

Postnatal Overfeeding in Rodents by Litter Size Reduction Induces Major Short- and Long-Term Pathophysiological Consequences^{1,2}

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Abstract

Numerous studies have demonstrated that the early postnatal environment can influence body weight and energy homeostasis into adulthood. Rodents raised in small litters have been shown to be a useful experimental model to study the short- and long-term consequences of early overnutrition, which can lead to modifications not only in body weight but also of several metabolic features. Postnatal overfeeding (PNOF) induces early malprogramming of the hypothalamic system, inducing acquired persisting central leptin and insulin resistance and an increase in orexigenic signals. Visceral white adipose tissue, lipogenic activity, and inflammatory status are increased in PNOF rodents, while brown adipose tissue shows reduced thermogenic activity. Pancreatic and hepatic glucose responsiveness is persistently reduced in PNOF rodents, which also frequently present disturbances in plasma lipids. PNOF rodents present increased circulating concentrations of leptin, elevated corticosterone secretion, and significant changes in glucocorticoid sensitivity. PNOF also influences nephrogenesis and renal maturation. Increased oxidative stress is also described in circulating blood and in some tissues, such as the heart or liver. At the cardiovascular level, a moderate increase in arterial blood pressure is sometimes observed and rapid cardiac hypertrophy is observed at weaning; however, during maturation, impaired contractility and fibrosis are observed. Myocardial genome expression is rapidly modified in overfed mice. Moreover, hearts of PNOF rodents are more sensitive to ischemia-reperfusion injury. Together, these results suggest that the nutritional state in the immediate postnatal period should be taken into account, because it may have an impact on cardiometabolic risk in adulthood. *J. Nutr.* 143: 553–562, 2013.

Introduction

Cardiovascular disease (CVD)³ is a major cause of morbidity and mortality in Europe and North America and its occurrence is also exponentially increasing in developing countries. Like the management of CVDs, the management of overweight and obesity is one of the great challenges of this century. The prevalence of obesity and overweight among adults aged 20–70 y in the United States in 2007–2008 was estimated at 68% (1). Alarming, the prevalence of obesity in children has almost

tripled during the past 30 y (2). Today, epidemiological studies on weight gain in populations provide worrying data that underline the need to reinforce both fundamental and clinical research in this field. It is indeed a crucial problem of public health, because overweight and obesity are major risk factors for the development of chronic metabolic diseases that ultimately lead to CVD (3).

As well as genetic aspects, poor eating habits and a sedentary lifestyle are usually recognized as the main factors contributing to the current obesity epidemic. However, the amplitude of the phenomenon indicates that other, perhaps underestimated, elements probably play a role in the occurrence of this epidemic.

Historically, the epidemiological work conducted by Barker (4) in the 1990s first raised the possibility that early-life events in humans can play a major role in the development of overweight and chronic diseases in adulthood. Indeed, in his work, he showed that newborns with a low birth weight, due to malnutrition during pregnancy, were more likely to develop an adult phenotype of overweight and cardio-metabolic disorders. This work was later corroborated by other studies conducted in humans or in experimental animal models that have established the notion of “fetal programming.” It is now completely accepted that the nutritional or hormonal environment during

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³ Abbreviations used: ARC, arcuate nucleus; BAT, brown adipose tissue; CVD, cardiovascular disease; DIO, diet-induced obesity; GLUT, glucose transporter; HPA, hypothalamic-pituitary-adrenal; IRS-1, insulin receptor substrate-1; JAK2, Janus tyrosine kinase 2; NPY, neuropeptide Y; Ob-Rb, leptin receptor type b; PAI-1, plasminogen activator inhibitor-1; PI3K, phosphatidylinositol 3-kinase; PNOF, postnatally overfeeding; PVN, paraventricular nucleus; SOCS-3, cytokine signaling-3; SOD, superoxide dismutase; STAT3, signal transducer and activator of transcription 3; WAT, white adipose tissue.

