as “maternal constraint” (504). Furthermore, while fetal growth in late gestation is normally regulated by fetal nutrient supply, large changes in maternal diet may have a relatively small impact on this supply if there is a large

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The margin of “safety” in the capacity of the placenta to ensure adequate transport of maternal nutrients to the fetus (196). A further important factor is the balance of macro- and micronutrients in the maternal diet. It has been shown, in a relatively well-nourished population, that a combination of high carbohydrate in early pregnancy and low protein intake in late pregnancy was associated with a reduced placental weight, birth weight, low ponderal index, and reduced placental weight (175, 176). It is also the case that common clinical causes of impaired fetal growth such as maternal hypertension associated with reduced uterine blood flow, or placental infarcts,

**FIG. 1.** A summary of the current mechanisms considered to underlie the developmental origins of adult disease.
resulting in a reduced placental transfer capacity, may severely limit fetal nutrient supply in the absence of any change in maternal nutrition. Thus there may not be a direct and obvious relationship between the level of maternal nutrition and fetal nutrition, and this may confound attempts to draw inferences on the role of maternal nutrition in determining the fetal growth trajectory.

In humans, size at birth has been associated with cord blood levels of insulin, insulin-like growth factor (IGF)-I, IGF-II and their binding proteins, and the IGF-II receptor (IGF2R), which acts to remove IGF-II within tissues (377). It has been proposed that genetically determined insulin resistance could result in poor insulin-mediated fetal growth, low birth weight, and insulin resistance in childhood and in adult life (198). For instance, a mutation in the glycolytic enzyme glucokinase in the fetus results in a ~500 g decrease in fetal size as a consequence of a decrease in fetal insulin secretion (197). Although such rare monogenic disorders cannot explain the variation in birthweight within a normal population, it has been argued that undefined polygenic genetic factors that increase insulin resistance both in utero and in adult life would produce the two phenotypes of a small, thin baby and an adult with insulin resistance (198). While it is theoretically possible that there is a common genetic cause of low birth weight and type 2 diabetes, studies of identical twins have shown that the effect of low birth weight can operate independently of a genetic change (433). Furthermore, continuing impairment in insulin secretion or action in early postnatal life would be unlikely to support the rapid weight gain or “catch up” growth that commonly occurs in those infants who were growth restricted in utero.

3. Interaction between prenatal and postnatal growth

“Catch up” and “catch down” growth have been defined on the clinical basis of a change in weight or length standard deviation score that results in significant centile crossing on standard infant growth charts. With the use of this definition in a contemporary well-nourished population, it was found that ~30% of all infants show catch up growth, 25% show catch down growth, and the remainder follow the same weight or length centile from birth (380). In this cohort the children who showed catch up growth had a lower birth weight, length, and ponderal index at birth; they had taller fathers and their mothers had lower birth weights and were more likely to smoke during pregnancy (380). It has been suggested that postnatal growth acts to restore infant size back towards their genetic growth trajectory by ~2 yr of age (502). Thus ~90% of infants who are born small for their gestational age show some catch up growth within the first 6 mo of life (248). In affluent communities, infants who were growth restricted at birth and show early postnatal catch up growth tend to overshoot their genetic target and have a higher body mass index (BMI), fat mass, and truncal fat distribution during childhood (380). It has been proposed that tissues that were previously exposed to low concentrations of insulin and IGF-I during fetal life may develop insulin

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resistance as a metabolic defense against hypoglycemia when exposed to relatively higher concentrations of these hormones during rapid postnatal growth and that the crucial time for the development of long-term consequences of fetal growth restriction is therefore the early postnatal period (78). Other commentators have argued, however, that given the biological interaction between the prenatal and postnatal environments, it may not be possible to separate the precise contribution of each of these environments to subsequent adverse health outcomes (191). Again, while rapid postnatal growth in the context of a reduced endowment of cell numbers or specific cell types within key body organs of the growth restricted fetus may produce detrimental outcomes for tissue function, it has been argued that “buying” survival to reproductive age at the expense of adaptations which are detrimental in much later life is a strategy with a positive evolutionary advantage. An alternative, but convergent, view is that the high rate of genetic mutation combined with suboptimal levels of nutrition during most of human history may have allowed the selection of a variety of genotypes that favor survival from fetal life through infancy to reproductive age and also maternal mechanisms that act to restrain fetal growth. Such genotypes may, however, confer an increased risk of insulin resistance and associated cardiovascular and metabolic disease (378, 379). Ong and Dunger (378) have revisited the thrifty genotype hypothesis as originally proposed by Neel (362) and argued that fetal metabolic adaptation to undernutrition could be related to thrifty genotypes that enhance these adaptive mechanisms and therefore in utero survival. Potential fetal thrifty genotypes include the insulin gene variable number of tandem repeats class III/III genotype (INS VNTR class III allele), which is associated with larger size at birth and has been associated with insulin-resistant states such as type 2 diabetes in adults (381). Similarly there may be a number of thrifty fetal genotypes with moderate but cumulative effects on fetal survival advantage, and their predisposition to adult disease risk may be enhanced by rapid growth after birth, obesity, and the associated exacerbation of insulin resistance (379). In contrast, mechanisms that restrain fetal growth and protect maternal survival may be inherited on mitochondrial DNA or maternally expressed genes such as IGF2R. Furthermore, Ong and Dunger (378) propose that larger postnatal size may have a major impact on improving infant survival and that this may explain the rapid, catch up growth that occurs in the growth-restricted fetus after birth. Whereas some genotypes might therefore promote growth in infancy, they may also predispose to insulin resistance, type 2 diabetes, obesity, and cardiovascular disease in later life. This is consistent with the recent findings that infants who are small for gestational age are initially insulin sensitive with respect to glucose disposal (32) but become more resistant by 1 yr of age (489). In this cohort there were significant associations of insulin VNTR genotype and insulin sensitivity and secretion at 1 yr of age (31).

Although there are importance associations between specific prenatal and postnatal growth patterns in causation of adult disease, there has been a reluctance to begin intervention studies in humans or to reduce or abolish the effects of constraint of fetal growth. This is not surprising given the history of unexpected consequences of attempts to improve maternal and fetal outcomes of pregnancy (68, 265, 458). The main conclusion that can be drawn from the recently updated systematic review of randomized trials of energy and protein intake in pregnancy (264) is that balanced protein energy supplements reduced perinatal mortality and the number of small for gestational age babies, but only has a small effect on birthweight (264).

4. Twin studies

There has been considerable debate about the usefulness or appropriateness of using twin pregnancies to investigate the fetal origins of adult diseases. Morley et al. (355) have argued that twin pregnancies should be used to study the early origins of adult disease, as “the nutritional supply line will be more stretched.” They also argue that a comparison of twins with singletons allow easier identification of maternal factors that influence later health. Others including one of the authors of this review have argued that there are too many problems associated with studies of twin pregnancies and that these limit the use of twins in unraveling of mechanisms underlying the fetal origins of adult diseases (415, 416).

Spontaneous twin pregnancy is relatively uncommon and poses greater problems than for singleton pregnancies for implantation, miscarriage, preterm labor, abnormalities and perinatal death, and survivor effects for one or both twins after birth (415). There may also be different mechanisms setting the initial growth rate of twins, and this can be compounded more often in monozygotic pregnancies by the presence of a shared circulation causing the twin-twin transfusion syndrome. It has not been possible to examine the role of birth order for twins in subsequent outcomes, yet it has been reported the first twin when born prematurely is more mature than the second. None of the recent studies has reported use of steroids in twin pregnancies, yet this may have been different for these pregnancies and newborns before or after birth. An example of the comparatively poor outcome of twin pregnancies is the different outcomes of singleton and twin pregnancies after in vitro fertilization (IVF) and advanced reproductive technologies (ART). In singleton pregnancy, ART is associated with increased incidence of preterm birth, growth restriction, and perinatal death. However, ART does not increase these out-
comes in twin pregnancies above the much higher rate observed for spontaneous twins compared with singletons (203). Despite these ranges of issues, the debate about the relevance of studies in twins as a test of the fetal origins hypothesis has not stopped publication of studies in monozygotic and dizygotic twin pairs. Because twins are generally smaller than singletons, the fetal origins hypothesis should indicate a higher all-cause mortality or deaths from ischemic heart disease in twins than in singletons, but this is not so (77, 512). However, mortality for dizygotic compared with monozygotic female twins was higher between 30 and 59 yr (77). In twins, as has been reported for singletons, there was a positive association between birth weight and blood pressure in infants (301). In keeping with these findings, blood pressure was lower in twins aged nine compared with singletons after appropriate adjustments had been made for gestational age (544). In this study smoking, which constrains growth, was associated with an increase in blood pressure. Furthermore, at age seven, there was no association between birth size in twins and blood pressure (569). It might be concluded from these studies that the mechanisms setting growth and cardiovascular outcomes are different in twins compared with singletons.

Although the setting of growth of twins in utero may be different, there are intertwin differences in growth rate that are likely to be due to growth retardation, as in singletons. The shorter twin has been found to be more likely to die from heart disease (512), and lighter, shorter twins or those with smaller head circumferences are more prone to acute myocardial infarction than external matched twins, although this relationship did not hold for intratwin pairs (222). Thus studies have examined whether being the smaller rather than the larger twin is consistent with the fetal origins hypothesis for risk factors for heart disease. It has been reported that there is no difference in blood pressure between larger and smaller twins (16, 569). In contrast, in female twins, higher blood pressure was found in the smaller twin, and there was a trend for this to increase with the intertwin difference in birthweight (434). The effect was greater when women taking antihypertensives were excluded from the analysis, and this raises the concern about the best way to account for those taking antihypertensives, since in singletons more adults who were light than heavy at birth require antihypertensive treatment. Dwyer et al. (117) reported that blood pressure decreased by 1.94 mmHg for every additional kilogram in weight at birth in singletons and by 7.0 mmHg in twins. They concluded that a principal causal pathway must include the fetoplacental unit.

In singletons, there are associations between birth weight and endocrine function with a common finding of hormone resistance. Glucose tolerance and insulin sensitivity have been examined in only a few studies. It has been reported that there is (237) or is no (318) difference in insulin sensitivity. In summary, there is more evidence against than support for the application of the fetal origins hypothesis to twin pregnancy in women.

This review first considers the epidemiological evidence for the association between the perturbation of the early nutritional environment and the major risk factors (hypertension, insulin resistance, and obesity) for cardiovascular disease, diabetes, and the metabolic syndrome in adult life. Second, we focus on those experimental studies that have investigated the critical windows during which specific perturbations of the intrauterine environment have major effects; the nature of the structural and functional adaptive responses of the embryo, fetus, and neonate to such perturbations which may result in a permanent programming of cardiovascular and metabolic function; and the role of the interaction between the pre- and postnatal environment in determining final health outcomes.

II. CARDIOVASCULAR DISEASE AND HYPERTENSION

A. Epidemiological Evidence for the Association Between Low Birth Weight, Cardiovascular Disease, and Hypertension

I. Cardiovascular disease

In 1987, Barker and colleagues (22, 388) found that lower birth weight and weight at 1 yr of age were associated with an increased risk of death from cardiovascular disease and stroke in a cohort of men and women born in Hertfordshire, England between 1911 and 1930. Early criticisms of the association of death rate from coronary heart disease with lower birth weight raised questions about completeness of follow-up (244), although it was recognized that loss of subjects from cohorts was inevitable with the requirement for follow-up over many decades (263). This criticism was largely answered in a study of 15,000 Swedish men and women with a 97% follow-up over a period of more than 50 yr. In this study the death rates from ischemic heart disease were increased in individuals in the lower quartiles of birth weight compared with those in the highest quartile (296). Also of note was the Nurses Study in the United States which includes 121,700 women who have been followed since 1976. In one retrospective study of more than 70,000 women from this group, there were strong negative trends between self-reported birth weight and nonfatal coronary heart disease and stroke (451). The association of cardiovascular death and birth weight has now been reported in many (156, 157, 335, 493) but not all studies (224), particularly those involving twins (512).

The importance of environmental influences that impair fetal and infant growth was raised when it was re-
ported that men with the lowest weights at birth and at 1 yr of age had the highest death rates from ischemic heart disease (25). Although the death rate increased with decreases in birth weight in both males and females, the effect of reduced infant growth on subsequent cardiovascular disease was present in men only (388). It was suggested that promotion of fetal and infant growth might reduce cardiovascular deaths, and a special emphasis was given to the importance of promoting growth in the first year of life of boys who weighed <7.5 lbs. (~3.5 kg) at birth (22). Although low weight gain in the first year of life was associated with increased cardiovascular mortality, it has also been shown in a cohort of Finnish men that increased weight gain as early as 3 yr of age is also associated with increased cardiovascular mortality (134). Men who died were obese with above average BMI at all ages from 7 to 15 years of age (135). These effects may be greatest in males born to mothers who were obese (152). Short birth length of female infants followed by increase in height was also associated with increased risk of death from coronary heart disease (151). These women tended to have tall mothers (151), which suggests that prenatal growth of these female infants was constrained. Thus the most adverse cardiovascular disease risk profile is found in men and women who were small at birth but became obese in adult life (136), and the effects of adult BMI on high blood pressure, type 2 diabetes, and insulin resistance were greater in individuals of low birth weight (133, 157). Whatever the mechanisms, the possibility of a biological link between birth weight and adult BMI complicates interpretation of studies that link birth weight and adult BMI to other adult outcomes such as cardiovascular disease, hypertension, and insulin resistance. Whilst many authors adjust for current BMI when evaluating associations between birth weight and adult outcomes, it has been argued that there may be statistical implications with this approach that should be taken into consideration when interpreting the interaction between birth weight, BMI, and cardiovascular or metabolic health outcomes (58, 322, 530).

2. Hypertension

In the late 1980s, a study in Sweden reported that the risk of increased diastolic blood pressure was significantly higher in a group of male army conscripts who had been small at birth. The authors concluded that being born small for gestational age might be a predictor of raised blood pressure in adult life (170) and, by implication, increased risk of cardiovascular death. Soon afterwards, the association between low birth weight and high blood pressure in adult life was extended to include the normal range of birth weight (22). In addition to raised blood pressure at age 10, children living in areas with higher rates of death from cardiovascular disease also had high resting pulse rates. The children and their mothers were shorter than those living in areas with low cardiovascular mortality, raising the possibility of environmental or inherited/genetic effects (22).

A small number of studies have examined the contribution of the placenta to the association between size at birth and blood pressure later in life. In cohorts in Preston in the United Kingdom and in Adelaide in Australia, blood pressure increased with decreasing birth weight and increasing placental weight (19, 349). In the Adelaide cohort, however, the effect of placental weight was no longer apparent when the blood pressure was measured when the subjects were ~20 yr of age. In this group, the amplification of blood pressure from 8–20 yr of age was greatest in those with lower birth weights (349). No support for an effect of placental size or birth weight was found in the New Zealand cohort followed from birth to 18 yr of age (545) or for other indices of fetal or placental growth in 8-yr-old Australians (58).

Four systematic reviews have examined the association between birth weight and blood pressure in later life (226, 227, 291, 292). The first review summarized data from 1,895 children and 3,240 adults. There was a negative relationship between blood pressure and birth weight, which amplified with increasing age in adult life. The number of subjects and studies increased rapidly to 66,000 in 34 studies (292) and to 440,000 in 80 studies (227). In the earlier reviews the association of blood pressure with birth weight was not evident in adolescence; however, with increased numbers, an attenuated relationship was found (227). The conclusion drawn by Huxley et al. (227) was that both birth weight and head circumference at birth were inversely related to systolic pressure and that accelerated postnatal growth was also associated with raised blood pressure. Many of the studies had been completed with automated blood pressure devices that calculate but do not measure diastolic pressure. It is uncertain how this might have effected the conclusions about diastolic pressure. Certainly with publication of this third systematic review, it seemed as though there could be little room for further debate about the existence of the association of blood pressure throughout life after infancy and birth weight. This conclusion was challenged, however, with the publication of a fourth systematic review (226), which was confined to 55 studies that reported regression coefficients of systolic blood pressure on birth weight. The authors reported “a clear trend (P < 0.001) towards a weaker association in the larger studies.” The regression coefficient was −1.9 mmHg/kg for studies with <1,000 subjects and −0.6 mmHg for studies with >3,000 subjects. In twins, the summary coefficient did not achieve statistical significance (226). It was also noted that the studies from the group in Southampton reported larger regression coefficients. Huxley et al. (226) concluded that “the claims for
a strong inverse association between birth weight and subsequent blood pressure may chiefly reflect the impact of random error, selective emphasis on particular results and inappropriate adjustment for current weight and confounding factors.” A similar note of caution has been expressed with an analysis, suggesting that there has been publication bias and that the association with birth weight is reduced when a correction is made for this factor (463).

The criticism about adjustment for current weight has been addressed (204), and problems with studies in twins were discussed in detail above. Others criticized the use of a single regression coefficient, which effectively excludes the phenomenon of tracking and catch-up. Other problems raised with the conclusions of the systematic review (226) included emphasis on large studies that included self-report of blood pressure and inclusion in these large studies of measurements of blood pressure of subjects who were taking antihypertensive agents (84). This latter point is important since the number requiring treatment decreases progressively with increasing birth weight (20, 132). In the large Shanghai Women’s study, the women who were above average weight at 15 and were of low birth weight were 4 times more likely to have hypertension than women of average weight with birth weights between 2,500 and 3,249 g. Women who were above average weight and below average height and who were of low birth weight had the highest odds of developing hypertension OR 7.64 (95% CI 2.85–20.44) (570). In a smaller Danish case-control study of juvenile obese men, body weight history after the age of 7 had no influence on blood pressure in addition to current BMI. The authors concluded that there was an adverse effect of low intrauterine weight or of a high gain in weight before the age of 7 on adult systolic blood pressure (462). In parallel with the worldwide series of epidemiological studies on the association between low birth weight and adult blood pressure, there have been a range of animal-based experimental studies that have investigated the mechanisms that could underlie the association between poor fetal growth and adult hypertension.

B. Perinatal Nutrition and the Programming of Hypertension

1. Fetal nutrient restriction and programming of hypertension in the guinea pig and rat

One of the first studies to provide experimental evidence for the fetal origins of adult disease hypothesis was carried out by Persson and Jansson (405) in which one uterine horn was ligated in pregnant guinea pigs to restrict fetal growth. This resulted in severe fetal growth restriction and relative hypertension in the restricted pups. In 1994, Langley and Jackson reported that feeding low-protein diets (60–120 g protein/kg diet) to pregnant rats resulted in an increase in blood pressure in the offspring, compared with control animals (277). This low-protein diet (the Southampton low-protein diet) is composed of casein, sucrose, starch, and corn oil and is supplemented with vitamins, minerals, and methionine (277). Subsequent studies showed that when rat dams are fed this low-protein diet (90 g/kg: 9% protein diet) during pregnancy, fetal growth is enhanced until day 20 of gestation (term = 21 day gestation), and this is followed by a period of growth restriction over the last 2 days of gestation such that the pups tend to be of low or low-normal birth weight (282). The offspring of rats fed the low-protein diet have raised systolic blood pressure by weaning at ~4 wk of age (277, 286). When dams were fed the low-protein diet for single weeks during pregnancy, the magnitude of the effect of the diet on postnatal blood pressure was greatest when the low-protein diet was consumed during the last week of gestation (287). Subsequent studies, using a low-protein diet (6%) from 12 days gestation or a low-protein (9%) diet throughout gestation also reported that the offspring developed hypertension by 8 and 21 wk of age, respectively (332, 516, 557). The hypertension associated with prenatal exposure to a low-protein diet appears to be a consequence of an increased peripheral resistance as the pulse rate tends to be lower, and there is no cardiac hypertrophy, suggesting that cardiac output is not elevated (232). Interestingly, a balanced reduction (a 50–70% decrease) in maternal nutrient intake produces a less consistent effect on later blood pressure than does the specific restriction of maternal protein intake (216, 390, 517, 519, 520, 555) (see Table 1). Furthermore, when different low-protein diets have been compared, it has been demonstrated that they elicit different programming effects on blood pressure in the offspring (278). Prenatal exposure to an alternative low-protein diet, the Hope Farm diet, results in normotensive, insulin-resistant offspring (321, 397, 398). The Hope Farm low-protein diet differs from the Southampton low-protein diet in fat source and fat (Hope Farm: soybean oil 4.3%, Southampton: corn oil 10%), methionine (Hope Farm: 0.2%, Southampton: 0.5%), and carbohydrate content (Hope Farm: 8% starch and 66.7% glucose, Southampton: 48.5% starch and 24.3% sucrose).

