Developmental Programming of Energy Balance and Its Hypothalamic Regulation

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Developmental programming is an important physiological process that allows different phenotypes to originate from a single genotype. Through plasticity in early life, the developing organism can adopt a phenotype (within the limits of its genetic background) that is best suited to its expected environment. In humans, together with the relative irreversibility of the phenomenon, the low predictive value of the fetal environment for later conditions in affluent countries makes it a potential contributor to the obesity epidemic of recent decades. Here, we review the current evidence for developmental programming of energy balance. For a proper understanding of the subject, knowledge about energy balance is indispensable. Therefore, we first present an overview of the major hypothalamic routes through which energy balance is regulated and their ontogeny. With this background, we then turn to the available evidence for programming of energy balance by the early nutritional environment, in both man and rodent models. A wealth of studies suggest that energy balance can indeed be permanently affected by the early-life environment. However, the direction of the effects of programming appears to vary considerably, both between and within different animal models. Because of these inconsistencies, a comprehensive picture is still elusive. More standardization between studies seems essential to reach veritable conclusions about the role of developmental programming in adult energy balance and obesity. **(*Endocrine Reviews* 32: 272–311, 2011)**

I. Introduction

The concepts of "nutritional programming," "fetal programming," "fetal origins of adult disease," "developmental origins of health and disease," "developmental induction," and "developmental programming" (1–5) were all conceived to explain the same phenomenon: a detrimental environment during a critical period of development has persistent effects, whereas the same environmental stimulus outside that critical period induces only reversible changes. Many epidemiological studies have shown an association between low birth weight and an elevated risk of developing several chronic diseases in adulthood (reviewed in Refs. 2 and 6–9). The Dutch famine, a unique "natural experiment" with a well-defined period of food shortage in an otherwise well-nourished population, has shown that maternal undernutrition during gestation compromises health in later life and that these long-term effects depend on its timing during gestation (10). It is assumed that low-birth-weight infants, who are not small *per se* but rather are small for gestational age (SGA), suffered from intrauterine growth restriction (IUGR) due to a low availability of nutrients. As adults, these subjects have an increased risk for insulin resistance, hypertension, and cardiovascular disease, collectively

Abbreviations: AgRP, Agouti-related protein; ARC, arcuate nucleus; BMI, body mass index; CART, cocaine- and amphetamine-regulated transcript; DMN, dorsomedial nucleus; HPA axis, hypothalamic-pituitary-adrenal axis; HPT axis, hypothalamic-pituitary-thyroid axis; IUGR, intrauterine growth restriction; LHA, lateral hypothalamic area; MC, melanocortin; MCH, melanin-concentrating hormone; α-MSH, α-melanocyte-stimulating hormone; Ob-R, leptin receptor; ORX, orexin; NPY, neuropeptide Y; POMC, proopiomelanocortin; PVN, paraventricular nucleus; SGA, small for gestational age; VMN, ventromedial nucleus; Y_xR, NPY receptor.

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called the "metabolic syndrome" or "syndrome X" (11). How early malnutrition should lead to conditions normally related with affluent environments has been the subject of much debate. It is now believed that adaptations that helped these IUGR or SGA infants survive during pregnancy may become detrimental in later life when nutrients are no longer scarce. It is said that these individuals are "programmed" for the metabolic syndrome. Whether the obesity that is part of the metabolic syndrome is also programmed has long been a matter of debate (12–15). In addition to a low birth weight, high birth weight (*e.g.*, through maternal obesity or maternal diabetes) is also associated with an increased risk of developing obesity in later life (13, 16–19). In this review, developmental programming will be discussed, followed by energy balance, its regulation, its normal development, and what is known about its programming.

II. Developmental Programming

The concept of developmental programming implies that characteristics of the environment encountered during early development can permanently alter physiology in later life (20). The perinatal level of nutrition has been proposed to be a particularly important feature (21). In early development, there is a window of plasticity, a period in which the organism can still develop in different directions. During this period, the developing organism has a large potential to adapt to its environment. Once the window of plasticity has closed, many of these adaptations will become fixed. Although epidemiological studies have mainly concentrated on the detrimental consequences of programming, it is not principally a harmful phenomenon. Being able to adjust your phenotype to the environment encountered in early life can be "evolutionary" adaptive if the environment is relatively stable (22, 23). A mismatch between the environment in early life and adult life may result in inappropriate adaptations in the organism.

This is thought to be the case for SGA infants in developed countries. The low nutrient availability during intrauterine development that causes these babies' growth restriction is usually not due to a low maternal energy intake, but instead is due to other causes such as placental insufficiency (24), drug use (including caffeine, alcohol, and smoking), and stress or illness (25), and hence does not give an adequate prediction of nutrient availability in postnatal life. SGA subjects that develop the metabolic syndrome in later life are a good example of "developmental programming gone bad" (2, 26). In contrast, in infants born to obese mothers, the enhanced availability of nutrients to the fetus is thought to allow increased fetal adipogenesis and simultaneously alter the systems that regulate energy balance while they are still plastic (26, 27). It is now well established that the perinatal environment can program similar changes in experimental animals (reviewed in Refs. 28–31).

III. Energy Balance Regulation

Energy homeostasis, or the process whereby stable energy reserves are maintained over long periods of time, is tightly regulated. To maintain neutral energy balance, energy intake, thermogenesis, and activity need to be regulated. These components of energy balance are regulated by at least two separate, but interrelated systems: 1) a short-term system that controls the initiation and termination of meals depending on the contents of the gastrointestinal tract; and 2) a long-term system that defends the stability of the energy reserves and thereby that of body weight (32). In addition, higher brain functions, such as motivation and reward, as well as environmental factors such as social influences and food availability alter our food intake and activity levels (33, 34). It is the task of the short-term and long-term regulatory systems to balance the energy reserves in the face of a changing environment. Manipulations of the gastrointestinal peptides involved in the short-term regulation of hunger and satiety were mostly shown to have little effect on food intake and body weight over a longer period, and therefore we rely predominantly on the long-term system to maintain neutral energy balance.

The first indications that an important part of the long-term regulatory system resides in the hypothalamus of the brain came from early studies reporting severe anorexia or obesity after lesions of distinct areas of the hypothalamus (35, 36). The hypothalamus consists of several distinct nuclei that produce specific neuropeptides and perform different tasks in the homeostasis of temperature, water, energy, sleep, reproduction, and other functions. In the brain, there are extensive connections to, within, and from the hypothalamus (37). The regulation of energy balance by the hypothalamus is a complicated process, and the following explanation is a simplified summary. It is also important to keep in mind that in addition to energy balance, these nuclei and peptides are involved in other hypothalamic functions as well. Although the majority of the literature cited concerns research in rodents, most of this discussion is also applicable to humans (38–43).

In short, neurons in the mediobasally located arcuate nucleus (ARC) receive information about the status of the energy reserves (*e.g.*, adipose tissue) through peripheral hormones that circulate in amounts related to body fat stores; the information is integrated and passed on to several other hypothalamic nuclei, including the paraven-

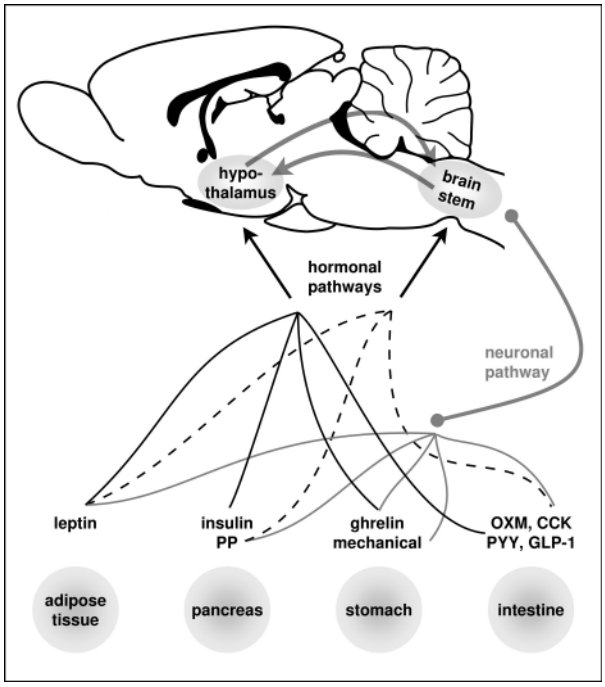

FIG. 1. A simplified overview of the regulation of energy balance. Peripheral signals of energy reserves reach the hypothalamus and brainstem via hormonal and neuronal pathways. The former pathway is more important in transferring long-term adiposity signals, whereas the latter handles the rapid transmission of short-term satiety signals, mainly through the vagus nerve. Several brain areas, including the hypothalamus and brainstem, then interact with each other to regulate intake and expenditure of energy. This review will focus on the interaction between long-term adiposity signals and the hypothalamus. See the text for further information. CCK, Cholecystokinin; GLP-1, glucagon-like peptide 1; OXM, oxyntomodulin; PP, pancreatic polypeptide; PYY, peptide YY. [Adapted from G. Paxinos and C. Watson: *The rat brain in stereotaxic coordinates*, Academic Press, New York, 1997 (599). © Elsevier 1997].

tricular nucleus (PVN) and the lateral hypothalamic area (LHA), and from there on to output functions. The process is also influenced by satiety hormones from the gastrointestinal tract (44) (Fig. 1).

A. Peripheral signals

Several hormones provide the brain with information on the status of energy balance. These can be divided into two categories: 1) hormones that are produced by the gastrointestinal tract and signal on satiety and hunger, or short-term information; and 2) hormones that signal the status of fat reserves of the body, the long-term signals. Leptin is the major peripheral hormone involved in long-term energy homeostasis.

1. Leptin

Leptin was first identified as the product of the gene that is defective in obese *ob/ob* mice and was named after "leptos," the Greek word for "thin" (45, 46). In both humans and rodents, leptin is produced by adipose tissue, in proportion to the body fat content, as an "adiposity signal" (47–52). The leptin receptor (Ob-R) Ob-Rb is highly expressed in the ARC and is also found in some other hypothalamic areas (53–55). In addition, short forms of the Ob-R (a, c–f), which probably act as leptin transporters, exist in the choroid plexus and other areas where substances may cross the blood-brain barrier (53–56).

Leptin gene expression and serum levels decrease upon negative energy balance in both humans and rodents (48, 51, 52, 57–59). However, its levels are disproportionately reduced during acute depletion (60) to initiate immediate action to restore these reserves. The central effects of leptin include reductions in food intake and body weight gain and increased energy expenditure (61–63).

2. Other peripheral signals

Insulin, produced by the β-cells of the pancreas, controls blood glucose availability. In energy balance regulation, it acts both as a short-term and as a long-term signal (47). It is released upon acute changes in energy levels such as meal ingestion, but its circulating levels are also directly correlated with fat reserves. In the brain, it acts on receptors in the ARC to reduce food intake and increase energy expenditure (reviewed in Ref. 47).

Ghrelin, produced by the stomach, indicates negative energy balance (64). Its levels rise before meal onset and decrease with feeding. Over longer time periods, ghrelin levels are inversely correlated to energy stores. Ghrelin acts on receptors in the hypothalamus to influence ARC neuron activity. Upon peripheral or central injection, ghrelin stimulates food intake and decreases energy expenditure (reviewed in 64).

