Nutritional manipulations in the perinatal period program adipose tissue in offspring

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Running title: perinatal nutrition and adipose tissue programming

Key words: adipose tissue, adipogenesis, gestation, lactation, epigenetics.

Disclosure statement: The authors have nothing to declare and no conflict of interest.
Epidemiological studies initially demonstrated that maternal undernutrition results in low birth weight with increased risk for long-lasting energy balance disorders. Maternal obesity and diabetes associated with high birth weight, excessive nutrition in neonates and rapid catch-up growth also increase the risk of adult-onset obesity. As stated by the Developmental Origin of Health and Disease concept, nutrient supply perturbations in the fetus or neonate result in long-term programming of individual body weight set-point. Adipose tissue is a key fuel storage unit mainly involved in the maintenance of energy homeostasis. Studies in numerous animal models have demonstrated that the adipose tissue is the focus of developmental programming events in a gender- and depot-specific manner. In rodents, adipose tissue development is particularly active during the perinatal period, especially during the last week of gestation and during early postnatal life. In contrast to rodents, this process essentially takes place before birth in bigger mammals. Despite these different developmental time windows, altricial and precocial species share several mechanisms of adipose tissue programming. Offspring from malnourished dams present adipose tissue with series of alterations: impaired glucose uptake, insulin and leptin resistance, low-grade inflammation, modified sympathetic activity with reduced noradrenergic innervations and thermogenesis. These modifications reprogram adipose tissue metabolism by changing fat distribution and composition, and by enhancing adipogenesis predisposing the offspring to fat accumulation. Subtle adipose tissue circadian rhythm changes are also observed. Inappropriate hormone levels, modified tissue sensitivity (especially glucocorticoid system) and epigenetic mechanisms are key factors for adipose tissue programming during the perinatal period.
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**Abbreviations**

BAT: brown adipose tissue

C/EBP: CCAAT/enhancer binding protein

FAS: fatty acid synthase

FR: food restricted

GC: glucocorticoid

GR: glucocorticoid receptor

11β-HSD1: 11β-hydroxysteroid dehydrogenase type 1

11β-HSD2: 11β-hydroxysteroid dehydrogenase type 2

IL-6: interleukin-6

LPL: lipoprotein lipase

LP: low protein diet

MR: mineralocorticoid receptor

NPY: neuropeptide Y

PGC1-α: PPARγ coactivator 1-α

PPAR: peroxisome proliferator-activated receptor

SCD1: stearoyl-CoA desaturase

SREBP1c: sterol regulatory element-binding protein 1c

TNF-α: tumor necrosis factor-α

TG: triglycerides

UCP1: uncoupling protein 1

WAT: white adipose tissue
Introduction

Increasing evidence suggests that adult-onset metabolic disorders may derive in part from events taking place during fetal and postnatal development. Epidemiological studies initially demonstrated that intrauterine growth retardation (IUGR) and low birth weight are associated with increased risk of metabolic pathologies during adulthood (24). For instance, the Dutch Famine Study showed that fetuses whose mothers were exposed to famine during early pregnancy displayed a higher adiposity in adulthood (98). Originally called the Barker hypothesis or fetal programming, these observations have led to the Developmental Origin of Health and Disease (DOHaD) hypothesis (6). This concept states that an adverse environment in utero or during infancy, including dysnutrition, can program or imprint the development of several tissues. It then may permanently determine physiological responses and ultimately produce energy balance dysfunction and diseases later in life. In humans born with low birth weight, postnatal hypercaloric nutrition, and more specifically rapid catch-up growth, are also important accelerators in the etiology of adult-onset diseases (38), suggesting that the degree of mismatch between the pre-and postnatal environments is crucial in metabolic programming (44).

Studies in animals have confirmed that maternal nutritional manipulations sensitize the offspring to long-lasting perturbation of the hypothalamus-adipose tissue axis associated with adult-onset obesity (reviewed in 18). The hypothalamus plays a pivotal role in the maintenance of energy homeostasis by controlling food intake and energy expenditure (Figure 1). The hypothalamus integrates peripheral information such as hormone, adipocytokine and nutrient levels by modulating neuronal populations which express appetite-regulating neuropeptides (4). Changes in hypothalamic neuropeptide expression can also influence energy expenditure such as adipose tissue lipolysis and/or thermogenesis via the sympathetic autonomic nervous system (39) (Figure 1).
The adipose tissue is mostly composed of mature adipocytes (specialized fat-storing cells) and a stromal vascular fraction that includes preadipocytes, fibroblasts, endothelial cells and a variety of immune cells. Several types of adipose tissue with different properties coexist in mammalian species. The first type of adipose tissue, the white adipose tissue (WAT), has an essential role as a long-term fuel reserve. WAT adipocytes are characterized by the presence of a single (unilocular) lipid droplet in which excess energy is stored under the form of triglycerides (TG) by simple esterification from dietary fatty acid. When energy is required, stored TG are hydrolyzed by lipolytic pathways, mainly driven by the noradrenergic innervations (69). Both lipogenesis and lipolysis are highly regulated cellular processes (Figure 2). WAT expansion involves adipogenesis, a two-step process by which preadipocytes are first recruited from precursor cells and then differentiated into adipocytes. Adipogenesis is driven by the expression of adipogenic and lipogenic transcription factors including peroxisome proliferator-activated receptor-γ (PPARγ), CCAAT/enhancer binding protein (C/EBPα, β, γ), the sterol regulatory element-binding protein 1c (SREBP1c) as well as the expression of specific lipid-metabolizing enzymes such as fatty acid synthase (FAS). Fully differentiated adipocytes are characterized by the production of the adipocyte-specific hormone, leptin (110). WAT expansion also relies on lipogenesis, a process by which acetyl-CoA is converted to fatty acids, and TG synthesis that convert fatty acids into TG in pre-existing or mature adipocyte. The transcription factor PPARγ is able to promote all processes involved in adipose tissue expansion (82).