One of the major differences between low-protein diets is the amount of the amino acid methionine. The Southampton low-protein diet contains significantly more methionine than other low-protein diets. Casein provides less than half of the cysteine required by rats during gestation, so extra methionine is added to the diet so that rats can produce cysteine by transulfuration of homocysteine. High concentrations of methionine may therefore lead to hyperhomocystinemia, due to its production as an intermediate in the conversion of excess methionine to cysteine (278, 445), and it has been shown that rats fed the Southampton low-protein diet have increased serum lev-
TABLE 1. *Impact of manipulation of the maternal diet or uteroplacental blood flow during pregnancy in the rat on blood pressure during postnatal life and on the structural and functional development of the kidney and vasculature*

<table>
<thead>
<tr>
<th>Manipulation</th>
<th>Timing</th>
<th>Fetal Growth and Birth Weight</th>
<th>Blood Pressure and Vascular Reactivity in Postnatal Life</th>
<th>Fetal and Postnatal Kidney Development</th>
</tr>
</thead>
<tbody>
<tr>
<td>9% Protein vs. 18% protein</td>
<td>0–4.25 days</td>
<td>↓ Birth weight (females) (266)</td>
<td>↑ SBP (females) 4, 11 wk (266)</td>
<td>↓ Kidney: body weight (males) 12 wk (266)</td>
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<td></td>
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<td>↓ Birth weight (males) (266)</td>
<td>↑ SBP (males) 4, 11 wk (266)</td>
<td>⇔ Nephron number 20–22 days gestation (266)</td>
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<tr>
<td>9% Protein vs. 18% protein</td>
<td>0–7 days</td>
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<td>↑ SBP (males) 4 wk (287)</td>
<td>⇔ Plasma ANG II 4 wk (287)</td>
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<tr>
<td>9% Protein vs. 18% protein</td>
<td>8–14 days</td>
<td></td>
<td>↑ SBP 4 wk (287)</td>
<td>↑ Nephron number 20 days gestation (286)</td>
</tr>
<tr>
<td>9% Protein vs. 18% protein</td>
<td>15–22 days</td>
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<td>↑ SBP 4 wk (287)</td>
<td>↓ Nephron number 20 days gestation (286)</td>
</tr>
<tr>
<td>9% Protein vs. 18% protein</td>
<td>0 days-term</td>
<td>⇔ Birth weight (48, 367)</td>
<td>↑ SBP 4 wk (283, 286, 287, 367, 471, 472)</td>
<td>⇔ Nephron number 20 days gestation (286)</td>
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<td>↑ SBP 6, 7, 12, 13, 19, 20 wk (48, 160, 280, 284, 286, 367)</td>
<td>⇔ Nephron number 22 days gestation (286)</td>
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<td>↑ SBP &gt;20 wk (284, 285)</td>
<td>⇔ Nephron number 4 wk (286)</td>
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<td>↓ HR 4, 12 wk ⇔ HR 20 wk (367)</td>
<td>↓ Renal size 4 wk (286, 367, 19 wk (286)</td>
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<td>↓ Endothelium-dependent and</td>
<td>↑ Plasma ACE 4, 13 wk (284)</td>
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<td>–independent vasorelaxation in vitro</td>
<td>⇔ Plasma renin 13 wk (284)</td>
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<td>12, 23 wk (48)</td>
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<tr>
<td>8.5% Protein vs. 19% protein</td>
<td>1 day-term</td>
<td>↑ Birth weight (557)</td>
<td>↑ MAP 21 wk (557)</td>
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<tr>
<td>12% Protein vs. 18% protein</td>
<td>14 days before mating-term</td>
<td>⇔ Birth weight (277)</td>
<td>↑ SBP 9 wk (277)</td>
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<td>⇔ SBP 12 wk (277)</td>
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<tr>
<td>9% Protein vs. 18% protein</td>
<td>14 days before mating-term</td>
<td>⇔ Birth weight (277)</td>
<td>↑ SBP 9, 21 wk (277)</td>
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<tr>
<td>6% Protein vs. 18% protein</td>
<td>14 days before mating-term</td>
<td>⇔ Birth weight (277)</td>
<td>↑ SBP 9, 21 wk (277)</td>
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<tr>
<td>Manipulation</td>
<td>Timing</td>
<td>Fetal Growth and Birth Weight</td>
<td>Blood Pressure and Vascular Reactivity in Postnatal Life</td>
<td>Fetal and Postnatal Kidney Development</td>
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<tr>
<td>6% Protein vs. 19–20% protein</td>
<td>12 days-term</td>
<td>↓ Birth weight (331, 332, 516)</td>
<td>↑ SBP 8, 40 wk (331, 332, 516)</td>
<td>↑ Glomeruli/kidney 8 wk (516)</td>
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<td>↑ Renal apoptosis 8 wk (516)</td>
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<td>↑ Plasma Na 8 wk (332)</td>
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<td>⇣ Plasma Na 40 wk (332)</td>
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<td>↓ Plasma renin 4, 8 wk (332, 516)</td>
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<td>↑ Plasma renin 36, 44 wk (332)</td>
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<td>↑ Plasma aldosterone 4, 8 wk (516)</td>
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<td>⇣ Renal NHE3, ENaC abundance 4 wk (331)</td>
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<td>↑ Renal BSC1, TSC abundance 4 wk (331)</td>
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<td>↑ Renal BSC1 mRNA 1 day, 4, 8 wk (331)</td>
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<td>↑ Renal TSC1 mRNA 4 wk (331)</td>
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<td>70% vs. 100% maternal intake</td>
<td>0–18 days</td>
<td>↓ Birth weight (390)</td>
<td>↑ MAP (males) 8, 14, 28 wk (390)</td>
<td>↑ MAP (females) 14, 28 wk (390)</td>
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<td>↓ Phenylephrine- and NA-induced vasoconstriction 3 wk (390)</td>
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<td>↑ U46619-induced vasoconstriction (males) 28 wk (390)</td>
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<td>↑ MAP (males) 14, 28 wk (390)</td>
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<td>↑ MAP (females) 14, 28 wk (390)</td>
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<td>↑ MAP (males) 14, 28 wk (390)</td>
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<td>↓ Phenylephrine- and NA-induced vasoconstriction 3 wk (390)</td>
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<td>↑ MAP (males) 14, 28 wk (390)</td>
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<td>↑ MAP (females) 14, 28 wk (390)</td>
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<tr>
<td>50% vs. 100% maternal intake</td>
<td>11 days-term</td>
<td>↓ Body weight 20 days gestation (216)</td>
<td>⇣ SBP, ⇣ DBP 14 wk (216)</td>
<td>↓ K+ -induced vasoconstriction 12-15 wk (216)</td>
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<td>⇣ NA-induced vasoconstriction 12–15 wk (216)</td>
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<td>↓ Endothelium-dependent vasorelaxation in vitro 12–15 wk (216); ↑</td>
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<td>Endothelium-independent vasorelaxation in vitro (216)</td>
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<tr>
<td>30% vs. 100% maternal intake</td>
<td>0 days-term</td>
<td>↓ Birth weight (517, 519, 520, 555)</td>
<td>↑ SBP 14 wk (517, 519)</td>
<td>↑ SBP 25 wk (520)</td>
</tr>
<tr>
<td>Bilateral uterine artery ligation vs. controls</td>
<td>14 days gestation</td>
<td>↓ Birth weight (402)</td>
<td>↑ SBP 30–56 wk (555)</td>
<td>↑ MAP 4–12 wk (402)</td>
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<td></td>
<td>↑ Phenylephrine-induced vasoconstriction 4–12 wk (402)</td>
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<td></td>
<td>↓ Endothelium-dependent NO-mediated vascular relaxation (402)</td>
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</table>
els of homocysteine during the first 4 days of pregnancy (406), although not in later pregnancy (406, 445). Because the fetus lacks cystathionine \(\beta\)-synthase, it is compelled to eliminate homocysteine through the synthesis of S-adenosyl homocysteine, which is then remethylated to S-adenosyl methionine. The remethylation of S-adenosyl homocysteine also increases the demand for methyl groups derived from tetrahydrofolate, reducing the availability of folates for deoxynucleoside triphosphate synthesis. The degree of DNA methylation has been shown to depend on dietary composition in adult rats (429), and it has been suggested that changes in methionine metabolism increase homocysteine production, which may in turn lead to changes in DNA methylation in the fetus and perturb key events in organogenesis and in embryonic vasculogenesis (492).

One of the consequences of the Southampton low-protein diet is a fall in maternal plasma and fetal body concentrations of the essential amino acid threonine (406, 446). Cystathionine and homoserine are by-products of cysteine synthesis and share the same oxidative pathway as threonine; therefore, the induction of transulfuration increases threonine oxidation (445). In one study it was reported that supplementation of the low-protein diet with threonine during the initial phases of pregnancy reduced maternal circulating concentrations of homocysteine (406), whereas in a second study it was reported that threonine supplementation resulted in increased concentrations of maternal serum homocysteine in later pregnancy (445). Furthermore, in the latter study, the endogenous methylation of DNA was greater in the liver (although not the heart or kidney) of fetuses from dams fed the low-protein diet and increased further when the diet was supplemented with threonine (445).

In a study in which nonessential nitrogen was added to a 9% protein diet in the form of glycine, alanine, or urea, the hypertensive effect of the low-protein diet during pregnancy on the blood pressure of the offspring was effectively reversed by supplemental glycine, but not alanine or urea (232). There is a large fetal requirement for glycine as it appears to be a conditionally essential amino acid for a number of metabolic compounds including nucleic acids, collagen, heme, and keratin (231). Any marginal decrease in glycine can be exacerbated if dietary methionine is increased as glycine is used in the detoxification of an excess of methionine by enhancing methionine breakdown via the transulfuration pathway (15), and methionine and homocysteine levels are influenced by glycine availability in the S-amino acid metabolic pathway. Supplementing the Southampton low-protein diet with glycine, which as well as folate supplementation results in a decrease in plasma homocysteine, results in a normalization of blood pressure in the offspring, which again suggests that hyperhomocysteinemia may be important in the programming effect of the Southampton low-protein diet.

In summary, it is therefore unlikely that protein undernutrition per se is the critical factor in the impact of the low-protein diet on programming of cardiovascular function. The balance of specific amino acids and other nutrients may be critical in determining DNA methylation and specific outcomes for the cardiovascular and metabolic systems and long-term health effects of maternal undernutrition in pregnancy (279).

It has also recently been demonstrated that deprivation of omega-3 polyunsaturated fatty acids from conception to 9 wk of age resulted in a maintained elevation of mean arterial blood pressure in rats at around 33 wk of age (534). It has been argued that it is feasible that increased blood pressure measured in adult offspring subjected to a low-protein diet may in some instances be the result of an omega-3 fatty acid deficiency as the corn oil

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**TABLE 1—Continued**

<table>
<thead>
<tr>
<th>Manipulation</th>
<th>Timing</th>
<th>Fetal Growth and Birth Weight</th>
<th>Blood Pressure and Vascular Reactivity in Postnatal Life</th>
<th>Fetal and Postnatal Kidney Development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilateral uterine artery ligation vs. controls</td>
<td>Late gestation</td>
<td>↓ Birth weight (234, 413)</td>
<td>↑ MAP 12–16 wk (234)</td>
<td>↑ Renal apoptosis 21 days gestation (413), ↑ Caspase-3 mRNA, ↓ Bcl-2 mRNA, ↓ IGF-I mRNA, ↑ Bax mRNA, ↑ p53 mRNA 21 days gestation (413) ↓ Glomerular number 21 days gestation-3 wk (413) ↓ Nephron number 2 wk (346) ↓ GFR 2 wk (346)</td>
</tr>
<tr>
<td>Partial artery ligation of one uterine horn vs. controls</td>
<td>17 days gestation</td>
<td>↓ Birth weight (346)</td>
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</tr>
</tbody>
</table>

SBP, systolic blood pressure; MAP, mean arterial pressure; HR, heart rate; DBP, diastolic blood pressure; GFR, glomerular filtration rate; IGF-I, insulin-like growth factor I. Reference numbers are given in parentheses.
used as a lipid source in some low-protein diets is deficient in omega-3 polyunsaturated fatty acid (PUFA).

Finally, although there are a range of models of nutrient restriction utilized to study the programming of hypertension, it has also been reported that arterial blood pressure is also raised in 6- and 12-mo-old female offspring of pregnant rats fed a diet rich in lard from 10 days before conception through pregnancy and lactation (254).

A further important issue that needs to be considered when reviewing the extensive literature on the programming of postnatal blood pressure in rats is that many of these studies have investigated the effects of perturbing maternal nutrition on blood pressure using tail cuff plethysmography which entails restraint of the animal. There is therefore a potential contribution of a programmed “stress response” contributing to the increase in arterial blood pressure in these studies which is discussed in the sections below.

2. Maternal nutrition in the preimplantation period

Interestingly, male offspring of pregnant rats fed the Southampton low-protein diet during the preimplantation period (days 0–4.5) also develop increased blood pressure at 4–12 wk of age (266). One possibility is that maternal undernutrition during the preimplantation period may act through epigenetic gene regulatory mechanisms. The genome of the preimplantation embryo undergoes extensive demethylation, and patterns of cytosine methylation are reestablished after implantation (447). These methylation patterns are then maintained throughout fetal and postnatal development. Maternal nutrition and, in particular, the availability of dietary methyl donors and cofactors during critical periods, including the preimplantation period might therefore influence DNA methylation and subsequent gene expression patterns (97, 531). There is therefore significant interest in the impact of maternal diets, which may result in increased serum homocysteine concentrations and/or functional folate deficiencies around the time of conception and implantation and their longer term consequences.

3. Interaction between prenatal and postnatal nutrition

There is also an interaction between the perinatal and postweaning diets in the induction of high blood pressure (412). When rats are exposed to a low-protein diet (8% protein) throughout gestation and lactation and then fed either standard lab chow or a highly palatable cafeteria-style diet from 70 days, there are additive effects of perinatal protein restriction and obesity (induced by the cafeteria-style diet) on blood pressure in these rats at 1 yr of age, suggesting that early protein restriction, and later obesity, are independent risk factors for the development of hypertension (412). Similarly in rats exposed to a 70% reduction in maternal energy intake throughout gestation, and fed either a control or hypercaloric (30% fat) diet from weaning, there were separate effects of prenatal undernutrition and the hypercaloric diet on systolic blood pressure at 14 wk of age (517). In an additional series of experiments, these authors measured systolic blood pressure in the same treatment groups before and after a 14-day period of treatment with IGF-I. IGF-I treatment alleviated the effect of early undernutrition on systolic blood pressure (520). It was suggested that this effect might be a consequence of the actions of IGF-I on insulin sensitivity, vasodilation, or improved renal function (520).

4. Maternal undernutrition and the programming of hypertension in the sheep

In sheep that had been severely undernourished for either 10 or 20 days from 105 days gestation (term 147 ± 3 days gestation), it was found that blood pressure increased with current weight and decreased with increasing birth weight at 5, but not 30 mo of age, and that prenatal nutrition did not affect systolic blood pressure in postnatal life when the current weight and birth weight were taken into account (375). Whilst these data could suggest that size at birth is more closely related to processes that determine postnatal phenotype than is maternal nutrition in late gestation, a number of caveats (size of the cohort, length of undernutrition, and follow-up period) were expressed about this conclusion by the authors (375).

Furthermore, in pregnant ewes which were undernourished (a 30% reduction) during the periconceptional/preimplantation period (60 days before and for 7 days after conception), there was an increase in fetal arterial blood pressure some 100 days later at 115–147 days gestation in twin pregnancies (123). Lambs born after a period of undernutrition (15% reduction) for the first 70 days of pregnancy also have a higher arterial blood pressure in postnatal life (200). The impact of periconceptional undernutrition on the cardiovascular system in the sheep therefore appears to persist into adulthood in the sheep, as in the rat. When sheep were fed 50% equivalent food intake of control ewes from 1 to 30 days gestation, it was reported that the offspring of the nutrient-restricted sheep had increased pulse pressure, a reduced rate pressure product, and a leftward shift in their baroreflex function curve in adult life (161). Baroreflex sensitivity during angiotensin II infusion was also blunted in sheep that had been exposed to early nutrient restriction in utero, but the tachycardia following a reduction in central blood pressure appeared potentiated, relative to controls. Thus periimplantation undernutrition in both the rat and sheep appears to result in an increased risk of hypertension later in life.
In summary, exposure to a period of maternal nutrient restriction during different stages of gestation results in the programming of high blood pressure in a range of species. The mechanisms underlying these programming events are likely to be different, multifactorial, and depend on the relative influence of fetal nutrient deprivation and the maternal and fetal neuroendocrine adaptations to undernutrition during critical periods of organogenesis and the development of fetal cardiorenal function. Evidence for some of the main candidate mechanisms underlying the intrauterine programming of hypertension is summarized in the following sections.

C. Prenatal Glucocorticoid Exposure and the Programming of Hypertension

1. Rat

Treatment of pregnant rats fed a low-protein diet with an inhibitor of maternal glucocorticoid synthesis (metyrapone) resulted in offspring that did not have raised blood pressure, and corticosterone replacement of metyrapone-treated dams restored the hypertensive effect of the diet in female offspring (280, 281). Treatment of pregnant rats with the synthetic glucocorticoid dexamethasone or with carbenoxolone, an inhibitor of the placental enzyme, 11β-hydroxysteroid dehydrogenase-2 (11βHSD2), which metabolizes corticosterone to the inert 11-dehydrocorticosterone, also results in a lower mean birth weight and persistent elevations of arterial blood pressure in the adult offspring (33, 303, 310). This effect requires the presence of the maternal adrenal, as carbenoxolone administered to adrenalectomized pregnant rats had no effect on birth weight or blood pressure (310). These data support the hypothesis that excess exposure of the fetoplacental unit to maternal glucocorticoids reduces birth weight and programs subsequent hypertension in controlling such exposure (119). It has also been shown that treatment of pregnant rats with dexamethasone for 48 h on days 13 and 14, 15 and 16, and 17 and 18 of gestation resulted in elevated blood pressures in male rats at 6 mo of age (384, 385).

Undernutrition of pregnant rats (50% reduction in energy intake) results in an increase in maternal and neonatal glucocorticoids (299), and similarly in the guinea pig, a 40% reduction in maternal energy in maternal nutrition also increased maternal and fetal plasma cortisol concentrations (116). Low-protein diets are also associated with a decrease in placental 11βHSD2 activity, which would in turn increase access of endogenous maternal corticosterone to the fetus (285). The dependence of low-protein diet-induced hypertension on glucocorticoids appears to extend beyond the prenatal period as adrenalectomy of 4-wk-old rats, exposed to a maternal low protein intake in utero, also resulted in a normalization of blood pressure to levels similar to controls (160).

2. Sheep

Treatment of the pregnant ewe with either dexamethasone (12 mg/day) or with cortisol (120 mg/day) for 2 days between 26 and 28 days gestation resulted in hypertensive offspring at 3–4 mo of age (105, 111). The hypertension induced by dexamethasone exposure at 26–28 days gestation amplified with postnatal age and was associated with an increased cardiac output that was attributable to an increase in stroke volume (107). Interestingly, this programming effect was specific in timing as the same dose of dexamethasone administered for 48 h between 59 and 66 days gestation or a lower dose of dexamethasone given for a longer period (25–45 days gestation) did not produce hypertension in the offspring (110, 111, 350). Similarly in sheep, single or repeated maternal injections of betamethasone between 104 and 125 days gestation or repeated injections of dexamethasone, starting on day 103 of gestation, did not result in hypertension in the offspring at 5–12 mo of age (347, 356). Thus the impact of exposure of the fetus to excess glucocorticoids on blood pressure in adult life appears to be greatest when exposure occurs in late gestation in the rat and early gestation in the sheep. These differences relate to differences in the critical windows of development of the kidney and key tissues in the cardiovascular system.

D. Targets for Programming: the Kidney and the Intrarenal Renin-Angiotensin System

1. Human

It has been proposed that maternal undernutrition may lead to permanent structural alterations within the kidneys that contribute to a propensity for adult cardiorenal disease. Brenner and colleagues (53, 54) originally proposed the theory that essential hypertension, including that associated with intrauterine growth restriction, is a consequence of a reduced total number of nephrons, leading to sodium retention. This theory was developed further by the proposal that reduction of nephron number is followed by increasing single-nephron GFR and where increased pressure within single nephrons is sustained, further by the proposal that reduction of nephron number is followed by increasing single-nephron GFR and where increased pressure within single nephrons is sustained, focal glomerulosclerosis occurs, resulting in nephron loss. The individual therefore enters a cycle in which mean arterial pressure continues to rise to maintain hemodynamic function with progressive and irreversible renal injury (55, 324, 325) (Fig. 3). In the human, nephrogenesis is complete by 32–34 wk, and therefore, a nephron deficit present at birth would persist through life. Two studies have reported that the nephron number in stillborn intrauterine growth restricted (IUGR) human fe-
tuses was significantly lower than in their normally grown counterparts (209, 330) and that there was a correlation between nephron number and increased glomerular volume with birth weight (330). Furthermore, ultrasound studies have shown that growth in the longitudinal plane of fetal kidneys was similar in small- and appropriate-for-gestational age groups; however, growth in the anterio-posterior, transverse, and circumference planes of the kidneys was significantly slower in the small-for-gestational-age group after 26 wk gestation. Differences in growth rate in the two groups were most marked between 26 and 34 wk and persisted until delivery when the anterio-posterior diameter was significantly smaller in the small-for-gestational-age group (259). These authors suggested that the 26–34 wk gestation could be the “critical period” during which the insult that leads to in utero programming for the development of adult hypertension occurs.

A recent study showed that in humans, the number of nephrons ranged from 227,327 to 1,825,380 and that there was a strong correlation with birth weight so that it was estimated that the number of nephrons increased by 257,426 for each kilogram increase in birth weight (223). Thus a glomerular origin of hypertension may extend across the normal range of birth weights and not be confined to growth-restricted individuals.

2. Rat and sheep

In the rat, nephrogenesis begins at around day 12 of gestation and is not complete until 8 days after birth (288) and involves rapid remodeling of structures and apoptosis (83, 260). Recently, it has been demonstrated in a model of uteroplacental insufficiency, induced by bilateral uterine ligation of the pregnant rat at 19 days of gestation, that there was a significant reduction in glomeruli number in the full-term fetal kidneys (413) (see Table 1 and Fig. 4). These studies are consistent with the finding that uteroplacental insufficiency reduced nephron numbers by up to 30% in the rat, rabbit, and piglets, and in these animals, the decrease in nephron number was associated with a parallel drop in GFR (27, 29, 346). Uteroplacental insufficiency in late gestation results in a decrease in Bcl-2 expression and a significant increase in Bax expression in the kidney (413). Bcl-2 is an antiapoptosis protein that attenuates the effects of cytochrome c release from the mitochondria and counters the effects of the proapoptosis protein Bax (Fig. 4). These changes were associated with an increase in apoptotic nuclei as measured by the transferase uridine nick end label technique (TUNEL staining), an increase in caspase-3 activity and in the expression of p53, a regulator of Bcl-2 transcription (413). Uteroplacental insufficiency specifically decreased CpG methylation of the renal p53 BstU I site promoter and induced relative hypomethylation from exon 5 to exon 8, which was associated with decreased mRNA levels of DNA methyltransferase 1 (DNMT1) (413). It has been shown that glutathione depletion causes genomic-wide DNA hypomethylation (298), and Pham et al. (413) proposed that the intrauterine environment associated with uteroplacental insufficiency leads to oxidative stress, which is known to reduce renal glutathione levels (413). Thus changes in CpG methylation may represent one important mechanism through which an altered intrauterine environment acts to alter the regulation of apoptosis during development of key organs such as the kidney with potential long-term adverse consequences.