Other peripheral signals, which are predominantly involved in the short-term regulation of the initiation and termination of meals, are produced in different regions of the gastrointestinal tract and include cholecystokinin, glucagon-like peptide 1, oxyntomodulin, pancreatic polypeptide, and peptide YY (65, 66). These are mainly secreted after eating and inhibit further food intake. Their actions on food intake are exerted via the vagus nerve, the brainstem, and the hypothalamus. It has also been shown that several of these peripheral signals interact with each other to regulate food intake. Another important function of these gut peptides is to control the proper processing of the nutrients ingested in a meal (reviewed in Refs. 65 and 66).

B. Peptides from the arcuate nucleus

The ARC is located mediobasally in the hypothalamus, close to the median eminence. This is a circumventricular organ where the blood-brain barrier is incomplete and blood-borne signals can easily reach the ARC neurons (67, 68). In addition, leptin, insulin, and ghrelin are actively

transported across the blood-brain barrier (69–71). Apart from these direct inputs from the periphery, the hypothalamus also receives information concerning energy balance from brainstem areas (66). The ARC integrates this information and drives other hypothalamic areas such as the PVN and the LHA (44).

The ARC contains two populations of neurons that are strongly involved in the regulation of energy balance. These two populations express different neuropeptides. A medial population coexpresses the orexigenic peptides neuropeptide Y (NPY) and agouti-related protein (AgRP) (72–75). The anorexigenic peptides α-melanocyte-stimulating hormone (α-MSH) and cocaine- and amphetamine-regulated transcript (CART) are produced by a more lateral population of ARC neurons (74, 76, 77). Both of these populations coexpress receptors for peripheral signals, including those for leptin (40, 78, 79), insulin (80, 81), and ghrelin (82, 83). Both the NPY/AgRP and the proopiomelanocortin (POMC)/CART neurons project widely throughout the hypothalamus and the brain (39, 72, 84, 85). Besides the ARC, CART is also expressed in several other hypothalamic nuclei, including the PVN and LHA (86).

These peptides exert their effects via various receptors. In the rat, four subtypes of the NPY receptor have been identified that have all been found in many different brain areas, including most hypothalamic nuclei (87, 88). Within the ARC, Y_1R and Y_5R mRNA and protein are found in many α-MSH/CART neurons (89, 90), whereas the mRNA and protein for Y_2R, which is believed to be an autoreceptor (91), was found in most NPY/AgRP neurons (89, 90). Of the five identified melanocortin (MC) receptors, the MC3 and MC4 receptors mediate the effects of α-MSH in the regulation of energy balance (92–95), although some debate previously existed over the involvement of the MC3 receptor (93). Both are highly expressed in hypothalamic nuclei, but MC4 is more widely expressed throughout the brain (96, 97). AgRP is an inverse agonist of the constitutively active MC receptors (98, 99). A CART receptor has not been identified yet (100), but specific CART binding has been reported in cultured cells from the hypothalamus, hippocampus, and nucleus accumbens (101).

1. Neuropeptide Y

NPY is one of the most abundant peptides in the brain (84). Negative energy balance, as elicited by fasting, has been shown to increase NPY peptide and expression levels (102–106). Leptin injections, mimicking positive energy balance, have been reported to lower NPY expression (62, 102, 106, 107), as well as the activity of NPY neurons (82), NPY secretion by the hypothalamus (107), and NPY levels in the PVN (108). In the hypothalamus, NPY stimulates food intake and body weight gain (109–111), increases white fat lipid storage, and reduces brown fat thermogenesis (112, 113). In contrast, NPY injections in most areas outside the hypothalamus did not have any effect on food intake (109).

2. Agouti-related protein

AgRP was discovered because of its resemblance to agouti, which in mice causes severe obesity when overexpressed (114, 115). As with NPY, levels of AgRP expression, peptide, and activity are increased by fasting and decreased by leptin (103, 106, 116–118). Administration of AgRP or other antagonists of MC receptors were shown to elevate food intake, body weight, and body fat and to reduce energy expenditure and brown fat thermogenesis (119–121). In contrast to the relatively short-lived effects of NPY, a single injection of AgRP will increase food intake for up to 1 wk (122, 123). These long-lasting effects of AgRP are proposed to be mediated by other routes than the MC receptors (122, 124).

3. α-Melanocyte-stimulating hormone

α-MSH is cleaved from the precursor polypeptide POMC, together with other peptides like β-endorphin and ACTH (125). POMC gene expression is reduced by fasting and stimulated by leptin (102–104, 106, 126, 127). In addition, leptin stimulates activity of POMC neurons and α-MSH release (76, 128, 129). Central administration of α-MSH or its agonist melanotan II decreases food intake, weight gain, and adiposity and increases energy expenditure, brown adipose tissue activity, and body temperature, but not locomotor activity (119, 120, 130–133). Within the hypothalamus, it reduced food intake when injected in the ARC, PVN, and LHA among others (134). In contrast to α-MSH, β-endorphin has been shown to increase food intake (133).

4. Cocaine-and amphetamine-regulated transcript

CART was identified when its expression levels were shown to be increased after administration of cocaine or amphetamine (135), although it was sequenced as a peptide with unknown function long before that (136). ARC CART mRNA levels are decreased by fasting and increased by leptin (102, 103, 137, 138). In line with an anorexigenic role, intracerebroventricular CART injections decreased food intake (137, 139, 140), and intrahypothalamic injection increased gene expression of the thermogenic uncoupling protein-1 in brown adipose tissue (141, 142). Chronic injections also reduced body weight gain and increased lipid oxidation (143, 144). Injections into distinct hypothalamic nuclei, however, have produced either increased or decreased food intake (139–142). These contradictory results and colocalization with

both orexigenic and anorexigenic neuropeptides have been interpreted to suggest that CART may play a modulatory role, with different effects depending on its localization (100).

C. Anorexigenic peptides from the paraventricular nucleus

The PVN expresses the anorexigenic peptides CRH and TRH in two distinct populations of parvocellular neurons (145, 146). A subpopulation of the latter coexpresses CART (40, 86). The PVN receives innervation from ARC NPY/AgRP, POMC, and CART terminals (85, 137, 147). In addition, receptors for these peptides are expressed in the PVN (88, 96), providing all the "machinery" for signaling from the ARC to the PVN. The PVN also receives some input from the LHA, dense projections from the dorsomedial nucleus (DMN), and indirect input from the amygdala (37).

Both peptides exert their effects via two receptors: the CRH_1- and CRH_2-receptors (148, 149) and the TRH_1- and TRH_2-receptors (150–152), respectively. CRH_1 (153, 154) and TRH_2 (155) are both widely expressed in different brain areas, whereas the expression of CRH_2 (156, 157) and TRH_1 (155) is mostly restricted to the hypothalamus.

1. Corticotropin-releasing hormone

CRH, known for its role in the hypothalamic-pituitary-adrenal (HPA) or stress axis, is also involved in the regulation of energy balance (158). PVN CRH neurons project to the median eminence, where CRH regulates the release of ACTH and β-endorphin from the pituitary (146, 159), and to some cell groups in the brainstem and spinal cord (160).

PVN CRH expression and peptide levels are decreased by food deprivation and increased by leptin (62, 104, 127, 161, 162). Furthermore, CRH expression is increased by injections of both α-MSH and CART (163, 164). Intracerebroventricular CRH has been shown to decrease food intake and body weight gain and to induce both locomotor activity and activity of brown adipose tissue (165–168). These data all point toward an anorexigenic and catabolic role for CRH.

2. TSH-releasing hormone

TRH stimulates TSH release from the pituitary. TRH terminals are found throughout the hypothalamus, in the median eminence, and the pituitary (145). Via thyroid hormone, which stimulates energy expenditure and thermogenesis, this hypothalamic-pituitary-thyroid (HPT) axis plays an important role in energy homeostasis (169). TRH expression and release are reduced by fasting, NPY, and AgRP, and increased by leptin, α-MSH, and CART (129, 170–174). Central and peripheral injections of TRH decrease food intake and increase body temperature (175–177). Despite increased food intake (possibly a compensatory response), chronic oral TRH causes a reduction in body weight (178). These data point toward an anorexigenic and catabolic role for TRH.

D. Orexigenic peptides from the lateral hypothalamic area

The LHA was recognized early on as a "feeding center" (35, 36). Two distinct cell populations express the orexigenic peptides melanin-concentrating hormone (MCH) and the orexins (ORXs), the latter especially in the perifornical area (179). Some MCH cells also contain CART (40, 86). The LHA is innervated by nerve terminals containing NPY, AgRP, and α-MSH (39, 179) and expresses receptors for these peptides (88, 96, 97). Apart from this input from the ARC, the LHA also receives input from the hypothalamic PVN and DMN and from some higher brain areas including anterior limbic cortical areas, the nucleus accumbens, and indirectly from the hippocampus (37). From the cell bodies in the LHA, MCH and ORX fibers project throughout the hypothalamus and to many different brain areas (180–182). MCH has been identified as the ligand for an orphan receptor by several groups simultaneously (183–187). Humans, but not rodents, have an additional MCH_2-receptor (188). The distribution of MCH_1 and the ORX_1- and ORX_2-receptors corresponds well with that of MCH (189, 190) and ORX (191, 192) fibers, respectively.

1. Melanin-concentrating hormone

MCH was first discovered in fish, as the peptide that causes melanosomes to contract and thereby has a skin-lightening effect (193). Thereafter, a similar peptide was found in rat brain (194), and the homologous peptide was identified (195). MCH expression was either decreased (as would be expected for an orexigenic peptide) or increased by injections of leptin (196, 197). Fasting has been shown to increase MCH expression (103, 197–199), but MCH neurons appear to be inhibited by NPY (200). Most studies have reported increased food intake after MCH injections (199, 201–203), although the opposite has also been reported (198). When injected chronically, the orexigenic effect of MCH faded, and body weight was not affected (204). Mice that lack MCH or its receptor show hyperactivity (205, 206). This suggests that MCH suppresses activity, in accordance with an orexigenic, anabolic role.

2. The orexins

ORX-A (or hypocretin 1) and ORX-B (hypocretin 2) were discovered and named simultaneously by two groups (207, 208). Negative energy balance has been shown to

increase LHA ORX expression and peptide levels (208–211), although unchanged expression levels were also reported (103, 197, 212). Leptin lowers LHA ORX expression and ORX-A levels (210, 213, 214). Although AgRP injections were shown to increase activity of ORX neurons (215), NPY either decreased or failed to affect their activity in two different paradigms (216, 217). Nevertheless, Y_1R and Y_5R antagonists suppress ORX-induced feeding, which is again more in line with the orexigenic role of the ORXs. Central ORX injections stimulate feeding (208, 218–220), supporting an orexigenic role. However, not all ORX aspects fit the profile for a truly anabolic protein. The results for ORX-B have been less consistent, and the outcome generally depends on the time of day (221). Furthermore, metabolic rate and activity seem to be stimulated rather than decreased by ORX (222–225), and continuous infusion of ORX altered the circadian rhythm of feeding without affecting total food intake or body weight (219, 226). Apart from its complicated role in the regulation of energy balance, ORX is also involved in the regulation of arousal and vigilance, and its primary function in energy balance may be to synchronize feeding behavior with other essential behaviors and the environment (227–229).

