Review Article

Potential mechanisms of metabolic imprinting that lead to chronic disease[1–3]

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ABSTRACT This review synthesizes a subset of human epidemiologic and experimental animal studies that suggest that early nutrition affects susceptibility to chronic diseases in adulthood. These studies provide evidence that biological mechanisms may exist to "memorize" the metabolic effects of early nutritional environments. However, hypothesis-driven investigations of potential mechanisms have been scant. Thus, our understanding of the biology underlying metabolic imprinting is incomplete. A working definition of *metabolic imprinting* is proposed, emphasizing the adaptive nature and limited ontogenic window of the mechanisms putatively responsible for these relations. Five specific candidate mechanisms of metabolic imprinting are elaborated: *1*) induced variations in organ structure, *2*) alterations in cell number, *3*) clonal selection, *4*) metabolic differentiation, and *5*) hepatocyte polyploidization. Last, experimental approaches for probing potential mechanisms with animal models are discussed. *Am J Clin Nutr* 1999;69:179–97.

KEY WORDS Metabolic imprinting, nutrition, development, early nutrition, chronic disease, obesity, cardiovascular disease, hypertension, type 2 diabetes mellitus, animal models, epidemiology, metabolism

INTRODUCTION

Humans and other animals are sensitive to external conditions that modulate ontogenic pathways and thereby exert persistent influences on diverse functional outcomes. This review synthesizes a subset of human epidemiologic and experimental animal studies suggesting that early nutrition affects susceptibility to chronic diseases in adulthood. Moreover, specific mechanisms are considered that may operate at the morphologic, cellular, or molecular level to store such information over a lifetime, and experimental approaches for probing these selected potential mechanisms are discussed.

Ongoing success in enhancing life expectancy and the desire to improve the quality of life at older ages have focused increased attention on diseases with adult clinical onset. The predicted global increase in the proportion of persons > 65 y of age (1), the ineffectiveness of treatment options for many diseases of old age, and rising medical costs each argues strongly for a greater focus on preventive strategies.

But at what age is it appropriate to begin targeting preventive approaches? Perinatal nutritional status is recognized to have a profound and persistent influence on neurologic development and cognitive function. Similarly, classic epidemiologic studies of survivors of the Dutch famine of 1944–1945 suggest that perinatal nutrition influences adult body mass index (BMI), often considered a proxy for body composition. More recent evidence that links low birth weight to an increased risk of chronic diseases in old age has generated renewed interest in preventive approaches initiated in the perinatal period.

Specifically, data from human epidemiologic studies and animal models strongly suggest that perinatal nutrition affects predispositions to adult obesity, cardiovascular disease (CVD), hypertension, and type 2 diabetes (*see* references 2–9 for reviews). For some, a focus on the perinatal period seems misplaced. Adults with modifiable risk factors may misconstrue discussions of predetermined risk as added excuses for not altering current behavior. Alternatively, the possibility of significantly reducing long-term risk via focused interventions in early life suggests an efficacious complement to prevention strategies undertaken at later life stages.

Understanding the underlying biology, however, is a prerequisite for the design of prospective clinical studies of the effects of perinatal nutritional status on adult disease and for the possible identification of interventions to improve health throughout peoples' lifetimes. As is reviewed below, such interventions may be particularly important in populations that experience high rates of low birth weight. Whatever the public health implications of data that link perinatal nutrition to adult disease may be, exploring the underlying mechanisms is in itself important. The underlying biology of these putative relations is at best only generally understood and is amenable to investigation. Accordingly, although salient findings from epidemiologic and animal studies linking early nutritional experiences to the expression of metabolic alterations in adulthood will be summarized, the principle focuses of this review are possible underlying biological mechanisms and the identification of experimental approaches that may help test mechanistic hypotheses.

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Links between nutrition and common morbidities

Adult obesity, CVD, hypertension, and type 2 diabetes are exceptional because of their high prevalence and complex interrelatedness. In the United States, data from the third National Health and Nutrition Examination Survey (NHANES III) indicate that one-third of US adults were overweight in 1991 (10). Recent estimates are even higher (11). Overweight puts individuals at elevated risk of CVD, hypertension, and insulin resistance and hyperinsulinemia. CVD is the most common cause of death in industrialized nations, especially among older age groups. One-third of US men and 10% of women develop CVD by age 60 y. CVD causes 70% of all deaths beyond the age of 75 y (12). Obesity and hypertension are established risk factors for CVD (13). More than 10% of Americans aged 65–74 y have type 2 diabetes (14). Hyperinsulinemia associated with type 2 diabetes and obesity-associated insulin resistance is a common component of syndrome X, which also encompasses dislipidemia, hypertension, and central obesity (15). Despite the common coexistence of those conditions, most epidemiologic studies that link perinatal nutrition to them, and animal studies that ascertain causality between them and specific nutritional conditions, consider obesity, CVD, hypertension, and type 2 diabetes individually. This review therefore treats each outcome individually, but also explores basic underlying biological mechanisms that may account for the concomitant occurrence of these morbidities.

Metabolic imprinting

The term *metabolic imprinting* is used in this review to describe the basic biological phenomena that putatively underlie relations among nutritional experiences of early life and later diseases. The term is intended to encompass adaptive responses to specific nutritional conditions early in 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 (that may differ quantitatively among individuals), and *4*) a dose-response or threshold relation between a specific exposure and outcome. Although previous workers have used the term *programming* to refer to the long-lasting effects of early nutritional experiences, *imprinting* more effectively conveys the salient features detailed in the first 2 parts of the definition adopted for this review. There is a clear historical precedent for this term. Konrad Lorenz (16) chose *imprinting* to refer to the setting of certain animal behaviors that resulted from early experience. Central to his definition was the fact that imprinting may only occur during "a narrowly-defined period in the individual's life" (the critical period) and, moreover, that the imprinted behavior "cannot be 'forgotten'!". These coincide with the first 2 parts of the definition of metabolic imprinting. The last 2 parts of the definition narrow the biological phenomena under consideration to specific relations appropriate for mechanistic characterization.

It is useful to distinguish metabolic imprinting from other biological phenomena that share similar qualities. Consider, for example, the developmental processes that enable the immune system to distinguish self from nonself. Early in development, fetal exposure to self antigens results in immunologic self-tolerance. Exposure to specific nonself antigens during this period results in persistent tolerance of them as well. Fetuses have an exceptional ability for tolerance induction, and this ability is reduced dramatically with age (17), suggesting a graded critical window that, although maximally sensitive in utero, extends into adulthood. Hence, the immunologic memory conveying tolerance to self and nonself antigens shares many of the hallmarks of imprinting phenomena.

Another relevant example is hormonal imprinting. If day-old male rats are injected with supraphysiologic concentrations of insulin, a permanent decrease in insulin responsiveness results that is correlated with a defect in insulin binding to hepatic membrane receptors. Such experiments suggest that early exposure to a hormone plays a role in the ontogeny of its cell-surface receptors (18), resulting in a sort of endocrinologic memory. Injecting adult rats with insulin has no such persistent effects, indicating that the critical window for imprinting insulin receptors does not extend into adulthood in rats.

EPIDEMIOLOGIC EVIDENCE OF METABOLIC IMPRINTING

By their nature, epidemiologic data can rarely provide definitive answers. This is especially true for the epidemiologic data pertaining to metabolic imprinting. The lengthy period of time between exposure and outcome, the imprecise quantification of exposure measurements and important covariates, and other inherent weaknesses of retrospective studies all limit our ability to draw causal inferences from these data. [These weaknesses were recently elaborated in an excellent review by Joseph and Kramer (19).] No single relation or data set is entirely convincing on its own for these reasons. However, the concordance of the evidence both within and across the various phenomena under consideration presents a picture of subtle metabolic imprinting effects that may synergistically exert a significant effect on human health.

Body mass index

We will review the imprinting of BMI first because of its important implications for the other outcomes of interest. Among the most compelling epidemiologic evidence of metabolic imprinting of BMI is the retrospective study of young male army draftees who were exposed perinatally to famine conditions during the Dutch famine of 1944–1945. Ravelli et al (20) studied 300 000 men aged 19 y and found that, compared with those born in non-famine-stricken control areas, men who were exposed in utero to famine conditions at some time during the first 2 trimesters experienced an 80% higher prevalence of overweight ($P < 0.0005$). Those exposed to famine for some period during the last trimester or the first 5 postnatal months experienced a 40% lower prevalence of overweight than in those not exposed ($P < 0.005$).

Others reported a high positive correlation between birth weight and adult BMI. These associations have supported speculations that birth weight is a proxy for adequacy of fetal nutrition, and thus that fetal nutrition modulates the risk of adult overweight. The high heritability of birth weight (21) and the well-established postnatal tracking of body size raise the question of whether maternal obesity may confound the association between birth weight and later body size. To address this question, Curhan et al (22) analyzed data from the US Nurses' Health Study. They reported that the women in high-birth-weight categories had increased odds of being in the highest adult BMI category. The odds were unaffected by adjustment for the respondent's characterization of their mother's figure at age 50 y, supporting the imprinting hypothesis. But recall errors and

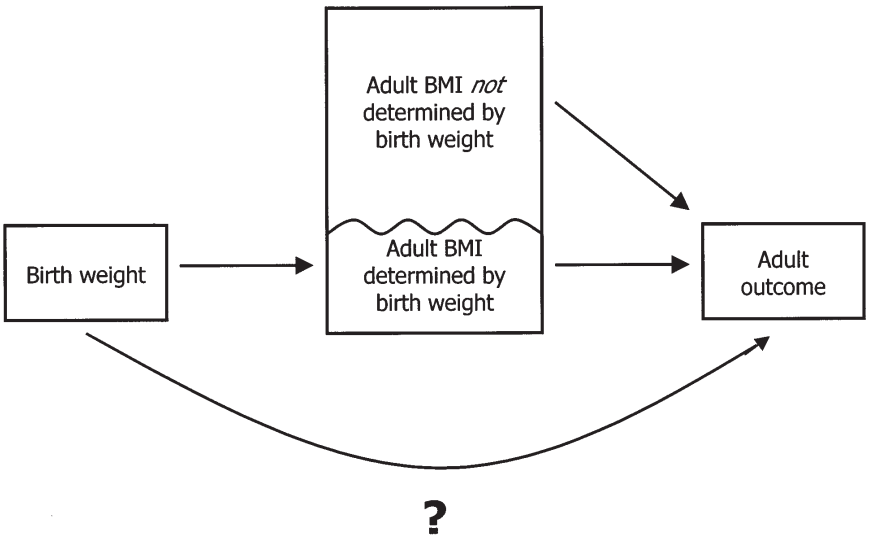

FIGURE 1. Conceptual model partitioning BMI into 2 components: one component is determined by birth weight and the other is independent of birth weight. Only by accurately partitioning BMI in this manner can the contributions of adult BMI be appropriately adjusted for in analyses linking birth weight to adult outcomes associated with BMI.

respondent biases limit the value of this approach. In a more robust study of 2880 monozygotic twins, Allison et al (23) found no correlation between intrapair birth weight differentials and BMI differentials at ≈40 y of age. These data appear contrary to the imprinting hypothesis. Because monozygotic twins are genetically identical, this design controls completely for the influence of genetics on birth weight. However, these results may be biased by the many social forces that tend to artifactually increase the similarity between monozygotic twins. Interestingly, the intrapair birth weight differential and later intrapair differentials in both height and weight were highly correlated, suggesting metabolic imprinting of body size but not BMI.