FIG. 3. A diagrammatic summary of how decreased nephrogenesis in early life may result in adult hypertension (53–55). GFR, glomerular filtration rate.
A model of maternal protein restriction throughout pregnancy has also been shown to produce a significant deficit in nephron number in the offspring in early postnatal and adult life (286, 516, 557), a decrease in renal function (GFR/kidney weight) (367, 557), and an increase in plasma Na concentration, which has been suggested to be a result of a primary Na retaining state as a consequence of a shift in the pressure-natriuresis curve to the right (332). Interestingly, a low-protein diet throughout pregnancy programs an increased expression of the glucocorticoid receptor (GR) and corticosteroid responsive Na\(^+\)-K\(^+\)-ATPase \(1_1\) and \(1_2\)-subunits in the kidney, liver, and lung in the offspring (37). There is also evidence that there is upregulation of two Na transporters, the thick ascending limb bumetanide-sensitive Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporter (BSC 1) and the distal convoluted tubule thiazide-sensitive Na\(^+\)-Cl\(^-\) cotransporter (TSC) before and during the early phase of the development of hypertension in this model (331). The prenatal programming of the Na\(^+\)-K\(^+\)-ATPase gene expression in these offspring may therefore contribute to Na retention and mediate a glucocorticoid-dependent programming of hypertension.

In the rat, prenatal dexamethasone for 2-day periods in late gestation results in a reduction in glomerular number, glomerulosclerosis, and hypertension (384, 385). Adult rats given dexamethasone on days 15 and 16 had more glomeruli with glomerulosclerosis than control rats. Male rats that received prenatal dexamethasone on days 13 and 14, 15 and 16, and 17 and 18 of gestation had elevated blood pressures at 6 mo of age, but the group treated on days 13 and 14 of gestation did not have a reduction in glomerular number, suggesting that a reduction in glomerular number is not the sole cause for the development of hypertension.

In sheep, the permanent metanephric kidney completes nephrogenesis by \(~130\) days of gestation (353), which is well before birth (term is 145–150 days). Sheep, programmed to become hypertensive after exposure to dexamethasone (0.48 mg/h, for 48 h at 26–28 days of gestation), also have a significantly lower nephron number and an increased glomerular volume in adult life (551). In these studies the low nephron number was associated with enlarged and dilated proximal tubules and greater accumulation of collagen in the tubular interstitium and perialventitia of the renal cortical vessels. The timing at which treatment with synthetic glucocorticoids is most effective in inducing subsequent hypertension is similar in the fetal rat (15–16 days) and sheep (26–28 days) in terms of the stage of fetal kidney development in these two species. At 15–16 days in the rat, the kidney is mainly mesenchyme, and at 27 days gestation, the sheep kidney comprises a mass of metanephric mesenchyme into which the ureteric bud has grown and branched once (106). Thus the hypertensive programming effect of glucocorticoid treatment early in gestation may be related in part to a "critical window" during which nephrogenesis commences in the permanent metanephric kidney (351).

While intrauterine growth restriction may be associated with a decrease in nephron number, it is clear that loss of a kidney in either early or late adult life is not necessarily associated with an effect on renal functional reserve or on blood pressure (76, 142, 327, 361). In contrast, a reduction of nephron number occurring during the period of active nephrogenesis is associated with changes in postnatal renal function and elevations in arterial blood pressure. Unilateral renal agenesis in humans results in a variable reduction in GFR in the adult, which is correlated with elevations in arterial blood pressure (103). Similarly in the rat, removal of a kidney in the first day of life results in hypertension in the adult (559), and unilateral nephrectomy at 100 days gestation in the sheep, in which nephrogenesis is completed by 130 days, results in compensatory nephrogenesis in utero and a reduced GFR and an elevated blood pressure in female offspring by 6 mo of age (113, 354). It has therefore been suggested that some aspects of compensatory growth and/or nephrogenesis that occur during a critical window in development are
essentially maladaptive leading to the subsequent development of hypertension (106). The response of the intrarenal renin-angiotensin system (RAS) has been implicated in this aspect of programming of postnatal hypertension.

3. The intrarenal RAS and the programming of hypertension

All components of the RAS, i.e., angiotensinogen, renin, angiotensin converting enzyme (ACE), angiotensin type I receptor (AT1R), and AT2R are expressed from very early in gestation in the rat, human, and sheep mesonephros and metanephros (61, 178, 549, 563), and it is well established that the intrarenal RAS plays an important role in the development of the kidney (186).

A) RAT. Blockade of the AT1R during the first 12 days of postnatal life in the rat, i.e., during the period of nephrogenesis, results in a decreased number of glomeruli, reduced renal function, and increased arterial pressure at 22 wk of age (558). Furthermore, in offspring of mothers fed a low-protein diet throughout pregnancy, there was a suppression of renal renin mRNA and renin concentrations as well as renin immunostaining and reduced tissue angiotensin II levels in the immediate postnatal period (557).

B) SHEEP. In pregnant ewes in which the growth and function of the placenta was restricted from conception, the ratios of renin/actin mRNA and angiotensinogen mRNA/18S rRNA levels were significantly reduced in the kidneys of chronically hypoxemic, growth-restricted fetuses compared with normoxic, well-grown fetuses in late gestation (568). The authors speculated that specific suppression of fetal renal renin and angiotensinogen expression could alter the activity of the intrarenal RAS and so affect growth and development of the kidney.

Maternal nutrient restriction in the sheep during early-mid gestation (28–77 days gestation) results in an increase in AT1R expression in the kidney, liver, lung, and adrenals of the newborn lamb (537). The authors noted that there was an associated increase in glucocorticoid receptor (GR) mRNA in these tissues and decrease in 11βHSD2 mRNA expression in the kidney and adrenals and suggested programmed increases in peripheral tissue sensitivity to glucocorticoids were responsible for the increase in AT1R expression in target organs including the kidney (537). Prenatal infusion of dexamethasone into pregnant ewes at 26–27 days gestation also resulted in an increase in mRNA levels for angiotensinogen, the AT1R and AT2R in the kidney of the sheep fetus at 130 days gestation. These steroid-induced changes may be associated with a premature maturation of the kidney with an associated early completion of nephrogenesis and reduction in overall nephron number (352). Thus inappropriate blockade of the renal RAS or a premature stimulation of the renal RAS in early development may each result in a nephron deficit and functional abnormalities in later fetal and adult life depending on the timing of the perturbation of the intrarenal RAS in relation to the stage of nephrogenesis (Fig. 5).

E. Targets for Programming: the Renin-Angiotensin System and Vascular Smooth Muscle

I. Rat

It is important to distinguish between effects of maternal or fetal nutrition on the intrarenal and circulating RAS as these systems do not necessarily change in parallel. The pressor response to increasing doses of ANG II is greater and more prolonged in rats exposed to a low-protein diet in utero (279). Furthermore, mature adult rats exposed to a low-protein diet in utero showed lowered blood pressure after treatment with the ACE inhibitors captopril (284) or enalapril (332), and this effect was reversible (284). Treatment with captopril from 2–4 wk after birth during the preweaning period also produced an irreversible decrease in blood pressure in protein-restricted offspring (472). Losartan, a specific AT1R antagonist, also reduced blood pressure in a similar irreversible manner when administered at 2–4 wk after birth in rats exposed to a low-protein diet in utero, and these data suggest that there may be an upregulation of the AT1R during the early postnatal period which plays a role in the development of the programmed hypertension (471). This upregulation could occur within the vasculature, kidney, brain, or heart, and in this context it is interesting that glucocorticoids are known to increase AT1R expression at different peripheral sites including the vasculature (460).

As discussed in the previous section, a low-protein diet during pregnancy is associated with a decrease in intrarenal RAS activity and treatment of newborn rats with losartan during the first 12 days of life resulted in an irreversible decrease in blood pressure in protein-restricted offspring (472). Losartan, a specific AT1R antagonist, also reduced blood pressure in a similar irreversible manner when administered at 2–4 wk after birth in rats exposed to a low-protein diet in utero, and these data suggest that there may be an upregulation of the AT1R during the early postnatal period which plays a role in the development of the programmed hypertension (471). This upregulation could occur within the vasculature, kidney, brain, or heart, and in this context it is interesting that glucocorticoids are known to increase AT1R expression at different peripheral sites including the vasculature (460).

As discussed in the previous section, a low-protein diet during pregnancy is associated with a decrease in intrarenal RAS activity and treatment of newborn rats with losartan during the first 12 days of life results in a decreased number of nephrons and adult hypertension (558). This suggests that the development of both the intrarenal and circulating RAS could each be perturbed as a consequence of the maternal low-protein diet during pregnancy and that there must be two critical periods during which a decrease in intrarenal RAS (late gestation and up to 12 days after birth) or an increase in the circulating RAS or activation of AT1R (from before birth and between 2–4 wk after birth) can result in hypertension. Alternatively, it may be that differences in the composition of the low-protein diet in the respective studies of Sherman and Langley-Evans (471) (9% protein, 10% corn oil, 0.5% methionine) and Woods et al. (557) (9% protein, 5% corn oil, 5% lard, 0.15% methionine) result in a
different relative contribution of activation of the circulating RAS and suppression of the intrarenal RAS to the hypertensive outcome in the adult rats in each study.

2. Sheep

Intrafetal infusion of either cortisol or of betamethasone and dexamethasone, for periods of up to 48 h at ~120–130 days gestation, results in an increase in femoral vascular resistance (99) and in arterial blood pressure in the sheep fetus (13, 501, 554). Furthermore, blood pressure responses to increasing doses of ANG II, but not norepinephrine, were increased in fetal sheep after a 48-h cortisol infusion at around 125 days gestation (501). Intrafetal infusion of cortisol also results in an increased expression of AT1 receptor mRNA within the fetal heart (right and left atrium and right ventricle) (465), and there is a greater hypotensive effect after blockade of AT1R in fetal sheep that have been infused with cortisol (149).

Maternal undernutrition (a 50% reduction in nutrient intake) during late gestation also results in an increase in maternal plasma concentrations of cortisol and an associated increase in fetal arterial blood pressure and in the pressor responses to increasing doses of ANG II (121). Thus increased exposure to cortisol during fetal life may result in an increased sensitivity to the vasoconstrictor actions of ANG II through either an increase in the expression of the AT1R or changes in the postreceptor-mediated events within the vascular smooth muscle. Alternatively, there may be an enhanced central action of ANG II resulting in enhanced stimulation of sympathetic tone and concomitant inhibition of vagal tone.

In such species as the human and the sheep, the response of the fetal hypothalamo-pituitary-adrenal (HPA) axis to intrauterine stressors may play an important role in the adaptive responses of the fetal cardiovascular system to the prevailing suboptimal intrauterine environment. Cordocentesis studies have shown that plasma cortisol concentrations are higher in IUGR human fetuses than in normally grown fetuses at 18–38 wk of gestation (118). In the sheep, the relative growth of the fetal adrenal is increased, and fetal plasma concentrations of cortisol are also higher in fetuses when the growth and function of the placenta has been experimentally restricted from conception when compared with their normally grown counterparts after 127 days gestation (343, 419). In these growth-restricted fetuses, infusion of an ACE inhibitor, captopril, decreased arterial blood pressure to a greater extent than in normally grown, fetal
sheep after, but not before, 135 days gestation (124), and these results suggest that the RAS plays a greater role in the regulation of arterial blood pressure in the placentally restricted than in the normally grown fetal sheep in late gestation. This enhanced hypotensive response to captopril in the placentally restricted fetal sheep during late gestation may therefore be a consequence of an interaction between cortisol and the RAS, although it is unknown whether the enhanced hypotensive response to captopril persists postnatally in this model of fetal growth restriction.

Repeated injections of betamethasone into pregnant ewes between 104 and 125 days gestation do not, however, result in an increase in blood pressure in the offspring between 6 and 12 mo of age (356). As summarized above, it is clear that exposure of the fetus to high concentrations of synthetic or maternal glucocorticoids during the period of nephrogenesis in the rat (late gestation and first week after birth) and sheep (26–27 days gestation) do result in an increase in arterial blood pressure in the offspring. It is possible that the fetal glucocorticoid responses to placental restriction or maternal undernutrition in the sheep during late gestation are examples of important fetal adaptations to an adverse intrauterine environment which do not result in an increase in arterial blood pressure that persists into postnatal life.

While maternal undernutrition, placental restriction, or exposure to an increase in exogenous, maternal, or fetal glucocorticoids during late gestation may be associated with an enhanced activation of the RAS, there is little evidence that maternal undernutrition during the periconceptional period or synthetic glucocorticoid treatment in early gestation result in a hypertension that is dependent on an enhanced activation of the circulating RAS. While mean arterial pressure was higher during late gestation in twin fetuses of ewes that were undernourished during the periconceptional period (60 days preceding and 1 wk after conception), there was no difference in the fetal blood pressure responses to either increasing doses of angiotensin II or to captopril (123). Similarly, in animals that had been exposed to dexamethasone treatment at 26–28 days gestation, there was no difference in the circulating plasma levels of renin, angiotensinogen, ACE, ANG I, and ANG II, in the fetal or postnatal arterial blood pressure responses to increasing doses of ANG II or to short-term blockade of peripheral AT1R between glucocorticoid- and non-glucocorticoid-treated groups (111, 352, 404). In this model, however, there was an upregulation of the gene expression for ATIR in the medulla oblongata and for angiotensinogen in the hypothalamus at 130 days gestation and for ATIR in the medulla oblongata at 7 years of age, suggesting that a permanent alteration in the brain RAS might be implicated in the programming of adult hypertension (550).

F. Targets for Programming: the Endothelium

1. Human

Endothelial-dependent vasodilation is mediated by a combination of nitric oxide (NO), prostacyclin, and endothelium-derived hyperpolarization factors that act directly to reduce vascular smooth muscle tone. Several studies have reported that, either endothelial-dependent or endothelial-independent vasodilation is impaired and that flow-mediated dilation is decreased in low-birth-weight individuals at 3 mo of age, in later childhood or in early adult life (177, 179, 295, 334).

2. Rat

Offspring from rats that were undernourished (30% global reduction) during the first 18 days of pregnancy had higher blood pressures from 60 days after birth, and the maximal vasoconstriction response to phenylephrine or to norepinephrine was reduced in the isolated femoral arteries for these pups at 20 days of age, although these differences did not persist into adulthood (390). These results are consistent with studies in offspring from dams fed a globally restricted diet (50% reduction during the second half of pregnancy) (216) or a low-protein diet (9% protein throughout pregnancy) (48) in which there was no effect of the prenatal undernutrition on the vasoconstrictor responses of the peripheral arteries, suggesting that there is no alteration in the $\alpha_1$-adrenoceptor-mediated constriction pathway. When rats were fed 50% of the normal intake diet for the whole of pregnancy, however, endothelium intact aortic rings from the offspring at 14 wk of age showed increased responses to norepinephrine (155). In these animals, there was also decreased vasodilatation of the aortic rings to acetylcholine and unaltered responses to sodium nitroprusside in the offspring exposed to undernutrition compared with controls (155). Intrauterine undernutrition resulted in decreased gene expression for endothelial NO synthase (eNOS) in male aortae and a reduction in eNOS activity in both male and female offspring (155). In this model there was also increased superoxide synthesis in small mesenteric arteries, which could contribute to reduced endothelial-dependent dilatation through reduction of NO or tissue oxidative damage.

In male offspring of dams fed a low-protein diet throughout pregnancy, vascular relaxation induced by the endothelium-dependent vasodilators acetylcholine or bradykinin and by the endothelium-independent vasodilator sodium nitroprusside and a phosphodiesterase type 3 inhibitor were reduced (48). Abnormalities in the NO-cGMP pathway may explain the defect in endothelium-dependent and -independent relaxation, and these authors therefore suggested that reduced vasodilation may be a potential mechanism underlying the elevated systolic
blood pressure observed in this model. This mechanism appears to be generalizable to other key vessels as maternal protein restriction is also associated with diminished NO-dependent relaxation of cerebral microvasculature, which is predominantly due to decreased soluble guanyl cyclase activity and cGMP levels (267).

It has also been shown that dietary protein restriction reduces the acetylcholine sensitivity and relaxation in small mesenteric arteries from the pregnant dams and that acetylcholine-induced NO release is also significantly reduced in the mesenteric artery in protein-restricted pregnant dams compared with control dams (51, 261). Glycine supplementation to pregnant dams fed a protein-restricted diet reversed this vascular dysfunction and the impaired release of NO (NO) from the vessels (51). These authors found exposure to a low-protein diet in utero did not decrease the levels of eNOS mRNA, which indicates that changes in eNOS protein levels, eNOS activity, and/or substrate deficiency account for the endothelial dysfunction. While glycine supplementation normalizes the elevated blood pressure observed in the adult offspring of pregnant rats fed a low-protein diet (232), it is not known whether this is an indirect consequence of the changes in the maternal vascular responsiveness during pregnancy or a direct consequence of changes initiated in the fetal vasculature during the period of maternal undernutrition. The molecular mechanisms by which changes in the fetal and postnatal vasculature are programmed by alterations in maternal nutrition are not understood, although as discussed, maternal homocysteine metabolism may ultimately affect the degree of fetal DNA methylation and disturb embryonic vasculogenesis (492). Endothelial cell phenotypes are dependent on a range of factors including laminar flow, and vascular endothelial growth factor (159, 510) and undernutrition during pregnancy may alter the phenotypic modulation by these factors resulting in permanent changes in endothelial and vascular smooth muscle differentiation (49). Recent data also suggest that dietary protein restriction in pregnancy programs vasodilator dysfunction in isolated resistance arteries of female offspring when they become pregnant, which may in turn lead to “transgenerational” programming (511). It has been speculated that such programming may be dependent on the modification of genomic DNA in the offspring through imprinting mechanisms, although it is also possible that vascular dysfunction present in the offspring of low-protein-exposed dams may influence vascular function in subsequent generations through a nongenetic mechanism (49).

Maternal diets that are rich in fat may also result in endothelial dysfunction in offspring, resulting in reduced endothelium-dependent vasodilatation, when compared with animals adapted to a control diet in utero and then exposed to the fat-rich diet in adult life (253). The authors considered that this study provided evidence that predictive adaptive responses prevent endothelial dysfunction in the offspring of fat-fed dams, if offspring are raised on the same diet but do not prevent development of raised blood pressure (253).

3. Sheep

One concern in relation to the studies of offspring of protein-restricted pregnant dams has been that dysfunction of the vascular endothelium may be a consequence, rather than a cause, of the hypertension. Accordingly, there have been a number of studies of manipulation of nutrition of the pregnant ewe during early, mid, and late gestation to study the direct influence of undernutrition on vascular function, before the emergence of hypertension. In one study, ewes were nutritionally restricted by 30% (global restriction) or by 30% of the recommended protein intake (protein restricted) for ~12 days before and 70 days after conception (366). The femoral vascular response to norepinephrine, the maximal relaxation, and sensitivity to acetylcholine were all reduced in protein-restricted fetuses at 70 days gestation. Similarly, the response to sodium nitroprusside was also reduced in the protein-restricted group (366). These data are consistent with the observation that a 50% global restriction of maternal nutrient intake across the same periconceptional window resulted in a blunted endothelial-dependent and non-endothelial-dependent vasodilatation at ~130 days gestation (389).

When dexamethasone is administered to pregnant ewes as 3 weekly courses (4 injections of 2 mg at 12-h intervals), starting at 103 days gestation, there was an enhanced sensitivity to the endothelial vasoconstrictor, endothelin-1, abnormal endothelium-dependent relaxation, and normal endothelium-independent relaxation in femoral arteries at 120 days gestation (348). When ewes were allowed to give birth following the maternal dexamethasone treatment in late pregnancy, it was found that whilst arterial blood pressure was not elevated in the lambs at 5 mo of age, the vascular sensitivity to endothelin-1 (ET-1) and acetylcholine-induced relaxation were increased with no change in the endothelium-independent vasodilatation (347). These authors speculated that after prenatal dexamethasone treatment there would be a limited ability to increase compensatory vasodilatation after 5 mo of age, resulting in the eventual emergence of hypertension in these animals. Whether the levels of circulating glucocorticoids following maternal treatment with exogenous glucocorticoids and their vascular consequences are relevant to the changes in endogenous glucocorticoids measured following maternal undernutrition...
at different stages of pregnancy or in response to placental insufficiency is not clear. It is the case, however, that any persistent or programmed vascular effects are important in the context of the administration of single or repeat doses of synthetic glucocorticoids to pregnant women in threatened preterm labor.

G. Targets for Programming: the Heart

1. Rat: cardiomyocyte hyperplasia, hypertrophy, and apoptosis

One target for prenatal programming of cardiac function is the final number of cardiomyocytes within the heart. Growth of the vertebrate heart during embryonic and fetal life is characterized by hyperplasia of myocardial cells; these cells increase in number to a value characteristic for each species. In rodents, binucleation of cardiomyocytes occurs over postnatal days 4–12 (82, 305, 382, 487) after which myocardial cells lose the capability of dividing, and further growth of the heart is due to myocardial cell hypertrophy and nonmuscle cell hyperplasia. This hypertrophic growth results in a 30- to 40-fold increase in volume of individual myocardial cells during normal postnatal growth and maturation. The transition from hyperplastic to hypertrophic growth is related to formation of binucleated myocardial cells as a result of karyokinesis without cytokinesis (382).

Whereas the impact of a range of intrauterine perturbations, including uteroplacental ligation, global nutrient restriction, and low-protein diets on nephrogenesis has been intensively studied, there have been fewer studies on the associated changes in cardiomyocyte number or size in the heart of the growth-restricted fetus. In pregnant rats exposed to chronic hypoxia (10.5% O2) between days 4–12 and days 15 and 21 of gestation, there was an increase in the percentage and size of binucleated myocytes and an increase in apoptotic cells in the fetal heart (14). There was evidence that the increase in apoptosis in the fetal rat heart may be mediated by an increase in Fas and a decrease in Bcl-2 proteins. Chronic hypoxia also resulted in an increase in β1-adrenoreceptors and a decrease in heat shock proteins, and the authors speculated that these changes may also play an important role in apoptosis in the fetal heart (14). The increased cell death in the fetal heart following exposure to hypoxia may lead to cardiac hypertrophy, resulting in the asymmetric enlargement of the fetal heart in hypoxic animals. These changes in cardiomyocyte development appear to confer no immediate adaptive benefit; rather, they may represent a disruption in the normal pattern of cardiomyocyte development, which renders the postnatal heart more vulnerable in situations where an increased cardiac work load is required.