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embryonic and fetal periods plays an essential modulatory role, as it guides genome expression. However, in the immediate postnatal period, this genomic plasticity remains, although perhaps at a lower level. In most mammals, the development of several organs is not complete at birth and continues during the immediate postnatal suckling period (5,6). Thus, environmental stimuli of a physical, mental, or nutritional nature can influence genomic expression in the offspring, theoretically to improve adaptation to their environment. However, in the longer term, these changes may be inappropriate and even deleterious, because they predispose the adult organism to metabolic disorders. Metabolic malprogramming of vital regulatory pathways permanently affecting appetite and growth dynamics may thus occur as a response to an inappropriate nutritional experience during immediate postnatal life, a period that may be considered a vulnerable time for offspring.

Numerous studies have demonstrated that early postnatal environments can influence body weight and energy homeostasis into adulthood. Overnutrition during the perinatal period has been associated with susceptibility to overweight, obesity, and related comorbidities (7). Although infancy has not been the target of overweight/obesity prevention, several observations have shown that rapid weight gain in the early stages of life may influence weight later in life as well as the future development of CVDs in adulthood (7,8).

In rodents, early overnutrition can be induced by reducing litter size, as first demonstrated by Kennedy (9) in 1957. In this context, the pioneering studies published by Plagemann et al. (10–13) in the 1990s showed in the rat that litter size reduction after birth led to significant increases in dietary intake due to the greater availability of breast milk in the critical immediate postnatal period. Therefore, rodents raised in small litters have been shown to be a useful experimental model to study the consequences of early overnutrition (14) (Fig. 1). These disturbances can lead to the development of short- and long-term modifications not only in body weight and food intake but also in several biochemical and metabolic features (12,13,15,16).

The aim of this review is to summarize all of the pathophysiological consequences of postnatal overfeeding (PNOF) in rats and mice (Fig. 2).

Does PNOF Induce Overweight or Obesity?

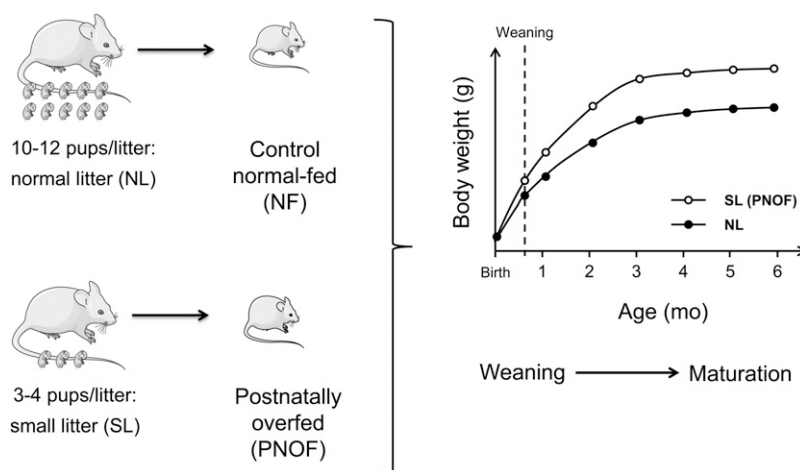
Raising rat or mice pups in small litters reduces competition for milk during the suckling period and therefore leads to overnourishment,

because the total calorie intake for each pup is increased (17). Some studies have observed that milk consumption in small litters increased in rats (18,19) and that the milk was richer in lipids, especially TGs (20). Due to this increase in milk consumption, rodents raised in small litters are overweight at weaning, but levels may vary among strains, geographical origin, and litter size from 56% to 10% in Wistar and Sprague Dawley rats to nearly 30% in mice (C57Bl/6 or Swiss).

In the postweaning period, we and others demonstrated that PNOF rodents maintained their body weight gain throughout life (14). Depending on the study, the increase in body weight may vary considerably during maturity, with some studies showing no permanent increase in body weight (21,22), whereas the vast majority reported a 10–25% increase in body weight at 12 mo in rats; PNOF mice were 20–30% heavier than control litters (Fig. 1). One of the key elements of overweight in adult PNOF rodents seems to be related to a persisting increase in food consumption into adulthood (13,14,18,23), because limiting food intake to small-litter rats after weaning inhibits weight gain and prevents the proliferation of fat cells (24).

