

REVIEW

Nutritional programming of hypothalamic development: critical periods and windows of opportunity

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Obesity is increasing at an alarming rate throughout the world, particularly among children. Epidemiological and experimental data have suggested that suboptimal nutrition and growth during prenatal and/or postnatal life can have a significant role in the development of obesity and related diseases. Similarly, exposure to malnutrition during perinatal life can result in lifelong metabolic disorders. Although the precise biological mechanisms governing metabolic programming have not been fully elucidated, there is growing evidence that obesity and other metabolic diseases may result from a change in the underlying developmental program of the hypothalamic pathways that regulate energy balance. The hypothalamus undergoes tremendous growth beginning in the embryonic period and continuing through adolescence, and an alteration in perinatal nutrition can affect various developmental processes, including neurogenesis and axon growth, which can lead to abnormal hypothalamic development. Metabolic hormones, particularly leptin, are capable of transmitting signals to the developing hypothalamus in response to alterations in the nutritional environment and may underlie potential maladaptive responses to early metabolic perturbations. A better understanding of the optimal perinatal hormonal and nutritional environment during hypothalamic development may help ameliorate and reverse the metabolic malprogramming of the fetus and/or neonate.

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INTRODUCTION

During the past three decades, we have witnessed an alarming increase in the prevalence of obesity in countries that have adopted a Western lifestyle, which includes both a sedentary lifestyle and the overconsumption of energy-rich, nutrient-poor food.^{1–3} Such lifestyle changes also affect children, which raises major health concerns because obese children and adolescents are more likely to become obese adults.^{4,5} A growing body of evidence has reported large disparities between population groups with regard to obesity, and different individuals exposed to the same obesogenic environments display different responses in terms of obesity predisposition. There appears to be a genetic basis for a subpopulation of obese patients; however, genetics alone cannot explain why obesity has increased so rapidly in recent decades. During the past few decades, evidence has accumulated that suggests that the perinatal environment can substantially perturb developmental events and contribute to deleterious health outcomes in the developing offspring. In particular, epidemiological and animal studies have revealed that changes in the hormonal and nutritional environments during critical periods of development may increase the susceptibility for the development of obesity later in life.^{6–9} Perinatal factors that contribute to lifelong obesity include both maternal and/or postnatal underfeeding and overfeeding (see refs 6–8,10 for review). The observation that similar adverse metabolic outcomes are associated with both a surplus and a paucity of energy supply during important periods of development suggests that the programmed metabolic dysregulation observed in these individuals may be tied to the same biological mechanism.

The hypothalamus is a critical regulator of energy balance and glucose homeostasis, because it contains sets of neurons that are devoted to metabolic regulation and can directly respond to peripheral hormonal and nutritional signals (Figure 1). Classical experiments using physical lesions of specific hypothalamic nuclei and more recent studies using conditional, neuron-specific gene targeting methods have revealed the importance of specific subpopulations of neurons within the arcuate nucleus (ARH), ventromedial nucleus (VMH), dorsomedial nucleus (DMH), paraventricular nucleus (PVH) and lateral hypothalamic area (LHA) in the regulation of energy balance and glucose homeostasis (for review, see refs 11–14). The ARH appears to be a predominant site for the integration of peripheral blood-borne signals, including hormones and nutrients. In particular, neurons located in the ARH, such as the neurons that produce both neuropeptide Y (NPY) and agouti-related peptide (AgRP) and the neurons that contain proopiomelanocortin (POMC)-derived peptides, directly respond to peripheral hormonal signals, such as the adipocyte-derived hormone leptin. Both NPY/AgRP- and POMC-containing neurons project extensively to other key hypothalamic nuclei, including the PVH, DMH and LHA. These target nuclei contain sets of neurons that have crucial roles in energy balance regulation and neurons that produce anorexigenic peptides, such as galanin, enkephalin and dynorphin in the PVH, as well as neurons that express orexigenic neuropeptides, such as orexins and melanin-concentrating hormone in the LHA.