In the rat, fetal hypoxia induced by maternal hypoxia between 15 and 21 days of gestation also increases the susceptibility of the adult heart to ischemia-reperfusion injury (306). Ischemia-reperfusion-induced myocardial apoptosis was ~40% higher and myocardial infarction was ~60% higher in adult hearts that had been exposed to prenatal chronic hypoxia. The expression of heat shock protein 70 (hsp70), which plays an important role in the protection against ischemia, and eNOS were both lower in the adult hearts that had been exposed to prenatal hypoxia. Following prenatal hypoxia there is also an upregulation of β2-adrenoceptor expression (306) and isoproterenol sensitivity (206), which may serve as a compensatory mechanism and maintain left ventricular function at the resting level.

2. Sheep: cardiomyocyte hyperplasia, hypertrophy, and apoptosis

In the heart of the normal fetal sheep, myocyte size, intercapillary distance, and myocyte myofibrillar and mitochondrial volume densities increase, whereas capillary density, the myocyte-to-capillary ratio, and the myocyte matrix volume density decrease with increasing gestational age (485). In the fetal sheep at 77 days gestation (term ~147 days gestation), 2% of the myocytes were binucleated, which increased to 50% at 135 days gestation and 90% at 4–6 wk after birth (60). Before 110 days gestation, cardiac growth appeared to be due to myocyte hyperplasia, as approximate myocyte numbers and ventricular free wall weight increased at the same rate. After 110 days gestation, the approximate myocyte number per gram ventricular free wall weight decreased, which suggests that myocyte hypertrophy, as well as hyperplasia, was occurring in association with the appearance of a greater proportion of binucleated cells after that time (60). By 4–6 wk of age, there was marked hypertrophy of myocytes and an apparent reduction in myocyte number (60). It has been shown that the ratio of binucleated to mononucleated cells can be altered by environmental factors during fetal life. For example, an experimental systolic pressure load applied to the right ventricle in fetal animals stimulates cardiomyocyte hypertrophy, hyperplasia, and binucleation simultaneously, increasing the percentage of myocytes with two nuclei and the total number of myocytes (17). Thus changes in the pattern of placental function or maternal nutrition which increase fetal blood pressure may result in a change in the developmental profile of cardiomyocytes. In sheep maternal nutrient restriction (50% global nutrient restriction) between 28 and 78 days gestation alters the expression of a range of genes that have been implicated in either cardiac hypertrophy or inhibition of cardiac remodeling (192). A number of circulating and intracardiac agents and growth factors have been implicated in the regulation of cardiomyocyte hyper-

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plasia and hypertrophy, and these may be important in determining the response of the heart to intrauterine deprivation. It has recently been reported that ANG II stimulates hyperplastic growth among mononucleate myocytes, whereas phenylephrine, but not ANG II, stimulates hypertrophy of the binucleate myocytes (499). When IGF-I is overexpressed in the mouse heart using a transgenic approach, heart weight is increased, as is heart weight-to-body weight ratio, apparently due to an increase in cardiomyocyte number rather than cell size (448). It has also been shown in the sheep fetus that IGF-I activation of the IGF-I receptor leads to an increase in heart weight due to hyperplasia rather than hypertrophy (498). The authors of this study speculated that an important consequence of fetal growth restriction and an associated decrease in IGF-I expression in fetal tissues (257) may be a reduction in the total number of cardiomyocytes at birth with associated risks for heart disease in adult life (498). Again, a developmental deficit in cardiomyocyte number may be part of the overall biological “trade off” for a fetus in a situation where a poor substrate supply requires it to grow more slowly and may limit the subsequent functional capacity of the heart to respond to a range of postnatal demands. One important compensatory response, however, that may occur in utero is a matching of myocardial hypertrophy with an increase in myocardial vascularization.

3. Myocardial growth and vascularization

The impact of chronic hypoxia on myocardial growth and vascularization appears to vary depending on the mechanism used to achieve maternal and/or fetal hypoxia. Capillary tubes in the embryonic myocardium are initially formed by vasculogenesis from precursor cells that migrate from the liver region. The primary coronary plexus develops as a result of capillaries coalescing into microvessels and the formation of new vessels from pre-existing capillaries (508). The process of coronary vessel development is influenced by growth factors, extracellular matrix, and the mechanical forces of the beating heart. In fetal sheep whose mothers were exposed to high altitude between 30 and 139 days gestation, long-term hypoxia did not increase either capillary volume density or capillary-to-fiber ratio, or stimulate cardiac fiber hypertrophy in the fetal hearts (304). These authors suggested that ventricular hypertrophy is required for angiogenesis to occur. In fetal sheep exposed to chronic anemia (isovolemic hemorrhage for at least 10 days during late gestation), there is an increase in the fetal heart-to-body weight ratio, and the minimal capillary diameter was increased and the intercapillary distance was decreased in both right and left ventricles of anemic fetuses compared with controls (333). Furthermore, there was an increase in vascular endothelial growth factor (VEGF) mRNA and protein and in hypoxia-inducible factor 1α (HIF1α) protein in the ventricular tissue from the anemic fetuses (333). Thus in chronic fetal anemia there is evidence that cardiac hypertrophy stimulates HIF1α and VEGF expression to result in an increase in myocardial vascularization (333).

There is evidence that coronary conductance can be modified in utero by anemia (high flow and fetal hypoxia) and that this remodeling persists into adulthood. In fetal sheep exposed to chronic anemia (isovolemic hemorrhage for at least 10 days during late gestation), remodeling of the coronary vasculature occurs, and there is a significant increase in coronary conductance (95). In these animals after birth, whilst resting coronary blood flow is normal, there is an increase in maximal coronary conductance at 28 wk of age (94). In contrast, there was no effect of in utero anemia on the vascular reactivity of the mesenteric arteries in these animals. This provides evidence that the oxygen and hemodynamic environment during fetal life may alter the architecture of the coronary circulation for life (94). These authors also reported that animals exposed to a 3-wk period of induced anemia in utero responded differently to acute hypoxemia when tested under general anesthesia at 28 wk of age (56). In response to acute hypoxemia there was a doubling of the left ventricular end-systolic elastance in animals that had been exposed to in utero anemia, but not in control animals (56). Thus the responses to in utero anemia appear to confer both immediate and predictive adaptive benefit.

4. Glucocorticoids and cardiac programming

There is emerging evidence that prenatal exposure to glucocorticoids may also alter subsequent cardiac development. Sheep that become hypertensive after exposure to 2 days of dexamethasone treatment at the end of the first month of gestation have a significant increase in cardiac output at 40 mo of age (107). This increase in cardiac output is due to an increase in stroke volume, rather than heart rate, and there is also a rightward shift of the baroreflex curve in these animals. These animals also show left ventricular (LV) hypertrophy, a reduced cardiac functional reserve, and increased LV type 1 collagen content in adult life (109). In rats, there is also a significant downregulation of UCP-2 and UCP-3 protein expression and an upregulation of GLUT-1 protein expression in the hearts of adult male offspring of dexamethasone-treated mothers (274, 275), and it has been proposed that such changes may be linked with changes in cardiac fuel selection in later life (275).

There is therefore emerging evidence that intraruminal substrate restriction and associated changes in neuroendocrine, hemodynamic, metabolic, and growth factor factors may alter patterns of cardiomyocyte hyperplasia,
apoptosis and hypertrophy, myocardial development, fuel selection, and vascularization and therefore result in programmed changes in cardiac structure and function. The dissection of which of these factors and patterns of heart growth during the perinatal period render the heart more vulnerable to infarction, and damage in later life is a critical area for further investigation.

H. IUGR, the Sympathoadrenal System and the Programming of Hypertension

1. Human

There is some evidence for a role for programming of the sympathetic nervous system (SNS) in a cohort of 449 adults in which it was found that there was a direct relationship between adult pulse rate and birth weight (147). The authors concluded that, although the resting pulse rate is an imperfect index of activity of the SNS, these findings were consistent with the hypothesis that elevated SNS activity established in utero may be one mechanism linking small size with increased blood pressure in adult life.

2. Sheep and pig

It is well established that during acute hypoxemia, blood flow to the brain, heart, and adrenal glands is increased and that blood flow to the gastrointestinal, renal, and peripheral vascular beds decreases (193). This redistribution of fetal cardiac output is also maintained with prolonged hypoxemia in pregnancy presumably as a consequence of the action of vasoactive hormones (193). Clearly, the redistribution of the fetal cardiac output as an adaptation to chronic hypoxemia is of critical importance for the maintenance of the relative growth and optimal function of key fetal organs including the heart and the brain which occurs in placentally restricted fetuses (343). The vasoconstrictor responses to acute fetal hypoxemia and asphyxia are reduced by sympathectomy and α-adrenergic blockade (173, 243), but it is not yet clear whether the redistribution of fetal cardiac output during chronic hypoxemia is dependent on an increase in fetal sympathetic activity and whether this has longer term consequences for the programming of hypertension. This is important in considering whether the fetal neuroendocrine adaptations to the suboptimal intrauterine environment are simply immediately adaptive, or whether there is any “predictive” response that alters the development of the sympathetic innervation of the peripheral vasculature and persists into postnatal life. Plasma norepinephrine concentrations are significantly higher in chronically hypoxic, growth-restricted fetal sheep than their control counterparts between 110 and 140 days of gestation, and for every 1-mmHg decrease in arterial Po2, norepinephrine increases by 0.4 pmol/ml during basal conditions in both placentally restricted and control fetal sheep (476). Studies of total body catecholamine kinetics before and after birth in spontaneously hypoxic fetal lambs indicate that such increases in circulating catecholamine levels are related to an increased sympathoadrenal activity (484). One possibility is that placental restriction and the presence of chronic hypoxemia throughout late gestation is a stimulus for hyperinnervation of fetal vessels and tissues by sympathetic, postganglionic neurons. Alternatively low Po2, or other factors associated with placental restriction, reflexly stimulates catecholamine synthesis and secretion in developing sympathetic neurons. Ruiljenbeek et al. (455) have used the chicken embryo as a suitable model for studying the effects of hypoxia on cardiovascular development as it is possible to study the effects of adverse conditions in this model without the interference of maternal endocrine, metabolic, or cardiovascular factors. Chronic exposure of the chick embryo to moderate hypoxia leads to growth restriction, an increase in arterial sympathetic innervation, and reduced endothelial vasodilator function at embryonic day 19 (456, 457). While there is an increase in sympathetic nerve density and neuronal norepinephrine reuptake at embryonic day 19, these effects were not present when chickens, exposed to hypoxia in ovo, were studied at 3–4 wk postnatal age (455). This may be because sympathetic neuronal development in this model can continue past the hatching stage. In this model, mean arterial pressure and heart rate at 14–15 wk of age were also not affected by chronic in ovo hypoxia, but isolated femoral arteries were more sensitive to electrical stimulation and pharmacological stimulation of periartrial sympathetic nerves (455).

In the growth-restricted sheep fetus, although there is an inverse relationship between circulating norepinephrine and arterial Po2, there is a direct relationship between plasma epinephrine concentrations and arterial Po2 and between the level of expression of the epinephrine-synthesizing enzyme phenylethanolamine-N-methyltransferase (PNMT) in the fetal adrenal and Po2 (1). In the pig, plasma norepinephrine, but not epinephrine, concentrations are increased in low-birth-weight animals at 3 mo of age (431). In male 12-mo-old pigs, the norepinephrine response to hypoglycemia was reduced, whereas the epinephrine response to hypoglycemia was increased in animals of low birth weight (431). In contrast, in female 12-mo-old pigs, basal epinephrine concentrations were reduced, and there was no increase in the epinephrine response to hypoglycemia in the low-birth-weight group. The authors suggested that the sex-related differences in catecholamine secretion may be associated with the failure of low-birth-weight females to catch up in body weight by 12 mo of age (431). Thus there appears to be a dissociation of the responses of the separate components of the sympathoadrenal system to a restriction of fetal...
substrate supply, which may have cardiovascular and metabolic consequences in postnatal life.

3. Rat

Tonkiss et al. (509) used radiotelemetry to measure the impact of exposure to an olfactory stimulus (ammonia) in male offspring of dams fed a low-protein diet (509). Although basal blood pressure was similar in controls and offspring of the dams fed the low-protein diet, after exposure to the stressor the blood pressure responses were greater in the low-protein group. This suggests that the maternal low-protein diet may have generated an augmented sympathetic stress response in these offspring.

III. INSULIN RESISTANCE AND TYPE 2 DIABETES

A. Epidemiological Evidence for the Association Between Low Birth Weight and Type 2 Diabetes

Worldwide, there is a pandemic of type 2 diabetes mellitus, which will have its greatest effect in developing communities. For example, it has been suggested that there will be more than 60 million people with type 2 diabetes in India alone by 2020. Type 2 diabetes is particularly prevalent in Indians who are more insulin resistant than Caucasians with higher proportions of body fat for a given BMI. In these communities much of the type 2 diabetes will be part of the insulin resistance or metabolic syndrome, which includes glucose intolerance, dyslipidemia, obesity, and hypertension. It has been proposed that obesity-linked type 2 diabetes is a disease of insulin resistance combined with pancreatic β-cell dysfunction (313). In early obesity, an increase in β-cell mass and function might compensate for peripheral insulin resistance, but as obesity continues, there is a failure in such compensatory adaptation and the β-cell mass becomes inadequate. Obesity is associated with chronically elevated fatty acids and glucose intolerance, and the hyperlipidemia and hyperglycemia eventually contribute to β-cell dysfunction and a decrease in β-cell mass that characterizes the onset of type 2 diabetes in obese patients (313).

There is an association between fetal and infant growth and subsequent impaired glucose tolerance and type 2 diabetes in elderly men (188). The odds ratio for impaired glucose tolerance was 6.6 in men who weighed <5.5 lbs. (~2 kg) at birth compared with the reference group with birth weights >9.5 lbs. (~4.5 kg). Similarly, the odds ratio for impaired glucose tolerance for weight at 1 yr was 8.2 for those <18 lbs. (~8.5 kg) compared with those who weighed >27 lbs. (~12.5 kg) (188). The prevalence of metabolic syndrome in men and women fell progressively with increasing birth weight. The odds ratio for developing the metabolic syndrome was 18 (95% confidence intervals 2.6–118) in those who weighed 5.5 lbs. or less (24). Since then there have been many studies showing a negative relationship between birth weight and impaired glucose tolerance (420, 442, 452).

The relationship between birth weight and impaired glucose tolerance may be U-shaped or reversed J-shaped (341, 452). In the American Nurses study of 69,526 women, the relationship was described as a reverse J-shape, which became an inverse or negative association with adjustment for adult BMI. Adjustment for other factors including ethnicity, childhood socioeconomic factors, and adult lifestyle factors did not alter this relationship (452). Follow-up of the offspring of mothers exposed to famine in Holland in 1944–1945 showed that impaired glucose tolerance was greater in those exposed to the famine during mid and late gestation. Impairment of glucose tolerance was greater in offspring of thin mothers and in those who became obese (442).

A recent study has also reported that adolescents born preterm who were randomized to a lower nutrient diet that was suboptimal in supporting postnatal growth had lower fasting 32–33 split proinsulin concentrations (a partly processed form of insulin that in high concentrations may indicate insulin resistance or pancreatic islet secretion at a maximal rate) when compared with those given a nutrient-enriched diet (479). The authors suggested that these dietary effects were probably the result of the diet affecting the neonatal growth rate and that reduced growth rate during this period programs a lower insulin resistance and propensity to type 2 diabetes.

1. Gestational diabetes

Gestational diabetes in women is a common antecedent of type 2 diabetes mellitus; ~30% of women with gestational diabetes later develop type 2 diabetes. This may (454) or may not (393) have a selective advantage in evolutionary terms. Birth weight has a U-shaped relationship to the risk of developing gestational diabetes. Adjustment for BMI reduced the odds ratio for developing gestational diabetes. This adjustment left a strong negative relationship of birth weight with the risk for gestation diabetes (229). The association of low birth weight with the risk for development of gestational diabetes had been found previously (126, 428, 543). In the last decade, type 2 diabetes is being diagnosed more frequently in pregnancy raising concerns for the outcome for the offspring (143). Some of this increase may be due to consumption of high glycemic diets in pregnancy, which are associated with maternal obesity and high birth weight (79). Carbohydrate sources are categorized by the magnitude of their induced glucose response using a comparative scale termed the glycemic index (552). “Aboriginal” or low
glycemic carbohydrate (a diet containing ~55% of energy in the form of low glycemic carbohydrate) (79) should be preferred as a diet high in protein or fat may impair glucose tolerance in the offspring (473). Although minor hyperglycemia and maternal weight gain in pregnancy also contribute to high birth weight, more emphasis was placed on maternal obesity (289). It has also been argued that the lower birth weight may be due to mother’s insulin resistance and that common genetic factors contribute to the lower birth weights and to the risk for cardiovascular disease and diabetes in adults (198, 293).

B. Targets for Programming: Pancreatic Growth and Function

1. Pancreatic development

Morphogenesis of the endocrine pancreas follows a similar sequence in all mammals, although there are differences in the timing of specific events between species that are important in considering issues of critical windows during which nutritional perturbations may produce long-term consequences for pancreatic structure and function (153). The mass of endocrine tissue in the perinatal pancreas is dependent on three processes: neogenesis of the endocrine cells from the pancreatic duct epithelia, proliferation of the cells which are committed to endocrine differentiation, and apoptosis of those endocrine cells in the developing islets (153).

2. Rat

In the rat fetus during late gestation, pancreatic islet cell mass increases rapidly due to both β-cell replication and recruitment and maturation of undifferentiated β-cell precursors within the pancreatic ducts (208, 249). After birth, the growth rate of all islet cells, including β-cells, declines within 3–4 days and continues to decline thereafter so that the rate of mitosis in adult pancreatic β-cells is low (146). A wave of apoptosis occurs in neonatal rat islets with the main phase of remodeling occurring around weaning at 2–3 wk of age (461). The total pancreatic β-cell mass is not substantially changed however, and this suggests that a new population of β-cells derived from replication or neogenesis compensates for this loss. Initially insulin release from the β-cells within the “fetal type” islets is poorly responsive to glucose, but very responsive to amino acids. The process of developmental apoptosis deletes many of these β-cells, and they are replaced with new islet cells derived from a second wave of neogenesis that are sensitive to glucose with acute first phase insulin release. This developmental change prepares the individual for postnatal metabolism (207).

3. Human

In the human fetus, early budding of the endocrine cells from the pancreatic duct starts at ~10 wk gestation, and the development of the functional endocrine pancreas continues throughout pregnancy and the phase of remodeling of the islets continues from late gestation onwards for at least 4 yr (153).

4. Intrauterine nutrition and fetal pancreatic development in the rat

The effects of poor fetal nutrition during pregnancy on pancreatic development have been intensively studied using a variety of approaches including uterine artery ligation, which results in a decrease in the maternal-fetal transfer of nutrients (98, 370), a 30–50% decrease in maternal energy intake which results in a decrease in fetal nutrient supply (163, 216, 218, 219, 555), or an isocaloric low-protein diet (8 vs. 20%), which results in normal fetal glycemia but a decrease in specific amino acid concentrations including fetal plasma taurine concentrations (449). Each of these intrauterine challenges results in changes in fetal pancreatic development, and there is an interaction between these prenatal changes and the pattern of pancreatic development in postnatal life, which depends on the diet of the rat pup during neonatal and postnatal life (see Table 2).