Whatever the mechanism, the possibility of a biological link between birth weight and adult BMI complicates the numerous studies that link birth weight to other adult outcomes such as CVD, hypertension, and insulin resistance. Acknowledging the strong correlation between overweight and these other outcomes, many authors adjust for BMI when evaluating the associations of outcomes of interest with birth weight. There are 2 reasons for this. First, one may wish to reduce the error variance in outcome by using BMI as a covariate. However, doing so may lead to misleading interpretations (24) because BMI does not follow the same distribution in different birth weight categories. Second, because adult BMI may be an intervening variable between birth weight and adult outcome, one may wish to correct for this to determine the magnitude of the birth weight effect that is not mediated by BMI. However, this would require that the variance in BMI be partitioned into 2 components, one associated with and the other independent of birth weight (**Figure 1**). Although this conceptual model has theoretical appeal, it is unclear how such partitioning could be estimated.

To understand the effect of potential confounding by adult BMI, consider an outcome that is positively associated with BMI, such as CVD. If the association between birth weight and adult CVD is not adjusted for BMI, one would expect a positive association between birth weight and adult CVD based solely on the correlation between birth weight and adult BMI. Conversely, adjusting for adult BMI may have the opposite effect. In considering the model partitioning variance in BMI (Figure 1), one may assume that the component of BMI not associated with birth weight is most strongly related to lifestyle factors that would predict adult CVD risk. Hence, when comparing 2 individuals of the same adult BMI (ie, adjusting for BMI), the person with low birth weight would be expected to have a greater component of BMI not associated with birth weight (**Figure 2**). Accordingly, this person's BMI-associated risk of CVD would be higher. This rather simplistic model disregards potential interactions between birth weight and adult BMI and CVD risk, but nonetheless illustrates the potential for BMI adjustment to lead to incorrect interpretations. Thus, although it may be informative to analyze such relations by adjusting for BMI, the problems with such an approach suggest that associations adjusted and not adjusted for BMI should be reported. This has seldom been done.

Other investigators have reported data suggesting that early postnatal nutritional influences may also influence adult body composition. This approach avoids the troublesome use of birth weight as a proxy for gestational nutritional sufficiency. However, associations between later body habitus and early predictors such as rate of weight gain in infancy (25) or infant feeding mode (26) generally have not provided substantive evidence of metabolic imprinting. Nonetheless, the seemingly paradoxical findings from the Dutch famine cohort are consistent with the hypothesis that both the prenatal and neonatal periods imprint BMI. The cohort that was exposed to famine throughout the last trimester of gestation and the first few months of postnatal life experienced a significant decrease in birth weight and a lower prevalence of overweight in young adulthood. On the other hand, there was no birth weight effect in the cohort exposed to famine only during the first 2 trimesters (27). If we consider only birth weight as a predictor of adult BMI, it seems inconsistent that the cohort in whom birth weight was unaffected had an elevated prevalence of overweight in adulthood. However, if compensatory refeeding after liberation resulted in infantile

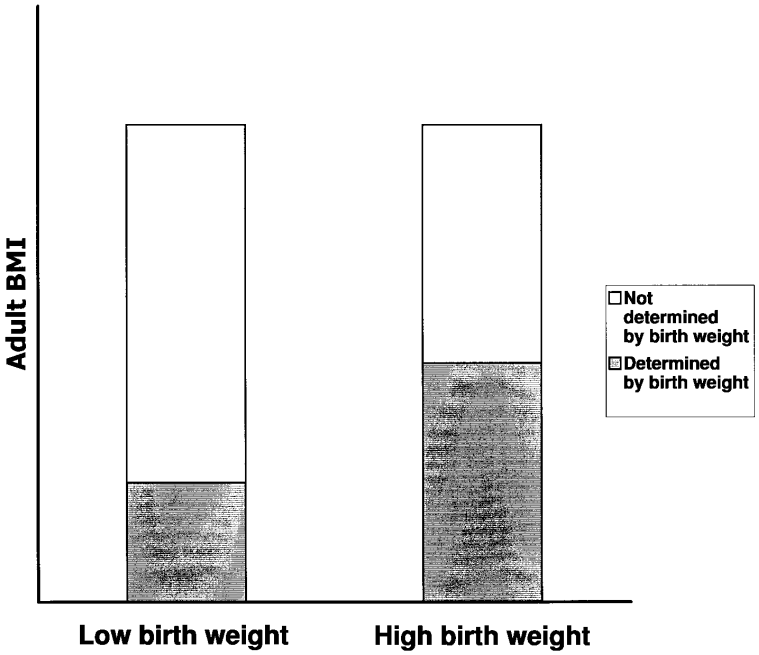

FIGURE 2. Illustration of how controlling for adult BMI might cause an inverse relation between birth weight and cardiovascular disease risk. Controlling for adult BMI effectively compares individuals of the same adult BMI. Compared with individuals with low birth weight of the same adult BMI, individuals with high birth weight would, on average, have a smaller component of BMI that is not associated with birth weight. If we assume that the component of BMI not associated with birth weight is most strongly related to lifestyle factors that predict adult cardiovascular disease risk, then individuals with high birth weight would have a lower risk of adult cardiovascular disease.

overnutrition, an elevated prevalence of later overweight among individuals exposed to famine conditions in early gestation would be consistent with the findings of studies suggesting imprinting of BMI in the early postnatal period.

In summary, data from several epidemiologic studies suggest that adult BMI may be imprinted both prenatally and postnatally. The most compelling data, drawn from the retrospective Dutch famine study, suggest that the critical window for BMI may extend throughout the perinatal period. The relation between birth weight and later BMI, whether reflective of imprinting or not, complicates the many epidemiologic studies reporting associations between birth weight and adult outcomes associated with BMI.

Cardiovascular disease

The evidence for early nutritional influences on later CVD was reviewed recently by Leon and Ben-Shlomo (28). The first indication that perinatal circumstances might affect susceptibility to coronary heart disease (CHD) in adult life arose from ecologic studies (29–31). These showed a strong correlation between birthplace and later CHD risk. Specifically, high rates of perinatal mortality appeared to be predictive of elevated adult CHD mortality. The simplest explanation of this relation is that of "continuity of circumstance." In other words, the same general poor environment that leads to high perinatal mortality may also act at later ages to predispose a population to elevated CHD mortality, as suggested by Ben-Shlomo and Smith (32).

Stronger links between perinatal nutrition and adult CVD were suggested by retrospective cohort studies of fetal and infant growth (based on records of birth weight and weight at 1 y of age) and adult incidence of CVD morbidity and mortality. Much of that evidence was derived from records that were collected by an exceptionally thorough midwifery system operating in Hertfordshire, United Kingdom, from 1911 to 1930. Osmond et al (33) linked mortality data to those records and tracked the individuals still living in the area. Analyses of the Hertfordshire data on >15 000 individuals indicated that CVD mortality was inversely related to birth weight in both men and women, and that it was similarly related to weight at age 1 y in men but not in women (33). However, because those data at 1 y of age were not adjusted for birth weight, it is unclear whether the relations with weight at age 1 y indicate postnatal imprinting of CVD susceptibility or merely reflect the primary relation with birth weight. The relations between birth weight and CVD mortality in the Hertfordshire cohort were corroborated in part by morbidity data on a subsample of men (n = 290) who still lived in Hertfordshire. Fall et al (34) found an inverse relation between weight at age 1 y and prevalence of CHD, but no such trend was found with birth weight. This is inconsistent with an inverse relation between CHD mortality and birth weight.

Barker et al (35) reported relations between birth weight and CVD mortality in a cohort of 1586 men born in Sheffield, United Kingdom, from 1907 to 1924. CVD mortality was inversely related to birth weight in this cohort. Furthermore, ponderal index at birth (given as oz/in^3) was inversely associated with CVD mortality.

As reported by Leon and Ben-Shlomo (28), the inverse relation between birth weight and adult CHD was confirmed in 4 of 5 retrospective studies: in 121 000 women aged 30–55 y in the US Nurses' Health Study (36); in 517 men born in Mysore, India, between 1934 and 1954 (37); in 1335 men born between 1920 and 1924 in Uppsala, Sweden (38); and in 1258 men aged 45–59 y in Caerphilly, South Wales (39). However, concern remains regarding the inferences that may be drawn collectively

from them. One source of uncertainty is the potential for biases imposed by the relatively high proportion of each birth cohort that was lost to follow-up as a result of migration or nonparticipation (19). This is a valid concern only if the relation between fetal growth and CVD differs between those who migrated and those who did not. Although such a difference is not intuitive, it is plausible. For example, a recent study suggests that these associations may be confounded by correlations between maternal CVD risk factors and probability of migration. Specifically, in a cohort of individuals born in Oxfordshire, United Kingdom, between 1965 and 1986, risk of migration was positively related to social class and inversely related to maternal systolic blood pressure (SBP). Also, the data suggested an increased risk of migration in the group with the lowest birth weight (40).

Another potential confounding variable acknowledged in all of those studies is socioeconomic status (SES), consistent with the alternative continuity of circumstance hypothesis. SES is associated with both birth weight and CVD risk. Although adjusting for SES does not alter the findings of most studies, such adjustments may often be inadequate, raising the possibility of significant residual confounding. For example, several studies that purported to adjust for social class at birth were unable to replicate well-established relations between social class and birth weight (19). Such unexpected results suggest that these studies may not have used a meaningful measure of social class. Conversely, in the Mysore, India, study (37), in which a broad range of well-defined social classes was represented, prevalence of CHD was correlated highly with social class. When the analyses were adjusted for social class, the effect of birth weight on CHD was no longer significant.

The relation between birth weight and adult BMI suggests another potential confounding factor in the association between birth weight and adult CVD risk. As discussed earlier, without adjustment for BMI, one would expect a positive correlation between birth weight and adult CVD because of the correlation between birth weight and adult BMI. Conversely, adjustment for adult BMI is expected to have the opposite effect. Note that inverse correlations between birth weight and adult CVD were found both in studies of CVD mortality (in which no BMI adjustment is possible) and in studies of CVD prevalence (some of which use BMI adjustment). Also, in one study that reported BMI-unadjusted and -adjusted data (39), the findings were not affected by the adjustment, supporting the theory that the birth weight–BMI association does not result in a spurious link between birth weight and adult CVD.