Because of the importance of the hypothalamus in the regulation of eating and energy balance, it has been suggested that impairments of hypothalamic development during perinatal life may result in lifelong metabolic dysregulation (see refs 6, 7, 10,

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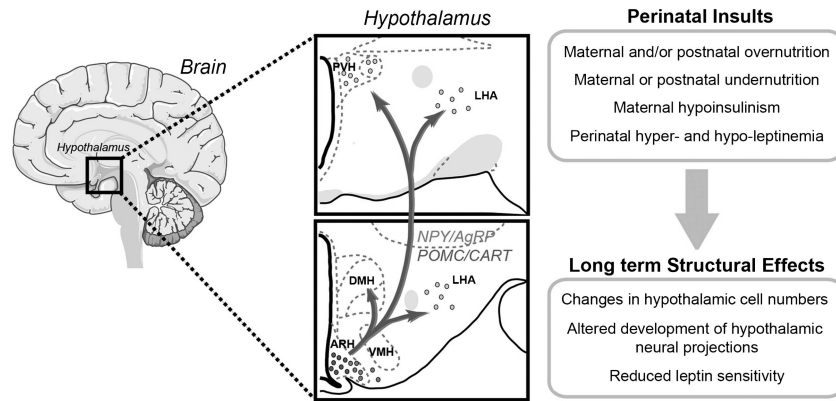


Figure 1. Developmental programming of hypothalamic appetite-related pathways. The developmental programming of hypothalamic neural systems by the perinatal environment represents a possible mechanism by which alterations in maternal and/or postnatal nutrition predispose the offspring to obesity. The development of the ARH, which contains a subset of neurons that are devoted to metabolic regulation, such as NPY and AgRP-co-expressing neurons and POMC-containing neurons, appears highly sensitive to changes in the nutritional environment. Structural defects have been observed in the hypothalamus of animals subjected to an altered nutritional environment, such as changes in cell numbers, perturbation in the formation of axonal connections and attenuated leptin sensitivity. These effects appear to be mediated, to some extent, by abnormal leptin secretion and/or signaling during critical periods of fetal and/or postnatal development.

15–17 for review). The present review will summarize the developmental and structural alterations that have been observed in the hypothalamus in response to changes in the early nutritional and hormonal environment. In addition, we discuss how alterations of a diverse array of hypothalamic neural systems may have enduring effects on the regulation of appetite and energy balance.

CRITICAL PERIODS FOR HYPOTHALAMIC DEVELOPMENT

During brain development, there are critical periods during which particular experiences affect brain maturation and function. Although certain experiences are essential for orderly brain development to proceed, some adverse experiences will cause deleterious effects in the developing neural system. Critical periods exist because of the temporal and regional emergence of vital developmental processes. The developmental processes that produce a functional hypothalamus can fall into two broad categories: the determination of cell numbers, which involves neurogenesis, neuron migration and cell death, and the formation of functional circuits, which includes axon growth and synaptogenesis (see refs 18,19 for review) (Figure 2). Experiments using the thymidine analog bromodeoxyuridine to label newly formed cells have shown that the majority of neurons in the hypothalamus are born between embryonic day (E) 12 and E14 in mice and between E12 and E17 in rats^{20–22} (Figure 2). In nonhuman primates (NHPs), hypothalamic neurogenesis occurs in the first quarter of gestation^{23,24} (Figure 2). Reports on human fetal chemoarchitecture and cytoarchitecture have also suggested that early hypothalamic neurogenesis is limited to the 9th and 10th weeks of gestation.^{25–29} Part of the complex process of hypothalamic development includes the proper migration of neurons from their sites of origin (that is, the proliferative zone of the third ventricle) to their final positions in the mature hypothalamus, and this developmental process primarily occurs during late gestation in rodents.³⁰

In both rats and mice, the hypothalamus is relatively immature at birth and continues to develop during the first 2 weeks of postnatal life (P), as neurons send axonal projections to their target sites and form functional synapses (Figure 2). Axonal labeling experiments in mice have indicated that ARH axons reach their nuclei between P6 and P16.³¹ In contrast, efferent projections from the DMH to the PVH and LHA are fully established by P6.³¹ Synapses, which give rise to most neurological cell-to-cell