In the model of uteroplacental insufficiency first developed by Wigglesworth (541), bilateral uterine artery ligation in the pregnant rat from either 18 or 19 days gestation results in diminished arterial PO2, plasma glucose, and branched chain amino acid concentrations and fetal growth. In this model there is a decrease in fetal pancreatic endocrine tissue and β-cell mass and in fetal insulin concentrations (98, 370). Global energy restriction (50–70% reduction) either throughout pregnancy or during late pregnancy alone results in a decreased nutrient supply to the fetus, a decreased fetal whole body glucose utilization rate, and fetal growth restriction (163, 216, 218, 219, 300, 555). In these fetuses, there is a reduction in pancreatic cell number, in β-cell mass, islet number, pancreatic insulin content, and circulating fetal insulin concentrations (163, 219, 548). Exposure of the fetal rat to a low-protein diet throughout pregnancy also results in a decrease in the number of β-cells and insulin content in the fetal pancreatic islets as a consequence of a decrease in the proliferation and an increase in apoptosis of the islet cells (34, 89, 345, 408, 486). The primary impact of protein restriction is on the proliferation of existing β-cells (408). This is different from the impact of a global energy restriction where cellular neogenesis is predominantly affected (163). Exposure to a low-protein diet during pregnancy also results in a decrease in the vascularization of the pancreas and a decrease in the insulin response of the fetal pancreas to arginine and taurine by
TABLE 2. Impact of manipulation of the maternal diet or uteroplacental blood flow during pregnancy in the rat on pancreatic development, body growth, glucose tolerance, and insulin sensitivity in postnatal life

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<td>↓ Neonatal pancreatic insulin cells (98)</td>
<td>↑ Glucose uptake into skeletal muscle (male) 12 wk (398)</td>
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<td>Birth weight (98, 475)</td>
<td>↓ Fetal and neonatal plasma glucose and insulin (98, 370)</td>
<td>↑ Glucose uptake into skeletal muscle (male) 12 wk (398)</td>
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<td>Body weight 7 wk (475)</td>
<td>↓ Plasma insulin and glucose 1 wk (475)</td>
<td>↑ Skeletal muscle insulin receptors 12 wk (male) (398)</td>
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<td>Body weight 26 wk (475)</td>
<td>↓ Acute first phase insulin response to glucose 1–26 wk (234, 475)</td>
<td>↑ Adipocyte insulin receptors 6 wk (470)</td>
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<td>↑ Insulin-stimulated glucose utilization (female) (220)</td>
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<td>↓ Glucose tolerance 60 wk (190)</td>
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<td>↓ Insulin-stimulated skeletal muscle glucose uptake 60 wk (396)</td>
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<td>↑ Basal adipocyte glucose uptake 60 wk (391)</td>
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<td>↑ Insulin inhibition of isoproterenol-stimulated lipolysis 60 wk (391)</td>
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<td>↑ Plasma glucose and development of diabetes 68 wk (410)</td>
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<td>8% Protein vs. 19/20% protein</td>
<td>0 Days-term + 8% protein lactation + 8% protein postweaning</td>
<td>Fetal weight (89, 210)</td>
<td>↓ Islet insulin content 12–13 wk (441)</td>
<td>↓ Glucose uptake into skeletal muscle (male) 12 wk (398)</td>
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<td>Birth weight (98, 475)</td>
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<td>↑ Skeletal muscle insulin receptors 12 wk (male) (398)</td>
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<td>↓ Insulin-stimulated skeletal muscle glucose uptake 24 wk (466)</td>
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TABLE 2—Continued

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<td>Streptozotocin (low dose)-induced diabetes vs. controls</td>
<td>Streptozotocin administered: Before mating (252)</td>
<td>↑/↓ Birth weight (359, 373)</td>
<td>↑ Islet proliferation and size (450)</td>
<td>↓ Islet insulin response to glucose (3)</td>
<td>↓ Glucose tolerance 10–12 wk (females)</td>
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<td>1-Day gestation (450)</td>
<td>↑ Body weight (females) 0–10 wk (373)</td>
<td>↑ Fetal and neonatal pancreatic insulin content (252, 373)</td>
<td>↑ Plasma insulin response to oral glucose 10 wk (373)</td>
<td>↓ Insulin sensitivity (166)</td>
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<td>2 Days gestation (359)</td>
<td>↑ Body weight (males) 3–6 wk (373)</td>
<td>↑ Neonatal plasma insulin (252, 373)</td>
<td>_PP Pancreatic mass (7)</td>
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<td>5 Days gestation (373)</td>
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<td>↑ Fetal pancreatic insulin response to glucose in vitro (252)</td>
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<td>Streptozotocin (high dose)-induced diabetes vs. controls</td>
<td>Before mating (184)</td>
<td>↓ Birth weight (64, 252, 359)</td>
<td>↓ Fetal pancreatic insulin content (252)</td>
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<td>2 Days gestation (359)</td>
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<td>↓ Fetal plasma insulin (252)</td>
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<td>Islet hypertrophy and β-cell hyperplasia and degranulation (5)</td>
<td>↑ Islet insulin response to glucose (3)</td>
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<td>↓ Fetal insulin response to glucose in vivo and in vitro (252)</td>
<td>↓ Plasma insulin (214, 217)</td>
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Reference numbers are given in parentheses

a mechanism located at the exocytosis step in the insulin secretion cascade (73, 74, 210, 486). Levels of the amino acid taurine are reduced in maternal and fetal plasma of protein-restricted dams (47), and supplementation of the low-protein diet with taurine (2.5%) in the drinking water of the pregnant rat restored normal insulin secretion of islets isolated from the fetuses after in vitro stimulation with either taurine, methionine, leucine, or arginine (73). Taurine is derived from cysteine, and supplementing with taurine will also reduce the demand for cysteine, which in turn will reduce the flux through the transulfuration pathway and decrease homocysteine production.

5. Intrauterine nutrition and postnatal pancreatic development in the rat

After bilateral uterine ligation in the rat, although there was no difference in β-cell mass, islet size, or pancreatic weight between growth-restricted and control animals at 1 and 7 wk of age, by 15 wk, the relative β-cell mass was 50% that of controls, and by 26 wk of age, it was less than one-third that of controls (475) (see Table 2). If the offspring of protein-restricted dams are maintained on a normal diet after birth, the islets remain small and irregular in shape with reduced amounts of β-cells (34). At 4 mo of age, pancreatic and islet blood flow are not reduced in those offspring exposed to a low-protein diet only during pregnancy (228). There appears to be conflicting evidence as to whether the insulin secretory responses to either amino acids or glucose of islets from postnatal animals exposed to low-protein diets only in utero are or are not impaired in postnatal life (211, 441).

In vivo, basal plasma glucose and insulin levels are normal in offspring that were exposed to a low-protein diet during pregnancy, but the insulin response to an oral glucose challenge was abnormally low in adult female offspring and remained lower in these animals when they became pregnant (211). In offspring born after exposure to a 5% protein diet in utero, glucose-stimulated insulin release from islets in vitro was also reduced if these animals had been fed sucrose or a high-fat diet after weaning (546).

In a recent series of studies (35, 36), dams were fed either a control diet, a low-protein isocaloric diet (5% protein), an energy-restricted diet (50% of control levels), or a energy-restricted, low-protein diet (5% protein, 50% energy levels) from 14.5 days gestation. There was a 50% decrease in pancreatic β-cell mass in all three restricted groups at 21.5 days gestation. A low-protein diet, either associated or not with energy restriction, increased β-cell insulin content at this gestational age, and fetal plasma taurine levels were the main predictor of fetal plasma insulin concentrations. Interestingly, when the offspring were studied at 8 wk of age, β-cell mass was only significantly decreased in the low-protein isocaloric diet group.
when compared with the two energy-restricted groups. The authors concluded that the last week of pregnancy is a critical window for the effects of a low-protein diet on the programmed development of the pancreas in the rat and that the decrease in β-cell mass growth can be partially recovered after birth, but that this relative recovery depends on the pattern or type of maternal food restriction experienced during the last week of pregnancy. The three nutrient-deprived groups showed no major impairment in glucose tolerance, glucose utilization, or glucose production at 8 wk of age, although the authors argued that such an impairment may become more evident with ageing (35) (see Table 2).

Juvenile offspring of rats that were 50% food restricted from day 15 of pregnancy and during lactation have a significantly reduced β-cell mass, but normal β-cell proliferation and insulin content at 3 wk of age (163). β-Cell mass is not fully restored in adult life even when rats are fed a normal diet after weaning (162). At 8 mo of age, these offspring also had a 40% lower insulin content, and nonfasting plasma glucose concentrations were increased (164, 219).

When an isocaloric low-protein diet is continued through pregnancy and into the postnatal period, pancreatic β-cell mass and insulin content are reduced with smaller islets and a decrease in β-cell proliferation and an increase in β-cell apoptosis in rat offspring at 3 wk of age (408). Interestingly, the timing of the neonatal wave of apoptosis is not altered by continuing exposure to a low-protein diet during lactation (408). In animals fed an isocaloric low-protein diet either throughout pregnancy and postnatally or during the postnatal period alone, the pancreas had fewer but larger islets, and there was a reduction in the amount of β-cells within each islet in both these groups (34). When the low-protein diet is continued through pregnancy and into postnatal life, there is also a decrease in islet blood vessel density and in pancreatic and islet blood flow, and pancreatic insulin content is reduced (228). There is also a decreased insulin secretory response to both glucose and amino acids of islets from 3-mo-old offspring, and this was associated with a decrease in activity of the mitochondrial glycerophosphate dehydrogenase in islet homogenates from these animals (441). The insulin response to an oral glucose challenge is also decreased at 3 mo of age in these rats (211). The persistence of decreased pancreatic function in postnatal life when the postnatal nutritional environment is matched to the prenatal nutritional environment would suggest that poor maternal nutrition has an adverse effect on pancreatic functional capacity as a consequence of the occurrence of an irreversible developmental deficit (Fig. 6).

A range of candidate mechanisms underlying the changes in pancreatic development induced by deficits in nutrition during the fetal and neonatal periods have been proposed. These include a decrease in stem cell number (225), changes in pancreatic growth factor expression (including IGF-I and IGF-II and VEGF and fibroblast growth factor-7), alterations in stimulus-secretion coupling within the pancreatic β-cell, or an alteration in pancreatic innervation (207). Proteome analysis of fetal pancreatic islets in culture identified that 70 out of a total of 2,810 protein spots were changed due to exposure to a low-protein diet (490). These proteins included those that may play a role in proliferation of islet cells, insulin secretion, the control of apoptosis, and susceptibility of the islets to cytokine action (490). The normal transient increase in islet cell apoptosis in the neonatal rat is temporally coincident with a loss of expression of IGF-II in pancreatic islet cells. Endogenous IGF-II acts as a survival factor that prevents apoptosis in isolated islets from 5-day-old rats (407), and there is a reduced pancreatic expression of IGF-II mRNA in fetal and neonatal rats exposed to a low-protein diet during gestation and lactation (408). A decrease in pancreatic IGF-II expression may be a consequence of a deficit in the levels of specific amino acids, as supplementation of the pregnant rat given a low-protein diet with taurine can reverse the decrease in IGF-II immunostaining within the fetal islets (47). Taurine supplementation also restored the normal volume and numerical density of vessels and the number of cells immunopositive for VEGF and the VEGF receptor, fetal liver kinase-1 (Flk-1), in the pancreatic endocrine cells of fetuses and pups given taurine. Thus taurine appears to play a role in islet vasculogenesis (46). The expression of IGF-II or other pancreatic growth factors may also be regulated by exposure to maternal glucocorticoids.

An isocaloric low-protein diet or food restriction (50%) during the last week in gestation results in an overexpression of the fetuses to maternal corticosterone (283), which disrupts the subsequent development of the HPA in the newborn pups (299). In undernourished pregnant rats, fetal β-cell mass increased after maternal adrenalectomy, and infusion of an inhibitor of corticosteroid synthesis resulted in a further increase (43). Treatment of pregnant rats with dexamethasone during the last week in pregnancy reduces the insulin content of the fetal pancreatic β-cells, and administration of dexamethasone to organ cultures of mouse embryonic pancreas results in a reduction of the transcription factor pancreatic duodenal homeobox-1 (Pdx-1) and an increase in the expression of the transcription factor C/EBPβ (CCAAT/enhancer-binding protein β), which may compromise the differentiation and later function of the β-cells (469).

There is a reduced β-cell proliferation in the pancreas during pregnancy in those female offspring who were nutrient restricted during their own gestation (42, 211). Whereas there is normally an increase in pancreatic islet mass in well-nourished pregnant rats, the islet mass in previously malnourished rats remained similar to that in...
nonpregnant rats, throughout pregnancy (42, 211). In this model, perinatal malnutrition appears to impair subsequent adaptation to pregnancy by decreasing β-cell proliferation in the head of the pancreas at a critical time (~12 days gestation) during pregnancy (41). The fetuses of these maladapted dams also show a decrease in the β-cell mass, islet number, and insulin content at 20 days gestation, and this was associated with decreased expansion of the epithelial cell population expressing the homeodomain protein Pdx-1 (41). Thus there can be a persistence of abnormalities of pancreatic development across generations.

6. Maternal diabetes, fetal growth, and pancreatic development in the rat

While a decrease in maternal and fetal nutrition results in permanent changes in the structure and function of the endocrine pancreas, maternal hyperglycemia also alters pancreatic development. The experimental induction of diabetes using streptozotocin (STZ) has dose-dependent effects: low doses of STZ result in mild gestational diabetes associated with fetal macrosomia (2, 4, 215, 373), whereas high doses induce insulin-deficient diabetes associated with fetal growth restriction (4, 64, 214, 215, 359).

Mild maternal diabetes in rats leads to an increase in the fetal pancreatic insulin content, enhanced insulin secretion in response to glucose, and greater proliferation of islet cells (88, 252). Young adult macrosomic offspring show accelerated growth during the first 10 wk of life and higher plasma insulin and glucose concentrations following an oral glucose challenge (373). Macrosomic male and female animals were also less sensitive to the actions of insulin at 10 wk of age (167). The adaptation of these offspring to pregnancy is deficient, and the pregnant animals display gestational diabetes (4, 6, 7, 213). The fetuses of these dams show the typical features of islet hyperplasia, β-cell hyperactivity, hyperinsulinemia, and macrosomia and have an impaired glucose tolerance in adult life (4).

Severe diabetes leads to a decrease in fetal pancreatic weight with an increase in the proportion of pancre-
atic endocrine tissue and a decrease in pancreatic and circulating insulin (5, 39, 252, 315). The β-cells of the severely hyperglycemic fetal rat do not secrete insulin in vitro or in vivo with only arginine inducing a sustained monophasic insulin secretory response (38). If the offspring are cross-fostered by a normoglycemic rat, they appear to have a morphologically normal endocrine pancreas that is hypertrophic as a consequence of an increased number of small neogenic islets. The β-cell mass is normal, and plasma insulin concentrations are either normal or elevated at 3 mo of age (7, 214, 217). In vivo and in vitro stimulation of β-cells results in an enhanced insulin secretion (7), and these rats are markedly resistant to the actions of insulin (184, 214, 459). The insulin resistance in the offspring of STZ-induced diabetic rats can be partially restored by near normalization of maternal glycemia with islet transplantation during pregnancy (459).

As in perinatal protein-restricted offspring, the offspring of severely diabetic rats develop signs of glucose intolerance during pregnancy with higher glucose and lower insulin concentrations than normal pregnant rats (214). The fetuses of these mothers (the third generation offspring) also display islet hyperplasia, β-cell degranulation, hyperinsulinemia, and hyperglycemia and are macrosomic (2, 214), and in adult life, these third generation offspring exhibit impaired glucose tolerance with high glucose levels (513).

7. Postnatal nutrition and pancreatic development in the rat

The neonatal period is also a critical window in the context of the programming of pancreatic development. There were persistent defects in glucose-stimulated insulin secretion from isolated islets in rats assigned to small litters, and a range of islet genes were differentially expressed in this group beyond weaning and into adulthood (529). These genes included mitochondrial ATP synthase, calcium ATPase, cholecystokinin, and neuronatin. It has also recently been shown that artificial rearing of normally grown 4-day-old rat pups on a high-carbohydrate milk formula up until day 24 results in a leftward shift in insulin secretory capacity, increased hexokinase activity, increased expression of preproinsulin, and alterations in the number and size of the islets (491). These adaptations are programmed and are expressed in the adult, and it has also been shown that female rats raised on the high-carbohydrate intake during the neonatal period transmit the hyperinsulinemia and adult onset obesity phenotype to their progeny.

8. Fetal undernutrition and glucose tolerance in the rat

Uterine artery ligation in late gestation resulted in offspring that were glucose intolerant and insulin resistant at an early age (234, 475). Uterine artery ligation results in an elevated fasting blood glucose as well as lower insulin and higher glucose levels in response to a glucose load in both male and female offspring at 3–4 mo of age (234) (see Table 2). The first phase insulin secretion in response to glucose but not the insulin secretory response to arginine was also impaired in both male and female offspring, before the onset of hyperglycemia (475).

In rats exposed to a 50% decrease in maternal energy intake from day 15 of pregnancy until the end of lactation, there was a significant decrease in insulin secretion during an oral glucose tolerance test at both 4 and 8 mo of age, but glucose tolerance at these ages was only moderately affected (42). During early life (2–3 mo of age) offspring exposed to low protein during pregnancy and lactation have a better glucose tolerance than do controls. By 15 mo, however, the glucose tolerance of the low-protein group is significantly worse than that of controls, and by 17 mo, the low-protein offspring have frank diabetes (190, 220, 276, 409, 470). Thus the changes in insulin secretion in response to protein restriction are not associated with alterations in glucose tolerance in the offspring until later in life, and this appears in part to be related to compensatory changes in insulin sensitivity of the peripheral tissues (384, 398).

There are separate and additive effects of prenatal undernutrition (70% reduction in energy intake) and post-weaning overnutrition (a 30% hypercaloric diet) on plasma insulin concentrations resulting in a marked hyperinsulinism at 100 days of age. Offspring exposed to prenatal undernutrition developed hyperglycemia on a hypercaloric diet, which suggests that the increase in plasma insulin concentrations was not sufficient to maintain glycemic control (521).

Rats exposed to excess glucocorticoids during the third week of pregnancy also become hyperglycemic, glucose intolerant, and hyperinsulinemic in adult life, and this has been shown to be a consequence of the actions of glucocorticoids on the fetus or neonate, rather than on maternal behavior during the suckling period (369).

9. Fetal undernutrition and glucose tolerance in the sheep and pig

There is a relative paucity of studies of the impact of poor fetal nutrition on pancreatic development in species such as the sheep, pig, and human in which the periods of pancreatic development are less discrete than in the rat.

In growth-restricted human fetuses, there is a decrease in fetal insulin immunoreactivity and in the endocrine mass of the pancreas (514). Interestingly, periconceptional undernutrition (50% restriction of maternal nutrient intake from 60 days before until 30 days after mating) resulted in an increase in maternal and fetal plasma taurine concentrations in fetal sheep during late gestation and an en-
hanced fetal insulin response to glucose, but not to arginine (376). Thus the periconceptional period (up to 30 days gestation) may be a critical window for fetal pancreatic development in the sheep and/or an increase in circulating taurine in pregnancy may be associated with a maturation of fetal pancreatic β-cells in a species in which there is a fourfold increase in the responsiveness of the fetal pancreas to glucose during the last half of gestation (9). Nutritional deprivation during the final stages of pancreatic development results in a diminished insulin responsiveness in human and sheep fetuses (67, 364). In the sheep, prolonged maternal hypoglycemia (14 days) during late gestation results in a decrease in glucose- and arginine-stimulated insulin secretion (308). Furthermore, in this model, a reduction in two parameters of pancreatic β-cell responsiveness, insulin secretion in response to arginine and the time to reach maximal insulin concentration in response to acute hyperglycemia, persist even after fetal euglycemia has been restored for up to 5 days (308).

In the sheep and human, fetal hypoxemia and hypoglycemia are associated with an increase in circulating cortisol concentrations in the late-gestation sheep fetus (118, 419), and administration of betamethasone to the pregnant ewe in late gestation (either 4 doses administered between 104 and 125 days gestation or one dose administered at 104 days gestation) resulted in changes in glucose metabolism in the offspring (356). After repeated glucocorticoid administration, there was an increase in the insulin, but not glucose, response to a glucose challenge in lambs at 6 mo and resting glucose concentrations and blood glucose concentrations at 3 h after a glucose challenge were increased in these animals at 1 yr after birth. One-year-old sheep that had been exposed to a single administration of betamethasone also had a greater blood glucose response to a glucose challenge (356), and it was also demonstrated that these effects occurred independently of any effect of glucocorticoids on fetal growth (356). In contrast, exposure of fetal sheep to dexamethasone for 48 h at either 27 or 64 days of gestation did not result in any change in insulin or glucose concentrations in response to a glucose challenge or in the insulin sensitivity of net whole body glucose or amino acid uptake in adult life (165). Thus it appears that as in the rat, there is a critical period in late gestation in the sheep during which glucocorticoids act to result in changes in postnatal glucose metabolism. Glucocorticoid exposure early in gestation in the sheep results in postnatal hypertension but not impaired insulin-regulated glucose homeostasis, whereas glucocorticoid exposure in late gestation in this species results in altered postnatal glucose metabolism but no change in arterial blood pressure (111, 165, 356).

There is limited information on the impact of fetal growth restriction on postnatal pancreatic function or glucose metabolism in the sheep. In twin lambs born 20% lighter than their co-twins, plasma glucose and insulin tolerance were not adversely affected during at 1, 3, and 6 mo of age, although in this study, the lighter twins were relatively large (~4 kg) at birth (80).

A study in pigs on the effect of natural variations in birth weight on subsequent glucose tolerance found that poor glucose tolerance was observed in low-birth-weight (<1.47 kg) pigs at 12 mo, but not at 3 mo of age (430). In this study glucose intolerance at 12 mo of age was associated with low BMI and disproportionate body shape at birth. At 12 mo, the body weight of low-birth-weight pigs was no longer different from their high-birth-weight (>1.53 kg) littermates, due to the increased fractional growth rate very early in postnatal life during suckling, and between 3 and 12 mo of age. This “catch up” growth in the first month of life was also directly associated with impaired glucose tolerance at 12 mo of age. Naturally occurring low birth weight in pigs was associated with reduced basal insulin concentrations at 12 mo of age; however, a greater insulin response to glucose administration in low-birth-weight pigs suggests that their glucose intolerance in adulthood is not due to reduced insulin secretion. Low basal glucose concentrations in the low-birth-weight group at 12 mo suggested that glucogenesis is impaired rather than enhanced in these animals, at least during fasting conditions (430).