Although these methodologic issues are acknowledged, the preponderance of data suggests an inverse association between birth weight and adult CVD risk, and thus supports the prenatal imprinting of metabolic risk factors for CVD. The qualitative and quantitative similarities of this association among diverse cohorts underscore the generalizability of the association and likely basic biological underpinning. There is, however, little epidemiologic data suggesting that imprinting of adult CVD risk occurs in the early postnatal period.

Blood pressure

The association between fetal growth and adult blood pressure is well documented and suggestive of metabolic imprinting. A recent compilation of numerous studies supports the hypothesis that adult SBP is related inversely to birth weight over a wide range of birth weights (41). The earliest report of this relation focused on a 1946 British birth cohort. In that retrospective study, 3322 individuals were followed up to assess the influence of "social and family factors, smoking and body mass" at 36 y of age (42). Social class and birth weight were both correlated inversely with SBP at age 36 y; surprisingly, the authors did not emphasize the significance of the independent association with birth weight. In a later report, Barker et al (43) studied 449 English men and women identified in birth records from 1935 to 1943. In that sample, adult SBP was correlated inversely with birth weight and directly with placental weight. Further studies have corroborated this inverse correlation between birth weight and adult SBP in different populations, including young Croatian adults (44), elderly British women (45), 50-y-old Swedish men (46), and American women aged 30–55 y (22). However, in a retrospective study of 32 580 Israeli adolescents, Seidman et al (47) found a slight positive correlation between birth weight and SBP at age 17 y.

Generally, adjustment for current BMI strengthens the already significant inverse association between birth weight and adult blood pressure. Thus, it is unlikely that this effect only reflects tracking of body size. Nonetheless, analyzing the data by current weight or BMI category has led to disparate results. For example, Leon et al (46) found that the birth weight–blood pressure correlation was strongest in men in the upper tertile of BMI. These authors suggested that excess adiposity in adulthood potentiates the detrimental infuence of lower birth weight on blood pressure. But others have not confirmed this interaction. Other reports state that the inverse correlation between birth weight and SBP is maintained across all current BMI categories; however, inspection of those data often indicates otherwise. Examination of several studies that categorized the data by current BMI suggests that the middle BMI category shows this relation most robustly.

The greatest weakness of this relation is its potential confounding by factors that are difficult to measure, and therefore to adjust for. First, because birth weight and SBP are associated positively and negatively, respectively, with SES, a large component of the birth weight–SBP relation could be mediated by an effect of continuity of circumstance via SES. Many studies include a proxy for SES in their models and find that the relation between birth weight and adult SBP remains significant and is often unattenuated by this adjustment. However, considering the difficulty in assessing SES, especially over a lifetime, residual confounding is likely (19).

Another potential confounding factor is maternal blood pressure. The simplest explanation for a relation between birth weight and adult blood pressure is that maternal blood pressure affects infant birth weight via some physiologic mechanism during gestation and also influences the offspring's adult blood pressure genetically. This possibility has been discounted on the basis of data showing that infant birth weight is unrelated to the blood pressure of pregnant women at their first antenatal visit (43). However, a recent study (48) found that maternal 24-h ambulatory blood pressure measurements at different stages of pregnancy are correlated inversely with infant birth weight. This finding suggests that genetic endowment remains a potential confounding factor between birth weight and blood pressure.

To summarize, retrospective studies in diverse populations have found that birth weight is inversely correlated with adult blood pressure. Although each of the studies has some weaknesses, together they support a biological link between intrauterine growth and adult blood pressure. It remains unclear

whether this connection reflects imprinting of blood pressure or some other phenomenon. No epidemiologic evidence currently suggests the imprinting of blood pressure postnatally.

Impaired glucose tolerance and type 2 diabetes

Several large, retrospective cohort studies have identified an inverse correlation between birth weight and measures of glucose tolerance in adulthood. This literature was reviewed recently by McKeigue (49). The first published investigation of this relation measured plasma insulin and glucose concentrations (fasting and after an oral glucose load) in 408 men aged 64 y from the Hertfordshire birth cohort (50). After adjustment for current BMI, odds ratios for impaired glucose tolerance (IGT) or diabetes declined monotonically from 6.6 to 1 as birth weight category increased from ≤2.5 to ≥4.3 kg. This group later found a similar relation in another English cohort of 266 men and women aged 50 y (51). The odds ratios for IGT or newly diagnosed type 2 diabetes were 3.5-fold higher in men and 12-fold higher in women with birth weights ≤2.5 kg relative to those whose birth weights were >3.4 kg. Furthermore, intravenous insulin tolerance tests conducted in 103 members of this cohort showed that adult insulin resistance was likewise related inversely to ponderal index (kg/m^3) at birth (the relation with birth weight was not significant) (52).

The relation between birth weight and adult glucose tolerance was corroborated further in a cohort of 413 Mexican American men and women with a mean age of 31 y from the San Antonio Heart Study (53). Specifically, fasting insulin concentration was related inversely to birth weight in women ($P = 0.001$), but no such relation was found in men. In a study of 1179 Pima Indians 20–39 y of age, McCance et al (54) related birth weight to IGT. Unlike others, they found a strong U-shaped association between birth weight and IGT and type 2 diabetes. Age-adjusted prevalence of type 2 diabetes in the lowest and highest birth weight categories was twice that in the middle birth weight categories. This relation appeared to hold across quartiles of age and across tertiles of current BMI. Furthermore, unlike previous studies, the association was of similar magnitude in men and women. Recently, Lithell et al (55) reported an association between birth weight and IGT in a cohort of 1333 Swedish men aged 50–60 y. They found that glucose concentrations 60 min after intravenous glucose infusion were significantly inversely correlated with birth weight. Moreover, after adjustment for current BMI, significant inverse relations were found between birth weight and fasting serum insulin and glucose concentrations.

These compelling studies are characterized by the same potential for confounding that is common to studies relating birth weight to the other outcomes that have been discussed. First, there is the problem of adjusting for current BMI. In all cases, such adjustments markedly enhanced the relation with birth weight, and in some studies, the unadjusted relation was not statistically significant. As discussed earlier, adjustment for a variable that may be in the causal pathway may lead to inappropriate conclusions. Also, it seems questionable that SES is adjusted for adequately in most studies. Several studies that used some proxy measure of SES to adjust for its effects reported no effect of the adjustment on the relation between birth weight and adult glucose tolerance. However, only the San Antonio study replicated the well-established inverse relation between SES and glucose intolerance (53). This suggests that other researchers either did not measure SES adequately or had too homogeneous a sample to detect its effect. Furthermore, genetics may also explain the association between birth weight and type 2 diabetes or IGT. For example, a fetus's genetic tendency for insulin resistance may reduce fetal growth directly and subsequently predispose the individual to glucose intolerance throughout life. In contrast with what is observed in maternal gestational diabetes, reduced fetal weight was shown in a rat model of streptozocin-induced fetal diabetes (56). This demonstration is consistent with this alternative explanation.

Other approaches to investigating potential prenatal imprinting of glucose tolerance have supported the inverse and in some cases U-shaped relations between birth weight and adult IGT. For example, Pettitt et al (57) studied offspring of Pima Indian women who were diabetic during the index pregnancy. To control for genetic influences on diabetes, the prevalence of diabetes in the 10–24-y-old offspring from diabetic pregnancies was compared with that in the offspring of prediabetic women (who developed diabetes at some time after the index pregnancy). Even after the mother's age of onset of diabetes was controlled for, the age-adjusted prevalence of diabetes in offspring of a diabetic pregnancy was twice that in offspring of prediabetics. These results are consistent with those of McCance et al (54) in suggesting that prenatal overnutrition may persistently increase an individual's susceptibility to glucose intolerance. In a recent retrospective study of 702 adults from the Dutch famine cohort, Ravelli et al (58) tested the hypothesis that prenatal undernutrition causes IGT in adulthood. They found that plasma glucose concentrations 2 h after a glucose load were higher ($P = 0.006$) in individuals who had been exposed to famine prenatally than in individuals born before the famine. Of individuals with prenatal famine exposure, the highest 2-h glucose concentrations were found in those who were exposed during late gestation. Because these individuals also had the lowest mean birth weight, these findings are consistent with the studies linking low birth weight to adult IGT.

Hence, as with the other outcomes discussed here, evidence suggests an association between prenatal nutritional environment and type 2 diabetes and IGT in adulthood. Specifically, studies indicate that either prenatal undernutrition or overnutrition may impair glucose tolerance in adulthood. This apparent discordance may be consistent with distinct imprinting mechanisms operating at both extremes of prenatal nutritional sufficiency. It is interesting that most studies found this relation to be much stronger in women than in men. Because alternative hypotheses about the mechanism underlying this association have not been examined adequately, it is unclear whether the insulin axis is imprinted in utero. No epidemiologic evidence suggests postnatal imprinting of type 2 diabetes or IGT.

To summarize, there is substantial epidemiologic evidence that birth weight is linked to adult BMI, CVD, blood pressure, and glucose tolerance. However, inherent weaknesses of long-term retrospective studies and insufficient consideration of alternative hypotheses undermine inferences that attribute these associations to intrauterine metabolic imprinting. In all of the outcomes, the evidence for prenatal imprinting is stronger than that for postnatal imprinting. This, of course, does not refute the occurrence of postnatal effects, but may simply reflect the lack of a simple and universally recorded index of postnatal nutrition analogous to birth weight. Many of the weaknesses of epidemiologic data can be addressed by the controlled conditions of animal models. Accordingly, the numerous animal models used to

examine metabolic imprinting phenomena merit consideration. The following discussion focuses on investigations that model the nutritional exposures and outcomes most relevant to the epidemiologic observations that have been discussed.

ANIMAL MODELS OF METABOLIC IMPRINTING

Body composition

Long-term effects of intrauterine undernutrition have been studied extensively in the rat model. Stephens (59) cross-fostered the offspring of initially well-nourished dams either provided free access to food or restricted by 50% during pregnancy and followed these progeny to assess body weight and body composition at 130 d of age. This design allowed the effects of restriction limited to the intrauterine period to be distinguished from potential carryover effects of gestational restriction mediated via maternal lactation. Despite a 20% decrease in birth weight in the prenatally restricted animals, their body weights equaled those of the control group by the end of the weaning period, and no effect on body weight or composition was detected thereafter.