E. Downstream events

There are basically three output pathways through which hypothalamic signaling can eventually alter the intake and expenditure of energy. The first pathway influences behavior through integration of signals from many brain areas and, ultimately, the activation of motor neurons (33). A final effect of increased hypothalamic orexigenic activity may be the initiation of a meal. The second pathway, the neuroendocrine route, influences energy balance through the secretion of hormones. The HPA and HPT axes are part of this pathway. In the HPA axis, CRH from the PVN stimulates the release of ACTH from the pituitary, which in turn induces glucocorticoid release from the adrenal glands (37). These can indirectly influence feeding behavior and energy expenditure (reviewed in Ref. 230). In the HPT axis, TRH from the PVN, via the release of TSH from the pituitary, induces the release of thyroid hormone by the thyroid gland, which stimulates energy expenditure and thermogenesis (reviewed in Ref. 169). The third pathway is via the autonomic nervous system. Several hypothalamic nuclei, especially the PVN, innervate neurons in the brainstem and the spinal cord that are part of the autonomic nervous system, both sympathetic and parasympathetic (37). Via these sympathetic and parasympathetic pathways, energy expenditure can be regulated, for example by influencing the heart rate and thermogenesis by adipose tissue and skeletal muscle (reviewed in Refs. 32, 231, and 232).

F. Mutual connections

When reflecting on the regulation of energy balance, it is important to bear in mind that the pathways and processes described above are much simplified. The main route for information is from the periphery to the ARC and in turn via the PVN or LHA to the output systems (Fig. 2A). However, other brain areas (hypothalamic and otherwise) are involved in the routing of peripheral signals as well, with ample feedback between the different brain areas. In several respects, the regulation of energy balance is much more complex than the relatively straightforward pathways described above. A few examples are given below.

First, the different populations of cells in the ARC influence their own and each other's activity. Both NPY/AgRP and POMC/CART cells express receptors for NPY and MCs, albeit different subtypes: NPY/AgRP neurons express Y_2R and the MC3 receptor, whereas POMC/CART neurons express Y_1R, Y_5R, and the MC4 receptor (89, 90, 233). Through these receptors, NPY inhibits the POMC/CART cells (128, 234); NPY and AgRP stimulate each other's release and (at least *in vitro*) can be stimulated by α-MSH and CART (235); whereas CART reduces α-MSH release (140).

Second, the PVN and LHA may also receive peripheral input directly, through leptin receptors in these nuclei, although these receptors are not necessarily colocalized with the four peptides of our interest: CRH, TRH, MCH, and ORX (236–239). For example, leptin also influences motivational brain areas directly, via receptors in these areas (reviewed in Refs. 33 and 240).

Third, there is feedback within and between the PVN and LHA. CRH and TRH neurons are contacted by each other's axons (241), as are MCH and ORX neurons (242), and ORX stimulates both ORX and MCH neurons (200, 243). Furthermore, MCH and ORX have been shown to stimulate CRH neurons and release (244, 245), whereas MCH reduces TRH release (246). CRH, in turn, has been shown to activate ORX neurons (247).

Fourth, besides this mutual influence of the PVN and LHA peptides, they also project back onto the ARC neurons. ORX axons, for example, terminate on both NPY and POMC neurons (220, 248). Via these terminals, ORX stimulates NPY neurons and inhibits POMC neurons (82, 220). MCH has been shown to have similar effects on the ARC as ORX (201). Moreover, CRH receptors have been identified in NPY neurons in the ARC (249), and a CRH receptor agonist has been shown to inhibit medial ARC neurons (250).

In addition, as mentioned before, there are many more brain areas involved in the regulation of energy balance than these three hypothalamic nuclei. Among these are the

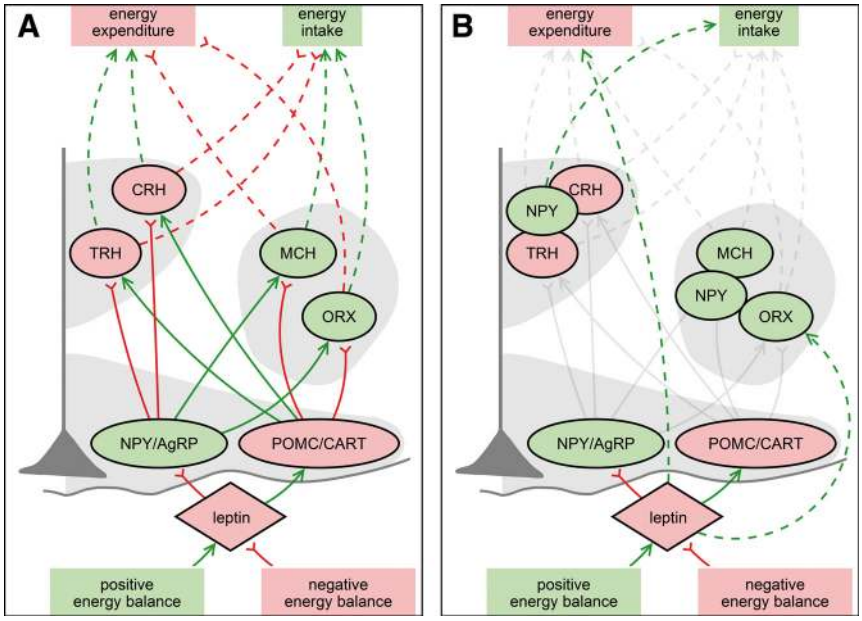

FIG. 2. Simplified diagrams of the hypothalamic regulation of energy balance in adult (A) and juvenile (B) life. A, The main pathway for energy balance regulation is from peripheral input (leptin) to the ARC (with NPY, AgRP, POMC, and CART), via the PVN (CRH and TRH) and LHA (MCH and ORX), to the output systems. B, Energy balance does not appear to be tightly regulated in the neonatal period. The few connections that have been reported to be in place are summarized in this figure. The adult connections are not yet present and/or active and are shown in *light gray*. *Green arrow*, Positive effect; *red inverted arrow*, negative effect; *solid line*, direct connection; *dashed line*, indirect connection; *green* (*red*) *nodes* lead to more positive (negative) energy balance when stimulated/active. See the text for details.

ventromedial nucleus (VMN), the DMN, and the medial preoptic area (251–255). Furthermore, the caudal brainstem is not only a relay station between the periphery and the hypothalamus; it is also known to be capable of performing part of the regulation of food intake independently of the forebrain (256). In addition, the higher brain areas that deal with the reward, cognitive, and social aspects of food intake are not only output areas for the hypothalamus, but also send information back to the hypothalamus (33).

Lastly, although the peptides that have been mentioned here do play an important role in the regulation of energy balance, many other substances are involved. Some of these are hypothalamic neuropeptides, such as galanin, galanin-like peptide, malonyl-coenzyme A, neurotensin, and neuromedin U (257–261). Naturally, the classical neurotransmitters, glutamate and γ-aminobutyric acid, are also present and functional in the hypothalamus (262–264). Moreover, the hypothalamic nuclei and peptides discussed here are involved in many other processes besides energy balance. These include the immune system (leptin, the MCs), bone formation and remodeling (leptin, NPY, CART, MCH), blood pressure and cardiovascular regulation (leptin, NPY), kidney function (NPY), reproduction (NPY, MCH), stress (NPY, CART, CRH, MCH), pigmentation and pain sensation (the MCs), reward and addiction (CART, MCH), anxiety (MCH), and the wake-sleep cycle (MCH and ORX) (100, 158, 227, 265–273).

This section is not meant to be exhaustive, but intends merely to give an impression of the complexity of the regulation of energy balance (summarized in Fig. 3). However, despite the many interconnections, the main pathway is believed to be from the peripheral input to the ARC (with NPY, AgRP, POMC, and CART), via the PVN (CRH and TRH) and LHA (MCH and ORX), to the output systems (as depicted in Fig. 2A). Although at first sight these peptides all seem to fulfill one of two functions (orexigenic or anorexigenic), subtle differences between these peptides are revealed upon closer inspection. We have concentrated on evidence from rodents. Although some variation in the details exists (38, 274), the regulation of energy balance is very similar in different animal species, including humans.

G. Ontogeny

To program a certain system or function, an environmental stimulus must occur during a period in development when the system or function is still plastic. In rodents, the energy balance-regulating system is structurally and functionally immature at the start of postnatal life. The basic anatomy of the rat hypothalamus is established prenatally, with its nuclei expressing specific neuropeptides being recognizable before birth (275), but the majority of connections between the hypothalamus and its input and output systems (276, 277), and those within the hypothalamus itself (27, 278), develop only in the first weeks after birth. This rapid postnatal development is also reflected in overall brain growth: in neonatal rats, total brain weight increases by a factor 5 between birth and weaning (279).

Developing rat pups go through some major transitions. Whereas the fetus receives mainly glucose, lactate, and amino acids via the placenta, at birth the source of energy changes to high-fat mother's milk (280). Only a few weeks later, the pups are weaned and make a more gradual transition to the high-carbohydrate, low-fat adult diet

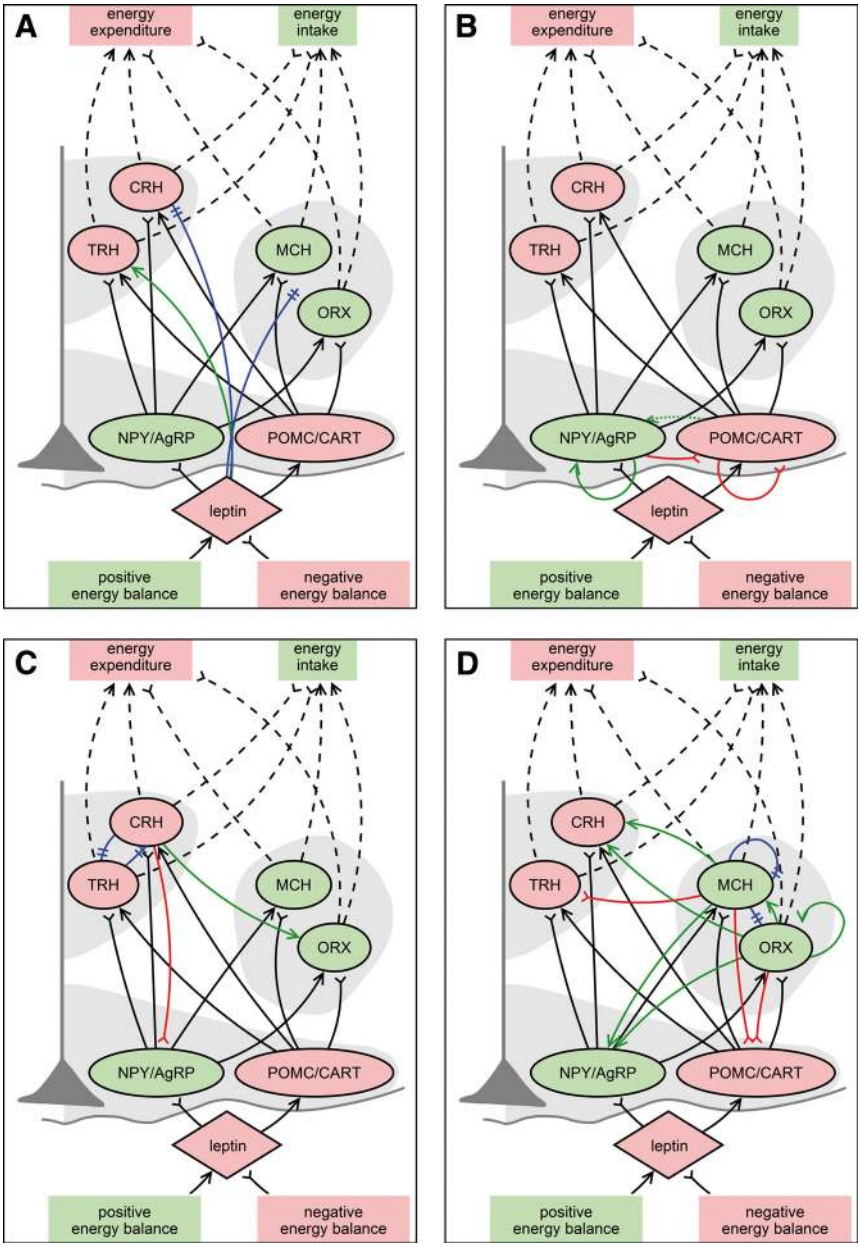

FIG. 3. Simplified diagrams of the hypothalamic regulation of energy balance. Besides the main pathway (see Fig. 2), these schematics show additional connections from leptin (A), the ARC (B), the PVN (C), and the LHA (D). Note that in A, the connection to the LHA does not contact MCH or ORX neurons directly. *Green arrow*, Positive effect; *red inverted arrow*, negative effect; *blue crossed line*, unspecified effect; *solid line*, direct connection; *dashed line*, indirect connection; *dotted line*, probable connection; *green* (*red*) *nodes* lead to more positive (negative) energy balance when stimulated/active. See the text for details.