C. Targets for Programming: the Liver and Glucose Tolerance

1. Rat

Bilateral uterine artery ligation of the pregnant rat in late pregnancy results in fetal growth restriction and overt diabetes in adult life, characterized by fasting hyperglycemia and hyperinsulinemia (475). Oxidative phosphorylation is decreased in growth-restricted fetuses, and hepatic cellular energy and redox states are uncoupled resulting in less ATP generated per unit of glucose (371). These changes were associated with enhanced mRNA levels of the glucose transporter, GLUT-1 in the liver of the fetal and neonatal rat after uteroplacental restriction in late pregnancy (268). Recent experiments using this model have demonstrated that hepatic peroxisome-proliferator-activated receptor-γ coactivator-1 (PGC-1) is increased in the livers of the offspring at birth and at 21 days of life (273). PGC-1 is a transcriptional coactivator of nuclear receptors that control the hepatic expression of key gluconeogenic enzymes including glucose-6-phosphatase, phosphoenolpyruvate carboxykinase (PEPCK), and fructose-1,6-bisphosphatase (562). Concurrent with the increased PGC-1 gene expression, there was also an increase in the mRNA levels of each of these enzymes in the liver at birth and on day 21 in the growth-restricted
animals, whereas hepatic glucokinase mRNA levels were significantly decreased. These findings suggest that an increase in PGC-1 expression and subsequent hepatic glucose production contribute to the insulin resistance observed in this model of intrauterine programming. It has also been demonstrated that NADH-ubiquinone oxireductase subunit 4L mRNA (ND-4L), adenine-nucleotide translocator-2 (ANT-2), glucose-6-phosphate dehydrogenase (G6PD), and mitochondrial malate dehydrogenase (MMD) are increased in the liver of fetuses after induction of uteroplacental insufficiency (269). These changes persisted into the newborn period, and hepatic ornithine transcarbamylase (OTC) and phosphofructokinase-2 (PFK-2) mRNA levels were also elevated in the growth-restricted group. Further studies have found that there is also altered expression of hepatic fatty acid metabolizing enzymes in newborn and juvenile rats after the experimental induction of uteroplacental insufficiency in late pregnancy (270). In adult male offspring, serum triglycerides, hepatic malonyl coA levels, and acetyl-CoA carboxylase mRNA levels were significantly increased, whereas carnitine palmitoyltransferase 1 (CPT1) and the β-oxidation-trifunctional protein (HADH) mRNA levels were significantly decreased. In contrast, adult female offspring demonstrated no significant changes in these variables (270). It was argued that the growth-restricted male fetus is programmed towards an increase in hepatic fatty acid synthesis or decreased fatty acid oxidation, resulting in an increased supply of hepatic triglycerides which may act to decrease skeletal muscle insulin sensitivity and spare glucose for the growth of key organs, such as the brain. These authors also reported that progeny (F2 generation) of rats that were exposed to uteroplacental insufficiency are growth restricted, independently of which parental rat experienced the intrauterine deprivation, and they suggested that the metabolic changes initiated by uteroplacental insufficiency alter gene expression by inheritable epigenetic phenomena, such as DNA methylation. Thus there is a “metabolic imprint” on the fetal DNA that endows the IUGR individual with a potential evolutionary advantage (273).

Adult female rats subjected to a reduction in maternal intake during the perinatal period are resistant to the actions of insulin as evidenced by the decreased infusion rates of glucose to maintain euglycemia (219). This resistance to insulin was found to be the result of a decreased responsiveness of the liver, i.e., a dampened suppression of glucose production during hyperinsulinemia, whereas insulin action at the peripheral tissues remained normal (219). Furthermore, these effects were present when rats were malnourished during the fetal period alone, and this suggests that fetal malnutrition is the main determinant of hepatic insulin resistance in this model (219).

There is a significant effect of a low-protein diet during pregnancy and lactation on hepatocyte proliferation, liver growth, and morphology in the offspring (59, 130). There was a decrease in cellular proliferation and in IGF-I production in hepatocytes derived from fetuses exposed to a low-protein diet through gestation (130). In parallel in these fetuses, there was a decrease in circulating IGF-I and an increase in the abundance of 29- to 32-kDa IGF binding proteins (IGFBPs). The livers in these offspring at 3 mo have fewer, but larger, lobules, and there is a loss of the initial insulin suppression of glucose output in vitro, despite an increased expression of the insulin receptor in the liver at this age (59, 397). In the offspring of dams fed a low-protein diet during either pregnancy alone or during pregnancy and lactation, there was a decreased activity of hepatic glucokinase: a high K_m hexokinase IV which regulates hepatic glucose uptake (100, 101). There was also an increased activity of hepatic PEPCK, a flux regulating enzyme of gluconeogenesis in these offspring, and there were parallel changes apparent in the mRNA levels of glucokinase and PEPCK in the low-protein exposed male offspring. There were no changes, however, in the activities of either glucokinase or PEPCK in the livers of offspring that were fed a control diet throughout pregnancy and then suckled mothers fed a low-protein diet only during the period of lactation. When offspring exposed to a low-protein or control diet throughout gestation and lactation were fed a highly palatable diet postweaning, the increased nutrition and enhanced weight gain in adult life led to a significant increased activity of glucokinase and decreased activity of PEPCK in both groups (100). These changes in hepatic glycolytic and gluconeogenic enzyme activity were still present in 11-mo-old offspring that had been weaned onto a normal laboratory chow diet and could not be attributed to abnormal glucagon or insulin concentrations, because the ratios of these hormones were comparable between the control and low-protein groups (101). Nevertheless, the relative changes present as a consequence of exposure to protein restriction in the pre- and postnatal periods were maintained, despite the effects of enhanced nutrient intake. Therefore, although the offspring of protein-restricted rat dams have permanently changed activities of key hepatic enzymes of glucose metabolism, the effect of programming is not to prevent further metabolic and endocrine control but to alter the “set point” about which this control takes place. The authors suggested that rat pups exposed to early maternal malnutrition have their metabolic control point shifted in the direction of poor nutrition, since the activity of a glucose-utilizing enzyme is decreased and the activity of a glucose-producing enzyme is increased (100). In further studies, using detailed mapping of metabolic function within the liver, it was reported that there was a diminution in the perivenous uptake of glucose in the livers of offspring.
exposed to a low-protein diet through pregnancy and lactation (59), and it was proposed that fetal programming may operate in part through changes in glucokinase expression in the immediate perivenous region. These changes are consistent with the thrifty phenotype hypothesis in that there are predictive adaptive responses to a poor nutritional environment before birth which have adverse consequences when the nutritional environment in the postweaning period is relatively better than that experienced in the prenatal period. One additional point is that whilst the effects in these studies have been attributed to a decrease in protein content of the maternal diet, as glucose was used to replace protein in the diet, the programming stimulus could be related to the effects of increased glucose intake, rather than protein restriction. These findings are of interest in the context that patients with type 2 diabetes also show decreased hepatic glucokinase activity (66) and that mutations in the glucokinase gene or in the genes for transcription factors involved in the control of glucokinase expression are responsible for some cases of maturity onset diabetes of the young (522, 561).

Euglycemic hyperinsulinemic clamp studies have also demonstrated the existence of an insulin resistance in the liver of adult offspring of either the mildly or severely diabetic rats (167, 214) (see Table 2).

Administration of dexamethasone to rats in the third week of pregnancy, but not earlier, results in a permanent upregulation of hepatic PEPCK mRNA expression and activity (368). Changes in local glucocorticoid action may mediate the observed metabolic changes in glucocorticoid-exposed offspring as it has been shown that expression of the GR is upregulated in the liver of these animals (Fig. 6) (368).

2. Sheep

Relatively few studies have investigated the impact of poor maternal or fetal nutrition on the structural and functional development of the liver in non-litter-bearing species. It has been shown that cortisol is a major regulator of fetal gluconeogenesis during late gestation (154). Furthermore, there is a premature increase in hepatic cytosolic PEPCK activity and fetal glucose production in chronically hypoglycemic and hypoinsulinemic fetal sheep. It is not clear, however, whether this persists into the newborn period following intrauterine deprivation (360). Experimental restriction of placental growth and function results in fetal hypoxemia and hypoglycemia and a relative decrease in liver weight in fetal sheep once fetal body weight falls below ~3 kg in late gestation (343). In these fetuses there is also an increase in the expression of 11βHSD-1 mRNA in the liver in late gestation. This suggests that there is increased hepatic exposure to cortisol in the growth-restricted fetus, which may be important in the reprogramming of hepatic physiology that may occur after growth restriction in utero (344). When maternal nutrient intake was reduced by 30% from 26 days gestation until 135 days gestation, there was no impact on the absolute or relative fetal liver weight at any gestational age between 45 and 135 days gestation in the sheep (387). A 50% reduction in maternal nutrition between 28 and 78 days gestation, however, resulted in a relative increase in fetal liver weight at 78 days gestation (523), and although this effect did not persist into the neonatal period (537), there was an increase in GR, but not 11βHSD-1 mRNA, expression in the neonatal liver (537). The effects of maternal nutrition during early to mid pregnancy on fetal growth outcomes in the sheep can be variable, however, and are in part dependent on the body condition and fatness of the ewe before the imposition of the nutrition restriction (180, 525). There is a need for further studies in large animals that normally bear one or two fetuses to determine the contributions of maternal nutritional stores before conception, poor maternal and/or fetal nutrition, and the maternal and fetal endocrine responses to these challenges on glucose homeostasis in later life.

D. Targets for Programming: Skeletal Muscle and Insulin Sensitivity

1. Skeletal muscle development

Embryonic cells with the potential to form muscle are present during gastrulation (171), and when dissociated cells from the epiblast layer of the primitive streak stage embryos are cultured at high density, they express a muscle-specific transcription factor and differentiate into skeletal muscle (171). The major commitment to the myogenic lineage occurs, however, in the mesodermal precursor cells in the somites. A family of myogenic regulatory factors (myoD, myf5, myogenin, and MRF4) have been identified that play a central regulatory function in the development of skeletal muscle (323). The final stage in the formation of muscle fibers is biphasic. An initial wave of synchronous fusion of myoblasts gives rise to a population of primary fibers. In rats primary fibers form around day 14 of gestation (542), and in humans, this occurs between 6 and 8 wk of gestation. A second wave of myogenesis then results in the asynchronous appearance of secondary fibers on the surface of each primary fiber between 17 and 21 days gestation in the rat and between weeks 8 and 18 of gestation in the human (18, 542). The final stage in establishment of adult muscle continues by fiber conversion and hypertrophy in the postnatal period when neural and growth factor influences are important in determining muscle characteristics (329).
From studies on polytocous species it has been suggested that the number of primary fibers is genetically determined, whereas the number of secondary fibers, which vary between littermates, appears to be determined by local and environmental signals, including nutrition (114, 115). In these species maternal starvation or substrate restriction during pregnancy leads to a reduction in the number of fetal fibers (115, 547), which appears to be irreversible (436), and secondary fibers are preferentially affected (115, 527). There is evidence in the sheep that preimplantation events can alter the primary and secondary fiber area within the plantaris muscle at around 125 days gestation. After in vitro culture of the sheep embryo, the secondary-to-primary fiber ratio was $\sim 15$–25% greater in the cultured group than in control lambs at this age (339, 340). The sensitivity of fetal myogenesis to procedures applied before implantation support the concept that there is a pool of cells specified to become muscle before the onset of somitogenesis.

Given that muscle represents the major site of postprandial glucose disposal, it is not surprising that changes in the mass, type, growth patterns, and functional characteristics of the muscle fibers during the perinatal period are important in the programming of insulin sensitivity and diabetes (Fig. 6).

2. Rat

In the rat model of uteroplacental insufficiency, it has been demonstrated that pups are insulin resistant early in life and develop diabetes by 6 mo of age. In this model, the extent to which there is catch up growth after birth depends on the severity of the intrauterine growth restriction and the level of postnatal nutrition (70). After bilateral uterine ligation at 17 days of gestation, there is no catch up growth up to $\sim 100$ days after birth in male or female offspring (70, 221). After bilateral uterine ligation at 19 days gestation and cross-fostering to nonoperated mothers after birth, however, intrauterine growth-restricted rats demonstrated accelerated growth between 7 and 10 wk of age and by 26 wk of age were obese (475).

Glycogen content and insulin-stimulated 2-deoxyglucose uptake were significantly decreased in muscle from the growth-restricted rats (466). Mitochondria from the muscles of these rats exhibited defects associated with a chronic reduction in the supply of ATP available from oxidative phosphorylation, and these authors suggested that impaired ATP synthesis in muscle compromises energy-dependent GLUT4 recruitment to the cell surface, glucose transport, and glycogen synthesis (466). It has also been reported that PGC-1 mRNA levels were increased in the skeletal muscle of perinatal and postnatal growth-restricted rats (272). Furthermore, in isolated skeletal muscle mitochondria from 21-day-old rats, CPT1, the trifunctional protein of beta-oxidation (HADH), and uncoupling protein 3 (UCP3) mRNA levels were significantly increased in the intrauterine growth-restricted rats compared with controls (271). Despite increased CPT1 gene expression and HADH $\alpha$-subunit gene expression and function, skeletal muscle triglycerides were also significantly increased, and the authors speculated that, at this age (preweaning), an equilibrium is reached between increased hepatic synthesis and increased skeletal muscle mitochondrial lipid oxidation, which subsequently results in only a moderate increase in plasma triglycerides. The authors suggested that the adaptations observed in the skeletal muscle of the growth-restricted group might be beneficial by leading to an enhanced skeletal muscle mitochondrial lipid oxidation and subsequent glucose sparing to maintain brain growth (271). Interestingly, it has subsequently been shown that PGC-1 (a transcriptional coactivator that affects gene expression of key lipid metabolizing enzymes) is upregulated in perinatal hindlimb skeletal muscle and in juvenile extensor digitorum longus (272).

When maternal energy intake is restricted by 50% during both pregnancy and lactation, offspring have a lower body weight from fetal life onward, whereas the offspring of rats that were food restricted only during pregnancy increased their body weight above control values (219). When maternal energy intake is restricted to 30% of control levels, however, offspring have a lower body weight than controls throughout postnatal life, independently of the level of postnatal nutrition (517). While adult female rats are resistant to the actions of insulin following perinatal malnutrition, this is primarily a consequence of a decreased responsiveness of the liver as peripheral glucose utilization in the basal state and during hyperinsulinemia remained normal (219).

In rats, protein restriction through gestation or immediately after birth results in a reduction in skeletal muscle mass (102). In young adult life, there is an improved glucose tolerance and isolated muscle strips from low-protein animals took up more radiolabeled glucose than those from controls, and insulin had a significantly greater effect on stimulation of glucose transport into preincubated low-protein muscle strips (395). This increase in insulin sensitivity was associated with a twofold increase in insulin receptors in muscle membranes from the low-protein group compared with the control group (395). By 15 mo of age, however, there was a decrease in the insulin sensitivity of glucose uptake in skeletal muscle from the group exposed to the low-protein diet in utero (396). The impaired insulin action was not associated with changes in the expression of either the insulin receptor or GLUT-4, which suggests that the molecular defect responsible for the insulin resistance is down-stream of the insulin receptor. The muscle from the low-protein-exposed group expressed significantly less of the zeta-isomform of protein kinase C, an isofrom that is posi-
Offspring of severely diabetic rats have a lower body weight from fetal life onwards, and the glucose metabolic index in the various skeletal muscles of these adult offspring was lower under basal postabsorptive conditions and during physiological hyperinsulinemia than in control rats (214, 217). When doses of STZ of between 15 and 35 mg/kg are administered at 3 days of gestation, fetal plasma glucose and insulin concentrations are each increased. Although fetal body weights at a dose of STZ of 25 mg/kg tended to be heavier, this effect was not consistent, and therefore, studies were performed on those offspring in the 35 mg/kg group, which were of equivalent body weight to control animals (505). The postnatal growth of these pups was dependent on how they were reared during the postnatal period (505). When pups were reared by diabetic mothers, there was a loss in body weight and postnatal growth restriction. In contrast, the newborns of diabetic mothers that were raised by control mothers were heavier in postnatal life and developed obesity by 180 days after birth (505). In these studies, a maternal diabetic environment caused no change in skeletal muscle GLUT4 mRNA and protein levels, although there was a higher amount of GLUT4 associated with the skeletal muscle sarcolemma in the basal state. In contrast to control pups, however, there was no change in the subcellular distribution of GLUT4, after insulin administration to newborn pups born to diabetic mothers. Thus in utero exposure to the metabolic consequences of gestational diabetes resulted in a resistance to insulin-induced translocation of GLUT4 in skeletal muscle. Suckling of pups exposed to diabetes in utero by diabetic mothers, however, led to a relative amelioration of the GLUT4 defect in skeletal muscle, and the authors suggested that extension of the period of nutrient restriction beyond the suckling phase may be required for a complete reversal of the defect (505).

The potential role of glucocorticoids in the programming of insulin resistance has been investigated in the rat. Offspring of rats treated with dexamethasone during the last week of gestation show incomplete catch up growth after birth and remain lighter than control animals from birth until 160 days of age. GR expression is programmed in skeletal muscle by prenatal dexamethasone exposure during the last week of gestation in a fiber-type manner, being downregulated in soleus muscle (predominantly type I fibers) but not in extensor digitorum longus (predominantly type II fibers) (81). Although glycogen storage was reduced in the quadriceps in the dexamethasone-treated group, there was no decrease in glucose uptake into either quadriceps or soleus in these offspring, and it therefore does not appear that a diminished glucose uptake into muscle contributes to the hyperglycemia in this model (81). These data are of interest in the context of the finding that expression of the ligand binding GRα in human skeletal myoblasts is positively associated with features of the metabolic syndrome including obesity and insulin resistance (536). The higher expression of GRα expression in the myoblasts was also associated with an increased GRα-mediated upregulation of 11βHSD1, which has 11-oxoreductase activity and generates cortisol from cortisone (536).

3. Sheep and pig

The impact of maternal undernutrition during pregnancy and the immediate postnatal period on skeletal muscle development varies between altricial, litter-bearing, and precocial non-litter-bearing species. Greenwood and colleagues (181, 183) reported that skeletal muscle weight was reduced in low-birth-weight lambs compared with high-birth-weight counterparts and that the rates of gain in several skeletal muscles, including semitendinosus, were persistently lower in low-birth-weight lambs, as were rates of gain in DNA and RNA. Myofiber numbers in the majority of muscles collected were not different, however, between low- and high-birth-weight lambs, and therefore, in this species the capacity for postnatal growth of muscle may be constrained by decreased mitotic rates of fetal myosatellite cells during late gestation (183) and low muscle DNA content at birth (182). Thus the myofiber number in fetal sheep muscles appeared to be established before the adverse consequences of inadequate fetal nutrient supply on skeletal muscle growth and development became apparent, whereas the proliferation of myonuclei may be influenced by fetal nutrition in late pregnancy. In normally grown (birth weight >4.3 kg) and severely growth-retarded male lambs (<2.9 kg) that were reared artificially on sheep milk replacer from birth until a live weight of 20 kg, there were no differences in plasma glucose concentrations between the small and normally grown lambs during the first 2 mo of postnatal life, but plasma insulin concentrations increased rapidly during the early postnatal period in low-birth-weight lambs feeding ad libitum and from 2 wk of age. At 20 kg, plasma insulin concentrations were persistently higher in the low-birth-weight lambs (180).

A recent study in the pig found that at 3 mo of age, thinness at birth and rapid catch up growth in the first month of life were associated with increased insulin sensitivity in males, whereas at 12 mo, early postnatal catch up growth was associated with insulin resistance in males and females (432). Thus intrauterine growth restriction in a number of species may be followed by a high level of energy intake in the immediate postpartum period and a period of increased insulin sensitivity before the emergence of insulin resistance.
E. Targets for Programming: the Adipocyte and Insulin Sensitivity

1. Rat

As discussed above, a 50% decrease in maternal energy intake during pregnancy and lactation results in fetal hypoinsulinemia and growth restriction and a decrease in adult weight. While there is a development of whole body insulin resistance in the adult offspring, this is not a consequence of a decrease in glucose uptake by either the skeletal muscles or the adipose tissue; rather, it is the result of less inhibition of hepatic glucose production by insulin (218) (see Table 2).

Restriction of maternal protein intake during pregnancy and lactation results in an increased glucose tolerance at 6 wk of age and an increased expression of insulin receptors in epididymal adipocytes (470). The improved glucose tolerance is maintained into young adult life, and consistent with an increase in insulin sensitivity, the offspring have lower circulating triglyceride concentrations (321, 400) and reduced circulating β-hydroxybutyrate concentrations, despite higher nonesterified fatty acid concentrations (400). In young adult life, mesenteric adipocytes from low-protein offspring show an enhanced glucose utilization during euglycemic hyperinsulinemia, and insulin receptor expression is increased in epididymal (220, 395) and intra-abdominal but not subcutaneous adipocytes at 3 mo of age (392). Basal glucose uptakes were higher in adipocytes from epididymal, intra-abdominal, and subcutaneous fat depots; however, insulin-stimulated uptake of glucose into these adipocytes is smaller in low-protein offspring compared with controls. There was no difference in GLUT4 protein expression in the adipocytes from the low-protein offspring. The magnitude of insulin inhibition of isoproterenol-stimulated lipolysis is smaller in epididymal and intra-abdominal adipocytes from low-protein offspring at 15 mo of age, suggesting a selective resistance to insulin in these adipocytes (Fig. 6) (391, 392, 399). At 15 mo of age, insulin receptor expression was similar in adipocytes from low-protein offspring and controls, suggesting that the molecular alteration that leads to insulin resistance must therefore be at the postreceptor level (391). At 3 and 15 mo of age, insulin-stimulated phosphatidylinositol 3-kinase activity was reduced, and this is associated with a reduction in the level of the p110β subunit in adipocytes from low-protein offspring (391, 395). It may be that this catalytic subunit of phosphatidylinositol 3-kinase is required to mediate the antilipolytic action of insulin. At 15 mo, insulin-stimulated protein kinase B was also reduced in the adipocytes from low-protein offspring, which may explain the insulin resistance observed in the adipocyte at this age (391).

In the offspring of STZ-induced severely diabetic mothers, there was a tendency for the glucose metabolic index to increase in white adipose tissue during hyperinsulinemia, in contrast to the decrease that occurs in the glucose metabolic index in skeletal muscles during the hyperinsulinemic euglycemic clamp (217, 218). When STZ is administered early in pregnancy, there is an increase in fetal plasma glucose and insulin concentrations, and birth weight is not decreased (505). As discussed when these pups are reared by diabetic mothers, they are smaller in later life than control pups. When pups are raised by control mothers, however, they become heavier in postnatal life and develop obesity by 180 days after birth (505). In these studies, a maternal diabetic environment resulted in no change in GLUT4 mRNA and protein levels in the white adipose tissue in the offspring. As in the skeletal muscle, there was a higher amount of plasma membrane-associated GLUT4 in the adipose tissue of diabetic pups with a lack of insulin-induced translocation. This effect persisted into adulthood, independently of the postnatal diet, although suckling of the pups exposed to diabetes in utero by diabetic mothers led to a relative amelioration of the GLUT4 defect in the adipose tissue (505).

When fetal macrosomia was induced by administration of STZ at 5 days gestation (166), the offspring were obese and hyperinsulinemic at 70 days of age, and the adipocytes of the epididymal fat from obese males and periovarian fat from obese female had a higher lipid content and significantly larger cell size when compared with control rats. The adipocytes of the macrosomic pups also showed an attenuated response to insulin-stimulated glucose conversion to lipid and fatty acid compared with controls. The authors concluded that a postreceptor deficit most likely accounted for the abnormality in glucose metabolism in these obese rats (166).