The second type of adipose tissue, the brown adipose tissue (BAT), is specialized in the dissipation of energy through the production of heat (55). In contrast to white adipocytes, brown adipocytes contain numerous (multilocular) smaller lipid droplets and a much higher number of mitochondria. Brown adipocytes are characterized by high expression of uncoupling protein 1 (UCP1), a BAT-specific marker, fatty-acid-activated transcription factor
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PPARα and PPARγ coactivator 1-α (PGC1-α) (101). BAT also differs from WAT by its cell origin. Indeed, brown adipocytes have been shown to arise mainly from a Myf5-positive myoblastic lineage, pointing at a close developmental relationship between brown adipocytes and myocytes (63). However, the BAT utilizes its own developmental transcription factor pathway (i.e., PRDM16, PGC1-α, PPARα and PPARγ) that drives preadipocytes differentiation into brown adipocytes (101).

Beside classical white and brown adipocytes, a third type of adipocytes known as “brite” (brown-in-white, or also “beige”) have been detected within some WAT depots. While brite adipocytes possess many of the morphological and functional characteristics of brown adipocytes, they display unique genetic markers and do not express myogenic markers. This suggests that brite adipocytes have a distinct origin from brown adipocytes (9). Several studies in rodents showed that prolonged cold exposure, treatment with β-adrenergic agonists (via β3-adrenoreceptor activation) and endurance exercise resulted in browning of white adipocytes (i.e, enhanced brite adipocytes) (112). Thus, the existence of white-to-brown adipocyte transdifferentiation indicates that there is a pool of potential convertible or recruitable cells, sparsely distributed within WAT (9).

In addition to its role in adipose tissue lipolysis and thermogenesis, the sympathetic system may also control fat cell number via inhibition of adipocyte proliferation (17). Finally, adipocytes function as endocrine cells as they produce adipocytokines, hormones and appetite-regulating related peptides that act as peripheral endocrine signals to regulate hypothalamic energy homeostasis (114). Some adipocyte-secreted factors such as leptin can also act in an autocrine/paracrine manner to regulate adipocyte metabolism (58).

The timing of adipose tissue development determines the window of vulnerability to potential environmental insults, and this window markedly differs between species. In
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rodents, adipose tissue adipogenesis is particularly active during the perinatal period. In rats, these processes occur primarily during the last week of gestation (embryonic day 15), accelerate during early postnatal life until pups are completely weaned (postnatal day 21). In fetus and neonates, adipocyte stem cells are still plastic and potentially very sensitive to maternal factors (110). In contrast to rodents, adipose tissue growth and adipogenesis essentially takes place before birth in bigger mammals such as sheep or primates (82). Despite this difference, altricial and precocial species share several mechanisms of offspring programming.

Here, we provide an overview of the impact of perinatal nutritional manipulations on adipose tissue properties in offspring. We also focus on developmental programming mechanisms underlying long-lasting perturbation of adipogenesis and adipocyte metabolism that may predispose to obesity.
Malnourished offspring display altered adipogenesis and fat accumulation

Intrauterine growth-restricted offspring

Two models of maternal undernutrition have been mainly described in the literature: maternal low-protein diet (8% instead of 20% protein) and global maternal food restriction ranging from 20 to 70% of control intake. Both protocols usually produce IUGR and reduce birth weight of the resulting pups.

Rat offspring of low-protein diet (LP) fed-dams during gestation and lactation have persistent smaller adipocytes (37). Although exposure to a maternal low-protein diet did not affect the capacity of in vitro preadipocyte cultures to divide or store fat in perinatal period (fetuses, neonates and weaning), adult offspring from LP dams exhibited increased rate of cultured preadipocyte proliferation (11, 121). This is likely a consequence of aberrant gene expression resulting from the altered responsiveness of adipocytes and/or preadipocytes to hormones and growth factors such as TGF\(\alpha\) and IGF-II) (48). Indeed, the mRNA expression levels of adipogenic transcription factors such as C/EBP\(\alpha\) and PPAR\(\gamma\) were increased in the WAT of adult rat offspring from LP dams (48).

In addition, adult rat from LP dams exhibited increased adipose tissue expression of miRNA-483-3p, known to regulate early and late stages of in vitro differentiation and the capacity of adipocytes to store lipids. Thus, we speculate that, in vivo, the programmed changes in miRNA expression levels could contribute to the inhibition of adipocyte hypertrophy (i.e., lipid storage) that would affect other tissues by promoting ectopic TG storage, lipotoxicity and associated metabolic diseases (37).

In contrast to maternal LP procedure, low birth-weight rat offspring from 50% food-restricted dams (FR50) during gestation from day 10 to term (28) or from 70% food-restricted dams (FR70) throughout the gestation (19, 75) showed persistent hypertrophic adipocytes.
Indeed, when provided standard nursing and postweaning diet, IUGR newborns demonstrate rapid catch-up growth, hyperphagia and adult obesity (28). Increased adipocyte size was observed in offspring of FR50 dams as soon as weaning (28).

Prior to the onset of overt obesity, offspring from FR dams showed increased adipogenesis signaling cascade, including enhanced expression of adipogenic and lipogenic transcription factors and enzymes, leading to adipocyte hypertrophy. As soon as postnatal day 1, IUGR offspring from FR50 dams exhibited enhanced protein contents of the key adipogenic factor PPARγ and its upstream regulatory transcriptional factors (C/EBPβ, C/EBPδ and C/EBPα) (119). Interestingly, IUGR newborns at postnatal day 1 showed increased adipose tissue protein contents of some PPARγ corepressors such as SIRT1, an NAD⁺-dependent histone deacetylase and SMRT (silencing mediator for retinoid and thyroid hormone receptor) as well as the coactivator SRC1 (steroid receptor co-activator 1), suggesting a key role of coactivator in modulating PPARγ activity (30). Accordingly, at both postnatal day 1 and 21, primary cell cultures from IUGR offspring displayed increased preadipocyte proliferation and adipocyte TG accumulation (119). In addition, it has been shown that adipocytes from IUGR offspring retain the basal phenotypic characteristic of programmed upregulation of adipogenic PPARγ in cell culture, suggesting that the adipocyte itself exhibits programmed adipogenesis/lipogenesis, independent of the IUGR hormonal milieu (30).