(280, 281). At the same time, the pups have to make the transition from obtaining all energy and fluids from the dam by suckling to the two separate processes of feeding and drinking (282). As will be described below, different mechanisms appear to regulate these different types of ingestive behavior.

Rat pups as young as 1 d old already regulate their milk intake according to how deprived they are (283, 284). The only cue that suckling rats have been shown to use to regulate their milk intake is the distension by gastrointestinal fill (285, 286). This response is mediated primarily by vagus nerve activity (285, 287), and hence by the brainstem rather than by the hypothalamus. Other signals that influence food intake in adult rats, such as the nutritional value of the stomach contents, serum leptin levels, and manipulations of levels of glucose and free fatty acids, do not affect intake in suckling rats (286, 288, 289). It appears that the regulation of energy balance in the suckling pup is limited to optimizing energy intake for growth, and intake is only restricted by a full stomach to prevent gross overeating. Therefore, in suckling pups there seems to be only short-term regulation of milk intake, with no long-term regulation (290).

Thermoregulation and the regulation of adult forms of ingestion then develop in the early postnatal period. From d 1 on, pups can already regulate their temperature by moving toward or away from a heat source (291), whereas mechanisms for thermogenesis develop over the first 2 wk of life (288). In the first 10 d of life, gastric distension is the only cue that terminates intake. From then on, the nutritive value of the gastric content starts to play a role (reviewed in Refs. 276 and 290). At this same age, pups first start to adjust their intake according to their level of fatty acids (reviewed in Ref. 282), whereas a similar response to glucose levels does not appear until the age of 4 to 5 wk (284, 290). Another major development event is the differentiation between feeding and drinking; young pups simply increase their intake when they are dehydrated, and only from around the age of 20 d they will reduce their milk intake when dehydrated, a phenomenon called dehydration anorexia (reviewed in Refs. 276 and 290).

The development of the regulation of energy balance is accompanied by changes in mRNA and protein levels of the reviewed peptides. The ontogeny of these peptides is summarized in *Sections III.G.1–4* and depicted in Fig. 2B.

1. Leptin

In rats, leptin can be detected in fetal plasma on d 19 of gestation (292). In nearly full-term fetuses (d 20–21), leptin levels strongly resembled those of the pregnant dams (292, 293). Leptin mRNA is already expressed by rat adipose tissue at birth, and its expression and serum levels are immediately regulated by the nutritional status of the neonatal pup (294). In addition, Ob-Rb, the leptin receptor, has been shown to be expressed in the fetal brain as early as d 14 of gestation (295, 296).

During the lactation period, leptin undergoes some major changes. A first, relatively small increase in serum leptin levels can be detected in rat pups 1 to 2 d old (293, 297), followed by a second and larger peak around d 7 to 12 (297–299). Interestingly, this leptin surge is unrelated to changes in body weight and fat content in the neonatal period (297, 300). The high leptin levels do coincide with elevated leptin mRNA in neonatal adipose tissue, suggesting that the peak originates from the pups' own leptin production (301). Both the neonatal pituitary, which has high leptin expression during this period (298), and the dam's milk (302) may contribute to the leptin surge. In concert with the changes in leptin levels, hypothalamic levels of Ob-Rb and its mRNA rise significantly between birth and weaning (297, 303).

Leptin's functionality in the regulation of energy balance appears to be partial in the neonatal period. In rats as young as 1 wk old, leptin injections are found to reduce gain in body weight and especially in fat mass, without any effect on milk intake (289, 304–306). Instead, these effects seem to be the result of an increase in energy expenditure (306, 307). Leptin is effective in increasing POMC and decreasing NPY mRNA in the ARC of rats in this neonatal period (305), and a robust positive relation between leptin levels and fat mass has been reported on d 10 (308). The exact timing of the development of this system seems to differ between mice and rats. In mice, serum leptin levels were not altered after milk deprivation on d 8 (300), energy expenditure was not yet increased by leptin injections on d 9 (309), and daily leptin injections in the second week of postnatal life were not found to affect hypothalamic neuropeptide expression (310).

During the period of partial functionality in energy balance regulation, leptin has a neurotrophic role. In the absence of leptin, general brain development and that of the hypothalamic circuitry specifically are impaired (311, 312). Leptin shares this property with insulin, which is also implicated in brain development. The neurotrophic actions of insulin include stimulation of neurite outgrowth, protein synthesis, and neuronal survival (313–315). Leptin's neurotrophic effects may actually persist until adulthood because leptin administration in *ob/ob* mice significantly alters the synaptic input on both NPY and POMC neurons in the ARC (316). Furthermore, the lining of the third ventricle has been shown to contain neural progenitor cells that can be induced by neurotrophic factors to proliferate and differentiate into functional hypothalamic neurons (317, 318). This residual plasticity of the hypothalamic circuitry in adulthood provides an additional route by which environmental signals (including leptin) can regulate energy balance (319).

2. ARC peptides

The four reviewed peptides that are expressed by the ARC (NPY, AgRP, POMC, and CART) are already expressed in the prenatal rat brain (295). However, ARC projections to other hypothalamic nuclei only develop during the early postnatal period (320). During this period, there are also dynamic changes in the levels of the peptides and their gene expression.

a. Orexigenic ARC peptides. NPY peptide is detected in the rat fetal midbrain as early as d 13 or 14 of gestation (321–323). NPY mRNA levels rise during gestation to reach near adult levels around birth (75, 324). Like leptin, NPY gene expression is elevated during the lactation period, with a peak around d 16 (324, 325). At the same time, NPY mRNA is transiently expressed in hypothalamic areas that do not produce NPY in adulthood. Suckling rat pups express NPY mRNA in the DMN, PVN, LHA, and perifornical area, albeit at lower levels than in the ARC (324, 326). Alongside the developmental changes in NPY mRNA, NPY peptide levels show a rapid postnatal rise and in the ARC reach adult levels by the time of weaning (327, 328). Immunohistochemistry studies have shown that the number of cell bodies containing NPY peptide rises gradually until birth, with declining numbers afterward (321, 323). After d 10, NPY cell bodies can only be visualized when axonal transport is chemically blocked by colchicine administration—a finding that is consistent with the simultaneous increase in NPY-immunoreactive fibers throughout the hypothalamus (321–323). In a more recent study, by staining for NPY and AgRP peptide simultaneously, the origin of these postnatally developing fibers was proven to be the ARC (329). Indeed, the developmental pattern of AgRP resembles that of NPY, with increasing expression during the first postnatal weeks and a peak around d 16 (329, 330).

In the neonatal period, NPY and AgRP already appear to have some functionality. Maternal deprivation has been shown to increase expression in the ARC already on d 2

(NPY) and at least from d 11 (AgRP) (326, 331). Furthermore, NPY injections into the PVN increased intake of water and milk as early as d 2; on d 15 the pups showed a preferential increase in milk intake (332). As mentioned, intrahypothalamic fibers in the neonatal rat are still incomplete, and NPY is expressed in several hypothalamic nuclei. Therefore, NPY may exert most of its actions locally at the site of expression, rather than after being axonally transported from the ARC to other hypothalamic regions.

b. Anorexigenic ARC peptides. POMC mRNA is first detected in the midbrain on d 13 of gestation (333). During the lactation period, hypothalamic POMC expression is either stable (334) or increases toward weaning (310). ARC POMC expression then increases significantly between weaning and young adulthood (335, 336). Hypothalamic POMC peptide has been detected as early as d 12 of gestation (337, 338), with α-MSH, the cleaved product, only appearing between d 15 and d 19.5 of gestation (337, 339, 340). Postnatally, POMC and α-MSH protein in the ARC go through a rapid increase, to peak around d 21 to 28 (337, 340). There is only limited information about early CART ontogeny. One study in mice has reported low levels of hypothalamic mRNA on postnatal d 5, with near adult levels on d 10 and 22 (310). However, the developmental patterns reported by this study for NPY, AgRP, and POMC were different from those found in most other studies.

In contrast to NPY, α-MSH does not seem to have much functionality early in life. In 1-wk-old rat pups, many PVN neurons are responsive to NPY, whereas only a few show a response after administration of an α-MSH agonist (341). At the age of 4 to 5 wk, however, the number of PVN neurons responsive to NPY has decreased, whereas the number of neurons responsive to melanotan II has increased dramatically (341). This phenomenon may ensure a high intake in neonatal life by minimizing anorexigenic signaling in early life.

3. PVN and LHA peptides

Less detailed information is available about the development of the peptides of interest in the PVN and the LHA: CRH, TRH, MCH, and ORX. Gene expression is detected in the fetal rat brain for all four peptides (342–346). The peptide is generally also detected in the hypothalamus before birth (343, 346–348). Neonatally, there is a gradual increase in expression and protein levels of most peptides, and adult levels are generally reached around the time of weaning (207, 344–346, 349–353), although ORX and TRH peptide levels may keep on rising between weaning and young adulthood (347, 354).

Functional tests are reported for ORX. In the neonatal period, leptin administration increases ORX mRNA in the LHA (353), where the normal effect in adults would be inhibition of expression (210). Interestingly, the neonatal leptin administration that increases ORX expression does not affect body weight and blood glucose levels, whereas 24 h of milk deprivation reduces body weight and blood glucose levels but does not affect ORX expression levels (353). Therefore, the neonatal leptin effect on ORX mRNA may be interpreted to reflect a developmental role, rather than an effect on energy balance regulation (353).

4. Development in humans and rats

If we want to extrapolate data and conclusions from animal studies to the human situation, it is important to consider the respective timing of the ontogeny of the relevant systems in humans and rats. At birth, humans are further in their development, and many developmental events that occur in the early postnatal period in rats take place in the third trimester of human pregnancy (27, 355–357). NPY immunoreactivity is first detected in the human ARC at about 21 wk gestation (41), and in nonhuman primates, NPY/AgRP projections to the PVN increase dramatically during the third trimester of gestation (358) and seem to be nearly complete by birth (27, 278). Therefore, caution is needed in extrapolating findings from one species to another.

IV. Energy Balance Programming

As has been shown in *Section III.G*, a large part of the development of the energy balance-regulating system occurs in the perinatal period in both man and rat, although the exact timing of developmental events differs between the two species. With the knowledge of the previous section, one can imagine that the perinatal period with its rapid development may be a critical period and that during this critical time-window, the organism is vulnerable to environmental influences. One can also imagine that different timing of an external stimulus, relative to the stage of development of the organism, can produce different outcomes. Also, different types of stimulus (*e.g.*, undernutrition *vs.* overnutrition, global *vs.* specific nutrients, maternal *vs.* fetal/neonatal) may produce different outcomes. Therefore, in this section, we will discuss developmental programming of energy balance according to the type and timing of the stimulus.