The effect of the timing of prenatal energy restriction on adult body composition was probed in greater depth as several workers sought to model the relations between perinatal restriction and adult BMI suggested by the Dutch famine data (20). Indeed, several groups found that when rats were energy restricted during the first 2 trimesters and then allowed free access to food, their offspring became 10–20% heavier and fatter than the progeny of nonrestricted controls (60, 61). Curiously, most studies showed this effect only in male offspring. The increased adiposity of those animals appeared to be attributable to adipocyte hypertrophy rather than to hyperplasia (62). Jones et al (63) postulated that the increased adiposity seen in the adult offspring of prenatally restricted rats could be because of the compensatory hyperphagia that follows a period of restriction rather than because of the restriction per se. Accordingly, the offspring of 2 groups of dams that were energy restricted during the first 2 trimesters were studied (63). In one group (RA), mothers were allowed free access to a stock diet from the third trimester through lactation, whereas the others (RP) were pair fed (with a never-restricted control group) from the third trimester onward to prevent compensatory hyperphagia. At 200 d of age, the RA male progeny were heavier and fatter than the male progeny of the nonrestricted controls. However, the mean body weight and composition of the RP progeny were similar to those of the controls. These findings suggest that maternal compensatory hyperphagia was the cause of the offsprings' later elevated adiposity. Unfortunately, it is unclear whether the effect of the dams' compensatory hyperphagia was mediated gestationally or during the suckling period. As in the other studies, the increased adiposity in the RA males correlated with increased adipocyte size, not number.

Litter manipulation in rodents has also been used extensively to study the long-term effects of postnatal under- and overnutrition. In this model, pups are redistributed shortly after birth to either small or large litters, resulting in overnutrition or undernutrition during the suckling period, respectively. This model provided some of the earliest evidence for a critical window in the imprinting of body size and body composition. Widdowson and McCance (64) showed that rats undernourished during the suckling period followed a permanently diminished growth trajectory, whereas those undernourished to a similar extent after weaning quickly compensated for the period of slowed growth and resumed the growth trajectory of their normally nourished littermates. Subsequently, several studies showed that relative to rodents raised in normal-sized litters, those suckled in small litters were heavier and fatter as adults and those suckled in large litters were smaller and leaner throughout life (65, 66). The persistent effects on weight and adiposity ranged from 10% to 40% higher or lower than control values. Studies focusing on changes in adult rodent adipocyte morphology associated with diverse litter sizes concluded that the effect on adiposity was mediated via changes in adipocyte number rather than size (67–69). This finding is opposite that found in offspring of rodents restricted during gestation.

In another model examining the long-term effects of postnatal nutrition on later adiposity and adipocyte morphology, Lewis et al (70) artificially fed baboons formulas of different energy densities to effect normal nutrition, 31% overnutrition, or 40% undernutrition throughout the suckling period. At 5 y of age, female baboons that had been overnourished in the preweaning period were 40% heavier than their normally fed counterparts. The increased weight was correlated with elevated adiposity; the mean weight of 10 different fat depots in the 5-y-old postnatally overnourished female baboons was 5 times that of the normally nourished females. Although no body weight effect was seen in the males at 5 y of age, the mean weight of the 10 adipose depots in the postnatally overnourished males was 3 times that of the control males. In both males and females, the increased adiposity was primarily attributable to an increase in adipocyte size, not number, in contrast with the association found in rats suckled in small litters.

Cumulatively, these animal studies suggest that nutritional status during the early postnatal period has profound and persistent effects on body weight and adiposity. On the other hand, fetuses seem to be resistant to metabolic imprinting of weight and adiposity resulting from intrauterine undernutrition. It would be interesting to test in an appropriate animal model the putative long-term effects of overnutrition that are limited to the prenatal period, as is suggested by the epidemiologic relations between birth weight and later adiposity.

Cardiovascular disease

Although many of the established risk factors for CVD, such as obesity, hypertension, and insulin resistance, have been considered in this review as separate metabolic imprinting outcomes, abnormal lipid profiles have not. Hypercholesterolemia and elevated ratios of LDL to HDL are well-accepted major causal risk factors for CVD (13). Hence, to avoid overlap with other areas, this section will focus on studies suggesting metabolic imprinting of blood lipid profiles or lipid metabolism.

Most of the epidemiologic evidence for metabolic imprinting of CVD is based on birth weight, implying the importance of prenatal nutrition. Unfortunately, the vast majority of the animal model studies related to metabolic imprinting of CVD are based on postnatal nutritional treatments or on combined prenatal and postnatal treatments. In one study that examined the long-term effects of prenatal nutrition on adult plasma lipids, Lucas et al (71) provided rats a protein-restricted diet during pregnancy, lactation, or both and cross-fostered the offspring to independently assess the effects of exposure to the deficient diet during

these 2 periods. Plasma total cholesterol, HDL-cholesterol, and triacylglycerol concentrations were all 20–50% lower in the 6-mo-old offspring of animals exposed to the low-protein diet during both gestation and suckling and in offspring of animals exposed to the low-protein diet during suckling only than in controls. Only the decrease in plasma triacylglycerol concentrations was significant in the group exposed to a low-protein diet during gestation only. These results, of course, are contrary to expectations based on the inverse relation between birth weight and adult CVD in humans.

In 1972, Reiser and Sidelman (72) published a highly controversial study of the hypothesis that ingestion of diverse concentrations of cholesterol in mother's milk imprints cholesterol metabolism in the progeny. In their small rat study, the dams' diets were varied to affect milk cholesterol concentrations. Serum cholesterol concentrations in male offspring fed a high-fat, high-cholesterol postweaning diet (cholesterol-challenge diet) were related inversely (through 32 wk of age) to the cholesterol concentration in their mothers' milk. At 32 wk of age, the serum cholesterol concentration of male offspring who had consumed the highest concentration of milk cholesterol during the suckling period was only 60% of that of control animals whose mothers had been fed a stock diet during lactation. In a later study that attempted to replicate this result, Kris-Etherton et al (73) obtained elevated milk cholesterol concentrations by feeding a high-fat, high-cholesterol diet to dams from day 18 of pregnancy though lactation. At 26 wk of age, the male progeny of these dams had 25% higher serum cholesterol concentrations than control animals. Note that these studies are not necessarily contradictory, because the former varied the dams' diet only postnatally, whereas the latter applied the dietary treatment starting from late gestation.

Results of subsequent studies of the persistent effects of perinatal maternal fat and cholesterol intakes were generally consistent with the findings of Kris-Etherton et al. Rats whose mothers were fed a high-fat diet from late gestation through lactation showed greater elevations in serum cholesterol than did controls in response to a cholesterol-challenge diet in adulthood (74, 75). Strictly speaking, however, the study of Reiser and Sidelman, in which the dams' diets were varied during lactation only, has apparently not been repeated.

Hahn (76) attempted to identify metabolic alterations that underlie metabolic imprinting of cholesterol homeostasis. He studied long-term effects of litter size manipulations in rodents on hepatic and adipose tissue enzyme activities. At 60 and 300 d of age, male rodents who were suckled in small litters (SL; 4/litter) had elevated plasma cholesterol concentrations and plasma insulin concentrations that were nearly twice as high as animals suckled in large litters of 18 (LL). Also, the activity of hepatic β-hydroxy-β-methylglutaryl-CoA (HMG-CoA) reductase (the rate-limiting enzyme in cholesterol biosynthesis) was 50% higher in LL than in SL animals at 60 but not at 240 d of age. The elevated hepatic HMG-CoA reductase activity of the LL animals at 60 d of age is intriguing given their decreased plasma insulin relative to the SL animals. A decrement in plasma insulin would be expected to down-regulate HMG-CoA reductase expression (77).

Rodents are generally considered a poor model for human CVD because of their relatively low serum LDL concentrations and their resistance to diet-induced atherosclerosis (78). Therefore, one might ask whether rodent data suggesting metabolic imprinting of cholesterol metabolism are relevant to metabolic imprinting of CVD in humans. Transgenic mouse models of atherogenesis have shown that although normal rodent lipoprotein metabolism differs quantitatively from that of humans, the biochemistry of atherogenesis is qualitatively similar (78). Hence, the rodent studies discussed here are likely to be relevant to metabolic imprinting of CVD in humans.

Mott et al (79) examined the long-term effects of preweaning cholesterol intake on cholesterol homeostasis in baboons. In this model, infant baboons were either breast-fed by their mothers or fed one of several formulas that varied in cholesterol concentration. After weaning, animals were assigned to 1 of 4 diets that varied in cholesterol content and ratios of polyunsaturated to saturated fat. In adulthood, few differences were found in serum cholesterol concentrations or cholesterol metabolism between the groups fed different concentrations of cholesterol during infancy. Mean serum triacylglycerol and HDL-cholesterol concentrations in the adult baboons were 10–15% lower in the mother-fed group than in all of the formula-fed groups ($P = 0.05$). Also, animals fed by their mothers had a 15% higher ratio of VLDL + LDL to HDL ($P = 0.08$), indicating a more atherogenic lipid profile. Accordingly, on necropsy at 8 y of age, the breast-fed baboons tended to have more extensive atherosclerotic lesions than did their formula-fed counterparts, but this trend was not statistically significant.

Cumulatively, the results of those studies indicated that adult cholesterol metabolism may be influenced by prenatal and early postnatal nutritional manipulations. Whereas the strongest epidemiologic data suggest prenatal imprinting of CVD susceptibility, most animal models have investigated the persistent effects of postnatal treatments. One study that did examine the long-term effects of prenatal restriction indicated an apparently protective effect against CVD risk factors (71). This was unexpected because of the inverse association found in humans between birth weight and adult CVD morbidity and mortality. Mott et al's (79) results in artificially fed baboons suggest that heretofore unidentified factors in mother's milk, rather than cholesterol, may imprint cholesterol metabolism during the suckling period. Such unknown factors may explain the inconsistent results found in different investigations of milk cholesterol intake during the suckling period and adult serum cholesterol homeostasis in rodents.

Blood pressure

Relatively few animal studies have examined the association between perinatal nutritional status and adult blood pressure. One study that explored this relation used unilateral uterine artery ligation in guinea pigs to create intrauterine growth retardation in half of the littermates (80). The intrauterine growth-retarded group included animals whose birth weight was reduced by ≥20% relative to their normal littermates. When the animals were 3–4 mo old, heart rate, but not blood pressure, was related inversely to birth weight. An analysis of 12 pairs of littermates showed a significant correlation between intrapair birth weight differentials and intrapair adult blood pressure differentials ($r^2 = 0.34$, $P < 0.05$).

Langley and Jackson (81) reported a long-term effect of maternal gestational protein intake on the blood pressure of female progeny in the rat model. From before conception to delivery, dams were fed diets containing 6%, 9%, 12%, or 18% protein by weight. After delivery, all dams were provided a stock diet. At 9 wk of age, SBP in the female offspring was cor-

related inversely with the dams' protein intake during pregnancy (r^2 = 0.27, P < 0.005). When combining blood pressure data from all ages (9, 12, 15, and 21 wk), SBP tended to increase with decreasing percentage of maternal protein intake. In a later study that used a similar design, Lucas et al (71) found that gestational protein restriction in rats (8% compared with 20% protein by wt) had the opposite effect on blood pressure in both male and female offspring at 6 mo of age. Recently, Woodall et al (82) studied the effect of maternal energy restriction (to 30% of ad libitum intake) during pregnancy on SBP of offspring. SBP was measured in the progeny at 30, 40, 48, and 56 wk of age, and was 5% higher (P < 0.05) in the restricted animals at every age except for 40 wk.