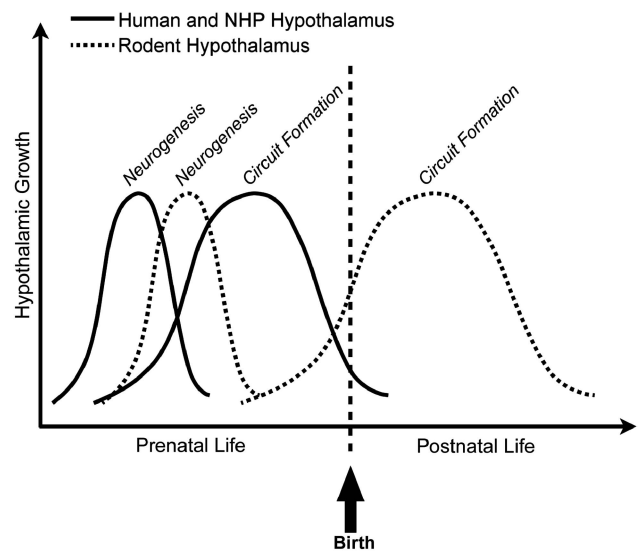


Figure 2. Time lines of hypothalamic development. The formation of a functional hypothalamus occurs in two major phases: the determination of cell numbers, which includes neurogenesis, and the formation of functional circuits, which includes axon growth and the formation of functional synapses. In rodents, humans and NHPs, neurogenesis primarily occurs during early to mid-gestation. The considerable importance of postnatal hypothalamic axon growth in rodents, however, differs from that in humans and NHPs, where hypothalamic neural projections develop almost entirely during fetal life.

communication, are formed after neuronal projections are established. In the rodent hypothalamus, synapses mature gradually from birth to adulthood.^{32,33} The considerable importance of postnatal hypothalamic development in rodents differs from that in humans and NHPs, where the hypothalamus develops almost entirely during fetal life (Figure 2). In both humans and NHPs, hypothalamic feeding circuits appear to primarily develop during intrauterine life. Work from Grove and colleagues³⁴ has shown that NPY/AgRP fibers innervate the PVH in Japanese macaques as early as on gestational day 100 (that is, the late second trimester), and a mature pattern of projections is apparent

at gestational day 170. Similarly, in human fetuses, NPY-immunoreactive (IR) fibers are detected in the ARH and the PVH as early as at 21 weeks of gestation.²⁹

Together, these data indicate that the rodent hypothalamus develops in two distinct environments: an intra-utero environment in which cell number is determined and an extra-utero environment in which hypothalamic neuronal connectivity is established. In contrast, the hypothalamus of humans and NHPs primarily develops during intrauterine life. These data also suggest that the disruption of hypothalamic development during these important developmental periods can result in severe structural and functional hypothalamic abnormalities.

PRENATAL INFLUENCES

Maternal obesity/high-fat feeding

In the United States, studies have estimated that one in five women are obese when they conceive.³⁵ As maternal obesity contributes to many negative outcomes in the infant, including obesity and diabetes, a number of studies have specifically attempted to evaluate the consequences of maternal obesity on hypothalamic development. The most widely used approach for studying the consequences of maternal obesity in both rodents and NHPs has been feeding pregnant dams with a high-fat diet (HFD; 30–60% of calories from fat). The offspring that are born to HFD-induced obese females become progressively overweight irrespective of whether the diet was only fed during gestation or during gestation and lactation. In addition, the offspring of HFD-induced obese females become hyperphagic, glucose intolerant and display an increase in adiposity.^{36,37} Notably, a short exposure to an HFD during pregnancy without the development of metabolic syndrome also predisposes the offspring to obesity, which includes an increase in body weight, fat mass and food intake.³⁸ These observations suggest that maternal obesity *per se* is not required for metabolic programming. Indeed, short transient exposures to nutritional and hormonal challenges during gestation are sufficient to induce long-term metabolic alterations in the offspring. The model of diet-induced obesity (DIO) developed by Levin³⁹ is well suited for the study of the underlying biological processes that contribute to the development of obesity in humans because Levin's DIO rats share several features with human obesity, including polygenic inheritance. Similar to the animals that are born to HFD dams, the offspring of mothers that are genetically predisposed to DIO are obese, hyperphagic and glucose intolerant when fed a high-energy diet, whereas the offspring that are born to diet-resistant (DR) dams do not show these effects.^{40,41}

The capacity of maternal obesity to alter cell proliferation in the developing hypothalamus has recently been shown in rodents. Maternal high-fat feeding in rats increases hypothalamic cell proliferation and results in higher numbers of orexigenic neurons in the PVH and LHA,³⁸ including galanin, enkephalin, dynorphin, melanin-concentrating hormone and orexin neurons. In general, rodent studies have indicated that the adverse effects of a maternal obesogenic environment are similar on hypothalamic cell numbers when they occur either during gestation or during gestation and lactation. For example, offspring of HFD mothers cross-fostered with control mothers during lactation exhibit similar changes in orexigenic cell numbers and metabolic outcomes compared with pups that are raised by HFD mothers during pregnancy and lactation.³⁸