The increased expression of adipogenic factors was accompanied with elevated expression of lipogenic factors (SREBP1c, FAS, leptin) in obesity-prone weanling pups (28, 104) and adult offspring (28, 75) from FR dams. The propensity for fat storage and adiposity in IUGR offspring may be due to higher desaturation index highlighted by elevated stearoyl-CoA desaturase (SCD1) mRNA expression levels in WAT (102). In addition, the expression
of genes associated with lipogenesis (FAS, leptin) occurred in a depot-specific manner in adulthood (75).

Other animal models of IUGR have been developed. In particular, uterine artery ligation in the pregnant dams reduces the blood flow to the fetuses and is used as a model for placental insufficiency. This model is characterized by global nutrient reduction and oxygen restriction, resulting in low birth weight that persists into adulthood. Uteroplacental insufficiency leads to a gender- and depot-specific marked adiposity in adult rat offspring. As reported in offspring of undernourished rat dams (28), PPARγ mRNA expression levels were enhanced in WAT of juvenile offspring prior to the onset of overt obesity (61).

The energy supply mismatch between overfeeding-induced postnatal catch-up growth and fetal nutrient restriction results in offspring with exacerbated fat accumulation. In particular, pronounced adipocyte hyperplasia and hypertrophy were observed. Cultured preadipocytes from overfed (i.e., reared in small litter increasing milk intake) juvenile rats from LP dams showed enhanced proliferation and increased leptin mRNA expression levels (10, 13). In agreement with these findings, it has been previously reported the increased proliferation and differentiation of preadipocytes from normal birth weight rats that were overfed during the suckling period (34). Overfed (i.e., small litter rearing and/or postweaning hypercaloric diet) adult offspring from undernourished dams displayed marked adiposity with a global increase in adipogenic and lipogenic genes (SREBP1c, C/EBPα, PPARγ, FAS, leptin) in a depot-specific manner (12, 47, 75). The in vitro and in vivo appearance of a second population of smaller adipocytes suggest an active adipogenesis via the recruitment of fat cells precursors present in WAT (12, 13).

In addition, mRNA expression levels of many peptide precursors, including adipogenic neuropeptide Y (NPY) and peptide YY systems, showed marked changes in rat offspring of perinatally FR dams in a depot-specific manner (27, 75). The meaning of these
observations is currently a matter of debate and may be interpreted as an adipogenesis predisposition. Consistent with this hypothesis, mouse offspring from LP dams that were predisposed to develop obesity exhibited elevated circulating and fat cell NPY system in a sex-specific manner (53).

In sheep, which have a similar profile of adipose tissue cell development as humans, a programmed effect on adipocyte development is implicated because of increased PPARγ (82). Indeed, PPARγ and leptin mRNA expression levels were lower within reduced fat depots in low birth-weight lamb (45). After a period of accelerated postnatal growth, adult offspring from undernourished dams during gestation presented overt obesity with increased PPARγ mRNA expression levels in WAT (82). Thus, as described in rodents, the undernourished sheep model also revealed strong pre-and postnatal impacts of perinatal undernutrition growth, food preferences, fat depositions patterns and increased adiposity in offspring (85).

Data obtained from altricial and precocial species suggest that undernourished offspring, especially when fed an obesogenic diet later in life, are vulnerable to adipogenesis and fat accumulation. This may be advantageous to survival under conditions of poor nutrition as stated by the thrifty phenotype hypothesis. Thus, more than fetal growth retardation, the adverse effect of a rapid postnatal catch-up growth subsequent to it (due to enriched postnatal diet), might be a key determinant of programmed adiposity at adult age.

Finally, maternal reduced nutrition also modifies WAT circadian rhythms in offspring. Adult mice from LP dams cross-fostered to control lactating dams had abnormal feeding circadian rhythms before the onset of obesity. They exhibited enhanced WAT mRNA expression levels of lipogenic and clock genes coinciding with the period of maximum food consumption (109). In accordance with dysregulated light/dark-phase food intake rhythm (75), we also found that the daily transcriptional profile of several clock genes was modified in WAT offspring from FR70 dams throughout the gestation (unpublished data). Although the
underlying mechanisms remain unclear, several studies have described the association between the disruption of the circadian clock and metabolic dysfunction (79).

**Maternal overfeeding**

Models of maternal overnutrition and obesity rely on feeding the dams a high-fat (HF) or high-energy/cafeteria (fat and sugar) diet before (preconceptional period) and/or during gestation and/or lactation. In rodents, studies on maternal overfeeding have reported variable effect on birth weight, ranging from low to normal to high values (51, 67, 80). The discrepancy between these studies may be related to the complex interaction between maternal diet composition and genotypic susceptibility of individual rodent strains. It also may be attributed to other confounding maternal abnormalities, such as the presence of diabetes. However, offspring of obese animals are consistently prone to increased adiposity in adulthood in a gender-dependent manner (7, 100). Consequently, understanding the underlying mechanisms of programmed obesity in offspring has received increasing attention over the past years.