Unfortunately, none of those studies in rats reported correlations between birth weight and blood pressure at older ages to compare with relevant epidemiologic studies. A major common weakness of the dietary restriction studies is that cross-fostering was not used to distinguish the long-term effects of prenatal and residual postnatal exposure to maternal dietary restriction. Although restricted dams were allowed ad libitum access to control diets after delivery, milk quantity and quality may be reduced after prolonged and severe dietary restriction (83). Hence, without cross-fostering restricted progeny to unrestricted dams, it is unclear whether some of the long-term effects on blood pressure are mediated by diet during the early suckling period. But overall, with the exception of the study by Lucas et al (71), these studies are consistent in their support of the general hypothesis that prenatal undernutrition causes a permanent elevation in blood pressure.

Glucose tolerance

Numerous animal studies have explored the relation between perinatal nutrition and adult indicators of glucose tolerance. Hoet's group (84, 85) studied the effects of maternal low-protein diets on the glucose metabolism of adult progeny. Pregnant rats were fed diets containing 20% or 8% protein. After birth, the progeny of dams fed the low-protein diet were fostered by control dams to limit the period of restriction to gestation only. Growth of these recuperated animals was similar to that of the control group through 80 d of age. Nonetheless, a glucose tolerance test at 70 d of age revealed IGT correlated with blunted insulin secretion in the female, but not the male, recuperated animals. More recent studies with this same model showed that glucose tolerance is elevated in young recuperated rats but lower in older recuperated rats relative to controls (86). Accordingly, enhanced glucose tolerance and a 100% increase in adipocyte insulin receptor number were found in male recuperated rats at 6 wk of age (87). In vitro studies also showed increased glucose uptake in skeletal muscle in 3-mo-old male recuperated animals, which correlated with elevated concentrations of glucose transporter 4 in the plasma membrane fraction of the tissue and elevated insulin binding to glucose transporter 4 (88).

Long-term effects of prenatal exposure to hyperglycemia have been modeled by streptozocin-induced gestational diabetes in rats. Streptozocin was administered to pregnant rats on the first day of gestation (89), causing dramatic hyperglycemia throughout gestation. The female offspring of these animals had IGT at 80 d of age. This persistent effect of gestational hyperglycemia was later confirmed in both male and female offspring (90). Because birth weight is elevated dramatically in progeny of streptozocin-treated dams, these results appear consistent with the epidemiologic data suggesting a U-shaped relation between birth weight and adult IGT.

Several groups have investigated the long-term effects on glucose metabolism associated with various postnatal nutritional states. For example, rodents suckled in litters of different sizes have persistent alterations in fasting serum insulin, but not in glucose concentrations (69, 76). Fasting serum insulin concentrations in adulthood had a dose-response relation to nutritional adequacy during the suckling period: they were 2-fold higher in animals suckled in small litters and reduced by half in animals suckled in large litters relative to those in normal litters (65). Also, glucose tolerance tests conducted at 18 wk of age showed a hyperinsulinemic response in SL animals and a blunted insulin response in LL animals relative to controls suckled in normal-size litters (65). Patel's group (91, 92) used gastrostomy feeding to study persistent effects of feeding a high-carbohydrate diet throughout the suckling period. Compared with animals artificially fed a high-fat diet with a composition similar to that of rat milk, the high-carbohydrate diet group had 80% higher plasma insulin concentrations at 54 and 100 d of age. They also had IGT at 78 d of age (91) and altered insulin release from isolated pancreatic islets at 250 d of age (92). In another model, rats fed a low-protein diet during the postweaning period (from 3 to 6 wk of age) showed a normoglycemic hypoinsulinemic response to a glucose tolerance test at 12 wk of age (93). This suggests increased tissue sensitivity to insulin resulting from early postweaning protein deprivation. Thus, the critical window for the imprinting of insulin sensitivity apparently extends beyond the suckling period in some mammals.

Despite the abundance of data from animal models in this category, the implications of these studies in assessments of epidemiologic associations between prenatal nutrition and later glucose tolerance are unclear. Protein undernutrition of pregnant rats results in altered glucose tolerance in the progeny. The decreased glucose tolerance of older recuperated animals is generally consistent with the association between low birth weight and IGT in adult humans. Unfortunately, these studies have not reported differences in birth weight between controls and gestationally restricted animals. However, other studies in rats subjected to a similar degree of gestational protein restriction reported a significant decrease in progeny birth weight (94). It would be interesting to determine whether gestational energy restriction alone (which clearly results in reduced birth weight) has long-term effects on the glucose metabolism of progeny that are similar to those resulting from protein restriction of pregnant dams. In any event, it is clear from these various animal models that perinatal nutritional status can profoundly affect glucose tolerance and insulin sensitivity in adulthood.

Overall, the data from animal models of metabolic imprinting support the observed epidemiologic associations. The specific relations between perinatal treatments and adult outcomes in these models are not always concordant with expectations based on the epidemiologic literature. However, because most of the animal literature is based on rodent models, differences in perinatal developmental stages between rodents and humans make such comparisons difficult. Nonetheless, the general premise that biological mechanisms exist that result in the perinatal nutritional state imprinting metabolism is upheld. It is appropriate, therefore, to consider the nature of potential mechanisms that account for these putative effects of early diet.

POTENTIAL MECHANISMS

How is it that specific tissues are affected permanently by their nutritional biochemical milieu during development? What specifically is "memorized" by the tissues? These questions become even more intriguing when one considers that most of the tissues putatively involved in imprinting undergo continuous cellular turnover. Hence, cells exposed to specific stimuli perinatally must pass the effects of that exposure on to their progeny.

Generating a usefully concise list of potential mechanisms of metabolic imprinting requires establishing inclusion criteria for candidate mechanisms. The first 2 criteria derive from the definition of metabolic imprinting. First, it is an adaptive process. Accordingly, only mechanisms consistent with adaptive responses to early nutritional environments are considered. (In the context of this review, note that adaptive responses to nutritional conditions during development may in fact prove maladaptive if the organism's nutritional environment is later modified or if its homeostatic conditions are sufficiently changed at later life stages.) Second, imprinting occurs during a limited period of susceptibility. Hence, only mechanisms operating within a limited ontogenic period were included. For these reasons, responses to clearly pathologic insults are not considered. For example, perinatal exposure to toxins that permanently alter metabolism by compromising liver function would not be considered to result in metabolic imprinting. The last criterion stipulates that a potential mechanism must explain a specific putative imprinting process, and not simply be a reflection of the outcome. For example, whereas some have proposed altered cell size as a mechanism of perpetuating the effect of early nutrition, cell size is in most cases likely to be indicative of, rather than a determinant of, cellular metabolism. Nonetheless, this last criterion is the most difficult to apply because almost any biological mechanism may, on close study, be broken down into a subset of component mechanisms. Hence, candidate mechanisms are most likely limited in their scope by incomplete knowledge of upstream component mechanisms.

Others have proposed mechanisms that may be consistent with these criteria. For example, on the basis of studies showing that perinatal undernutrition in rats permanently reduces pancreatic islet vascularization, Hoet's group (95) suggested that metabolic effects of early nutrition may result not only from alterations at the cellular level but also from changes in organ structure. Considering the central role the endocrine system plays in regulating metabolism, Csaba's (96) theory of "hormonal imprinting" may be relevant to metabolic imprinting. His studies suggested that the concentrations of hormones and hormone analogues present at critical developmental periods permanently affect the hormonal responses to specific stimuli, tissue sensitivity to specific hormones, or both. Dorner (97) put forth the concept that abnormal nutritional environments during the period of brain differentiation could lead to persistent metabolic disturbances. The idea that early nutrition may, for example, "set" the hypothalamus and thereby regulate appetite, growth, and behavior has since been considered by several authors.

Several years ago, Lucas (8) put forth the most complete list of potential mechanisms by which perinatal nutrition might persistently affect an organism's structure or function. He proposed that such nutritional programming could occur via 4 general mechanisms: *1*) undernutrition at a critical stage may fail to fuel a growth process, *2*) the early nutrient environment may permanently alter gene expression, *3*) the early nutrient environment may affect clonal selection, or *4*) the early nutrient environment may affect cell number.

Lucas's list of mechanisms and the suggestions of others discussed above are a useful starting point, but are often not sufficiently specific to guide experimental characterization. For example, "failing to fuel a growth process" is conceptually too broad, and "permanently altering gene expression," though certainly central to metabolic imprinting, underlies nearly all candidate mechanisms. Admittedly, formulating a useful and concise list of specific potential mechanisms of metabolic imprinting is challenging because of the interrelatedness and nonexclusive characteristics of the likely candidates. On the basis of the criteria outlined above, we propose the following mechanisms as among the most useful to consider in future research: *1*) induced variations in organ structure, *2*) alterations in cell number, *3*) clonal selection, *4*) metabolic differentiation, and *5*) hepatocyte polyploidization.

The physiologic and molecular bases of these 5 potential mechanisms are reviewed first. Then these mechanisms are considered in the context of examples of putative metabolic imprinting phenomena from the animal and human literature. Last, some experimental approaches are proposed to determine which of these mechanisms may play dominant roles in mediating metabolic imprinting in specific animal models.

Organ structure

This mechanism is restricted to gross morphologic alterations occurring during organogenesis to distinguish it from other candidate mechanisms that may result in altered organ structure via changes at the cellular or subcellular level. Morphologic outcomes consistent with this potential mechanism are, for example, alterations in organ vascularization or innervation and changes in the juxtaposition of different cell types within an organ. As such, altered organ structure is the only mechanism of metabolic imprinting considered in this review that operates above the cellular level.

How might altered organ structure occurring during organogenesis permanently affect the organism's metabolism, and thus lead to imprinting? The most obvious examples pertain to the ability of individual cells to generate and respond to external signals within the organism. If patterns of organ vascularization are permanently affected by nutrition during organogenesis, this could affect the cells' responses to blood-borne nutrients or hormonal signals. Or, within the liver, for example, the specific juxtaposition of hepatocytes, endothelial cells, and Kupfer cells determined during organogenesis could have a persistent influence on hepatic metabolism.

During gastrulation, the 3 embryonic germ layers, the endoderm, mesoderm, and ectoderm, are formed. The subsequent process of organogenesis involves inductive interactions among those different layers to direct changes in cell position, shape, and differentiation that result in the formation of the organ rudiments. For example, the mammalian liver forms from a mutually inductive interaction between endoderm and mesoderm (98). Organogenesis begins very early in human embryonic development. By 5 wk of gestation, a human embryo develops rudiments of most organs, and by 8 wk organogenesis is nearly completed (99). However, the endpoint of organogenesis is difficult to define. Cellular differentiation continues into postnatal development and transforms the fetal organ rudiments into functioning organs.