Therefore, PNOF rodents could be used as a model to study the consequences of long-term moderate overweight in opposition to the usual models of genetic- or diet-induced obese rodents. Indeed, several experimental models of obesity in rats and mice have considerably improved our understanding of the mechanisms involved in the cardiovascular and metabolic consequences of overweight (25). Some of these genetic models correspond to conditions in which obesity is induced by a deficiency in leptin signaling, either by the total lack of leptin (*Zucker falfa* rat, *ob/ob* mouse) or its receptor (*db/db* mouse). Mutant rodents are phenotypically indistinguishable from their unaffected littermates at birth but gain weight rapidly throughout their lives, reaching a weight 3 times that of unaffected rodents, a feature characteristic of the highest grades of obesity in humans. Unfortunately, monogenic forms of obesity in humans are very sporadic events and are accompanied by severe metabolism abnormalities, sometimes with dramatic early consequences. Another method to induce major overweight in experimental animals is to use lipid- or carbohydrate-enriched diets, which rapidly lead to major body weight gain; experiment animals sometimes reach twice the weight of controls. Likewise, the conditions of this diet-induced weight gain correspond to severe grades of obesity but are probably poorly representative of conditions leading to simple overweight. Therefore, PNOF in

FIGURE 1 Schematic diagram representing the model of PNOF by litter-size reduction in rodents (rats and mice) and its consequences on body weight. Control litters of 10–12 pups/dam were considered NF, whereas SL of 3–4 pups induced PNOF. At weaning, depending on strains, PNOF Wistar and Sprague Dawley rats weighed 10–56% more than their NF counterparts, whereas the PNOF mice were nearly 30% heavier than their control NF littermates. In the postweaning period, PNOF rodents maintained their body weight gain throughout life. Depending on the experimental study, the increase in body weight may vary, the vast majority reporting a 10–25% increase in body weight during adulthood in rats, whereas PNOF mice were 20–30% heavier than control litters at maturity. NF, normally fed; PNOF, postnatally overfeeding; SL, small litter. Figures were produced using Servier Medical Art (www.servier.com).