A rat model of maternal obesity based on intragastric feeding of HF diet demonstrated that maternal obesity at conception programs increased adiposity in offspring despite normal birth weight. Indeed, a greater percentage of large adipocytes associated with increased PPARγ were observed in adulthood suggesting enhanced adipogenesis (102). In agreement with these findings, fetuses from obese mice fed a cafeteria diet before mating and throughout gestation exhibited larger adipocytes (83). A sheep model of maternal overfeeding during late gestation was also associated with higher WAT mass in the fetus with enhanced PPARγ and leptin mRNA expression levels sensitizing to postnatal adiposity (81, 82). Hence, it appears that regulators of the adipogenic program may be susceptible to fetal programming.
In a rat model of maternal obesity induced by HF diet before mating and during pregnancy, newborns had similar body weights than pups born from control diet-fed mothers (49). Maternal HF diet during lactation had a significant impact on adiposity of the offspring resulting in accelerated catch-up growth and early obesity, apparent at the end of the lactation period (30). Indeed, weanling rats of overfed dams prior to and throughout pregnancy and lactation exhibited an increase in leptin mRNA expression levels in WAT (65). In rodent models, offspring of obese dams consistently shows fat expansion suggesting enhanced adipogenesis which may be attributed, at least in part, to upregulated PPARγ (7, 8, 30, 65, 100). Indeed, in contrast to IUGR newborns, neonates of overfed rats exhibited downregulated PPARγ corepressors (SIRT1, SMRT, NeoR: nuclear receptor corepressor) with unchanged PPARγ coactivator (TIF2: transcriptional intermediary factor 1), suggesting a key role of these PPARγ corepressors in adipose tissue programming (30). In agreement with these findings, overweight adult offspring from obese mice fed a cafeteria diet before mating and throughout gestation displayed marked adiposity in a gender-dependent manner. The adipocyte hypertrophy, more pronounced in female, was accompanied by reduced lipolytic adrenoreceptors and elevated PPARγ mRNA expression levels (100).

In adulthood, obese rat offspring of cafeteria-diet-fed dams during gestation and lactation presented an increase in adipose tissue TG content with elevated lipogenic enzyme activities (lipoprotein lipase (LPL)). They also had abnormalities in fatty acid composition, particularly enhanced proportions of saturated and monounsaturated fatty acids as well as decreased polyunsaturated fatty acids (8). We speculate that, as observed in IUGR offspring, the propensity for increased fat storage in offspring of overfed dams may be due to higher desaturation index with elevated SCD1 activity within WAT (118).

Overall, although offspring from obese dams gain more fat mass than those from control dams independent of lactation and/or postweaning diet, overnutrition during these
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periods always worsens adipogenesis programming. These findings highlight the importance of postnatal diet in modifying adiposity (7, 8, 65, 100).

The signals that mediate the effects of maternal metabolic disorders in overfed offspring have not been fully identified, but hormone such as insulin or leptin and nutrient such as glucose may influence perinatal development (see programming mechanisms).

Maternal hyperglycemia is associated with fetal and early postnatal hyperglycemia, which in turn may cause elevations in perinatal insulin levels, leading to an increase in fetal growth and adiposity (107). In rodents, gestational diabetes (GD) is mostly induced in early pregnancy by injections of streptozotocin, a pancreatic islet toxin leading to maternal insulin deficiency and hyperglycemia. This model is not equivalent to human GD which is characterized by insulin resistance and compensatory hyperinsulinemia. For example, a recent study showed that a mouse model of maternal GD leads to overweight in adult offspring associated with larger adipocytes. These findings indicate that perinatal exposure to a diabetic milieu characterized by increased glucose and/or insulin levels can program developmental processes such as adipogenesis (107).

Altered feeding in the neonatal period

A few animal models have been developed to precisely delineate the impact of altered feeding in neonates. These models shed light on the importance of energy intake and milk composition during the lactation period for adipose tissue programming. Because adipogenesis mostly takes place after birth in rodents, models based on postnatal dietary manipulations of the nursing pups have been extensively used to study offspring programming. In particular, either postnatal over-(small litters) or undernutrition (large litters) can be achieved immediately after birth by controlling litter size to rapidly modify milk intake and, thus, postnatal growth and body weight until weaning.
Neonatally overfed offspring reared in small litters showed a long-lasting obese phenotype. Overfed rat offspring displayed increased body weight and plasma insulin as early as 10 days of age. They also presented higher preadipocyte and stromal cell numbers within WAT. At this age, adipose tissue expansion arose only from adipocyte hypertrophy (i.e., enhanced LPL activity), since hyperplasia occurred only at 15 days of age (34). In later life, higher fat mass and hypertrophic adipocytes are associated with a depot-specific elevation of lipogenic gene expression, especially the glucocorticoid (GC) system (14, 15). In line with these findings, a model of mice reared in small litters exhibited marked upregulation of genes involved in the lipid droplet growth (66). These results indicate that overfeeding in early life increases fat storage capacity through a simultaneous increase in adipocyte precursor proliferation and differentiation.

Other animal models suggest that the propensity to higher adipogenesis in offspring may be due, at least in part, to elevation of plasma insulin levels in the immediate postnatal period. For example, obesity-prone rat offspring artificially raised by intragastric canula (“pup in the cup” model) under a high-carbohydrate milk formula (in contrast to rat milk wherein the major source of calories is fat) exhibited hyperinsulinemia, hypertrophied adipocytes with enhanced lipogenic enzyme activities (106). Thus, as observed in offspring of diabetic dams, the altered hormonal environment of neonatal rats (i.e., increased insulin levels) could be an important cue for the adipose tissue programming that may predispose for adult-onset obesity.

On the contrary, neonatally underfed offspring reared in large litters presented persistent lower adiposity with downregulation of genes involved in the adipocyte lipid droplet growth (66). Additional rodent models of maternal undernutrition only during the suckling period have been developed, confirming the phenotype of persistent lower body weight with adipocyte atrophy in the offspring (36, 92). In particular, adult offspring from FR30 dams only during suckling period exhibited beneficial gene expression programming
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(i.e., decreased expression of adiponenic genes) (92). Although underlying mechanisms remain elusive, reduced energy intake during suckling period may have a long-term protective effect providing resistance to diet-induced obesity (36, 92, 93). Overall, these findings fit with the notion that promoting catch-up growth in low birth weight offspring may not be beneficial for their long-term outcome related to adiposity.