During the limited period of organogenesis, the fates of cells depend on externally derived inductive signals from adjacent

cells and from morphogen gradients that originate at more distant sites. Hence, it is reasonable to expect that the local concentrations of diverse nutrients, their metabolites, or both modulate the end result of this process. For example, many cell-cell interactions critical to inductive interactions and cell migration are mediated through proteoglycans at the cell surface. Imbalances in macronutrients from which these components are derived could affect these processes, resulting in permanent alterations in organ structure. As another example, retinoic acid, a derivative of vitamin A, serves an important regulatory role during normal organogenesis. It binds to ligand-activated transcription factors that then regulate the expression of genes involved in morphologic development (100). Thus, local concentrations of this nutrient-derived transducer assist in the establishment of organ structure.

Cell number

Of all the potential metabolic imprinting mechanisms, a permanent alteration in cell number is the simplest and easiest to put forward. During development, organs can increase in size either by increasing the number of cells (hyperplasia) or by increasing cell size (hypertrophy). Different tissues go through diverse, limited periods of hyperplastic and hypertrophic growth. The rate of cellular proliferation is directly dependent on nutrient availability (the supply of building blocks) and may be indirectly dependent on the organism's general nutritional status via hormonal signals that control cellular proliferation. Nutrient limitations or excesses during critical periods of hyperplastic growth that affect rates of cell division may lead to permanent changes in cell number, regardless of later nutrient availability.

This phenomenon has been examined most exhaustively for the central nervous system. For example, in a rat model, Winick and Noble (101) showed that severe malnutrition during the period of brain cell hyperplasia resulted in permanent deficits in brain cell number, whereas malnutrition during later periods of brain cell hypertrophy did not have permanent effects. Conversely, if nutrient delivery or the hormonal milieu is conducive to rapid cellular proliferation, the organ may have a permanently increased number of cells. Because an organ's metabolic activity is limited by cell number, a permanent effect on cell number could permanently affect metabolism.

Multinucleation of muscle tissue may be viewed as a special category of hyperplastic growth and hence warrants comment in this section. During myogenesis, myoblasts proliferate, align, and fuse to form myotubes. These multinucleated syncytia then develop into muscle fibers. Once a myoblast fuses with a myotube it no longer proliferates (102). Hence, the rate of myoblast hyperplasia during the limited period of myogenesis determines the degree of nucleation within muscle fibers. Although the total number of myocytes may not be affected, the metabolic activity of the myocytes will be limited by their degree of nucleation, analogous to an effect on cell number.

Clonal selection

Cellular proliferation in all organs involves the initial multiplication of a finite population of founder cells. Founder cells in specific organs are not necessarily identical to each other. Although cellular proliferation generally precedes terminal differentiation, successive generations of cells undergo limited differentiation in the early proliferative stage. As cellular proliferation proceeds, genetic and epigenetic modifications likely occur within individual cells that distinguish them from others in subpopulations of rapidly dividing cells. Distinguishing characteristics may offer selective advantages to specific clones competing for the available nutrient supply. Thus, for example, an incorrect base pairing during DNA replication may result in subtle effects on a cell's metabolism. This change would then be passed to all its progeny.

Such subtle differences among proliferating cells are the basis for clonal selection. Clonal selection is comparable with Darwinian evolution operating at the cellular level, but rather than through survival of the fittest, clonal selection works by disproportionate population growth of the most rapidly proliferating cells. Hence, 2 similar, heterogeneous populations of rapidly dividing cells may develop very distinct metabolic characteristics as a result of diverse microenvironmental conditions. If, for example, the nutrient environment is deficient in structural fatty acids, cells with a slightly more efficient or more active lipogenic pathway could disproportionately populate a tissue. Thus, it is possible that variations in nutritional status during development could lead to permanent alterations in organ or tissue metabolism via this mechanism.

Metabolic differentiation

The most complex and probably most developmentally relevant potential mechanism of metabolic imprinting is through an effect on metabolic differentiation. Greengard (103) defined enzymic differentiation as "the process whereby, in the course of prenatal or early postnatal development, the different organs of an animal acquire their characteristic, quantitative pattern of enzymes." Metabolic differentiation represents the process whereby individual cells develop a stable quantitative pattern of basal and inducible gene expression. As such, metabolic differentiation pertains not only to enzymes, but also to transcription factors, hormones, hormone receptors, transmembrane transporters, and other elements. Also, metabolic differentiation stresses the importance of the ontogeny of a tissue's capacity for adaptive as well as basal gene expression.

Metabolic differentiation should be distinguished from cellular differentiation. Cellular differentiation is the developmental process by which multicellular organisms develop a limited number of distinct and stable cell types (99). For the most part, developmental biologists have focused on the problem of cellular differentiation from a morphologic perspective, and it is from this perspective that the differentiated state is recognized most often as distinct and stable. Thus, although a human adult may have trillions of cells, there are only ≈200 different cell types, and a differentiated cell is generally committed to its specific cell type. Differentiated cells may be characterized as being one type or another based on structural features, or in some instances by qualitative protein markers. A fundamental feature of metabolic differentiation is that quantitative as well as qualitative differences in gene expression may distinguish one cell from another. For subtle metabolic differences among cells of a similar type to be important, a cell's quantitative pattern of gene expression, once established during development, must be stable. In other words, cellular memory, or the faithful transmission of determined states to progeny cells, must be maintained quantitatively (104).

Generally speaking, cellular differentiation is characterized by the stable ability to express a limited number of genes, constitutively or in response to given stimuli. This stability is maintained through epigenetic mechanisms. Epigenetics is defined as the study of "mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA

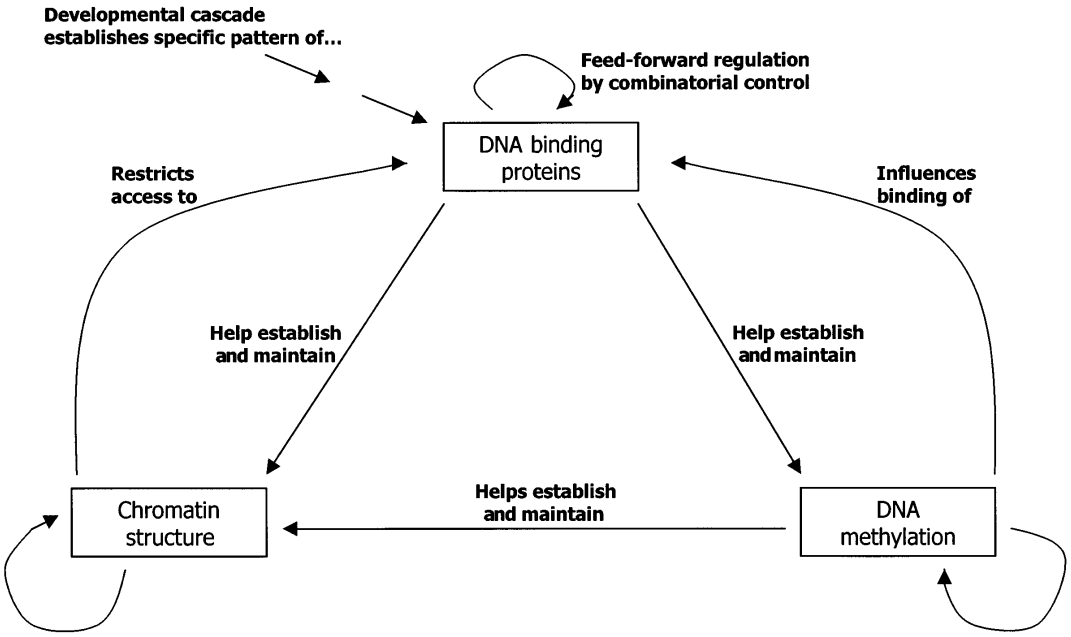

FIGURE 3. Schematic diagram showing how various epigenetic mechanisms work synergistically to maintain cellular memory with high fidelity in mammals.

sequence" (105). Epigenetic phenomena play important roles in mammalian development. Several reviews (106–109) discuss potential molecular mechanisms that underlie epigenetic phenomena such as genomic imprinting (whereby the maternal or paternal allele of specific genes are stably repressed) and X chromosome inactivation (which mediates X chromosome dosage compensation in mammals). It is likely that the same epigenetic mechanisms that mediate these well-studied phenomena also contribute to metabolic differentiation. Three epigenetic mechanisms of metabolic differentiation that might mediate metabolic imprinting are discussed below: *1*) the autoregulatory pattern of DNA binding proteins, *2*) the persistent modulation of chromatin structure, and *3*) DNA methylation. Note that these epigenetic mechanisms are not independent of one another, but likely function together to maintain the vast array of differentiated tissues that characterize higher organisms (**Figure 3**).

Autoregulatory pattern of DNA binding proteins

Eukaryotic organisms use combinatorial control of gene expression to efficiently regulate thousands of different genes in a precise, cell-specific manner. The basis of combinatorial control is that most genes are not regulated by a single transcription factor binding to their promoter. Rather, a specific combination of transcription factors is often required to activate or repress transcription. This dramatically increases the amount of complexity that can be dictated by a given number of gene regulatory proteins. Additionally, some of these transcription factors bind to their own promoters and to the promoters of other cell-specific transcription factor genes. Thus, once such a feed-forward transcription factor is expressed in a cell, it will perpetuate its own transcription as well as that of auxiliary transcription factors appropriate to that cell type. Because there are numerous copies of each transcription factor in each nucleus, when cells divide the factors are partitioned between the 2 daughter nuclei and hence perpetuate their specific autoregulatory pattern in the progeny cells.

This simple positive feedback mechanism is a common strategy of multicellular organisms. For example, many of the gene regulatory proteins that function combinatorially to establish body patterning during *Drosophila* development stimulate their own transcription (110). Also, the products of the genes regulating vertebrate muscle cell differentiation, such as MyoD, activate their own transcription as well as that of numerous downstream genes (111). Thus, through positive-feedback autoregulation and combinatorial control, a relatively small number of gene regulatory proteins can perpetuate a vast number of differentiated cell types. To the extent that early nutrition influences the developmental cascade that establishes cell-specific patterns of gene regulatory proteins, imprinting may occur by this mechanism.

Chromatin structure

DNA in the eukaryotic nucleus is packaged in a highly compact configuration with histone proteins and other DNA binding proteins. This protein-DNA complex is called chromatin. Two general types of chromatin are identified: *1*) euchromatin, an open configuration allowing rapid gene transcription; and *2*) heterochromatin, the closed and inactive configuration. The fundamental unit of chromatin structure is the nucleosome, which consists of about 200 base pairs of DNA wrapped around a histone octamer. Neighboring nucleosomes assemble into higher-order structural configurations corresponding to more condensed and presumably transcriptionally inactive DNA.