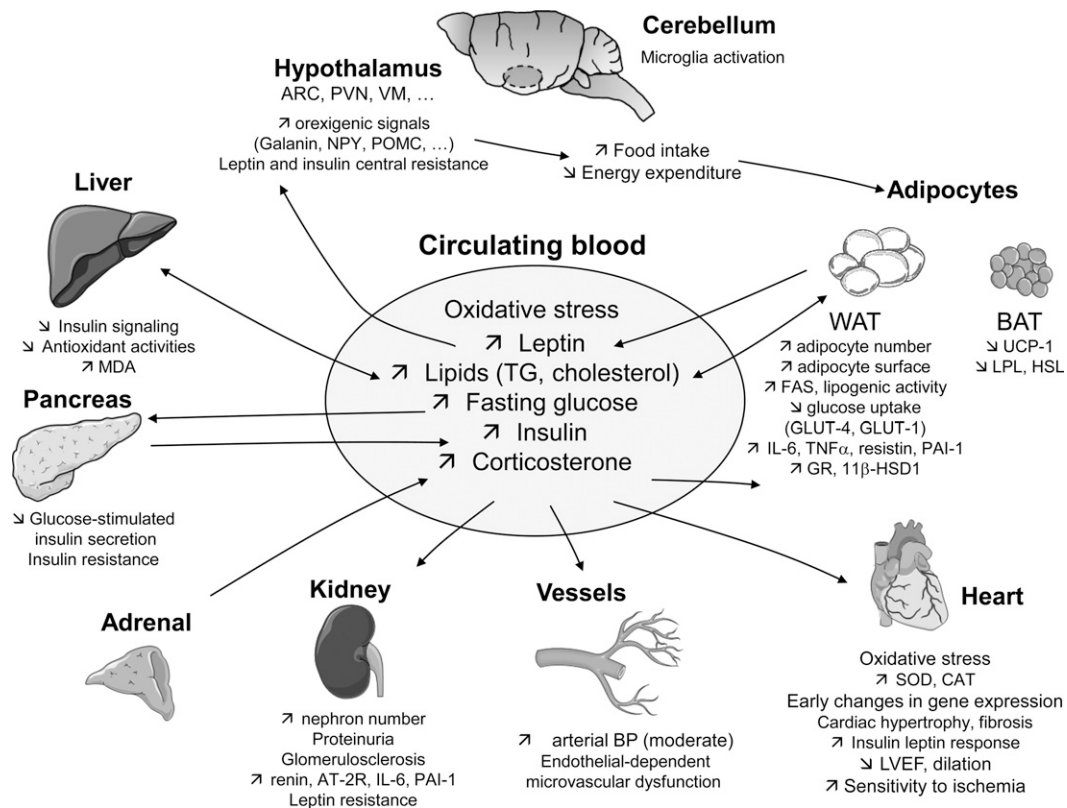


FIGURE 2 Pathophysiological consequences of PNOF in rodents. ARC, arcuate nucleus; AT-2R, angiotensin II receptor type-2; BAT, brown adipose tissue; BP, blood pressure; CAT, catalase; FAS, fatty-acid synthase; GLUT-1 and GLUT-4, glucose transporter-1 and -4; GR, glucocorticoid receptor; 11 β -HSD1, 11 β -hydroxysteroid dehydrogenase type 1; HSL, hormone-sensitive lipase; LPL, lipoprotein lipase; LVEF, left ventricular ejection fraction; MDA; malonaldehyde; NPY, neuropeptide Y; PAI-1, plasminogen activator inhibitor-1; POMC, pro-opio-melanocortin; PVN, paraventricular nucleus; SOD, superoxide dismutase; UCP-1, uncoupling protein-1; VM, ventro-median; WAT, white adipose tissue. Figures were produced using Servier Medical Art (www.servier.com).

rodents, for which adult body weight does not exceed 30% more than that of controls, might be a tool of choice to study the consequences of moderate overweight in youths and adults.

How Is PNOF Different from Overfeeding in Mature Animals?

Indeed, the use of high-calorie or high-fat diets in mature animals is the most common way to induce obesity in experimental rodents in order to explore its consequences on several biological or physiological functions (26,27). This model of diet-induced obesity (DIO) is extremely useful due to its close resemblance to the generally advanced cause of obesity in humans, i.e., the excessive intake of high-calorie foods and a sedentary lifestyle. However, as previously evoked, one of the main differences between PNOF and DIO is that high-fat diets generally induce a major increase in body weight and fat mass (i.e., obesity), whereas PNOF induces only moderate overweight. Moreover, after weaning, the body mass of PNOF rodents increases to a greater degree than that in their control littermates even though they have the same standard diet. This is very interesting, because the ingestion of high quantities of fat in DIO may induce confounding changes of both the gastro-intestinal tract physiology and gut microbiota (28). Additionally, DIO might be responsible for the deregulation of lipid metabolism that induces lipotoxicity, the effects of which are important contributing factors in cancer, diabetes, and CVD (29). Finally, in several studies, the increase in body weight and adiposity

induced by high-fat diets is completely or partially reversed by switching back to regular chow (26), a situation that is not encountered in PNOF rodents, which stay overweight when fed a standard diet. Therefore, PNOF is a very interesting model to study how nutritional changes in a critical time window might generate long-term moderate overweight and induce malprogramming of several metabolic and physiologic features.

PNOF Has an Early and Permanent Effect on the Central Nervous Organization and Endocrine System

Hormones, which regulate all the fundamental processes of life, are essential environment-dependent organizers of the developing neuroendocrine-immune network. As highlighted by Plagemann (30), when hormones are present in nonphysiological concentrations, induced by alterations in the intra-uterine or neonatal environment during critical periods of perinatal life, they can act as endogenous functional teratogens, leading to developmental disorders and diseases throughout life.