Perinatal malnutrition programs modifications in adipose tissue noradrenergic innervation and thermogenesis in offspring

Several studies have shown that maternal nutrient restriction impairs sympathetic activity in WAT offspring. Weanling rats from FR50 dams during the last week of gestation and lactation exhibited elevated circulating norepinephrine level that could participate, via chronic β-adrenergic stimulation, to the remodeling of WAT into a thermogenically active BAT. Indeed, a marked increase in UCP1, PGC1α and PPARα mRNA expression levels, markers of brown adipocytes and/or adaptive thermogenesis were observed in fat pads of offspring. Interestingly, the development of UCP1-positive multilocular adipocytes in WAT depots early in postnatal life and their disappearance shortly after weaning is physiological in rodents. This phenomenon is also affected by genetic variability, as it is particularly pronounced in obesity-resistant as opposed to obesity-prone animal strains (117). We speculate that maternal undernutrition may delay the maturation of offspring WAT during critical developmental time-windows and favor the acquisition of brite adipocytes in gonadal WAT. These phenotype changes might occur in order to increase thermogenesis, as a compensatory mechanism to overcome difficulties in maintaining their body temperature after birth (27). This transient phenomenon may extend beyond the postnatal period. Indeed, adult mice offspring of LP dams still showed elevated UCP1 mRNA expression levels in BAT (115). Adult rat offspring from FR20 dams during the first 12 days of pregnancy exhibited a
gender-dependent greater adiposity with enhanced adipocyte hyperplasia and hypertrophy. Indeed, only male rat offspring showed a reduction of noradrenergic innervation to WAT and BAT as well as modified adrenoreceptor subtypes ratio in a depot-dependent manner. These mechanisms might account, at least in part, for dysregulated adipocyte adipogenesis and/or lipolysis observed in these animals (43). Finally, adult rats reared in small litters exhibited reduced BAT mass and thermogenesis (i.e., lower UCP1 mRNA expression levels), modified lipolytic adrenoreceptor subtypes ratio and impaired sympathetic outflow activity that might affect lipolysis (116).

Taken together, these data suggest that offspring of malnourished mothers showed a reduction in sympathetic activity within adipose tissue that could presumably occur through an alteration in the hypothalamic control of sympathetic outflow. Thus, the functional impairment of the sympathetic innervation of WAT (Figure 2) as an inhibitor of fat cell proliferation (i.e., hyperplasia) and/or activator of lipolysis (i.e., hypertrophy) might be a key determinant of the adiposity in adult offspring.

Offspring of malnourished mothers exhibit a loss of insulin sensitivity in adipose tissue with increased pro-inflammatory markers

The adipose tissue of juvenile offspring from LP dams had improved insulin sensitivity associated with an increase of both basal and insulin-stimulated glucose uptake and higher mRNA expression levels of key components of the insulin signaling pathway (88). Offspring of malnourished dams also exhibited an increase in noradrenergic-stimulated lipolysis with modified adrenoreceptor subtype mRNA expression levels and a blunted response to insulin-reduced lipolytic action in WAT (88) (Figure 2). In contrast, adult offspring from LP dams underwent an age-related loss of glucose tolerance leading to frank diabetes that paralleled impaired insulin signaling pathway in adipocyte (87). There is
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growing evidence that adipose tissue dysregulation and inflammation have a critical role to play in the pathophysiology of the metabolic syndrome. Obesity and diabetes are associated with chronic inflammatory states in which several peripheral adipocytokines (i.e., tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6)) contribute to impair insulin sensitivity (2). Consistent with this notion, adipose tissue of prenatally undernourished adult sheep exhibited up-regulation of key pro-inflammatory genes accompanied by a recruitment of macrophage within WAT. The increased macrophage content may result in cross talk between adipocyte and macrophage resulting in the down-regulation of glucose/insulin signaling pathways (103).

Other animal models of IUGR such as uteroplacental insufficiency were also associated with enhanced inflammatory mediators such as TNF-α in WAT. Interestingly, the increase occurred prior to the onset of overt obesity (61). In addition, a recent study suggests that obesity-prone rat offspring from FR70 dams throughout the gestation exhibits impaired adipose tissue leptin sensitivity (75). Indeed, hyperleptinemic adult offspring had reduced leptin receptor contents as well as phosphorylated Signal Transducer and Activator of Transcription 3 (pSTAT3) in WAT (Figure 2). Considering the anti-lipogenic leptin action on WAT (58), any suppression of the FAS gene expression in offspring was observed, but instead a marked increased expression (75).

Increased adipose tissue inflammatory markers were also observed in overnourished offspring. Indeed, fetuses of obese mice fed a cafeteria diet before mating and throughout gestation exhibited an increase in several pro-inflammatory markers in adipose tissue, suggesting macrophage infiltration in WAT. The level of GLUT-4 that mediates insulin-stimulated glucose uptake in WAT was decreased (83). Similarly, enlarged adipocytes of rat offspring reared in small litters displayed a postnatal induction of pro-inflammatory cytokines (i.e., TNF-α and IL-6) mRNA expression levels that were exacerbated under HF diet (14).
Reduced insulin-stimulated glucose uptake (i.e., impaired insulin signaling pathway and GLUT4 glucose transporter activities) was also observed in adulthood, suggesting an adipocyte insulin-resistant state (99).

Taken together, these data indicate that perinatal nutritional manipulations can influence the expression of pro-inflammatory genes within WAT, potentially contributing to the development of an early insulin- and/or leptin-resistant phenotype in adulthood.

**Perinatally malnourished offspring show programmed adipose tissue glucocorticoid metabolism**

Several studies demonstrated that the predisposition of increased adiposity in malnourished offspring may be due to local adipose tissue GC metabolism, rather than systemic GC status.

A primate model of maternal nutrient reduction in which offspring develop increased adiposity in adulthood was associated with elevated mRNA expression levels of glucocorticoid receptor (GR) and 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), enzyme that amplifies local GC actions by converting inactive GC metabolites to active GC (Figure 2) in fetal adipose tissue in a sex-specific manner (50).