A. Indicators of developmental programming of energy balance

To identify programming of energy balance, different approaches have been taken. There are basically three types of outcome that can be measured to investigate this phenomenon. An indirect way of looking at energy bal-

ance is to measure body dimensions and body composition. Because positive energy balance results in fat deposition and allows growth, these measurements can give an indication of enduring positive or negative energy balance in the (recent) past. Relevant parameters are body weight, body length, body mass index (BMI), fat mass and lean mass, and whether or not there is complete catch-up growth. These parameters are most apparent, and in humans are often the first indication that energy balance may be disturbed. Another way of investigating energy balance programming is to examine components of energy balance directly. Energy intake, resting energy expenditure, and activity-related energy expenditure together determine energy balance. These parameters may be somewhat less explicit in everyday life, but they can be studied relatively easily, also in the human situation. The third approach to investigate energy balance programming is to study the peptides and hormones that are responsible for the regulation of energy balance. Properties like gene expression, peptide levels, epigenetic modifications, and functional changes can be studied. Because these measurements require invasive techniques, this approach is less suitable for use in the human situation.

Naturally, a combination of the three approaches will generate the most complete description of the phenomenon of developmental programming of energy balance. With many new studies on the subject, our understanding of this phenomenon has much advanced in recent years. Now, various influences of the perinatal environment on energy balance parameters will be discussed—first, briefly for the human situation, and then in different rat models.

B. Programming of energy balance in humans

Epidemiological evidence suggests that the early environment can have a profound influence on energy balance. With these studies, it must be kept in mind, however, that in the human situation, the underlying cause of low birth weight or restricted fetal growth varies and is often unknown (25, 359). In addition, there are many confounding factors (*e.g.*, the living conditions) that may obscure the real effects of the early environmental influence.

1. Body dimensions and body composition

Although higher adult body weight and BMI have repeatedly been reported with increasing birth weight (13, 16–19), the notion that low birth weight and impaired fetal growth may also program increased adiposity is gaining recognition. Over the last decade or so, researchers have increasingly investigated effects on more refined indicators of obesity, such as body composition (lean *vs.* fat mass) and fat distribution (*e.g.*, waist-to-hip ratio, skinfold ratios). These studies have shown that the positive relationship between birth weight and adult BMI results mostly from a positive relationship with lean mass, but not with fat mass (18, 360–365). Moreover, low birth weight and impaired fetal growth have now been shown to be associated with a higher fat percentage in later life (10, 365–369) and with a detrimental distribution of fat (*i.e.*, more central, abdominal, and visceral) (363, 370–380). The fact that these studies were performed in diverse populations [from different European countries (Belgium, Finland, France, The Netherlands, Spain, and the United Kingdom), the United States (non-Hispanic white, non-Hispanic black, and Mexican-American), Brazil, Guatemala, and Jamaica], with different ages (from young children to old age), and in both sexes underlines the robustness of these associations. It is becoming more and more clear that low birth weight is not always a reliable proxy for impaired fetal growth. When, for example, early-gestation growth impairment is followed by prenatal catch-up growth, adult health can be affected without an effect on birth weight (see Ref. 10). Furthermore, the significance of the rapid postnatal catch-up that often follows perinatal undernutrition, rather than that of the undernutrition *per se*, has been stressed in recent years. Several studies have shown that rapid early growth (with the definition of early ranging from the first week of postnatal life to about 3 yr) increases the risk for later adiposity and obesity (364, 381–385). This at least partly removes the apparent paradox of the association of both low and high birth weight with metabolic syndrome and obesity. When both situations are characterized by perinatal overfeeding (even if this is postnatal-only in the case of SGA subjects and may be both pre- and postnatal after maternal obesity), the underlying mechanisms may also share some similarities.

Maternal obesity and gestational diabetes are increasingly common problems (386, 387). The newborns of those affected usually have greater birth weights than infants born to control mothers (388–392). Greater gestational weight gain is also associated with higher birth weight (393, 394). Even when their birth weight is not altered, the offspring of diabetic mothers often have an increased fat percentage (395). In older children, ranging from 2 to 10 yr of age in the different studies, more obesity was found in those that were born to obese or diabetic mothers (393, 396–401). Interestingly, this obesity-prone profile improved dramatically after bariatric weight loss surgery. Children that were born to obese mothers with substantial weight loss after surgery had lower birth weights without a higher risk for SGA, and their obesity rates in the ages of 2 to 25 yr were reduced to normal population levels (402). To summarize, more obese phe-

notypes with detrimental adiposity have been found after both prenatal undernutrition and overnutrition.

2. Energy intake and expenditure

Relatively few studies have directly assessed energy balance parameters in low- and high-birth-weight subjects. For energy expenditure, mostly neonatal data are available. These suggest that infants that are born SGA have higher energy expenditure than both premature appropriate-for-gestational-age very low-birth-weight infants (403–406) and at-term appropriate-for-gestational-age infants (407, 408). In a study on prepubertal children on the other hand, SGA subjects were reported to have reduced resting energy expenditure compared with at-term appropriate-for-gestational-age children (409). Energy intake was generally similar to that of premature infants of the same body weight (403, 405, 406). One study reported a higher intake per kilogram body weight in SGA infants, whereas those large for gestational age had a lower relative intake compared with control infants of the same postnatal age (410). In a more long-term study, a sample of prepubertal SGA children that did not catch up had a food intake below the recommended energy intake for their age (411). After gestational famine exposure, middle-aged subjects had a higher energy intake, consumed diets with a higher fat density, and had lower levels of physical activity than nonexposed persons (412, 413).

3. Peptides and hormones

In humans, measurements of the third category (that of the peptides and hormones that are involved in the regulation of energy balance) have largely been limited to the circulating hormones. Serum leptin levels have been investigated most thoroughly.

In neonates, several studies have found positive correlations of leptin with birth weight, birth length, and BMI (414–419). Because the strongest correlation was usually found with BMI, these associations most likely reflect the deficit in fat deposition in low-birth-weight infants and the excess in those born after fetal hypernutrition, respectively. However, a programming effect is suggested by the fact that subjects that were born with a low birth weight were found to have high leptin levels with respect to their BMI at several different ages (ranging from 4 months to adulthood) (420–423). Another report that suggests programming of leptin levels studied the influence of early nutrition in preterm infants (424). It was shown that adolescents that had received preterm formula had more leptin per kilogram fat mass than adolescents that had received a control diet in infancy (424). Besides altered leptin levels, a few studies have shown increased ghrelin levels in SGA subjects at birth (417, 425, 426) but not at the age of 1 yr (427). In contrast, high-birth-weight newborns were reported to have normal ghrelin levels (425). Children 2 to 25 yr of age that were born to obese mothers after bariatric weight loss surgery had higher ghrelin levels and lower leptin levels than those born before such surgery, a beneficial profile that corresponded to their improved body composition (402). Lastly, there is also some evidence (in neonates and children) that the HPT axis may be disturbed in SGA subjects (428, 429).

4. Evidence for developmental programming

Summarizing, there is quite some evidence that the early nutritional environment can have a permanent effect on the body dimensions of humans. The long-term effects observed at both sides of the birth weight spectrum seem to share their general direction: after the initial period of catch-up growth after perinatal undernutrition, both are associated with more obese phenotypes. Although direct measurements of energy balance and its regulation are still scarce, disturbances have been found, some of which seem to persist into adult life. Because these kinds of measurements are more invasive and some can only be performed postmortem, they are obviously not employed in humans on a large scale. That is why different animal models were designed to study these effects more closely.

C. Programming of energy balance in animal models

The use of experimental animal models has some substantial advantages over studies in humans. In contrast to the human situation, with animal models for perinatal restriction of growth and nutrition, the exact cause of the observed symptoms is known, and the degree of control over the subsequent environment is far greater. In addition, animal models permit the use of more invasive methods than in humans.

Experimental animal models for developmental programming have been designed in various species, including primates, sheep, guinea pigs, and rats (28, 31, 387, 430). In this review, we will focus on studies in the rat, although a few studies in mice are also included. In rats, both prenatal and postnatal manipulations of nutrition have been used to induce developmental programming of energy balance, including ligation of the uterine arteries; maternal diets with altered protein, fat, or energy content; and manipulations of litter size (28, 31, 387, 430). These different models produce different phenotypes. Here, we will first describe effects on the body dimensions and body composition of the major models that have been used in rodents. Then, the effects on energy balance and its regulation will be discussed.

1. Body dimensions and body composition

a. Nutritional manipulation of the dam. Prenatal manipulations of fetal nutrition, via the diet of the pregnant dam, exert long-term effects on the body dimensions of the offspring, with or without an immediate effect on birth weight of the pups. Two major types of this kind of manipulation are maternal low-protein diets and global maternal food restriction to different degrees (ranging from 30 to 70% of control intake). Perinatal overfeeding, on the other hand, can be induced by feeding the dams high-fat or high-energy (high on both fat and sugar) diets.

Whether a maternal low-protein diet actually reduces birth weight of the pups appears to depend on the exact composition of the diet and other details in the methodology because some studies (mostly using a low-protein Hope Farms diet) report lower birth weights (431–435), whereas others (mostly using the Southampton diet) have reported normal birth weight after maternal low-protein diet during gestation (436–439). After a maternal low-protein diet, body weight either stays reduced or normalizes to control levels, with the outcome apparently independent of birth weight and the experimental diet used during pregnancy (431–437, 439–442). Two studies have reported rapid catch-up growth with increased body weight (438, 443). Adult body composition after a maternal low-protein diet has mostly been reported to be normal (439–442), although some of these studies did report an altered fat percentage in either males or females. One study found increased leptin and triglyceride levels in males, but not females, with otherwise normal body weight and fat mass (442). This suggests that, although the body composition may be normal, its regulation can still be disturbed in these animals.

Maternal food restriction usually reduces birth weight of the resulting pups (301, 444–451), except when the food restriction is limited to the first 2 wk of pregnancy or in some cases when intake is only mildly restricted to 70% of control intake (452–455). After maternal food restriction, rats show either complete or incomplete catch-up growth (446–448, 452, 456) so that in rats with a low birth weight, adult body weight was reduced, normal, or elevated compared with that of controls (445, 449, 450, 457). Several studies have found normal body composition after prenatal maternal food restriction (301, 445–447, 453–455, 458–460). However, increased and decreased adiposity has also been reported. Within studies, these different outcomes can be attributed to sex differences, different effects at different ages, strain differences, and timing of the food restriction (445, 452, 453, 455, 458, 460). Between studies, the method of determining body composition (*e.g.*, BMI, weight of different fat pads, total lipid determination by carcass analysis, dual-energy x-ray absorptiometry) and the severity of the food restriction may explain a large part of the variation in outcome. One group that uses severe maternal food restriction (to 30% of control levels) has consistently found a persistent lower body weight, combined with increased fat mass and leptin levels in both males and females (449–451, 456, 457). Leptin levels usually reflected body composition (301, 445, 447, 449–451, 457, 459, 460), although in one study increased leptin levels appeared to precede the increased fat percentage (445). In summary, although studies using the Vickers model present a constant exception, most studies have found normal body composition after prenatal maternal food restriction.