Changes in the hypothalamic-pituitary axis and control of food intake. The hypothalamus is the primary center in the brain for the regulation of food intake and body weight homeostasis. Neuropeptide Y (NPY) plays a key role in this field, particularly by acting in the orexigenic arcuate-paraventricular axis. This hypothalamic system consists of NPY-expressing neurons in the hypothalamic arcuate nucleus (ARC) that project

to the paraventricular nucleus (PVN). The expression and release of NPY within this axis are inhibited by circulating insulin and leptin, which act as a satiety signal that leads to fasting, whereas a decrease in insulin and leptin concentrations leads to activation of the NPY system, thereby stimulating food intake.

The original experiments of Andreas Plagemann et al. (13,31) focused on alterations in hypothalamic energy homeostasis in PNOF rats. These primary observations showed leptin and insulin resistance in the ARC (32–34) as well as inhibition of neurons in the PVN and ventro-medial nucleus by NPY (11,22), agouti-related protein (34,35), cortico-trophin-releasing factor, and dopamine (36), leading to feeding and reduced energy expenditure (37). Morphometric studies showed that the number of NPY neurons in the ARC and the number of galanin neurons in the PVN (12) increased. Taken together, these observations strongly indicate disorganization and malprogramming of the hypothalamic NPY system, induced by overfeeding during the critical period of early postnatal life, which in turn induces acquired persistent hypothalamic hypo-responsiveness in terms of central resistance to leptin and insulin. Indeed, concerning insulin, early PNOF rats displayed elevated intra-hypothalamic insulin concentrations during early postnatal life (13). However, in PNOF rats, acquired hypo-responsiveness to insulin manifesting as central resistance to insulin was observed. The malprogramming and resistance of orexigenic as well as anorexigenic neurons in the hypothalamus might contribute to the occurrence of hyper-phagia, overweight, and hyper-insulinemia throughout later life (30).

Modifications of reproductive function. Recent studies suggest that inappropriate neonatal nutrition can cause perturbations in the hypothalamic neural circuits that control reproductive function. In female rats, the onset of puberty was significantly shortened in those raised in small litters. Conversely, neonatally undernourished female mice displayed delayed puberty and defective development of axonal projections of the ARC to the preoptic region, which affected the neuronal organization of neuronal projections containing kisspeptin, a key neuropeptide involved in pubertal activation and fertility (38). In female mice, PNOF also perturbed the development of neural projections and induced reduced reproductive performance during adulthood, as shown by a reduction in the number of litters per month. Altogether, these results indicate that early alterations in the nutritional environment can cause long-lasting deleterious effects on the control of reproductive function along with lifelong functional perturbations.

Modifications of the hypothalamic-pituitary-adrenal glucocorticoid axis. Experimental evidence suggests that glucocorticoids are involved in the pathophysiology of abdominal obesity and its associated complications. Changes in glucocorticoid signaling have been described in obese humans and rodent models of obesity. In juvenile rats, PNOF induced the accelerated maturation of the hypothalamic-pituitary-adrenal (HPA) axis together with the upregulation of adipose tissue glucocorticoid receptor mRNA (39). In adulthood, neonatally overfed rats presented elevated basal and stress-induced corticosterone secretion (40) and significant changes in visceral adipose tissue glucocorticoid signaling (39), with increased mRNA concentrations for glucocorticoid receptor and 11β -hydroxysteroid dehydrogenase type 1, an enzyme responsible for the conversion of corticosterone into cortisol (41), thus increasing the sensitivity of adipose tissue to glucocorticoids. It is strongly suggested that

a primary alteration in the HPA axis occurs during early nutritional manipulation. In turn, this permanent hyperactivity of the glucocorticoid HPA system may play a pivotal role in the subsequent development of metabolic abnormalities in adulthood. In the liver, the activity and expression of 11β -hydroxysteroid dehydrogenase type 1 was also permanently increased in rats raised in small litters (42). In addition, the expressions of glucocorticoid-inactivating enzymes, 5α -reductase type 1 and 5β -reductase, transiently increased during puberty. These modifications in hepatic glucocorticoid metabolism could contribute to increased tissue-specific glucocorticoid exposure and aggravate the development of metabolic disorders in adults, such as insulin resistance, dyslipidemia, and fatty liver.