The impaired organization of hypothalamic circuits is a common structural change that is associated with maternal high-fat feeding. In particular, ARH neural projections are not properly established in animals that are subjected to high-fat feeding/obesity during perinatal life. In both rodents and NHPs, the chronic consumption of an HFD during pregnancy induces a

reduction in the density of ARH AgRP-containing fibers that innervate the PVH.^{36,42} These structural abnormalities are associated with altered functional response of hypothalamic neurons to metabolic signals during adult life. For example, maternal HFD causes reduced hypothalamic activation of the signal transducer and activator of transcription 3 (STAT3) after leptin administration.³⁶ Consistent with these findings, administration of leptin fails to reduce body weight and food intake in animals born to obese dams.³⁶ In addition to influences of the maternal diet, the architecture of various hypothalamic pathways can also be influenced by the genetic background. For example, DIO rats that inherited obesity as a polygenic trait also display a diminished density of ARH axonal projections in the PVH.⁴³ In addition, DIO rats exhibit abnormal dendrite morphology in the VMH.⁴⁴ Moreover, a significant remodeling of synapses has been reported in the hypothalamus of DIO rats, with an increase in inhibitory inputs to POMC neurons in the ARH of DIO rats compared with DR rats.⁴⁵

Because many animal models of maternal obesity are associated with hyperglycemia and insulin resistance, it can be difficult to determine whether the developmental abnormalities of pups born to obese dams are caused by changes in the diets of the dams or whether they arise as a consequence of maternal diabetes. The manipulation of glucose levels without alterations in the diet can be performed experimentally by injecting streptozotocin, which is a pancreatic beta cell toxin. By using the streptozotocin animal model, we recently found that pups that are born to nonobese diabetic dams have a reduction in the density of arcuate orexigenic (AgRP)- and anorexigenic (aMSH)-containing fibers that innervate the PVH.⁴⁶ These data show that maternal diabetes alone can also cause long-term structural changes in the hypothalamus.

Prenatal malnutrition/altered growth

A number of reports have also focused on the effects of maternal malnutrition on hypothalamic development. Several rodent models of perinatal growth retardation have been validated, such as rodents born to dams that were either calorie restricted (CR) or protein restricted (PR) during pregnancy and lactation. The offspring that are born and raised by CR or PR mothers have a low birth weight and exhibit slow growth during the preweaning period. In contrast, the pups that are born to CR or PR dams but raised by lactating mothers fed *ad libitum* display rapid catch-up growth during the first postnatal week.^{47–49} Although adult animals born to malnourished mothers display normal body weight, they are hyperphagic, glucose intolerant and display an increased sensitivity to DIO.^{47,49,50}

In contrast to maternal high-fat feeding, maternal malnutrition is associated with a reduction in the number of orexigenic neurons in the offspring's hypothalamus, including a reduction of NPY-IR neurons in the ARH.⁵¹ However, whether this change in neuronal cell number is the result of a reduction in neurogenesis and cell migration or an increase in the number of cells undergoing apoptosis remains to be investigated. Nevertheless, the observation that the thickness of the cortical plate is reduced in the fetuses of PR dams supports the idea that maternal malnutrition reduces cell proliferation during embryonic life.⁵² In addition, various genes involved in brain development, including cell proliferation and differentiation genes, are downregulated by maternal PR.⁴⁹ Moreover, astrocytogenesis, which directly follows the neurogenic period, is affected by maternal diet. At weaning, the offspring of PR mothers have a reduced number of astrocytes and a reduction in the glia-to-neuron ratio.⁵³ These findings are particularly relevant because of the reported role of glial cells in brain development and synaptic plasticity.⁵⁴

The exposure of neonates to a nutrient-deprived environment affects hypothalamic axonal connectivity. The density of AgRP- and POMC-IR nerve fibers in the PVH is reduced in the rodents that are

born to CR or PR dams.^{49,55} Moreover, for intrauterine growth-retarded animals, the timing of catch-up growth is an important determinant for postnatal hypothalamic development. Early catch-up growth ameliorates the abnormal organization of hypothalamic neural projections and is highly beneficial for markers of brain development, including markers of cell adhesion and axon elongation, whereas late catch-up growth causes more detrimental neurodevelopmental effects in the hypothalamus.⁴⁹