Low birth weight lamb with reduced fat deposition from undernourished dams during gestation, exhibits changes in potential GC sensitivity directly related to the increase in postnatal WAT mass. Indeed, adipose tissue GR and 11β-HSD1 mRNA expression levels displayed a progressive postnatal increase that parallels active catch-up growth and fat expansion. In contrast, 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) that degrades active GC to inactive metabolites was reduced (45) (Figure 2).

In rats, 11β-HSD1, 11β-HSD2, GR and MR genes are co-expressed in WAT (75).

Similarly, hypertrophied WAT of overfed offspring reared in small litters displayed a
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Postnatal induction of adipose tissue GR and 11β-HSD1 mRNA expression levels. Postweaning HF diet exacerbated this profile (14). Interestingly, adult rat offspring from diabetic dams also showed an increase in 11β-HSD1 mRNA expression levels prior to the onset of overt obesity (40). As reported during postnatal catch-up growth in undernourished lamb (45), these observations emphasize the pivotal role of the GC WAT environment during the perinatal period on the subsequent development of obesity.

Finally, the ratio between adipose tissue 11β-HSD1 and 11β-HSD2 expression were modified in adult rat offspring from FR70 dams throughout the gestation and, thus, the local tissue ratios between active and inactive GC. In response to HF diet, the depot-specific upregulation of 11β-HSD2 mRNA, while 11β-HSD1 expression remains stable, might limit corticosterone concentration within its local environment, thus, diminishing responsiveness to GC. This may represent an adaptive mechanism that may counteract excess fat storage (75).

Programming mechanisms

Different opposite paradigms (undernutrition versus overfeeding) have been used to study the long-term effects of nutritional manipulations in the perinatal period, and both protocol result in similar outcomes on the adult offspring's adipose tissue. Thus, the perturbation of circulating factor levels as well as adipose tissue local factor levels, other than nutrients, induced by nutrition during neonate development may account for long-lasting adipogenesis perturbations.

Circulating factors

Hormonal factors that regulate adipose tissue functionality have received increasing attention over the past years. However, most of the studies have focused on indirect effects of hormone programming via the modulation of the hypothalamus activity.
Some studies have provided compelling support for the key role of inappropriate neonatal leptin levels in programming fat expansion. Based on studies in the ob/ob mice, leptin was found to act as a neurotrophic factor by promoting neuronal outgrowth from the arcuate nucleus to the upper hypothalamic nuclei during the early postnatal period. Thus, leptin is highly involved in the plasticity and hardwiring of the appetite (and probably the sympathetic outflow activity) regulatory circuits (16). Leptin also displays marked trophic effects on cultured hypothalamic progenitor cells (29). Perinatal leptin manipulations have long-term detrimental effects on offspring hypothalamus-adipose axis regulation, especially leptin resistance and susceptibility to increased adiposity in adulthood (18). Numerous studies using maternal nutrition manipulations have revealed a close relationship between altered postnatal leptin surge observed in undernourished rodent (25, 26, 91, 93) or overnourished sheep offspring (74) and fat accumulation in adulthood. Thus, early postnatal leptin blockage leads to long-term leptin resistance and increased susceptibility to diet induced obesity in rats (5) whereas administration of leptin during the postnatal period reverses obesity in prenatally undernourished adult rats (104). Finally, leptin directly activates adipogenesis by promoting differentiation of preadipocytes (13) whereas it shows antilipogenic effects on mature adipocytes (58).

Increased insulin levels might also be a key factor of perinatal programming. Insulin activates differentiation of hypothalamic neural progenitor cells in vitro and may also act as a neurotrophic factor (29). Consistent with these findings, adult rat offspring from gestational dams injected with insulin exhibited enhanced neurite outgrowth of fibers in the PVN (60). Moreover, intrahypothalamic insulin treatment of newborn rats, that became overweight in adulthood, resulted in morphological alterations on hypothalamic nuclei (96). Finally, insulin directly activates adipogenesis and lipogenesis whereas its inhibits lipolysis in mature adipocytes (97).
Increased GC levels could be a potent factor of perinatal programming. Maternal nutritional manipulations coincide with elevated perinatal circulating GC levels (71). Thus, it may lead to long-lasting disturbed hypothalamo-pituitary-adrenal axis feedback with permanent hypercorticosteronemia that may contribute to the susceptibility of obesity in adulthood (15, 70, 75). Indeed, increased postnatal GC levels may participate to altered neonatal leptin peak and further leptin-resistant state (120). Numerous studies have described programming effects on long-term hypothalamus-adipose axis in offspring after maternal excess GC exposure (54). Indeed, prenatal dexamethasone-exposed animals displayed persistent adipogenic gene profile in a depot-dependent manner (23, 33, 86, 104). Chronic GC exposure activates adipogenesis via both GR and MR primarily by regulating key adipogenic transcription factors (i.e. C/EBPα, PPARγ) (22, 77). Moreover, it may induce the expression of pro-inflammatory genes, favoring macrophage infiltration and providing the environmental conditions for inflammation (57). GC exposure also stimulates LPL activity whereas it may have anti-lipolytic effect, which favors lipid mobilization and TG accumulation (22, 94, 105). Finally, dexamethasone exposure affects the circadian expression of clock genes in WAT explants cultures (46).

Other factors (i.e., IGF1, ghrelin, neurotrophins, thyroid hormones, prolactin, inflammatory cytokines and catecholamines) might also be valuable candidates of adipose tissue programming (52).

Adipose tissue local factors: potential implications of the glucocorticoid system

Later in life, GC may still regulate adipose tissue differentiation, function and distribution. In excess, they cause visceral obesity-associated inflammation with multiple metabolic disorders, as typically observed in Cushing’s syndrome (3) or in rats chronically treated with dexamethasone (22). As observed in malnourished offspring, persistent modified
GC circulating levels and/or adipose tissue GC sensitivity, might account for obesity predisposition in adulthood (14, 15, 45, 70, 75).