Because a considerable part of the developmental events that occur *in utero* in humans take place after birth in rats, postnatal manipulations are also frequently used as a model. When the same maternal dietary manipulations that are used prenatally are either started or continued in the lactation period, the reductions in body weight are generally longer lasting, and less catch-up growth is reported (15, 431, 433, 434, 442–447, 461–467). Concomitantly, an obese phenotype is observed less frequently than with strictly prenatal manipulations (434, 442, 445–447, 460–462). There may be less catch-up growth after these postnatal manipulations because the condition is too severe to recover from (especially when prenatal and postnatal malnutrition are combined), or at weaning the animals may have reached the end of the time-window in which complete catch-up is possible. Alternatively, the fact that these animals do not seem to be "programmed for obesity" may reflect a different type of programming than with exclusive prenatal maternal dietary manipulation.

Besides maternal underfeeding paradigms, maternal overfeeding and gestational diabetes have also been induced in rodents. After maternal overfeeding before and/or during gestation, birth weight in the offspring can be either higher (468) or lower (469) than in control animals, but it is more often found to be unchanged (470–476). Interestingly, one study reported a lower birth weight specifically after a pregestational-only cafeteria diet (477). With maternal overfeeding continued into lactation, a substantial number of studies reported increased body weight by the time of weaning (468–471, 476, 478–480), although a reduced body weight was found in a study where the high-fat-fed dams lost more weight during lactation than the control dams (472). In later life, animals born to overfed dams had normal (475, 479, 481, 482) or elevated (469, 471, 476, 478, 480, 483, 484) body weight when fed on chow. A higher body weight was usually accompanied by increased adiposity (469–471, 476, 479, 480, 483, 484). In one study, the development of overweight was specific to animals that were born to control

dams but then cross-fostered to dams fed on hypercaloric diets (485). When transferred to an obesogenic diet themselves, some (471, 475, 481, 482), but not all (476, 478, 481, 482), of these animals showed an increased susceptibility to diet-induced obesity. In the studies by Levin and colleagues (481, 482), the adverse consequences of the maternal diet were mostly specific to animals from a strain bred for diet-induced obesity, demonstrating the importance of the interaction between perinatal nutrition and genetic factors.

In rodents, gestational diabetes can be induced by glucose injections in early pregnancy or injections of the pancreatic islet toxin streptozotocin, but it also occurs in the female offspring of rats that underwent uterine artery ligation (see *Section IV.C.1.b*) and in *db*/+ mice that are heterozygous for a silencing mutation in the leptin receptor (486–489). Mostly, birth weight is found to be increased in these models (486, 487, 489), although normal birth weight has been reported after maternal streptozotocin injections (488). Around weaning, body weight remained higher in the offspring of ligated dams (486), remained normal, or increased slightly after streptozotocin injections (488, 490). Cross-fostering to normal dams after birth did not influence growth (486), but normal pups that were cross-fostered to diabetic dams had lower body weights (491). Offspring of diabetic mothers was reported to be overweight with increased adiposity in adulthood (489, 492, 493).

b. Nutritional manipulation of the offspring. Uterine artery ligation in the pregnant dam reduces the blood flow to the fetuses (494) and is frequently used as a model for placental insufficiency, the most common cause of low birth weight in westernized countries (24). To approach the human IUGR situation as closely as possibly, often only pups that are growth restricted according to similar criteria as those used in humans are selected for studies (495). This obviously results in a birth weight that is by definition reduced (496–500). Nevertheless, studies that did not use pup selection have also reported a lower birth weight in rats born after uterine artery ligation (494, 500–502). The long-term effects on body weight seem to be dependent on the exact timing of the ligation. When performed on d 17 of gestation, the weight deficit is usually persistent (496, 503–506), whereas after ligation on d 19 of gestation, complete catch-up growth has been reported (498, 507, 508). Some studies also found a return to normal body weight after ligation on d 16 or 17 (500, 509). Newborn pups that were growth restricted by uterine artery ligation were shown to have a fat percentage that was either reduced or comparable to that of control pups (497, 501). Juveniles and adults that do not completely catch up in body weight have been shown to have normal BMI, fat percentage, and serum leptin levels (496, 510). The ones that do catch up to control body weight also have normal leptin levels when young (at an age when their body weight is still reduced) (498, 511). Rats that stay at the same body weight as control rats after catch-up have elevated leptin levels and increased fat mass in adulthood (498). The group that reported overweight in adulthood found normal or increased fat mass at the age that body weights were similar to those of controls (508, 512) and increased fat mass afterward (512, 513). In summary, when there is complete (or even overcomplete) catch-up in body weight, the animals' body composition is disturbed and shifted toward a more obese phenotype. If the catch-up growth stays limited, however, body composition remains normal. It seems likely that the capacity for true growth of organs and other lean tissue is curbed by the early growth restriction, and if there is catch-up beyond a certain point, any additional "growth" is in fat only.

A method to manipulate early postnatal nutrition that targets the offspring directly (rather than indirectly via the diet of the dam) is to manually adjust the number of pups nursed in a litter (514, 515). In this way, both neonatal under- and overnutrition can be achieved. By definition, birth weight is not affected by these manipulations because they take place after birth. Shortly after redistribution into litters of different sizes, differences in body weight become apparent. Rats that are raised in a small litter of only two to five pups receive more milk, resulting in a higher growth rate and body weight before weaning (250, 516–525). Although a few studies report normalization of body weight (518, 519, 526–528), this elevated body weight is generally found to persist into adulthood and middle-age (250, 516, 520, 521, 523, 526, 529–543). The opposite is true for rats that are raised in a large litter of 14 to 24 pups, which has less milk available per pup. These rats grow much slower during the lactation period and have a significantly lower body weight (496, 503, 516–522, 544, 545). Again, some studies report normalization (519, 526, 546), but most researchers find that body weight is persistently reduced (496, 504, 516, 518–521, 526, 533, 535, 539, 540, 542, 544, 545, 547). Already during the lactation period, the two models show marked effects on body composition: overfed small-litter pups have an increased fat percentage and leptin levels, whereas these are both decreased in underfed large-litter pups (517, 519, 520, 522, 524, 525, 545). Thus, a disproportionate part of the added growth in small-litter pups can be ascribed to adipose tissue. After weaning, when all animals are transferred to a normal feeding regime, body composition remains disturbed. In most small- and large-litter rats with persistent changes in body

weight, fat percentage and leptin levels also remain altered into adulthood and middle-age (496, 510, 516, 518, 520, 527, 529, 533–535, 537, 538, 540–543, 545). One study even reported an increased fat percentage in small-litter rats at an age when their body weight was no longer elevated (527). Apart from a few exceptions, the effects of neonatal litter manipulations are long-lasting and also rather consistent between studies. Neonatal overfeeding by raising rats in small litters causes an immediate rise in growth velocity, with persistent higher body weight and fat mass in adulthood, resulting in an obese phenotype. Neonatal underfeeding by raising rats in large litters, on the other hand, acutely reduces growth rate and causes a permanently lower body weight and fat mass, resulting in a leaner phenotype.

c. Response to a dietary challenge. This section has demonstrated that diverse manipulations of perinatal nutrition can bring forth different phenotypes. Even seemingly comparable manipulations have been shown to generate different long-term effects on body dimensions and body composition. What's more, some of these manipulations have been shown to alter the animals' susceptibility to diet-induced obesity (which is induced by feeding a hypercaloric diet, usually a high-fat diet). Again, there is considerable variation in the reports on this effect. A maternal low-protein diet either did not affect (548) or increased (15, 549, 550) the susceptibility to diet-induced obesity when the manipulation was prenatal. When the manipulation was restricted to the lactation period, less obesity was observed on a highly palatable diet (15). Several studies have reported a higher susceptibility to diet-induced obesity after prenatal maternal food restriction (301, 453, 454, 456, 457, 459, 551, 552), but unchanged obesity has also been reported (450, 451, 453, 454, 551, 553). Here, there seems to be a difference in susceptibility between the sexes, although this sex difference may be strain-dependent; Jones (453, 454, 551) reported increased diet-induced obesity in Sprague-Dawley males but not females, whereas Vickers (450, 451, 456, 457, 459, 552, 553) found higher susceptibility in Wistar females but not males. In rats that were neonatally overfed or underfed by raising them in small or large litters, conflicting results have also been reported. In rats with persistent differences in body weight, some studies found no difference between the two models in their susceptibility to diet-induced obesity (518, 533). One study, however, reported that diet-induced obesity was augmented in small-litter rats and diminished in large-litter rats (540). From these data, we can conclude that the effects of a dietary challenge are mostly consistent with the general phenotype. More diet-induced obesity is observed in those models that under baseline conditions showed more catch-up growth and increased adiposity.

2. Energy intake and expenditure

In the above-mentioned rodent models, energy intake and energy expenditure have been studied using a range of different parameters. Expenditure-related parameters include resting and total energy expenditure, (locomotor) activity, body temperature, and measurements of thyroid function and cellular metabolism. For energy intake, the variety is more in how the data are represented. Daily food intake is given per animal (raw data), per kilogram body weight (or some other approximation of body size), or adjusted for body size in a statistical test. The results of these different representations are not always easily compared. Especially when intake is divided by body size, the results can be distorted. Because energy requirements per kilogram body weight fall with increasing body size, this calculation systematically underestimates energy utilization by larger individuals (554). Therefore, such studies are excluded from this review; only studies that report raw food intake data or intake adjusted for body size in a statistical test are included.

a. Nutritional manipulation of the dam. One study that induced prenatal underfeeding by a maternal low-protein diet reported normal food intake in the adult offspring (442). The same study found reduced food intake when the underfeeding was (continued) during the lactation period. This was confirmed by others (433, 467), although some have also reported normal levels of food intake in these (prenatally and) postnatally malnourished rats (432, 461, 464). These data suggest a subtle decrease in food intake after protein malnutrition in the lactation period, whereas prenatal-only malnutrition probably does not affect long-term energy intake. On the expenditure side, in rats with postnatal exposure, increased thyroid function (pointing to increased basal metabolism) was found (463, 465), and normal-to-low activity levels have been reported after prenatal exposure (441). Taken together, these studies suggest that in prenatally malnourished animals normal levels of intake and reduced activity may lead to positive energy balance, whereas in postnatally malnourished animals a negative balance may result from their lower food intake and increased basal metabolism.

After maternal food restriction, food intake was usually found to be similar to that of control animals. However, when body size is taken into account, the effects on energy intake differ according to the timing of the malnutrition: prenatally or postnatally. When pups were exposed to the maternal diet postnatally, they often had reduced body size combined with normal food intake (445, 447, 464, 467), which results in an elevated relative energy intake.

With prenatal-only maternal food restriction, both body size and food intake were usually normal (301, 447, 452–454, 458), leading to a normal relative food intake. In a few cases, both body size and food intake were elevated (445, 453, 454), which also may point to a fairly normal relative energy intake. Measurements of energy expenditure were mostly performed in prenatally underfed rats; in postnatally underfed rats, one study reported a normal thyroid function (463). Using the Vickers' model of prenatal maternal undernutrition, female rats (that have a low body weight and high fat mass) were found to have reduced activity levels in adulthood (552, 555). Other studies using prenatal undernutrition have reported normal levels of activity (453) and normal body temperature and resting energy expenditure (301). These data are suggestive of normal total energy expenditure, which together with an unaltered food intake points to a normal energy balance for these rats that are prenatally exposed to maternal undernutrition.