IV. OBESITY AND THE METABOLIC SYNDROME

A. Epidemiological Evidence for the Association Between Birth Weight and Obesity in Adult Life

During the past two decades there has been a marked increase in the global prevalence of adult and childhood obesity, and currently >50% of all adults in the United States and the United Kingdom are overweight, i.e., have a BMI of >25 kg/m² (63, 148, 233, 372). An increase in the prevalence of obesity (BMI >30 kg/m²) is associated with an increase in a range of comorbidities including type 2 diabetes, high blood pressure, and ischemic heart disease (233). A range of epidemiological studies have shown that there is a relationship between birth weight and BMI in childhood and in adult life (140, 319, 401, 488, 544, 560). In this context it is of particular interest that epidemiological, clinical, and experimental studies have shown that there is a relationship between the prenatal nutritional...
environment and patterns of postnatal growth and adult adiposity.

1. High birth weight and adult obesity

The relation between birth weight and fatness, measured in childhood or adulthood, is generally positive, although a number of studies have reported that there is a J-shaped or U-shaped relationship between birth weight and adult fat mass, with a higher prevalence of obesity occurring at both low and high birth weights. There are associations between maternal and paternal birth weight with offspring birth weight, and where adjustments for maternal BMI have been able to be made (86, 87, 326, 401), the relationship between birth weight and adult BMI has diminished. A recent study in a large British cohort found a weak but positive relationship between birth weight and BMI at age 33 and that this relationship was largely accounted for by maternal weight, i.e., heavier mothers had heavier babies and these babies went on to have a high BMI in adult life (401). In contrast, paternal weight, gestational age, social class, parity, mother’s age, and mother’s smoking habits had no influence on the relationship between birth weight and BMI at 33 (401). It has been suggested that the influence of maternal weight on the relationship between birth weight and subsequent BMI may operate through an impact of high maternal and hence fetal nutrient supply. Interestingly, there is also recent evidence that a high birth weight may program a relatively greater proportion of lean mass in children and adolescents and that the positive associations between birth weight and later BMI may represent an association of birth weight with lean rather than fat tissue (480). The authors of this latter study noted that programming of more lean tissue than fat mass by a high birth weight might explain the paradoxical associations of a high birth weight with adult BMI (positive) and cardiovascular disease (negative).

In pregnancies complicated by maternal diabetes mellitus, gestational diabetes, or even mildly impaired glucose tolerance, the offspring are at risk of developing obesity and glucose intolerance (57, 112). In one long-term follow up of infants of diabetic mothers, 50% of newborn infants had weights greater than the 90th percentile for gestational age. After 5 years of age, relative weight increased markedly and by 8 years of age, half of the group whose mothers were diabetic in pregnancy had weights greater than the 90th percentile (474). This childhood obesity was correlated with maternal prepregnancy weight and independently with amniotic fluid insulin at 32–38 wk gestation.

2. Low birth weight and central obesity in later life

Central or truncal obesity is associated with the clustering of pathologies which defines the insulin resistance or metabolic syndrome (hypertension, dyslipidemia, hyperinsulinism, impaired glucose tolerance, or frank diabetes) (444). Whereas people who were small babies tend to have a lower BMI in adult life than people who were larger at birth, these individuals tend to have a more abdominal distribution of obesity, a significantly reduced muscle mass, and a high body fat content in adolescent and adult life despite their lower BMI (139, 290, 317, 318, 328, 374, 480). As summarized earlier in this review, there are also associations between small size at birth and measures of insulin resistance and the metabolic syndrome. In general, after adjustment for current BMI, there is an inverse relationship between birth weight and these outcomes. Exposure to a reduced nutrient supply in early pregnancy, as occurred in the Dutch Winter Famine in 1944–1945, also resulted in increased adiposity in later life. In people exposed to this famine during early gestation, there was an increase in body weight, BMI, and waist circumference at 50 years of age (443). Interestingly, Parsons et al. (401) found that those low-birth-weight babies who were most vulnerable to developing obesity were men who had been light and thin at birth and had experienced a period of rapid childhood growth. Thus men with a lower birth weight who had achieved more of their adult height by age 7 had a risk of obesity comparable with that for men with higher birth weights. Similarly, in an Indian study, the levels of cardiovascular risk factors were highest in children who were born small but had then grown large (30).

Maternal cigarette smoking in pregnancy has a dose response on childhood obesity (538). The association between smoking and obesity in the offspring was not affected by adjustment for early life factors including mother’s BMI, social class at birth, birth weight, infant feeding, childhood social circumstances, or adult factors including diet, physical activity, and social class (435). There are two genes, CYP1A1 and GSTT1, involved in the metabolism and detoxification of polyaromatic hydrocarbons in cigarette smoke. The presence of type A polymorphism of CYP1A1 and absence of GSTT1 are associated with a reduction in birth weight of ~1,200 g due to poor fetal growth and preterm birth (526). This may be an example of an environmental interaction with a genotype that may also affect obesity in later life.

B. Targets for Programming: the Appetite

Regulatory Neural Network

In the adult, appetite and energy balance homeostasis are primarily regulated by a complex neuronal circuitry located within the hypothalamus which receives nutrient, hormonal, and neural signals from a range of sources including fat cells, the pancreas, the gastrointestinal tract, and other brain regions. A range of neuropep-
tides including the orexigenic neuropeptides, neuropeptide Y (NPY) and agouti-related protein (AgRP), and the anorexigenic neuropeptides proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) are expressed within the hypothalamus and together act in synchrony to regulate energy balance (158, 464). NPY is a 36-amino acid neuropeptide that markedly stimulates appetite and is predominantly localized in the arcuate nucleus of the hypothalamus with low levels of expression within the dorsomedial nucleus (DMN). NPY neurons project to hypothalamic regions that play important roles in energy balance including the paraventricular nucleus (PVN), DMN, perifornical region, and the lateral hypothalamic area (185). The blood-brain barrier is effectively reduced within the area of the arcuate nucleus, and NPY neurons are therefore able to sense and respond to a range of peripheral metabolic signals including insulin, glucose, ghrelin, and the adipocyte-derived hormone leptin. NPY expression is downregulated by signals of increased energy stores, including insulin and the adipocyte-derived hormone leptin. A long form variant of the leptin receptor is highly expressed on cell bodies in the hypothalamic arcuate and DMN, and increases in circulating leptin concentrations during periods of increased food intake result in a corresponding decrease in hypothalamic NPY mRNA and a subsequent fall in energy intake (464). AgRP is an orexigenic peptide that is coexpressed with NPY in the arcuate nucleus and is an endogenous antagonist of the anorexigenic melanocortin receptors MC3 and MC4R in the PVN and other hypothalamic regions. There are additional orexigenic peptides including orexin A and B, galanin, and ghrelin. The POMC-derived peptide α-MSH is an endogenous anorexigenic peptide which acts at the melanocortin receptors to suppress food intake, and leptin acts to upregulate POMC expression within the arcuate nucleus and thereby limits energy intake (464). The neuropeptide CART is colocalized within POMC neurons in the hypothalamus and also acts to suppress food intake.

Central administration of NPY into the PVN significantly increases feeding activity, increases lipoprotein lipase activity in fat cells, decreases sympathetic nerve activity and thermogenesis in brown adipose tissue, and can lead to obesity (158, 464). Fasting or food restriction markedly increases NPY expression in the arcuate nuclei and NPY release into the PVN both in vivo and in vitro. NPY increases neuronal activity within the PVN through inhibition of presynaptic GABA release, whereas α-MSH and MC4R agonists suppress neuronal activity within the PVN.

1. Development of the appetite regulatory neural network: rat

In the rat, NPY neurons first appear in the arcuate and dorsolateral hypothalamus at ~14.5 days gestation (10, 245, 556), and NPY mRNA expression rapidly increases between 2 and 15–16 days after birth and returns to adult levels at ~30 days of age (185). There is also transient expression of NPY in the DMN, the perifornical region, and the lateral hypothalamic area during the postnatal period. POMC, AgRP, and MC4R mRNA are also all present within the rat hypothalamus through this postnatal period. Whilst NPY is present within the arcuate nucleus from late fetal life, NPY/AgRP projections from the arcuate nucleus to the DMN are not complete until some 10–11 days after birth, and projections to the PVN do not fully develop until 15–16 days (185). Microinjection of NPY directly into the PVN at 2 days after birth stimulates milk and water intake, indicating that NPY receptors are present and functional at this early age (65). During the first week after birth, there appears to be a relative dominance of NPY and α-MSH innervation of the PVN by efferents derived from the brain stem, rather than the arcuate nucleus, and it has been suggested therefore that vagal sensory information from the gut relating to gut fullness may be important in regulating feeding behavior during this period (185). Acute peripheral leptin treatment at postnatal day 10 decreases NPY mRNA expression in the rostral arcuate nucleus but has little impact on food intake, which is consistent with the lack of NPY projections within the hypothalamus during the early postnatal period (438). Interestingly, acute leptin administration also results in an increase in NPY mRNA expression in the caudal region of the arcuate nucleus, although chronic leptin treatment of rats between 3 and 9 days after birth inhibits the NPY response to acute leptin administration at 10 days after birth (438). In these rats acute leptin treatment increased POMC gene expression in the arcuate nucleus, whereas chronic leptin treatment did not alter POMC expression (438).

2. Development of the appetite regulatory neural network: human and sheep

In the human, the earliest stage that NPY immunoreactivity was found to be present in the hypothalamic arcuate neurons was at 21 wk gestation, and there are projections from the arcuate to the PVN at this stage of pregnancy (262). In other precocial species, such as the sheep or Meschian pig, NPY containing cell bodies are present in the fetal hypothalamus from as early as has been investigated (110 days gestation in the sheep and 30 days gestation in the Meschian pig) (403, 528). In the Meschian pig, there was an increase in NPY immunoreactivity in the hypothalamus throughout gestation (term = 114 days gestation), and as in the rat, there was a marked reduction in the number of NPY containing cell bodies between the day of birth and 20 days after birth (just before weaning) in this species (403). In both the fetal sheep and pig, NPY projections are detected in the PVN.
during late gestation (403, 528). Differences between species in the timing of the appearance of neuropeptide expression within those hypothalamic nuclei implicated in energy balance regulation in adult life, and the development of neuronal projections between these nuclei may be important in determining the impact of changes in fetal or neonatal nutrition on the subsequent development of appetite regulation and energy homeostasis.

3. Programming of appetite: rat

The perinatal period represents a critical window for the programming of postnatal appetite in the rat (386). Exposure of the rat to hyperglycemia and/or hyperinsulinemia in the fetal or neonatal period can result in permanent changes in body fat mass and in the neuronal circuitry regulating appetite in the adult brain. When mild hyperglycemia is induced by STZ-induced gestational diabetes from early pregnancy, macroscopic pups maintain an accelerated growth during the first 10 wk of age (373). Maternal diabetes leading to fetal hyperglycemia and normoinsulinemia resulted in a decrease in fetal brain NPY mRNA and peptide levels (477). In macroscopic, hyperinsulinemic pups at 21 days of life, the mean areas of neuronal nuclei and cytoplasm were significantly decreased within the PVN and VMN, and the mean area of neuronal cytoplasm was also decreased in the arcuate nucleus (421). There was a significant increase, however, in the number of NPY- and galanin-containing neurons within the arcuate nucleus of the adult offspring of the mildly diabetic pregnant dam (422, 423).

Early studies demonstrated that rats from small litters were heavier than those from large litters in adult life (386). One factor responsible for the larger body size was the greater voluntary food intake after weaning. Thus the amount of food consumed during suckling in the rat plays an important role in determining subsequent food intake and fasting in rats in later life (386). The early postnatal overnourishment of rats growing up in small litters of only three pups per litter leads to increased early weight gain and fat deposition, followed by hyperphagia and obesity combined with hyperleptinemia, hyperglycemia, hyperinsulinemia, and insulin resistance (425, 424, 426). In these animals there are also changes in the neuronal responsiveness to insulin and leptin in the hypothalamic arcuate nucleus and VMN. Insulin and leptin tend to exert greater inhibitory effects on orexigenic neurons in the arcuate nucleus in the young adult, postnatally overfed rat (92, 93). Neurons in the VMN also have a different responsivity to NPY and AgRP, and there are altered responses to both orexigenic (AgRP, MCH) and anorexigenic (αMSH, CART) neuropeptides in the PVN in the young adult, postnatally overfed animals (90, 91, 202, 307). Both mild gestational diabetes and a reduction in litter size are associated with perinatal hyperinsulinism, and there is evidence that exposure to hyperinsulinemia during fetal or early postnatal life results in increased adiposity and altered hypothalamic development. Male, but not female, offspring from dams treated daily with insulin between 14 and 20 days gestation are significantly heavier in adult life (240, 241). Daily insulin treatment between 8 and 11 days after birth results in a greater body weight gain, chronic hyperinsulinemia, impaired glucose tolerance, and hypertension that persists in adult life and is associated with morphological changes within the VMN (194, 195).

Rats exposed to undernutrition in utero may develop an increased body fat mass depending on the degree and timing of malnutrition and the nature of the postnatal diet. When rats are undernourished (50% decrease in energy intake) during the first 2 wk of pregnancy but refeed during the third week, the male offspring develop significant hyperphagia and obesity when maintained on a high-fat diet (12, 238, 239, 242). The obesity has a delayed onset (~50 days of age) and is characterized by increases in the proportion of body fat and adipocyte hypertrophy in the epididymal and retroperitoneal fat pads. At this level of maternal undernutrition, refeeding during the third week of pregnancy is critical for the induction of postnatal obesity (494). When maternal nutrition is restricted to 30% of control intake throughout the whole of gestation, although the offspring were smaller throughout postnatal life, there was an increase in the relative mass of the retroperitoneal fat pad in these animals at 100 days of age (517). Food intake in the offspring of the undernourished rats (cross-fostered on to ad libitum-fed mothers) was increased early in postnatal life, increased with increasing age, and was amplified by postnatal hypercaloric nutrition (517). Interestingly in these offspring, locomotor activity was also decreased before the development of maturity-onset obesity and was significantly reduced in male compared with female offspring (518). It is not yet clear whether there are or are not permanent changes within the hypothalamic appetite regulatory network in these animals.

Protein restriction maintained during gestation and lactation is associated with hypoinsulinemia, normal leptin concentrations, an increase in NPY levels in the arcuate nucleus, PVN and lateral hypothalamic area, and unchanged NPY levels in the VMN. There are, however, fewer neurons immunopositive for NPY in the arcuate nucleus of these offspring (427). These authors have therefore suggested that hypoplasia of neurons expressing the orexigenic peptides such as NPY and galanin is the result of perinatal hypoinsulinism, whereas hyperplasia of these neurons is a consequence of perinatal hyperinsulinism. Maternal uteroplacental insufficiency is also associated with an increase in fetal brain NPY mRNA levels, although these changes are not accompanied by an increase in NPY peptide levels (439). Enhanced exposure of
the brain to NPY in early development results in programmed changes in food intake in adult life (515). Intracerebroventricular administration of NPY in rats at between 2 and 7 days after birth results in a maintained hyperinsulinemia and relative hyperleptinemia with euglycemia in the 120-day-old female offspring (515). This perturbation was associated with a 50–80% suppression of NPY immunoreactivity within the arcuate nucleus and PVN, a decline in food intake and in body weight gain at 60 and 120 days in the adult female (515). It is not clear whether exogenous NPY administered in the postnatal period acts to suppress endogenous hypothalamic NPY concentrations throughout postnatal life or whether the indirect actions of neonatal hyperinsulinemia and/or hyperleptinemia in turn act to suppress hypothalamic NPY.

4. Programming of appetite: human and sheep

There is little information on the impact of decreases or increases in perinatal nutrition in precocial species, such as the sheep or human in which the appetite regulatory neuropeptide circuitry is present before birth. Maternal undernutrition (50% decrease in energy intake) from 115 days gestation results in an increase in NPY mRNA expression within the fetal sheep hypothalamus at 140 days gestation, and central NPY administration in the sheep fetus significantly increases fetal swallowing activity (129, 528). While there is evidence that low-birth-weight lambs have a higher relative voluntary food intake during the early postnatal period and are fatter at body weights up to 20 kg when compared with lambs with normal birth weights (181), there is no information on whether this relative hyperphagia is a result of an early programming of the appetite regulatory neuropeptides.

C. Targets for Programming: the Adipocyte and Adipocyte Hormone Secretion

Leptin, a polypeptide hormone (~16 kDa), is synthesized and secreted by adipose tissue and acts as a circulating signal of fat mass through binding to specific receptors at a number of central and peripheral sites to decrease food intake and increase energy utilization (8, 158, 464). Adult obesity is associated with relatively high circulating leptin concentrations, and the tendency to gain weight in some, although not all, nonobese populations with high basal leptin concentrations may indicate an underlying role for leptin resistance in obesity (75, 309, 316). It has been proposed that elevated plasma levels of leptin result in an uncoupling of the action of leptin at its receptors in the hypothalamus, thereby disrupting signal transduction pathways that are required for the suppression of appetite by an increase in circulating leptin (8, 256). The presence of functional leptin receptors on pancreatic β-cells (141, 256), and the observation that leptin directly inhibits insulin secretion (131, 230) led to the concept of an adipoinsular axis (255) whereby insulin stimulates adipogenesis and leptin inhibits the production of insulin in the pancreas. It has therefore also been proposed that leptin resistance at the pancreas may be a key mechanisms underlying obesity-associated hyperinsulinism that may contribute to the pathogenesis of adipogenic diabetes (467).

1. Leptin and the early programming of human obesity

In the human infant, there is a positive relationship between cord blood concentrations of leptin at delivery and birth weight or neonatal adiposity. Furthermore, in pregnancies complicated by maternal diabetes, the fetus is hyperglycemic and hyperinsulinemic, and cord blood leptin concentrations are increased in parallel with increases in infant adiposity (69, 235, 258, 337, 468, 503). Leptin is also synthesized and secreted by the human placenta (336). Linneman et al. (314) and Lepercq et al. (297) reported that a relatively low proportion (~5%) of placental leptin is secreted into the fetal circulation, and it has therefore been proposed that umbilical leptin concentrations can be taken as a marker of fat deposition in human fetuses. Hoggard et al. (212), however, reported that a higher proportion (~14%) of leptin is released into the fetal circulation (212). While plasma leptin concentrations are low in growth-restricted infants at birth, they increase to become higher in these infants at 1 yr of age compared with their normal birth weight counterparts (236). It has also been demonstrated that people with low birth weight also go on to have higher leptin concentrations in adult life compared with individuals at the same BMI but with a higher birth weight (417). In a study on the impact of postnatal nutrition after preterm delivery on leptin concentrations in 13- to 16-yr-old adolescents, it was found that the ratio of leptin to fat mass was significantly greater in the children who had received a nutrient-enriched preterm formula than in those who received a standard formula or banked breast milk (478). Human milk intake was also associated with lower leptin concentrations relative to fat mass in adolescence (478). The authors therefore concluded that programming of relative leptin concentrations by early diet may be one mechanism that links early nutrition with later obesity (478). In this study, however, there was no effect of birth weight of the preterm infants on circulating leptin concentrations in adolescence, and it was suggested that this may be because there is a separate programming effect of low birth weight on obesity which is exerted in later gestation and is not present in the preterm cohort (478).
2. Leptin and the programming of adult obesity in the rat

In rodents, the capacity of fetal adipocytes to synthesize leptin is low until relatively late in gestation; the placenta synthesizes little if any leptin (11, 250), but there is significant transplacental transfer of maternal leptin to the fetus. This transfer increases during late pregnancy potentially as a consequence of an upregulation of expression of the shorter isoforms of the leptin receptor which serve as leptin transporters (482, 483). When leptin is administered to pregnant dams on days 8, 10, and 12 of pregnancy, the adult offspring have reduced adipose tissue mass and skeletal growth with no change in food intake (365). Maternal protein restriction results in a significant decrease in maternal but not fetal leptin concentrations during late gestation (145). Administration of leptin (from day 14 of pregnancy and throughout lactation) to pregnant rats fed a low-protein diet prevented the diet-induced fall in placental 11βHSD2 activity (496). The male offspring of saline-treated dams gained more weight and had higher plasma leptin levels when transferred to a high-fat diet at 6 wk, but the offspring of leptin-treated dams did not (496). Whilst fasting blood glucose and intraperitoneal glucose tolerance at 6 and 12 mo of age were not affected by the high-fat diet, the offspring of the leptin-treated dams achieved this control without raised insulin levels. Thus administration of leptin to rats during late pregnancy and lactation makes their male offspring less susceptible to high-fat diet-induced weight gain and insulin resistance (496). It is possible that this is an example of an experimental induction of a “predictive adaptation” in the fetus, i.e., an increase in maternal leptin (normally associated with an increase in body fat) programs the ability of the offspring to respond better to a high-fat diet in the postnatal period.

In contrast, exposure of rats to maternal undernutrition (30% energy intake) during gestation leads to an increase in relative adiposity and hyperleptinemia in adult offspring (517). The combination of prenatal undernutrition together with postnatal hypercaloric nutrition leads to a major amplification of the hyperleptinemia. The amplified hyperleptinemia in the presence of hyperinsulinemia in the offspring on the hypercaloric diet suggests that there may be development of leptin resistance both at the hypothalamus and the pancreas, which explains the hyperphagia and hyperinsulinemia, respectively (52).