PNOF Modifies the Metabolic Level

Changes in adipose tissue. As previously reported, PNOF rodents display an early and long-lasting increase in body weight, which is mostly associated with increased fat mass, as we observed in 7-mo-old C57Bl/6 mice with the use of EchoMRI (43). Adipose tissue, a loose connective tissue composed of adipocytes, exists in 2 distinct forms: white adipose tissue (WAT) and brown adipose tissue (BAT).

WAT is generally thought to serve as a source of energy and, in rodents and humans, may be subdivided into subcutaneous and visceral (abdominal) fat. An increase in visceral WAT is observed at weaning and later in PNOF rats (18,21–23,39,44) and mice (45) and goes along with an increase in adipocyte surface (39,40,42). However, subcutaneous fat mass is also increased in young but not adult PNOF mice (23), indicating that in adulthood, higher body weight and fat mass are mainly due to increased visceral fat mass. This increase in adiposity does not seem to be related to long-lasting effects on energy balance, i.e., energy intake minus energy expenditure, in later life (46). At weaning and later, the higher fat contents in adipose tissue were associated with higher amounts of fatty acid synthase, an enzyme that catalyzes de novo synthesis of fatty acids, and lipogenic activities in retroperitoneal adipose tissue (21). In epididymal WAT adipocytes isolated from PNOF rats, impaired insulin-stimulated glucose uptake was observed and was associated with a significant decrease in glucose transporters GLUT-4 and GLUT-1 and insulin receptor-signaling pathway components such as insulin receptor substrate-1 (IRS-1), phosphatidylinositol 3-kinase (PI3K) expression, and Akt serine kinase activity (47). Additionally, in mesenteric adipose tissue, the mRNA expression of IL-6, TNF- α , TNF-receptor-1, resistin, and plasminogen activator inhibitor-1 (PAI-1) was increased in PNOF rats (40), revealing the inflammatory status of WAT.

In rodents, one key structure involved in the regulation of body weight is the BAT, the major thermogenic structure. Postweaning and 4-mo-old PNOF rats showed reduced thermogenic activity and lower levels of uncoupling protein-1 mRNA (41,48), and in 6-mo-old PNOF mice, mRNA for uncoupling protein-1 was decreased in BAT (45). When PNOF rats were exposed to acute cold, their BAT was less active and thermogenic responsiveness was reduced. At the molecular level, the expression of several transcriptional regulators (PPAR γ , C/EBP-1) and lipases, such as lipoprotein lipase and hormone-sensitive lipase, was reduced in the BAT, whereas the expression of the sympathetic β 3-adrenergic receptor and the response to the β -adrenergic agonist, isoproterenol, were decreased in PNOF rats. In PNOF rats, excess weight gain during the early postnatal period led to permanent reprogramming of adaptive thermogenesis in BAT.

Perturbations of glucose/insulin homeostasis. Numerous studies have focused on the effects of early overnutrition on the insulin-glucose axis in adults. Indeed, neonatally overfed rats and mice frequently display increased basal values of fasting glucose and plasma insulin and impaired glucose/insulin homeostasis (13,14,18,21,39,40,49). Glucose and insulin tolerance tests have suggested that pancreatic glucose responsiveness is persistently reduced in PNOF rats (13,18,21,40,42) and mice (49). These effects concerning the glucose-insulin balance seem to be at their highest level at weaning (50) but may decrease or disappear with maturation (21,23,51). However, several studies failed to demonstrate any differences in glycemia or insulinemia in PNOF rodents (22,34,44,45,47,48). Interestingly, whereas litter manipulation had a significant effect in male rats, PNOF females were found to be resistant to hypo-responsiveness to insulin (24).