Several studies support the notion that adipose tissue-specific amplification of GC results in higher adiposity. Elevated GR and 11β-HSD1 mRNA expression levels in WAT showing a greater ability to bind GC have been associated with the development of obesity in humans (69). In mice, overexpression of 11β-HSD1 in WAT resulted in visceral obesity (64) when fed HF diet whereas WAT overexpression of 11β-HSD2 protected them from HF diet-induced obesity (78). Consistent with these findings, gene expression of MR was up-regulated in WAT of obese mice (56). It has been reported that 11β-HSD1 inhibition decreased WAT mass, adipocyte hypertrophy and adipose tissue fibrosis (113) whereas it increased the expression of BAT-specific genes (including UCP1) in obese rats (73).

Epigenetic mechanisms

Nutritional manipulations during the perinatal period are now considered as transient environmental challenges that can permanently imprint the offspring genome to exert their metabolic effect in adulthood. Indeed, maternal perinatal food manipulations can cause epigenetic modifications such as gene promoter region methylation (i.e. the CpG sites), chromatin histone acetylation/methylation and changes in miRNA expression levels in offspring. Animal studies have also shown the potential importance of paternal diet in these cellular processes (reviewed in 72).

These nutritionally-induced epigenetic modifications may persistently affect transcriptional levels of key genes involved in adipogenesis to program long-term dysfunction. Indeed, recent advances in epigenetics suggest that both preadipocyte determination (i.e., commitment of pluripotent precursor cells to preadipocytes) and adipocyte differentiation (i.e., terminal differentiation of committed preadipocytes into mature
adipocytes) rely on an interwined network of coregulators and transcriptionals factors that display chromatin-modifying activities. A role for miRNAs, chromatin remodeling, histone modifications (acetylation and methylation) has been described at different stages of adipocyte differentiation. These cellular processes are involved in modulating the activity of key adipogenic transcription factors (especially GR, C/EBPα and PPARγ) (reviewed in 84). However, to our knowledge, only two studies have reported epigenetic modulations directly associated with programmed adipogenesis induced by maternal nutritional manipulations (37, 62).

Jousse et al. (62) showed that adult mice from LP dams presented hypomethylation of the leptin promoter in WAT and that this was associated with lower leptin contents. In addition, Ferland-McCollough et al. (37) showed that rat offspring from LP mothers exhibited an increase in miRNA-483-3p expression levels in WAT with a decrease in GDF3 (a member of the BMP/TGF-β family) protein content, a factor that impairs late stages of adipocyte differentiation. However, it is noteworthy that the deletion of JHDM2A (histone H3 lysine 9-specific demethylase JmjC-containing histone demethylase 2A) resulted in increased body weight, reduced BAT weight and impaired thermogenesis (59).

It has been shown that key factors controlling GC action are prime targets of epigenetic changes induced by perinatal nutritional manipulations. Periconceptional undernutrition leads to hypomethylation of hypothalamic GR promoter in the ovine fetus with enhanced GR mRNA expression levels (108). In rat offspring from LP dams, developmental induction of obese-prone phenotype involves hypomethylation of hepatic GR promoter associated with enhanced mRNA expression levels as well as altered DNA methyltransferase mRNA expression levels in liver. Increased expression was associated with an increase in histone acetylation and methylation (20). Consistent with these findings, an unbalance
maternal diet in pregnancy was associated with epigenetic changes in genes controlling GC action (i.e., GR and 11β-HSD2) and fetal growth in human offspring (32).

**Sexual dimorphism and transgenerational programming**

Nutritional manipulations in the perinatal period can predispose to obesity and imprint adipogenesis in offspring in a gender-dependent manner (7, 42, 43, 50, 61, 100). Reasons for these sex specific programming effects remain unknown, but could reflect direct interactions between nutritional signals and sex hormones in tissues of the developing fetus (1). For example, the consumption of HF diet during pregnancy appears to directly influence placental methylation (41) and placental gene expression (76) patterns only in female mice offspring. Thus, sex-specific differences in term of epigenetic modulations are associated with developmentally programmed phenotypes in animal models. It is possible that the postnatal hormonal milieu, which is different between male and female offspring, modify the programming of adipose tissue functionality induced by malnutrition. This may result in gender-specific outcomes in relation to different sex-steroids (35). Consistent with these findings, it has been shown that 17β-estradiol increases the proliferation of primary cultured preadipocytes from female rat whereas no effect was observed for male preadipocytes (31).

Finally, although underlying mechanisms remain unclear, it appears that the transmission of epigenetic alterations might extend beyond the malnourished first generation resulting in the transgenerational inheritance of obesity (21). Thus, acute programming of somatic tissues can result in long-term health outcomes in the first generation. In addition, germ cells, which contribute genetic and epigenetic information to the second generation, undergo reprogramming during embryonic development (35). Interestingly, data from the Dutch Famine Study reveal that starvation during pregnancy can have transgenerational consequences wherein second generation offspring have increased neonatal adiposity (90). In
rats, a study on maternal protein restriction during gestation and lactation pointed out that second generation body mass was increased only in males but that both sexes displayed glucose metabolism impairments (95).

**Conclusion**

The periods of gestation and lactation appear to be particularly sensitive time windows for the developmental programming of adiposity. During these periods, plasma levels of circulating factors as well as adipose tissue hormone sensitivity show perturbations in offspring of malnourished dams resulting in long-lasting adipose tissue programming (i.e., increased fat mass) (Figure 3).

To date, we are still far from having achieved the discovery of circulating factors as well as understanding the molecular mechanisms by which perinatal adverse nutritional conditions sensitize the fetus and neonate to increased risk of obesity. Epigenetic mechanisms might also be responsible for adipose tissue programming induced by maternal nutritional manipulations. Given that GR, C/EBP\(\alpha\) and PPAR\(\gamma\) are regulated by epigenetic modifications during adipogenesis (30, 84), it would be valuable to investigate these mechanisms in adipose tissue of malnourished offspring. Indeed, an increasing number of studies showed that dietary maternal supplementations (i.e., taurine, glycine, vitamin D and n-3 fatty acid) may alleviate adverse consequences of perinatal programming. In particular, folic acid (known as a methyl donor) appears to be a valuable candidate (20). Thus, in the future, a better knowledge of the epigenome changes in response to maternal malnutrition raises the exciting possibility that dietary supplementation may provide a therapeutic option using specific regimen for reversing adverse programming of adipogenesis in humans.
Perinatal nutrition and adipose tissue programming

Declaration of interest: The authors declare that they have no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding: This work was supported by grants from the French Ministry of Education and grants of the Conseil Régional du Nord-Pas de Calais.