Chromatin structure is highly correlated with gene expression during development (104). In differentiating chicken erythrocytes, both the α- and β-globin genes are maintained in an open configuration, whereas in all other cell types these loci are sequestered within regions of inactive chromatin (112, 113). Analogously, in differentiating oligodendrocytes, up-regulation of myelin-associated glycoprotein, a component of the myelin sheath, appears to involve chromatin remodeling (114). X chromosome inactivation in female mammals also suggests an impor-

tant function of chromatin in inhibiting gene expression. Within each cell, the active X chromosome is predominantly euchromatic, whereas the inactive X chromosome is almost completely heterochromatic (115). Also, tissue-specific regulation of several genes has been correlated with alterations in local chromatin structure. For example, in chickens, the liver-specific nutritional regulation of malic enzyme correlates with DNAase-hypersensitive regions (indicating open chromatin configuration) upstream of the transcription start site that are not found in other tissues (116).

For chromatin structure to play a role in cell memory, and hence metabolic differentiation, the specific chromatin structure within a given region of DNA must be stably propagated from progenitor cells to progeny. It is not obvious how this might occur. The histones and other DNA binding proteins associated with the nucleosome must detach from a region of DNA before the DNA polymerase can replicate it. Several models have been proposed to explain how specific regions of DNA are maintained in a stable, specific chromatin configuration (117, 118).

One such model is based on histone modification. Histone proteins may undergo several posttranslational modifications, such as acetylation, ubiquitination, and phosphorylation. Histone hyperacetylation is associated with regions of open chromatin configuration (119). Hence, histone acetylation could serve as an epigenetic marker to identify regions of DNA that are to be maintained in a transcriptionally active configuration. Although histones detach from DNA as the polymerase moves along the DNA, they may remain in the vicinity of the replication fork. If, after replication, these localized histones segregate evenly to the parent and daughter strands, the acetylated histones could serve as a signal for the acetylation of the additional histones required for formation of complete nucleosomes (**Figure 4**). In this fashion, a specific chromatin configuration established during development could be propagated through multiple rounds of cellular turnover. Thus, to the degree that early metabolic conditions modulate histone posttranslational modifications, early nutrition may have a lasting effect on specific gene expression via this mechanism.

DNA methylation

In mammalian DNA, most of the cytosine nucleotides in the sequence CpG are methylated to 5-methyl-cytosine. The specific pattern of cytosine methylation varies among cells in different tissues. The methylation pattern is maintained through cycles of DNA replication by a DNA methyltransferase that is highly selective for cytosines in the hemimethylated DNA generated when DNA methylated on both strands is replicated (**Figure 5**). Thus, this maintenance methylase allows a specific pattern of methylation to be transmitted to progeny cells with high fidelity, suggesting a relatively simple mechanism of cell memory.

DNA methylation is highly correlated with gene expression in various systems. Generally, the degree of methylation at the 5′ end of a gene is related inversely to its level of expression. However, methylation at specific sites at the 3′ end and within genes has been associated with gene activity. Also, tissue-specific gene expression has been correlated with the methylation of several genes. An example is glucose-6-phosphate dehydrogenase. Its expression in various human tissues is correlated with the level of methylation at specific sites at the 3′ end of the gene (120). Similarly, the rat phosphoenolpyruvate carboxykinase gene is undermethylated in adult liver and kidney (sites of phosphoenolpyruvate carboxykinase expression) relative to that in nonexpressing tissues such as spleen and heart (121).

This tissue-specific pattern of DNA methylation may arise via several pathways. Oocyte and sperm DNA are hypomethylated relative to DNA in somatic tissue. De novo methylation of the entire genome occurs early in embryogenesis (108). As cells differentiate, demethylation of tissue-specific genes correlates with their expression in appropriate tissues, leading to the methylation mosaic of the adult. Demethylation appears to precede gene expression in some instances. Other genes are expressed by overriding methylation and demethylation then occurs after initiation of gene expression (122), perhaps functioning to reinforce the gene's activity. Most of the evidence suggesting a role of DNA methylation in development and cellular differentiation is correlational, making it unclear whether gene activity causes demethylation or vice versa. However, in targeted gene knockout experiments, mouse embryos that lack maintenance methylase activity die early in gestation (123), indicating that DNA methylation is critical to mammalian development. Inasmuch as early nutrition affects gene expression during differentiation, cell-specific DNA methylation may be influenced, leading to persistent effects on gene activity.

Hepatocyte polyploidization

Cells that contain more than the normal complement of chromosomes are said to be polyploid. In adult humans, hepatocytes, cardiomyocytes, and megakaryocytes are substantially polyploid (124). Hepatocyte polyploidization is of special interest because it seems most likely to be relevant to metabolic imprinting. In humans and in rats the period of hepatocyte polyploidization occurs during postnatal development and does not continue

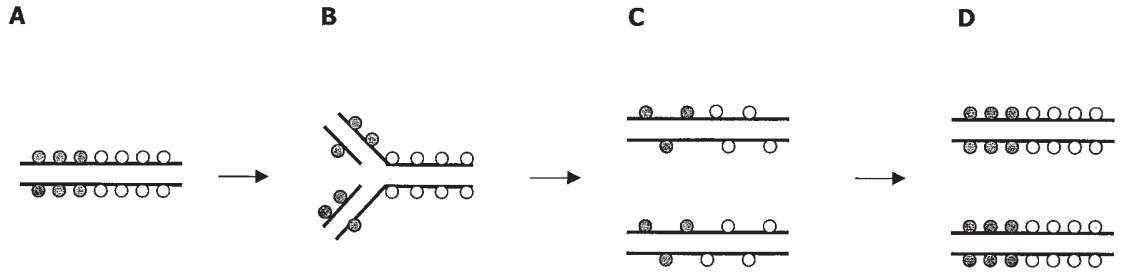

FIGURE 4. Hypothetical model by which histone acetylation could serve as a marker to maintain a specific chromatin configuration throughout DNA replication. Histones (circles) detach from DNA during replication but may remain in the vicinity of the replication fork (shading represents hyperacetylation). After the polymerase passes, the localized histones may bind to both DNA strands in the same region that they initially occupied (B and C). If the degree of histone acetylation in a region serves as a signal to regulate the binding of similarly acetylated histones, the original pattern of histone modification could be restored in the newly synthesized double-stranded DNA (D).

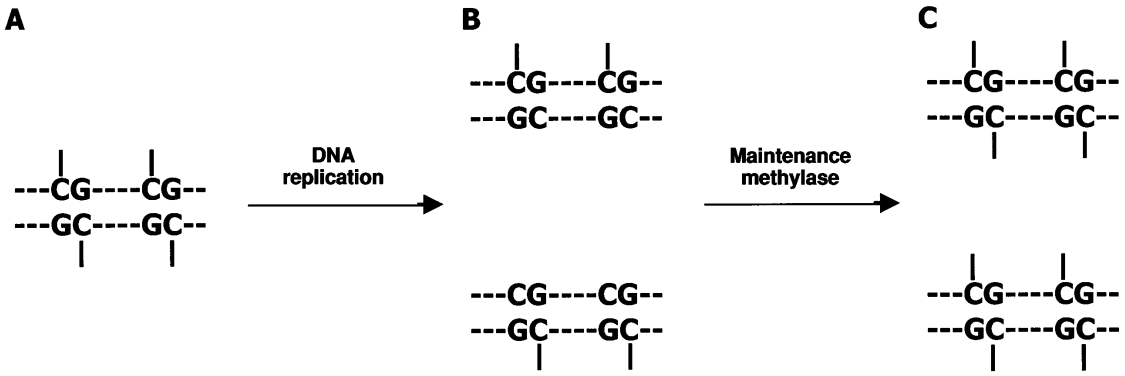

FIGURE 5. Propagation of a specific pattern of DNA methylation constitutes a relatively simple means of cellular memory. DNA replication results in hemimethylated DNA (B; the bars represent methyl groups). The maintenance methylase is highly specific for hemimethylated DNA, resulting in the restoration of the initial methylation pattern in the daughter strands (C).

through adulthood (125). During the suckling period, rat hepatocytes are almost exclusively diploid and mononucleated (2N). Initiation of polyploidization appears to coincide with weaning, such that by 4–5 wk of age, about half of the hepatocytes are binucleated (2N-2N). By 8 wk, a stable ploidy mosaic is established that is comprised predominantly of mononucleated tetraploid (4N), together with diploid (2N), binucleated tetraploid (4N-4N), and mononucleated octaploid (8N) hepatocytes (98). Hepatocyte polyploidization presumably serves to increase hepatic metabolic activity by enhancing both basal and inducible gene expression relative to a purely diploid organ. Accordingly, total RNA content (126), rate of RNA synthesis (127), and activity of specific enzymes (128, 129) correlate with the ploidy of individual rat hepatocytes.

The signals that determine the timing and extent of hepatocyte polyploidization remain unclear. However, because polyploidization is restricted to a limited period of postnatal development, it is possible that nutritional status during this period permanently affects liver ploidy and, hence, metabolism in adulthood. Divergent nutritional status limited to the suckling period in mice had dramatic and persistent effects on cardiomyocyte ploidy (130). For example, compared with mice suckled in litters of 16, those suckled in litters of 4 had 4 times as many diploid tetranucleated cardiomyocytes at 90 d of age. If early postnatal nutrition has similar persistent influences on hepatocyte ploidy, hepatic metabolism could be imprinted in this fashion.

ARE SPECIFIC MECHANISMS SUGGESTED BY THE LITERATURE?

Given these possible mechanisms, does the framework of available data suggest one that most likely leads to metabolic imprinting? Unfortunately, available data neither favor any specific mechanism nor any subset of the 5 mechanisms that have been described. There are several reasons for this.

The primary reason is that model systems used to test relevant hypotheses have not been probed at a level that allows this type of discrimination. For example, the most focused outcomes assessed to date are at the level of enzyme specific activity and protein quantity [and in a few cases, messenger RNA concentrations (131)]. Such outcomes are consistent with any of the 5 general mechanisms described in the preceding section. Another reason is that most metabolic imprinting phenomena have not been traced to the primary imprinting storage site or sites. For example, several investigators have used litter manipulation in a rat model to show that neonatal nutrition persistently affects serum insulin concentrations. This suggests imprinting of the endocrine pancreas or of various target tissues' insulin sensitivity. Other workers documented the permanent effects of the same treatment on the activity of hepatic enzymes that are influenced by insulin. However, no one has evaluated the relation between the insulin concentrations and enzyme activities to determine whether effects on hepatic enzyme activities are simply secondary to altered serum insulin concentrations or are due to metabolic imprinting within the liver. It is illogical to pursue molecular mechanisms of imprinted hepatic enzyme activity within the liver if the primary imprint occurs within the pancreas. Last, a major factor that limits the elucidation of mechanisms is the small effect size characteristic of most metabolic imprinting phenomena relative to normal interindividual variability of specific outcomes of interest. Subtle effects are notoriously difficult to probe at the molecular level.