POSTNATAL INFLUENCES

Postnatal overnutrition

Because of the importance of postnatal organ development, including the hypothalamus, in rodents, animal models of postnatal metabolic programming have been developed to specifically target the postnatal period. One approach that has proven extremely fruitful for the study of postnatal overfeeding is litter size reduction. Pups raised in small litters (SLs) display accelerated growth during the preweaning period. Accordingly, pups raised in SLs exhibit higher body weight as early as at 2 weeks of age, and these animals remain heavier throughout life.^{56–58} In addition, postnatally overfed animals show accelerated and exacerbated weight gain when fed an HFD.⁵⁷

Postnatal overfeeding can influence various hypothalamic developmental processes, including the determination of hypothalamic cell numbers and neuronal connectivity. Rats raised in SLs display higher numbers of neurons that produce the orexigenic neuropeptide galanin in the PVH.⁵⁹ In addition, postnatal overfeeding affects neuronal responses to neuropeptides. For example, PVH neurons of chronically overfed pups have been shown to display reduced electrophysiological responses to arcuate neuropeptides, such as NPY, AgRP, aMSH and CART.⁶⁰ A disruption in the development of ARH neural projections has also been reported in animals raised in SLs. Chronic postnatal overnutrition reduces the outgrowth of AgRP and aMSH axons to the PVH.⁵⁸

Postnatal malnutrition

Interest in postnatal malnutrition is particularly high given its prevalence in underdeveloped countries and the evidence that it can promote metabolic diseases. Rodent models that use different litter sizes can also be used to specifically study the role of postnatal malnutrition on metabolic and developmental outcomes. For example, raising pups in large litters (LLs) decreases milk availability and markedly attenuates preweaning growth. Interestingly, pups raised in LLs display rapid weaning-to-adult weight gain that is associated with an increase in adiposity and glucose intolerance.⁵⁸ A number of structural changes have been reported in the hypothalamus of postnatally malnourished animals. The densities of aMSH- and NPY/AgRP-IR fibers have been shown to be severely reduced in the hypothalamus of animals raised in LLs.⁵⁸ In addition, rats from LLs display an increased number of NPY-IR neurons and higher concentrations of NPY in the ARH at weaning as compared with normally fed animals.⁶¹ These observations indicate that the low density of NPY/AgRP fibers is due to alterations in axon growth as opposed to a reduction in cell number. The physiological and structural alterations that are induced by neonatal malnutrition do not seem to be restricted to the neural systems that are related to energy balance regulation. Interestingly, the effects of neonatal nutrition appear to be global and target a variety of neural pathways that are sensitive to feeding cues. For example, we recently found that postnatal underfeeding disrupts the normal development of neural projections containing kisspeptin, which is a key neuropeptide in reproductive function. This disruption can have long-term consequences that are associated with delayed puberty and fertility.⁶²

LEPTIN AS A POTENTIAL HORMONAL MECHANISM BY WHICH PERINATAL NUTRITION PROGRAMS HYPOTHALAMIC DEVELOPMENT

As discussed above, changes in perinatal nutrition during critical periods of life induce a plethora of structural changes in the hypothalamus. However, the precise biological mechanisms that underlie the perinatal nutrition-induced alterations in hypothalamic organization and function remain largely unknown. It is clear that a perturbation in perinatal nutrition causes a wide range of hormonal changes in both the dams and their offspring, and recent evidence has indicated that changes in perinatal leptin levels during critical periods of development may represent a likely cause for the perinatal nutrition-induced alterations in hypothalamic development. Maternal obesity/diabetes and postnatal overnutrition can increase leptin levels throughout postnatal life and cause leptin resistance (as evidenced by a reduction of leptin-induced pSTAT3 in the ARH) during critical periods of hypothalamic development.^{36,43,46,57,58} In contrast, maternal malnutrition (including CR and PR) during pregnancy and lactation or postnatal malnutrition blunts the naturally occurring postnatal leptin surge.^{47,49,58} Remarkably, daily leptin treatment during early postnatal life in pups born to malnourished dams normalizes their metabolic abnormalities,⁶³ which indicates that metabolic alterations are potentially reversible with leptin administration during specific phases of developmental plasticity, and that the developmental actions of leptin contribute to the adult phenotype of the organism. Taken together, these observations suggest that leptin has a role in the developmental responses to nutritional and metabolic changes. Consistent with this idea, mouse neonates that lack leptin (Lep^{ob}/Lep^{ob}) have a disrupted development of ARH axonal projections compared with wild-type mice, and these neurodevelopmental abnormalities persist throughout life.⁶⁴ Leptin treatment in Lep^{ob}/Lep^{ob} neonates restores a normal pattern of ARH connectivity; however, the same leptin treatment in adulthood has no effect on ARH projections,⁶⁴ which suggests that leptin primarily acts during a restricted neonatal period to exert its developmental effects on ARH neural projections.