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Figure legends

**Figure 1:** Schematic of the hypothalamus-adipose axis involved in regulation of food intake and energy expenditure (based on rat data). The hypothalamus integrates levels of circulating adiposity factors such as leptin. These signals act on hypothalamus to modulate neuronal populations that express appetite-regulating neuropeptides. Then, neuronal signals regulate the energy balance by modulating food intake and energy expenditure such as adipose tissue lipolysis and/or thermogenesis via the sympathetic autonomic nervous system.

**Figure 2:** Intracellular pathways of factors regulating adipogenesis, lipogenesis and lipolysis in the adipocyte. To simplify, only mechanisms that are primary targets of maternal nutrition manipulations have been represented. Triglycerides (TG) circulate in blood in the form of lipoproteins. Free fatty acids (FFA) that are released from lipoproteins, catalyzed by lipoprotein lipase (LPL), diffuse into the adipocyte. Intracellular FFA are converted to fatty acyl-CoA, and are then re-esterified to form TG using glycerol-3 phosphate (glycerol-3P) that is generated from glucose metabolism. FFAs may also originate from acetyl-CoA (de novo lipogenesis) driven by the lipogenic enzymes acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS). Lipolysis occurs via a cAMP-mediated cascade, which results in the phosphorylation of hormone-sensitive lipase (HSL), an enzyme which hydrolyzes TG into FFA and glycerol. These FFA are then free to diffuse into the blood. Leptin binding to its receptor (Ob-Rb) induces activation of Janus Activated Kinase 2 (JAK2), receptor dimerization, JAK2-mediated phosphorylation of intracellular part of Ob-Rb, phosphorylation and activation of Signal Transducer and Activator of Transcription 3 (STAT3). Activated STAT3 dimerizes and translocates to the nucleus to transactivate target genes.
Insulin binding to its receptor (InsR) induces receptor tyrosine autophosphorylation, activation of insulin receptor substrates (IRSs)/phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt/PKB) signaling pathways. Leptin and insulin action is both linked to the common PI3K signaling pathway. Insulin enhances the storage of fat as TG by increasing LPL and lipogenic enzyme activities. It also facilitates the transport of glucose by stimulating GLUT4 glucose transporter. In addition, phosphorylation and activation of cyclic nucleotide phosphodiesterases 3B (PDE3B) is a key event in the antilipolytic action of insulin, decreasing cAMP level in adipocyte. In contrast, leptin presents anti-lipogenic effects by suppressing expression and activity of lipogenic enzymes (i.e., FAS). Both hormones may also activate adipogenesis. Noradrenaline released from the sympathetic autonomic nervous system binds β-adrenoreceptor (β-AR) and activates lipolysis. Glucocorticoids (GC) bind intracellular glucocorticoid receptor (GR) and/or mineralocorticoid receptor (MR) and can also modulate adipogenesis and/or lipogenesis. This may be due either to an increase in circulating GC and/or to an increase in adipose tissue 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) activity that amplifies local GC actions by converting inactive GC metabolites (11-dehydrocorticosterone, 11DHC) to active GC (corticosterone) (in rodents) or inactive cortisol to active cortisol (in humans). 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) that degrades active GC to inactive metabolites is also found in adipose tissue.

**Figure 3: Schematic of the long-lasting effects of maternal nutritional manipulations on offspring’s adipose tissue.** Maternal nutritional manipulations may indirectly (via the hypothalamus) affect adipose tissue properties by modifying sympathetic outflow activity to tissue in offspring. The adipose tissue may also show reduced noradrenergic innervations. In addition, maternal nutritional manipulations may directly affect adipose tissue properties in offspring. Inappropriate circulating hormone levels, low-grade inflammation, modified tissue
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sensitivity (especially of the local GC system) and epigenetic mechanisms are key factors for adipose tissue programming. Overall, both indirect and direct actions lead to enhanced adipogenesis (i.e. hyperplasia), lipogenesis (i.e. hypertrophy) as well as reduced lipolysis and thermogenesis predisposing offspring to increased adiposity.
Food intake

Energy expenditure

Adipose tissue

Leptin action (via blood)

Noradrenergic action (via sympathetic autonomic nervous system)

Hypothalamus

Figure 1
Figure 2

Adipogenesis/Lipogenesis

Lipolysis

Triglyceride pool

FFA

Glucose

GLUT 4

Leptin

Insulin

Insulin receptor (InsR)

Leptin receptor (Ob-Rb)

GLUT 4

LPL

Lipoproteins (Triglyceride)

Glucocorticoids (GC)

11β-HSD1

Cortisone

11-DHC

Corticosterone

11β-HSD2

(active)

(inactive)

PKA

cAMP

Noradrenaline

B-AR

FKL

PDE3B

Akt/PKB

PDE3B

PDE3B

PI3K

IRS5

JAK2

JAK2

P STAT3

P STAT3

P STAT3

P STAT3

11β-HSD1

Cortisone

Corticosterone

11-DHC

11β-HSD2

Glucose

Acetyl CoA

Glycerol-3P

Fatty acyl-CoA

FFA

LPL

Glycerol-3P (ACC, FAS)

Adipogenesis/Lipogenesis

Lipolysis
Food intake
Energy expenditure
Adipose tissue

Leptin resistance
Impaired sympathetic activity
Programming mechanisms
Fat mass
Adipogenesis
Lipogenesis
Inflammation
Lipolysis
Thermogenesis

Figure 3