In juvenile pups born after maternal overfeeding, food intake may be normal (476, 480, 484), although dramatic overfeeding has also been reported (468). In adulthood, these animals are usually hyperphagic (469, 471, 476, 478, 480, 484). Moreover, rats born to cafeteria-diet-fed mothers showed a stronger preference to fatty and sugary foods themselves (483). In offspring of high-energy diet-fed dams, reduced activity levels and slightly increased diet-induced thermogenesis have been reported (479, 484). This will probably lead to lower total energy expenditure, with the reduction in locomotor activity only enhancing the obesogenic effects of the elevated food intake. An elevated food intake was also reported in rats born after gestational diabetes (493).

b. Nutritional manipulation of the offspring. Food intake was not widely studied after uterine artery ligation; one study reported decreased food intake (498), whereas another found an unaltered intake per kilogram body weight (508). In both studies, the experimental animals had similar body weight as controls (which nullifies the interpretational problems of the per kilogram representation). In both juvenile and adult rats, cellular metabolism was reduced (513, 556, 557), whereas locomotor activity has been reported to be normal (499, 500). Taken together, a reduced or normal food intake, lower basal metabolic rate, and probably normal activity-related energy expenditure suggest that energy balance may be either approximately normal (intake and expenditure both reduced) or more positive than in control animals (normal intake with reduced expenditure), respectively.

In virtually all small-litter rats that were heavier than controls, food intake was reported to be elevated throughout life (523, 526, 529–532, 536, 538, 539, 541, 543) although this was not always the case (537). In rats that would later lose their overweight, unchanged food intake was found in juvenile life (519, 527). Fewer studies have reported on the expenditure side of the balance. Rats raised in small litters were found to have a higher body temperature and resting expenditure (521), and in young animals, elevated total energy expenditure was reported (543). The latter study found that the elevation in energy expenditure was appropriate for the larger body size of the small-litter rats. Because both energy intake and expenditure are increased in these animals, the overall effect on energy balance depends on the relative sizes of the effects on intake and expenditure. These are difficult to compare between studies. On the other hand, large-litter rats were generally reported to have lower energy intake and expenditure than controls (521, 526, 539, 545, 558). Again, the fact that these measurements were taken in separate studies complicates interpretations about the overall effect on energy balance in these animals.

The foregoing paragraphs have shown that different models of perinatal malnutrition can have different effects on adult energy balance. They have also shown that, although there is a lot of information about the effects of these manipulations on components of the energy balance, the exact information needed to assess a directional change in energy balance is not always available. Furthermore, in the interpretation of these studies, it is vital to distinguish absolute measurements from adjusted data. Comparisons should only be made between data that are expressed in the same dimensions.

c. Response to leptin administration. A related parameter that marks the transition to the subject of the next paragraph is the anorexigenic effect of leptin. Peripheral leptin administration acutely reduces food intake in control animals, but not in adult rats that were previously subjected to prenatal or postnatal maternal food restriction or a postnatal maternal low-protein diet (456, 464, 559). In young adult small-litter rats, central injections of leptin are effective, in contrast to peripheral injections (537). This suggests that this leptin resistance may be due to impaired leptin transport, rather than an altered hypothalamic response (537).

3. Hypothalamic regulation

It has been known for quite some time that perinatal malnutrition can have profound effects on brain development (560). Nevertheless, studies investigating programming effects on the hypothalamic peptides that regulate energy balance are relatively scarce (compared with the other two categories of measurements discussed above). Most of these have studied relatively short-term effects.

a. Nutritional manipulation of the dam. Weanling rats subjected to a maternal low-protein diet during gestation and lactation were shown to have a reduced number of NPY immunoreactive cells in the ARC (561). This was combined with an increase of the concentration of NPY protein in the PVN and LHA and a tendency for an increased concentration in the ARC, whereas the NPY content of other hypothalamic nuclei was unaltered (562). This is suggestive of an increased orexigenic drive in these animals, provided that the PVN and LHA are fully responsive to NPY. In view of the slightly hypophagic phenotype of these animals (see *Section IV.C.2.a*), the responsiveness of these areas (or other regions further downstream) is probably reduced. Rats that were only exposed to a low-protein diet prenatally did not show changes in ARC gene expression of Ob-Rb, NPY, AgRP, POMC, and CART at weaning (563). In contrast, weanling pups that were subjected to the diet postnatally had increased expression of Ob-Rb, NPY, and AgRP and decreased expression of the anorexigenic POMC and CART (563), again suggesting an increased orexigenic drive. After fasting, NPY and AgRP mRNA were increased relative to control levels in weanling rats that were exposed either prenatally only or both pre- and postnatally, although the effects were stronger in the latter group (432). In adulthood, CART mRNA was found to be increased in animals that were exposed prenatally (but not in those exposed both pre- and postnatally), with no changes in expression of NPY, AgRP, and POMC (432).

Prenatal maternal food restriction has been shown to drastically increase hypothalamic mRNA levels of Ob-Rb at birth (559), an effect that was reversed by weaning to levels below normal (559, 564). In adulthood, hypothalamic Ob-Rb expression had normalized (459), but Ob-Ra expression was lower than in control animals (301), which points to a reduction in leptin transport. The latter is supported by a normal reaction to central injections of leptin, with a reduced reaction to peripheral leptin (301). In weanling rats, reductions in leptin, ghrelin, NPY, and α-MSH peptide levels, as well as NPY and POMC mRNA levels have been reported (564). Adult hypothalamic expression of the ARC peptide AgRP was reduced, whereas that of NPY and POMC was normal (459). Despite this, the PVN in these adult animals did receive a larger number of NPY and CART terminals (301). This was not reflected in a change in PVN CRH expression (565), although the PVN in juvenile rats did show increased neuronal activity and CRH mRNA levels (448, 566). When the maternal diet was continued postnatally, juvenile pups showed very low serum levels of leptin (567). This was accompanied by reduced POMC expression and axons, but surprisingly, hypothalamic NPY expression and its protein levels in the PVN were normal (567). There does not seem to be a predominant direction in which energy balance regulation is shifted, which is in line with the variation in the general phenotype described above for these animals.

After perinatal maternal overfeeding, changes have also been found in energy balance regulation. In two studies that found normal birth weight after maternal high-fat diet, serum leptin levels and hypothalamic gene expression for Ob-R, NPY, and POMC were either up-regulated or down-regulated at birth (473, 474). In the former case, hypothalamic Ob-R peptide and AgRP and MC4 receptor mRNA were also elevated (473). After a maternal cafeteria diet, a much more pronounced neonatal leptin surge was reported (480). By the time of weaning (when these animals were heavier than controls), serum leptin levels were increased (468, 470, 476, 478). In the hypothalamus, this resulted in down-regulated Ob-Rb mRNA (468), with normal to reduced NPY and AgRP and increased POMC expression (468, 470). But although the ARC response to elevated leptin signaling seemed roughly normal, the VMN showed reduced responsiveness (568). Fasting revealed more changes that were not seen under basal conditions: pups born to high-fat diet-fed dams showed increased elevations in mRNA levels of NPY, AgRP, and the Y_1 receptor but lacked the decrease in MC4 receptor expression that is found in control animals upon fasting (468). From weaning on, leptin levels in these animals were found to be normal to elevated (469, 471, 476, 478, 484), where the larger increases in leptin were usually in more overweight animals. Shortly after weaning, at a point that the rats born to overfed mothers had normal body weights, the number of projections from the ARC to the PVN containing AgRP was reduced, whereas projections containing α-MSH were normal (480). In addition, gene expression for the MC4 receptor was up-regulated in the VMN, and that for the leptin receptor Ob-R was down-regulated in the ARC (479), accompanied by a reduction in leptin sensitivity that remained at least until the age of 3 months (480). In adulthood, offspring of high-energy-fed dams showed enlargement of the VMN and DMN nuclei (482). In addition, hypothalamic gene expression was either reported to be normal for Ob-R and NPY and reduced for AgRP and POMC (471), or was up-regulated for NPY with normal expression of AgRP and POMC (476). Because in the former study, the reduction of POMC expression seemed stronger than that of AgRP, despite the conflicting details, both profiles could be expected to lead to more orexigenic signaling.

Hypothalamic alterations have also been found in pups born to or cross-fostered to dams with gestational diabetes due to streptozotocin injections. At weaning, despite normal serum leptin levels, postnatal-only exposure increased

the size of the PVN (491), whereas combined pre- and postnatal exposure led to a reduction in PVN and VMN size (490). At the same time, ARC peptide levels of NPY and AgRP were elevated, and those of POMC and α-MSH were reduced (488, 491), suggestive of increased orexigenic signaling. The up-regulation of NPY levels was also found in middle-aged animals (493).

b. Nutritional manipulation of the offspring. In rats that were prenatally growth restricted by uterine artery ligation, NPY mRNA and protein were both increased at weaning, whereas CRH levels were unaffected (511). In young adulthood, the number of ARC cells expressing NPY mRNA was normal, but the levels of expression were reduced (504). This suggests an increased orexigenic drive in the juvenile animals, which is in accordance with the complete catch-up growth reported for these animals (see *Section IV.C.1.b*). Lower NPY expression in the adult rats is concurrent with the incomplete catch-up growth that these animals display (see *Section IV.C.1.b*).

Weanling small-litter rats were shown to have reduced Ob-Rb expression (520), which is in agreement with the high serum leptin levels found in these rats. ARC NPY, AgRP, and CART mRNA levels were all increased, but this predominantly orexigenic signal did not seem to reach the PVN and LHA because expression of TRH, MCH, and ORX was unaltered (520). This was also suggested by the fact that NPY peptide levels in both the ARC and the PVN were normal (522). In young adulthood, expression of the ARC peptides NPY, AgRP, and CART is normal, as well as CRH and TRH expression in the PVN and MCH and ORX expression in the LHA (529, 537). At this age, leptin transport across the blood-brain barrier appears to be impaired (537), which indicates a state of leptin resistance. This leptin resistance seems to develop only after weaning because weanling small-litter rats are still responsive to peripheral injections of leptin (524). Taken together, these studies suggest that the obese phenotype of adult small-litter rats (see *Section IV.C.1.b*) may be at least partly attributable to central leptin resistance caused by high neonatal leptin levels and the resulting hyperproductivity of the ARC. Additionally, studies by Davidowa and colleagues (reviewed in Refs. 27, 278) suggest that in these rats, neurons of several hypothalamic nuclei have an altered response to many of the orexigenic and anorexigenic signals.

Interestingly, juvenile large-litter rats also show more orexigenic signaling. In the ARC, the balance between orexigenic and anorexigenic gene expression was shifted (569), or AgRP and NPY expression and NPY peptide levels were increased with unchanged CART expression (520, 522). This resulted in elevated PVN NPY peptide levels (522), but did not affect its expression of CRH or TRH, nor LHA ORX expression (520, 569). LHA MCH gene expression was shown to be transiently increased, with elevated levels at d 10, but not at 25 d of age (569). Unlike small-litter rats, juvenile rats raised in large litters had normal hypothalamic Ob-Rb expression (520). Instead, some of the short forms of Ob-R were expressed at increased levels (520). One study suggested that ARC NPY mRNA was no longer elevated in young adulthood (504), although there still seemed to be a small tendency toward higher expression levels. These results generally appear to be in agreement with the phenotype described above. The acute effects of juvenile food restriction seem to be mostly orexigenic, although apparently not enough to achieve full catch-up growth. Information on the long-term effects of this model is still largely missing.