A likely molecular mechanism underlying the trophic effects of leptin on hypothalamic circuits is the expression of the leptin receptor $LepRb$ by ARH neurons. The developing ARH contains a high level of $LepRb$ mRNA,⁶⁵ and the administration of leptin to mouse neonates results in the activation of major $LepRb$ signaling pathways, including pSTAT3, pERK and pAKT.⁶⁶ A clear role for ARH $LepRb$ signaling pathways in ARH development has recently been demonstrated. ARH neural projections are disrupted in leptin receptor-deficient mice ($LepR^{db}/LepR^{db}$) and in neonates specifically lacking $LepRb \rightarrow pSTAT3$ and $LepRb \rightarrow pERK$ signaling.⁶⁶ The findings that leptin induces neurite outgrowth in isolated ARH explants and that this effect is blunted if the explants are derived from $LepR^{db}/LepR^{db}$ mice also support the idea that leptin acts directly on $LepRb$ -containing ARH neurons to promote axonal growth.^{64,66} Notably, the ability of leptin to activate intracellular $LepRb$ signaling in ARH neurons is significantly reduced in neonates born to DIO or diabetic dams and in chronically overfed pups,^{43,46,57} which suggests that early leptin resistance may be responsible for the long-term changes in the organization of the hypothalamic feeding circuits that are observed in these animals. Consistent with this idea, *in vitro* experiments have shown that ARH neurons from DIO rats exhibit a severely diminished responsiveness to neurotrophic actions compared with ARH neurons from DR rats.⁴³

WINDOWS OF OPPORTUNITY

Critical periods should not only be conceived as a window of vulnerability but also as a window of opportunity. As with most

important developmental factors, perinatal nutrition can either be beneficial or detrimental toward hypothalamic feeding pathways and metabolism. The effects of perinatal nutrition depend on various parameters, such as the genetic background. For example, although neonatal malnutrition exerts detrimental effects on lifelong metabolic regulation and hypothalamic development in wild-type rodents, it has beneficial effects in DIO rats. Raising DIO rats in LLs confers an enhanced sensitivity to the anorectic effect of leptin and protects them from becoming obese.⁶⁷ Notably, DIO rats that are raised in LLs exhibit increased leptin binding to its receptor in the ARH at the time when ARH projections develop. Consistent with these findings, neonatally underfed DIO rats exhibit enhanced projections from the ARH to the PVH.⁶⁷ If nutritional intervention occurs at a later stage of development, however, the opposite phenotype is observed. For example, caloric restriction of DIO rats after weaning causes them to increase their food intake and become even more obese as soon as they are allowed to eat *ad libitum*, which differs from observations in non-CR rats.⁶⁸ These findings show that food restriction can either have beneficial or detrimental long-term metabolic effects in genetically predisposed obese rats depending on the timing of the intervention.

Exercise is known to reduce body weight in obese individuals, including humans and rodents.⁶⁹ Remarkably, only 3 weeks of postweaning exercise is sufficient to reduce food intake and slow weight gain in DIO rats, and these effects have been shown to persist at 10 weeks after exercise termination. Thus, short exposure to exercise early in life can have enduring beneficial effects on metabolism in DIO rats.⁶⁸ When obese rats are exposed to exercise as adults, however, their exercise-induced weight loss is transient, and the weight is regained when the exercise is terminated.⁷⁰ These data further illustrate the importance of the timing of the intervention.

Taken together, these data show that the immediate postweaning period and peripubertal life are important periods to design interventional studies to ameliorate and hopefully reverse the metabolic malprogramming of the fetus and/or neonate.

CONCLUSION

It is now well accepted that a disruption in neurodevelopmental processes can lead to disease later in life. Research during the past decades has provided valuable information on the role of perinatal nutrition in the susceptibility to disease development in adult life, including obesity. There is now clear evidence that nutritional changes during critical periods of life can also impair the development of the hypothalamic neural systems that are involved in energy balance regulation. One of the key mechanisms of the metabolic programming of the hypothalamus is leptin signaling during perinatal development. Approaches that are aimed at improving and extending leptin's neurotrophic effects beyond neonatal critical periods would enable more efficient strategies to promote proper hypothalamic wiring and could provide new therapeutic opportunities.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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