To narrow the search to the few mechanisms that are most likely to explain metabolic imprinting, an axiom put forth by William of Ockham, a 14th century philosopher, is likely to be helpful. He proposed that given several equally tenable explanations for a phenomenon, the simplest one is most likely to be correct. Hence, one might evaluate whether 1 or 2 of the simplest mechanisms, for example, alterations in cell number or organ structure, could possibly explain all or most of the phenomena of interest. The data do not make the choices evident. For example, energy restriction during the suckling period in rodents leads to permanent reductions in adipocyte number, suggesting that altered cell number could cause the permanent effects on adiposity in this model. However, the elevated adiposity consequent to prenatal energy restriction in rodents appears to be associated with increased adipocyte size, not number, which suggests altered gene expression either within the adipocytes or in other tissues affecting lipid metabolism.

Studies of the effects of perinatal nutritional treatment on enzyme adaptation to nutritional challenge later in life also suggest that metabolic imprinting cannot be explained purely by alterations in cell number or organ structure. For example, adult rodents that underwent various perinatal nutritional treatments may have physiologic indexes similar to those of their control counterparts when consuming a standard diet. However, their

responses to dietary changes, in terms of serum insulin (132), serum cholesterol (75), and adipose tissue enzyme activity (133) differ markedly from those of control animals. Metabolic alterations dependent on the adult diets suggest imprinting mechanisms that are more dynamic than a simple alteration in cell number.

Other characteristics of various metabolic imprinting phenomena may provide clues about the underlying mechanisms. The first of these relates to the timing of the organism's susceptibility to imprinting (the critical window). By identifying the specific critical window of the imprinting phenomenon and then considering the developmental processes occurring during this period, the list of likely mechanisms may be narrowed. Another intriguing attribute of many metabolic imprinting phenomena is sex specificity. Differences in growth patterns or endocrinologic development that are manifested during critical windows of sex-specific imprinting effects may suggest underlying mechanisms.

SUGGESTIONS TO GUIDE FUTURE RESEARCH

These considerations strongly support the need for research in animal models or perhaps simpler models that focus on the cellular level. Each of these approaches has advantages and drawbacks. Models of metabolic imprinting in, for example, yeast or bacteria are highly amenable to the study of specific candidate mechanisms at the molecular level. On the other hand, the complex developmental biology and integrated physiology of mammalian organisms favor more complex animal models of metabolic imprinting. Once specific mechanisms that underlie imprinting phenomena are identified in animals, the developmental biology of these mechanisms can be compared with that of humans. It should then become apparent whether similar phenomena may be relevant to humans. Hence, this discussion focuses on potential imprinting mechanisms that can be investigated in animals. Considering the extensive animal literature describing putative metabolic imprinting phenomena, one logical approach is to select a phenomenon that is well described and attempt to characterize the underlying mechanism or mechanisms.

Definition of metabolic imprinting: implications for research design

Few examples of putative metabolic imprinting meet the criteria of metabolic imprinting developed in anticipation of this review. This likely reflects the fact that the studies of metabolic imprinting remain at the descriptive stage. The first component of the definition is that imprinting phenomena should be characterized by a critical window. However, few studies have identified the critical windows for specific imprints.

The second component of the definition is effect persistence. Certainly, the specific effects of putative imprinting phenomena are of variable persistence. Determining the degree of effect persistence may offer insights similar to those gained by determining the critical windows of specific imprinting phenomena. For example, hepatocytes slowly turn over throughout life. Thus, clonal selection may continue to operate beyond the perinatal period of rapid hepatocyte proliferation, but at a much slower rate. Accordingly, metabolic imprinting mediated by hepatocyte clonal selection may be consistent with a gradual loss of the imprint.

The third component, effect specificity, also has obvious research implications. For example, though one may be interested in the effect of early nutrition on adult glucose tolerance, this outcome is in itself too broad to provide clues about a fundamental underlying mechanism. The more general the target effect, the greater the number of potential explanatory factors that will require investigation.

The last component of the definition, the dose-response or threshold effect that links treatment and outcome, is more than a means to bolster causality in epidemiologic studies. The dose-response nature of an imprinting phenomenon in an animal model provides information helpful in characterizing it and may suggest a specific mechanism or subset of mechanisms responsible for it. For example, consider the hypothetical dose-response curve in **Figure 6**. In this example, the adult outcome follows a linear dose response over a range of perinatal treatment levels, but beyond a specific severity of treatment, the outcome is no longer related to treatment level. Such a relation provides 2 important inferences. First, the linear dose-response region suggests an adaptive response over a range of treatment levels, consistent with metabolic imprinting. Second, the abrupt discontinuity at a certain treatment level suggests the breakdown of adaptive mechanisms or the involvement of new biological processes (possibly more toxic in nature). Hence, investigations of such metabolic imprinting phenomena would target the mechanisms operating in the adaptive region.

Threshold effects may also be consistent with metabolic imprinting. Responses of this type may be characteristic of a subset of the potential mechanisms considered above. For example, mechanisms such as alterations in cell number or clonal selection may affect outcomes over a broad range of treatment levels. However, metabolic differentiation that is controlled by several layers of epigenetic mechanisms may require that all be affected before persistent changes in gene expression are manifested. In this scenario, although each individual component underlying metabolic differentiation may follow an adaptive dose-response relation, no change in outcome may be seen until the most limiting component mechanism is affected.

Take an integrative approach at the whole-animal level

As mentioned previously, the primary imprint storage site is unclear in many examples of putative metabolic imprinting. This suggests that a hierarchical approach may be effective. That is, one must establish that the target outcome is not secondary to an upstream "primary" effect. Once a specific outcome is identified, one should consider the factors that regulate or influence that outcome to determine whether the effect is actually secondary to a primary effect in one of these ancillary factors. Hence, to study imprinting of a specific hepatic enzyme, one may consider the regulatory factors (such as serum insulin, glucagon, and substrate concentrations) that regulate the expression and activity of the enzyme. Each of those factors should be measured as outcomes. By examining concurrently the persistent effects of the perinatal treatment on the enzyme of interest and on its regulators, it should become clear whether the previously documented effect on hepatic enzyme activity originates in the liver or within another tissue.

Probe candidate tissues in vitro

The integrated nature of higher organisms makes it difficult to establish that a specific effect represents a primary imprint. The regulation of many physiologic variables relies on mutually coordinated inputs from different organs to allow adaptive homeostasis. A pertinent example is that of insulin sensitivity and secretion. Metabolic imprinting leading to an effect on serum insulin concentrations suggests a primary imprint affecting pancreatic

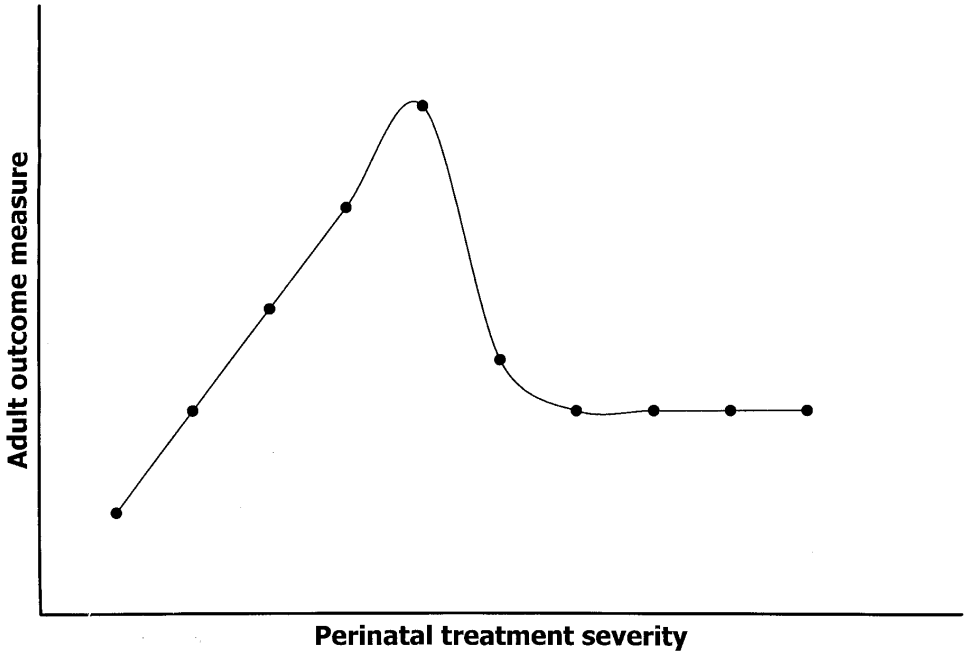

FIGURE 6. Hypothetical dose-response relation of a metabolic imprinting phenomenon. The specific adult outcome measure is plotted against perinatal treatment severity. In this example, the linear portion of the curve suggests an adaptive imprinting response. Beyond the discontinuity, different biological processes are likely involved.

insulin secretion, target tissue insulin sensitivity, or both. It is difficult to distinguish between these alternatives because chronic pancreatic hypersecretion of insulin may lead to tissue insulin resistance, and conversely, insulin resistance may lead to a compensatory increase in insulin secretion. It may be possible to identify the site of the primary imprint by assessing organ function under controlled conditions in vitro. For example, in vitro measurements may indicate that the imprinting phenomenon causes hypersecretion of insulin by isolated pancreatic islets under controlled glucose concentrations. Examination of skeletal muscle glucose uptake in vitro from those same animals may appear normal. Such data would strongly suggest that the endocrine pancreas is the primary storage site of the imprint in this example. Accordingly, investigation of potential mechanisms within the pancreas could commence.

CONCLUSION

Associations between human birth weight and adult chronic disease morbidity and mortality suggest that prenatal nutrition may permanently affect metabolism. However, the small magnitude of these individual effects coupled with the methodologic difficulties of eliminating biases and adequately adjusting for potentially important confounding factors in retrospective studies detract from our ability to attribute the associations to metabolic imprinting. Human epidemiologic data not predicated on birth weight, such as those from the Dutch famine cohort, generally support the metabolic imprinting hypothesis. Strong support for the biological plausibility of metabolic imprinting has come from animal models. These have shown that various prenatal and early postnatal nutritional states can have profound and persistent influences on outcomes related to body composition, lipid metabolism, blood pressure, and glucose tolerance.

Researchers in this field are faced with the challenge of elucidating the specific mechanisms that underlie the persistence of these effects in animal models. This will be a complex task. The integrated nature of mammalian physiology makes it difficult to identify tissues housing primary imprints that are responsible for specific phenomena, which is a logical first step. Indeed, effect persistence of some phenomena may depend on specific interactions among distinct organ systems. Analogously, once a specific tissue housing a primary imprint is identified, the functional interdependence among various candidate mechanisms of metabolic imprinting may make it difficult to determine which mechanisms play dominant roles. Nonetheless, comparing the developmental biology underlying specific mechanisms identified in animal models with analogous processes in human development is a necessary step toward determining putative effects of metabolic imprinting on human health. Without this fundamental knowledge, designing effective perinatal interventions for the prevention of chronic adult disease is unlikely. The significance of gaining this information is great, especially among populations that continue to experience high rates of low birth weight.

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