Although the studies described here have all used nutritional manipulations, perinatal nonnutritional manipulations have also been shown to program hypothalamic (an)orexigenic signaling. One example is neonatal stress, which has been shown to have long-term effects on levels of POMC, CRH, ORX, and ORX receptors (570, 571).

c. The neonatal role of leptin. As has been mentioned in *Section III.G.1*, leptin is not fully functional in the regulation of energy balance during the neonatal period. Instead, it seems to play a more developmental role. It is responsible for the proper development of intrahypothalamic connections (312, 572) that occurs during the early postnatal period (320). Even general brain development seems to depend on leptin because the brains of leptin-deficient mice show a variety of abnormalities that can be rescued by juvenile leptin treatment (311). In recent years, several researchers have hypothesized that altered neonatal leptin levels may play a key role in developmental programming (12, 15, 573–578). This hypothesis is supported by several recent studies that manipulated perinatal leptin levels.

Interestingly, the direction of the reported effects differed between these studies. Some researchers have found a beneficial effect of perinatal leptin administration on adult body adiposity (579, 580). Moreover, one study reported the absence of an anorexigenic reaction to peripheral leptin in adulthood when leptin action was blocked neonatally (581). In contrast, others have reported increased fat mass, leptin levels, and/or food intake (451, 582–584) and leptin resistance (301, 583, 584) in adult rodents subjected to perinatal leptin administration. Similarly, different effects of perinatal leptin on susceptibility to diet-induced obesity were reported. Some studies found perinatal leptin to be protective against diet-induced obesity (581, 585), whereas others reported in-

creased weight gain on a high-fat diet after neonatal leptin injections (301, 451).

Based on some of the positive effects mentioned above, several groups have investigated whether perinatal leptin administration might rescue the obesity-prone phenotype of rats that were programmed by perinatal nutritional manipulations. Their results have been mixed. Rats that were malnourished by a maternal low-protein diet throughout gestation and lactation had lower body weight in adulthood, similar leptin levels, and similar susceptibility to diet-induced obesity as controls (466). When the low-protein dams were infused with leptin during the perinatal period, weight gain on the high-fat diet was abolished (466). The effect of perinatal leptin on control rats was not investigated in this study, which hampers the interpretation of the results. Notably, the body weight of saline-treated low-protein pups appears to reach normal control levels on the high-fat diet. Another group has investigated the effects of neonatal leptin injections on the obese phenotype of rats subjected to prenatal maternal food restriction. These underfed rats have a higher baseline adiposity (at least the males), and both sexes are more susceptible to diet-induced obesity than control rats (451, 552). When prenatal undernutrition was followed by neonatal leptin injections, baseline adiposity was reduced in males, with no effect on diet-induced obesity (451), whereas in females, neonatal leptin reduced the effects of the high-fat diet to that found in controls, without an effect on baseline adiposity (552). Notably, neonatal leptin injections aggravated diet-induced obesity in control males, but not in control females. A third group attempted to rescue the obese phenotype of weanling rats raised in small litters by using neonatal leptin injections. In female small-litter rats, leptin injections reduced the fat percentage to that of control females raised in normal litters (524). In male rats, however, the fat percentage at weaning was not altered by leptin injections in small-litter animals, whereas it was significantly reduced by leptin in normal-litter males (525). Thus, neonatal leptin rescued the obese phenotype in weanling female small-litter rats, but not in males.

Summarizing, perinatal leptin supplementation can have beneficial or detrimental effects on energy balance and body composition in both normal and programmed rats. The outcome is probably determined by the exact timing and levels of leptin, as well as the phenotypic background of the animal. Therefore, we recommend extreme caution when investigating the option of providing infants with supplemental leptin as a proposed obesity-protective agent (586). An additional concern is the reduction in skeletal growth that was reported in some of the studies, resulting in reduced body length (451, 579), which is usually undesirable in the human situation.

d. Other programming candidates. Of course, leptin cannot be the sole factor responsible for programming. For that, the phenomenon is too widespread and its consequences too diverse. Examples of other proposed candidates are thyroid hormone, the IGFs, insulin, and glucocorticoids (587–589). These hormones are important regulators of fetal growth and metabolism, and their levels in the fetus depend on environmental conditions. Besides their own effects on fetal development, levels of thyroid hormone and the IGFs have been shown to be affected by IUGR (587, 588).

In the fetus, insulin correlates strongly with nutrient supplies and is mostly produced by the fetus itself in response to maternal glucose. The effects on the development of energy balance regulation in offspring of diabetic mothers mentioned earlier indicate a developmental role for insulin. Together with its previously mentioned neurotrophic actions (313–315) and the observation that early postnatal intrahypothalamic insulin administration induces altered hypothalamic organization (590), these data have identified insulin as an important candidate for developmental programming (591–593).

In normal pregnancy, the fetus is protected from maternal and environmental glucocorticoids by the placental 11β-hydroxysteroid dehydrogenase type 2, which catalyzes the transition of the biologically active cortisol into inactive metabolites (594, 595). Many factors that have been associated with low birth weight and later disease risk (such as hypoxia and stress) also reduce the placental activity of this enzyme and hence increase fetal exposure to glucocorticoids. Many studies have now found programming effects after excess glucocorticoid exposure (594, 595).

In addition to environmental factors that can influence programming, genetic factors have long been suggested to be causal in both low birth weight and later-life disease risks (596). Because of the technical difficulties of such studies, finding evidence for this hypothesis has been problematic. Recently however, large-scale studies have provided compelling support for a common genetic origin of alterations in birth weight and in later health (597, 598). Moreover, as we have seen above in the rare studies that have used animal models with different genetic backgrounds (482), the phenotype observed after nutritional manipulations can be modulated considerably by genetic factors.

In conclusion, leptin is an important candidate, but certainly not the only one, for the mechanistic underpinnings of developmental programming. It seems likely that the relative importance of the different candidates varies according to the environmental conditions. Whether experimental manipulation or naturally occurring, each set of

conditions is likely to elicit specific responses of the different candidates and hence bring about its specific phenotypical outcome.

4. Summary and implications

In this section, we have seen ample evidence for developmental programming of energy balance and its hypothalamic regulation in experimental animals. Studies have investigated outcomes in all three categories of measurements of energy balance (*i.e.*, body size and composition, energy intake and expenditure, and hypothalamic neuropeptides). Persistent changes have been found in all of these parameters in various rodent models with perinatal manipulations of nutrition. Nevertheless, the precise effects that have been reported differed considerably, not only between the models, but also between studies using similar models. Many of these apparent discrepancies can be explained by (small) differences between studies in the timing, nature, and severity of the manipulation or other subtle variations in their methods, such as the genetic background, sex, and age of the experimental animals. When we focus on the similarities between the studies discussed in this section, rather than their inconsistencies, a few generalizations can be made.

The long-term effects initiated by perinatal overnutrition seem to be quite consistent, independent of whether the exposure to overfeeding starts prenatally (*e.g.*, maternal diet) or postnatally (small litter size). Although the details vary, when an effect is found, it is generally a shift toward overweight, higher adiposity, more food intake, and more orexigenic signaling.

In the case of undernutrition, the exact perinatal timing combined with the degree of undernutrition seems to play an important role. Overweight in adulthood is seen more often after prenatal-only than after postnatal undernutrition. The thrifty phenotype, with a higher food intake, energy storage, and concomitant susceptibility to obesity, is proposed to be the result of an inappropriate "adaptive" response to an adult environment that turns out to be richer than expected (2, 6). There are several possible reasons why this thrifty phenotype is seen less often after postnatal undernutrition. First, postnatal underfeeding may fail to induce a predictive adaptive response, because of an insufficient degree of undernutrition to elicit such a response or maybe because the degree of undernutrition is so severe that it abolishes any adaptive response by disruption of developmental processes in the pups (such severe damage can also be imagined when prenatal undernutrition is continued into the neonatal period). Alternatively, it might be that in rodents the critical time-window of plasticity already closes before the postnatal undernutrition could induce any adaptive response. In case of prenatal malnutrition, postnatal growth may show either catch-up or lack of catch-up, presumably dependent on the adaptation of the organism to the insult. Unfortunately, in epidemiological and experimental studies alike, it is difficult to identify the contributions of different underlying causes to the observed phenotype.

The obesogenic effect of rapid catch-up growth and of perinatal overfeeding models may originate from programming of the set-points of energy balance regulation according to the circulating (high) levels in perinatal life of regulators such as insulin and leptin (589, 592). In most models of perinatal underfeeding, the hypothalamic effects observed in juvenile life (despite variations in the details) seem to be directed at promoting positive energy balance, and hence may be aimed at catch-up growth. These hypothalamic adaptations are also seen in the absence of adult overweight. The lack of obesogenic effects of this increased orexigenic signaling might be the result of irreversible damage to the hypothalamic or downstream circuitry that interferes with the putative predictive adaptive response. This could also be true for the disagreement between the studies using uterine artery ligation, where later ligation (*i.e.*, milder damage) results more often in an obese phenotype than ligation at an earlier time-point (*i.e.*, stronger damage) in gestation.

Although developmental events in the early postnatal period in rats resemble those in the third trimester of human pregnancy (355), rapid neonatal growth seems to program for later obesity in both species. Therefore, it might be postulated that the time-windows for hypothalamic plasticity may be similar (or at least extend until similar time-points) for both species. A striking difference between the evidence in humans and rodents is that in humans both extremes of perinatal nutrition (under- and overfeeding) seem to elicit consistent obesogenic effects, whereas in rodents the directions of long-term effects vary between higher and lower rates of overweight and obesity. This species difference may result from (interaction with) the obesogenic environment that most humans (unlike most experimental animals) encounter in later life. However, the fact that not all perinatally over- or underfed rodents are more susceptible to diet-induced obesity suggests that other factors must also play a role. Experiments by Levin and colleagues (482) suggest that the three-way interaction between perinatal nutrition, later obesogenic environment, and genetic background may explain many of the observed effects.

Because of its distinct role during development, leptin has been hypothesized to play a major role in programming of energy balance regulation. Evidence supporting this hypothesis has been published in recent years, but there is also strong evidence for the involvement of other factors besides leptin. With regard to the effects of neo-

natal leptin injections, the direction of its effects seems to vary strongly with a number of factors, some of which may even be unknown at present. Among these, the exact timing of administration, the initial state of energy balance, and the genetic background may be especially important. With so many aspects still unknown, proposals to supplement leptin as an obesity-protective agent seem rather premature and should be considered with extreme caution.

V. Concluding Remarks

In this review, we have presented the concept of developmental programming. It explains how changes in the environment during a critical time-window in early development can permanently alter the phenotype of an organism. In this manner, individuals can be "fine-tuned" to their expected future environment. Although there has been controversy on this subject, it is now generally believed that energy balance and its regulation can also be programmed. We have discussed a substantial number of studies that have investigated developmental programming of energy balance in different species, using different techniques, and from different angles. From these studies, we can conclude that early nutrition can truly program energy balance and its regulation in both humans and animals. The direction of the programming effects that were reported appears to be variable and dependent on the environment— both the perinatal and the adult environment. One thing that becomes apparent from the discussed animal studies is that developmental programming of energy balance does not necessarily entail detrimental changes; in some cases, the programmed changes were favorable, such as reductions in fat mass. In contrast, mostly adverse effects on adult body composition were reported in humans. This striking disparity, between metabolic effects with different directions in animal models on one hand and consistent detrimental effects in humans on the other, may result from (interaction with) the obesogenic environment that most humans (unlike most experimental animals) encounter in later life.

With the inconsistencies between animal studies, a comprehensive picture of the impact of perinatal nutrition on energy balance in later life has thus far remained elusive. If we intend to extrapolate conclusions between studies, and from animal models to the human situation, it is vital to identify the exact circumstances leading to each outcome and to standardize the variable methodology that researchers have used to investigate this subject.

Acknowledgments

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