Dexamethasone administration to the pregnant rat between days 15 and 21 of pregnancy results in offspring that are lighter and leaner than controls, smaller fat pads and relatively preserved muscle mass (81, 497). In these offspring there is an upregulation of GR expression in adipose tissue and increased plasma corticosterone levels, and while this combination would be expected to increase glucocorticoid-mediated effects on adipose tissue, there were no effects of prenatal dexamethasone on the glucose uptake of adipose tissue in the offspring at 6–7 mo. It has also been shown that manipulation of circulating corticosterone concentrations (by adrenalectomy with or without corticosterone replacement) and rosiglitazone treatment did not alter GR expression in the adipose tissue in these offspring, implying that neither hypercorticosteronemia nor hyperinsulinemia is sufficient to cause the changes in GR expression in the dexamethasone-programmed rats (81). Dexamethasone administration also results in maternal hyperleptinemia, decreased placental expression of mRNA and protein of the long form of the leptin receptor, decreased transplacental leptin transfer and fetal hypo leptinemia (482, 497). There were no differences in resistin (the fat cell hormone associated with insulin resistance), UCP-3 and leptin mRNA expression or in plasma leptin concentrations in dexamethasone-exposed offspring compared with controls at 6–7 mo of age (81), plasma leptin concentrations were increased by 12 mo, however, despite lower epididymal and parametrial fat pad weights in the male and female adults. At 12 mo of age, male, but not female, offspring of dexamethasone-treated pregnant rats were hyperinsulinemic, which suggests that preceding hyperinsulinemia may be permissive for or associated with the development of hyperleptinemia, but that an increase in prevailing insulin concentrations is not obligatory for the presence of hyperleptinemia in this model (497). While GR expression is upregulated in adipose tissue from the offspring of dexamethasone-treated pregnant rats, lipoprotein lipase mRNA is decreased, and there is an increase in the expression of PPARγ2 (the PPAR isoform most involved in adipocyte differentiation) in the intra-abdominal adipose tissue of these animals (81). These authors argue that these changes are unlikely to be mediated directly by increased glucocorticoid action and may either be independently programmed or be indirect consequences of insulin resistance (81). A reduced fat deposition may be explained in part by the hyperleptinemia and the downregulation of lipoprotein lipase expression consistent with a programmed attenuation of fatty acid uptake by visceral fat (81, 497).

3. Leptin, fetal nutrition, and the programming of obesity in the sheep

In the sheep and pig in which fat is deposited before birth, leptin is synthesized in fetal adipose tissue and is present in the fetal circulation through late gestation (72, 104, 127, 357, 358, 565, 566). Leptin is also expressed in the fetal sheep brain and liver, but in contrast to the human, leptin is not expressed in the sheep placenta (127, 507). Leptin is present in the circulation of the sheep fetus from as early as 40 days gestation, which is before the development of visible adipose tissue depots, and fetal...
plasma leptin may therefore originate from either the maternal circulation or from fetal tissues other than adipose tissue at this early stage of pregnancy (127). Circulating leptin concentrations are lower in the fetus than the pregnant ewe throughout late gestation (127, 357, 566). As the sheep placenta expresses the leptin receptor gene (507) and maternal and fetal plasma leptin concentrations are positively correlated throughout late gestation (566), it is possible that the placental leptin receptor may mediate the uptake of leptin from the maternal into the fetal circulation. Alternatively, maternal body composition or fatness either at the beginning or during pregnancy may determine the leptin synthetic and secretory capacity of both maternal and fetal adipose tissue. Importantly there is also a positive relationship between the relative abundance of leptin mRNA in fetal perirenal adipose tissue (which comprises >80% of the fetal fat mass) and fetal plasma leptin concentrations (566). Ultrastructural studies of adipose tissue in the sheep fetus have demonstrated that fetal adipocytes contain multiple lipid locules and an abundance of mitochondria and express UCP1, characteristic features of thermogenic or brown adipose tissue (168, 169, 500, 567). Fetal adipocytes also contain larger or dominant lipid locules, and there is a direct relationship between the relative mass of the “unilocular” component of perirenal and interscapular fat and circulating leptin concentrations in a cohort of fetuses in well-nourished pregnant ewes (357). This suggests that that circulating leptin concentrations may be a signal of the unilocular component of fetal fat, rather than total fat mass in fetal life, as it is in the neonate and adult.

When the dietary intake of adolescent pregnant ewes was increased from a moderate to a high plane at 50 days of pregnancy, maternal plasma leptin concentrations increased within 48 h, and circulating leptin concentrations in the ewe were correlated with indices of maternal body composition at 50–90 days after the change in diet (507). A moderate increase (55%) in maternal nutrient intake above maintenance requirements increased maternal plasma glucose and leptin concentrations during late gestation, but there was no concomitant increase in either total fetal fat mass or in fetal plasma leptin concentrations (357). Although this nutritionally induced increase in maternal and fetal plasma glucose and insulin concentrations did not result in an increase in fetal leptin concentrations, infusions of glucose resulting in chronic fetal hyperglycemia with hyperinsulinemia for a 14- to 20-day period in late gestation has been shown to increase fetal fat mass (495) and leptin mRNA abundance in fetal perirenal adipose tissue (104). In well-nourished ewes, there is also a significant relationship between fetal glucose and the relative mass of fetal unilocular fat and a positive relationship between fetal insulin concentrations and the relative abundance of leptin mRNA in fetal perirenal adipose tissue (358). A moderate increase in maternal nutrition also resulted in a strong reciprocal relationship between leptin and UCP1 expression in fetal perirenal adipose tissue in late gestation. Interestingly, leptin mRNA expression in fetal adipose tissue was also selectively increased in response to an experimentally induced four- to fivefold increase in fetal insulin concentrations with maintained euglycemia (104). Thus fetal glucose and insulin differentially regulate fetal fat deposition and leptin mRNA expression within the fetal perirenal adipose tissue depot during late gestation.

Intrafetal leptin infusion in the presence of normoglycemia and normoinsulinemia results in an increase in the proportion of multilocular tissue and a decrease in the proportion and relative mass of unilocular tissue in the perirenal adipose depot, and the relative abundance of leptin mRNA in perirenal adipose tissue was also lower in leptin-infused fetuses (567). These findings suggest that leptin may act as a signal of energy supply and have a “lipostatic” role before birth. It is therefore possible that leptin in the fetal circulation derived either from the maternal circulation (as in the rat in late gestation and potentially the sheep in early gestation) or fetal adipose tissue (as in the sheep and human in late gestation) acts centrally via leptin receptors located on neurons within the fetal hypothalamus. The central actions of leptin may in turn result in a stimulation of the sympathetic nervous system and a subsequent decrease in the proportion of the unilocular adipose tissue and in the abundance of leptin mRNA in the fetal fat depots.

A reduction in maternal nutrition during late gestation resulted in a fall in maternal plasma leptin concentrations, but there was no effect of maternal undernutrition on the fetal plasma concentrations of leptin or on the relative abundance of leptin mRNA in the fetal perirenal adipose tissue (127, 566). Prolonged periods (longer than 36 days) of fetal hypoglycemia and hypoinsulinemia do result in a suppression of leptin mRNA expression in the perirenal adipose tissue of the sheep fetus (104). It has also been shown that there is no difference in plasma leptin concentrations at birth in low- and high-birthweight lambs, which reflects the relatively low and similar proportion of body fat present in these low- and high-birthweight animals (128).

When pregnant ewes are undernourished during the period of maximal placental growth (28–80 days gestation) and fed at either maintenance or above maintenance levels from 80 days gestation, there is an increase in total, although not relative, fetal fat mass and in adipose tissue IGF-IR and IGF-IIIR mRNA levels at 140 days gestation (40). While nutrient restriction did not result in a change in leptin mRNA expression in fetal adipose tissue, there was a decrease in leptin mRNA abundance in fetal adipose tissue at 140 days gestation in ewes that were fed on a high plane from 80 days gestation (40). During the period of nutrient restriction, maternal plasma cortisol
Concentrations were reduced and GR and 11BHSD1 mRNA expression were higher in perirenal adipose tissue of newborn lambs born to ewes that were nutrient restricted between 28 and 77 days gestation and then fed at maintenance energy requirements. Future studies are clearly required to determine the interactions between maternal nutrition, fetal adiposity, and the roles of glucocorticoid exposure, the IGFs, leptin, and other adipocyte-derived hormones in the programming of postnatal obesity in animals in which fat is deposited and leptin is expressed within fat depots before birth.

V. THE HYPOTHALAMO-PITUITARY-ADRENAL AXIS: A TARGET AND MEDIATOR IN THE DEVELOPMENTAL ORIGINS OF ADULT DISEASE

That an increase in circulating glucocorticoids may play a role in early programming is also supported by the range of cited studies (106, 111, 303, 356, 368) that have demonstrated that administration of glucocorticoids at critical windows of development result in impaired renal development, hypertension, glucose intolerance, and insulin resistance in the offspring. As discussed, there is evidence in a range of animal models that perinatal undernutrition may result in an increased exposure of the developing embryo and fetus to enhanced corticosteroids derived either from the maternal circulation across the placenta (as in the low-protein diet in the pregnant dam) or as a consequence of stimulation of the fetal HPA axis (as in the growth-restricted sheep or human fetus) (118, 281, 419). In part, outcomes following glucocorticoid exposure are dependent on programmed changes in GR and 11BHSD1 and -2 within different target tissues. There is also evidence that there may be programming of the HPA axis itself during critical windows of development such that the set point of the axis changes resulting in altered basal and/or stress induced glucocorticoid responses in postnatal life (338). This neuroendocrine programming could in turn contribute to the association between low birth weight and cardiovascular and metabolic disease in later life. Epidemiological studies in three populations have found that adults who had lower birth weight had raised fasting plasma concentrations of cortisol, and elevated plasma cortisol concentrations were also associated with higher blood pressure (414, 418). The association between cortisol and blood pressure was most evident in those people who were obese, and the authors considered that the prenatal setting of the HPA axis is a potential cause for the hypercortisolemia. (418). A subsequent study in Finland found that there was an inverse correlation between fasting serum cortisol and birth weight, in people born before 39 wk gestation, whereas there were positive correlations between fasting serum cortisol and birth weight in subjects born after 40 wk (247). Although low birth weight was strongly associated with an enhanced suppression by dexamethasone in people born after, but not before 40 wk gestation, there was, however, no correlation between birth weight and the adrenal response to ACTH(1—24) in this cohort (246). In a group of young historically disadvantaged, urbanized South African adults, plasma cortisol concentrations and the cortisol response to ACTH were greater in those who had a birth weight below the 10th centile (302). In this group of adults, the activation of the HPA axis was not dependent on full catch up growth or on adult obesity (302). In two cohorts sampled across a 24-h period in everyday living circumstances, there was, however, no correlation between birth weight and serum or salivary cortisol concentrations, and it was proposed that the programmed effects on the HPA axis may influence the reactivity of the axis rather than resting cortisol secretion (138, 246).

There appear to be a number of critical windows in development and a range of potential agents including exposure to maternal and fetal stressors, nutrition, and antenatal administration of synthetic glucocorticoids and postnatal maternal care and behavior that are important in programming the subsequent reactivity of the HPA axis (71, 338). We focus primarily on the impact of maternal or fetal undernutrition and fetal exposure to excess glucocorticoids.

A. Maternal Undernutrition and the Programming of the HPA Axis in the Rat and Guinea Pig

Exposure of the fetal rat to maternal undernutrition (50% decrease) during the last week of gestation results in an increase in plasma corticosterone at 19–21 days gestation and in the relative weight of the adrenal at term (299). In the newborn period, there was a reduction in adrenal weight and in GR and in mineralocorticoid receptor (MR) expression in the hippocampus and CRH expression in the PVN and in circulating ACTH. Programmed alterations in expression and activity of the GR and MR within the hippocampus and hypothalamus may result in a resetting of the negative-feedback actions of glucocorticoids on the hippocampal-HPA (HHPA) and an alteration in the basal and stress responsiveness of this axis in postnatal life. When maternal circulating corticosterone concentrations were maintained at constant levels, the induction of undernutrition was not associated with changes in the postnatal HHPA axis, suggesting that maternal undernutrition during late gestation induces overexposure of the fetus to maternal corticosterone, which programs the development of the newborn HHPA axis (299). In rats exposed to a low-protein diet in utero, plasma corticosterone concentrations were normal.
whereas plasma ACTH concentrations exhibited a blunted diurnal pattern (283). GR binding capacity and numbers were increased in the hippocampus or brains of rats exposed to low protein in utero during fetal, neonatal, juvenile, and adult life (37), again highlighting that there may be an altered regulation of the HHPA axis in this model. The guinea pig is a species in which rapid development of GR and MR and brain growth occur before birth and in this species, 48 h of maternal fasting activates the maternal, but not the fetal HPA axis, and also decreases GR but not MR mRNA levels in the developing hypothalamus and limbic system. (312).

B. Undernutrition and the Programming of the HPA Axis in the Sheep

In the sheep, it has been demonstrated that maternal undernutrition (50% decrease in energy intake) during late gestation activates the fetal HPA axis (125), and 10 days, but not 20 days of maternal undernutrition results in an increase in the ACTH response to CRH plus AVP at 30 mo of age (44). In this latter study, there was also no relationship between birth weight and the degree to which HPA function was perturbed, and the authors speculated that there may have been in utero catch up growth in fetuses exposed to 10 days, rather than 20 days of maternal undernutrition which resulted in the programming of the postnatal HPA axis (44). In the pig, there are large variations in birth weight among littermates, and it has been shown that in pigs of low birth weight, adrenal size and stimulated cortisol concentrations are increased at 3 mo of age (431). At 12 mo of age, thinness at birth was associated with an enhanced adrenal response to insulin-induced hypoglycemia (431).

Intriguingly, maternal undernutrition imposed during the periconceptional period in the sheep (for up to 60 days before and 70 days after conception) alters HPA function during later fetal and early postnatal life (45, 120, 122, 199–201). The specific impact of early undernutrition on the programming of the HPA axis appears to be dependent on the degree and length of undernutrition. Maternal undernutrition (15% reduction in maternal energy intake) from 0 to 70 days gestation results in lower ACTH and cortisol responses to a CRH plus AVP challenge in late gestation and a decrease in CRH mRNA expression in the fetal PVN (201), although both ACTH and cortisol responses to CRH plus AVP were increased in lambs at 3 mo of age (200). Severe maternal undernutrition (70% reduction) imposed from before conception until 30 days gestation resulted in an earlier prepartum increase in fetal cortisol concentrations in singletons (45), whereas a more moderate nutrient restriction (30% reduction) imposed from before conception until the end of the first week after conception did not result in an earlier activation of the fetal HPA axis in singletons (122). In the latter study, however, moderate maternal undernutrition imposed before and during the preimplantation period resulted in higher plasma ACTH concentrations and in an increased plasma cortisol response to a CRH challenge in twin fetuses during late gestation, and these effects were not reversed by the provision of a control diet for the remainder of pregnancy (122). Taken together, these data suggest that the periconceptional and/or the preimplantation period are critical windows during which maternal undernutrition may act to alter the set point of the function of the HPA axis. Potential mechanisms for this effect include an impact of the maternal hormonal and metabolic responses to undernutrition on the epigenetic regulation of gene expression within the developing embryo (122). These data are supported by the finding of the emergence of a subpopulation of corticotrophs that secrete high amounts of ACTH in the pituitaries of fetuses that were exposed to a perturbed intrauterine environment early in pregnancy (62).

C. Glucocorticoid Exposure During Pregnancy and the Programming of the HPA Axis in the Rat

Exposure of the fetus to excess glucocorticoid concentrations can also result in long-term programming of the HPA axis (383). In the rat, dexamethasone exposure in the last week of gestation increases CRH mRNA in the PVN, reduces hippocampal GR and MR expression, and increases plasma corticosterone concentrations in offspring at 16 wk. Administration of dexamethasone throughout gestation, however, results in an increased GR expression in the amygdala (303, 535). In the guinea pig, 2 days of antenatal glucocorticoid exposure results in a reduction in basal and stimulated plasma cortisol concentrations and an increase in hippocampal MR expression in adult males (96). In adult females, GR expression was lower in the PVN, whereas GR expression was higher and MR expression lower in the hippocampus, and plasma cortisol concentrations were increased after prenatal glucocorticoid exposure (96). It has been proposed that the differential effects of glucocorticoids on GR expression between tissues and species may relate to the complexity of the GR promoter that has multiple tissue-specific alternate first exons (untranslated) that are spliced on to the common translated sequence beginning at exon 2 (383). There is also evidence that differential methylation of specific promoter sites on this exon may be implicated in the transmission of stable, long-term differences in GR expression over the life span (533).
D. Glucocorticoid Exposure During Pregnancy and the Programming of the HPA Axis in the Sheep

While maternal undernutrition in early pregnancy in the sheep results in changes within the fetal and postnatal HPA axis, administration of dexamethasone to ewes for 2 days at 27 days gestation did not alter plasma ACTH or cortisol responses to hemorrhage of GR and MR expression in the hippocampus or hypothalamus (108). Similarly, while administration of cortisol during the same gestational age window results in a transient increase in GR and MR expression within the hippocampus some 100 days later during late gestation, this was not sustained into the postnatal period (105). Prolonged low-dose dexamethasone treatment between 25 and 45 days gestation specifically suppressed hippocampal expression of GR and MR in the late gestation fetus, but again, these changes were not maintained into postnatal life (350). When betamethasone was administered to ewes or their fetuses at between 104 and 125 days, however, it resulted in changes in the responsiveness of the HPA axis to a CRH plus AVP challenge at between 6 and 12 mo of age (481). These data highlight the importance of timing in relation to glucocorticoid exposure in determining whether such exposure will have long-term consequences for the basal and stimulated function of the HHPA axis.

VI. CONCLUSIONS AND FUTURE DIRECTIONS

It is clear from the preceding sections that the nutritional environment during early life is a determinant of postnatal health. Whereas the dominant focus of experimental studies to date has been on defining the phenotypic consequences of perturbations of maternal nutrition, the emphasis has now shifted to determining those initiating mechanisms through which early nutrition and associated growth patterns result in cardiovascular and metabolic dysfunction (for summary see Fig. 7). The size and scope of this field has grown to include the interests of geneticists, physiologists, and evolutionary biologists and is informed by clinical, epidemiological, and experimental studies in equal measure. There are emerging areas of critical interest including the extension of studies of the nutritional environment of the early embryo to include a better understanding of the impact of the perturbations of the environment of the gametes and embryo.

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FIG. 7. The physiological mechanisms underlying the programming of the separate and combined elements of the metabolic syndrome.
A. Epigenetics, Imprinting, and Programming

Epigenetics refers to covalent modifications of DNA and core histones that regulate gene activity without altering the nucleotide sequence of DNA. The best-characterized epigenetic modification of DNA is the methylation of cytosine residues within CpG dinucleotides (531). As recently reviewed (531) given that early nutrition may influence the establishment and maintenance of cytosine methylation, future research will focus on those classes of elements in the genome that are particularly sensitive to nutritional regulation in early life. There is a growing body of evidence from studies of in vitro embryo culture that the methylation status of genomically imprinted genes, including IGF2, H19, IGFB2R, etc., can be altered with consequences for subsequent organ growth and function (531, 564). Importantly, the epigenetic lability of imprinted genes is not limited to the preimplantation period and includes the early postnatal period in rodents (529). Recent studies have also demonstrated that retrotransposons are elements within the genome that may also be epigenetically labile to early nutrition (532, 553).

B. Species Differences: Developmental Deficits Versus Developmental Plasticity

Although there has been considerable progress in the definition of critical windows for programming of cardiovascular and metabolic outcomes in the rat, there remains a lack of detailed information on such critical windows for programming in species, such as the human, sheep, and pig. In the rat, a predominant response to a poor intrauterine environment as summarized above is the loss of structural units in developing organ systems (e.g., nephrons, cardiomyocytes, and pancreatic \( \beta \)-cells) resulting in a permanent functional deficit, and it is also the

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**FIG. 8.** Potential consequences of environment-epigenetic interactions for the health of the next and subsequent generations.
case that maternal hormonal responses (e.g., corticosterone, leptin) may play a major role in driving developmental changes within the fetus. Some of these changes may represent developmental deficits that are inevitable in the face of a restriction of substrate supply, whereas the maternal adaptive responses may ensure that the immature fetal rat can survive the intrauterine challenge. These maternal adaptations to a change in nutrient supply may be of greater importance in species such as the rat, where there is relatively limited capacity for a fetal neuroendocrine response to poor intrauterine nutrition. The maternal adaptive responses may confer immediate survival benefit but result in poor postnatal outcomes as offspring move into an environment that is not characterized by nutrient restriction. The concept of a “maternal predictive adaptive” response has not been integrated fully into the developmental origins of adult health field. In the human, sheep, and pig, organogenesis and organ development occur over longer periods of time, and maternal, placental, and fetal adaptations to nutritional manipulations in pregnancy may play a more major role in prenatal programming compared with the rodent in which maternal and neonatal responses have a dominant influence.

C. Gender, Plasticity, and Programming

It has become clear from the epidemiological studies summarized above that the relationships between the patterns of growth in early life and cardiovascular disease outcomes in later life are different in males and females. As discussed, there is also a differential impact of early nutritional manipulations on male and female offspring in experimental studies in a range of animal species, and the physiological basis for such outcomes is not understood. Studies that allow a better understanding of the growth trajectory and aspects of the hormonal milieu of the male and female infant are required to determine to what extent these aspects of early development are directly relevant to programmed health outcomes.

Finally, this review has focussed on a limited array of programmed outcomes: those most relevant to the meta-

![Summary of the Developmental Origins of Health and Adult Disease](Image)
bolic syndrome and the triad of cardiovascular disease, type 2 diabetes, and obesity, and there are important and informative studies highlighting the impact of early life experiences in determining a range of other important health outcomes including malignancies, mental health, and musculoskeletal health (Fig. 9). There are two implications as the “developmental origins” field expands in its scope and focus. First, there is a clear requirement to reconcile the balance of contribution of the “thirsty phenotype” and “thrift genotypes” in the generation of adverse health outcomes after a period of nutritional deprivation in early life. Determination of these respective contributions will also inform the evolutionary context of why certain physiological adaptations that improve the likelihood of survival of a developing organism that is under duress may carry a consequence for poor adult health outcomes after reproductive senescence. It is clear that there will also be a period during which the use of terms such as programming, plasticity, and predictive adaptive responses may each be hotly debated particularly as experimental and epidemiological studies investigate the impact of relative overnutrition in prenatal life. This is a necessary challenge for a field that has had its scope and focus. First, there is a clear requirement to investigate the impact of relative overnutrition in prenatal life.

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