Some studies (51) showed defects in glucose-stimulated insulin secretion from pancreatic islets isolated from PNOF rats, supporting the hypothesis that the endocrine pancreas contributes to primary metabolic imprinting in this model. In fact, insulin secretion in pancreatic islets β -cells depends essentially on glucose uptake, a process that is driven by GLUT-2. Paradoxically, PNOF rats had an elevated GLUT-2 content in pancreatic islets (18), indicating that early postnatal overnutrition during a critical development period in life may induce permanent modifications in glucose-stimulated insulin secretion. Waterland et al. (51) used microarrays to identify genes showing differential expression in pancreatic islets isolated from rats. They observed early differential gene expression in PNOF at weaning. Some of these genes remained differentially expressed at 4 mo (51). These data indicate that even moderate overnutrition in the postnatal period may permanently alter gene expression in the endocrine pancreas, probably by influencing the epigenetic regulation of imprinted genes.

Recent studies of the liver have shown impaired insulin signaling in 6-mo-old rats that were raised in small litters. In fact, the hepatic expression of insulin-receptor- β , IRS-1, phospho-IRS-1, Akt1, and PI3-K significantly decreased (52). This impairment of hepatic insulin signaling might then reinforce the impairment of glucose/insulin homeostasis.

Modifications of plasma lipids and proteins. PNOF rats and mice frequently present disturbances in plasma lipids: elevated concentrations of TGs (13,19,39,49), FFAs (39,40,49), and total and HDL cholesterol (19,23,39,49). However, depending on the strain, litter size, age, and geographical origin of the animal, the lipid perturbations are not systematically encountered (42,44,47,48,53).

Small-litter rats presented higher levels of globulins in the immediate postweaning period and adulthood (6 mo) and circulating concentrations of albumin were higher at weaning and lower at adulthood (23). This increase in protein/albumin has also been described in 12-mo-old PNOF mice (49). In adult rats, the profile of lower albumin and higher globulin has been described in cases of chronic inflammation, which is reported to be associated with obesity.

Adipocytokines. It is well known that excessive central adiposity is a significant risk factor for CVD and may influence survival in individuals with coronary artery disease (54). Indeed, adipose tissue is able to secrete a plethora of peptides, called adipocytokines, which act in endocrine, paracrine, or autocrine modes to influence an array of biological functions, some of which are clearly involved in the development of CVD (55).

Leptin is produced primarily by WAT and concentrations of leptin are directly proportional to whole body adipose mass. Leptin plays a major role in the regulation of appetite and energy balance, but it also induces a variety of actions in the cardiovascular system. The increase in leptin concentrations in PNOF rats (14,18,22,23,34,39,41,50) or mice (49) is constantly reported in the literature and can be explained by the fact that they are related to the amount of fat mass. Though PNOF rodents have increased circulating concentrations of leptin, previous studies in rats suggested that PNOF may induce central leptin resistance due to lower leptin type-b (long isoform) receptor (Ob-Rb) expression in the hypothalamic nucleus (34,44,56). The signaling cascade of leptin in cells after its interaction with Ob-Rb involves PI3K, Janus tyrosine kinase 2 (JAK2), and signal transducer and activator of transcription 3 (STAT3). The JAK2/STAT3 pathway stimulates the suppression of cytokine signaling-3 (SOCS-3) transcription, a leptin-inducible inhibitor of the leptin signaling pathway. PNOF induced lower expression of both JAK2 and phosphorylated-STAT3 and higher expression of SOCS-3 in the adult rat hypothalamus, indicating central leptin resistance (23). Several studies suggested that hyperleptinemia during a critical period of development may be a key inducer of the malprogramming of energy homeostasis. Whereas leptin is a critical trophic factor for the hypothalamus during development, the inhibitory mechanisms that mediate the effects of leptin on food intake are not fully functional until at least postnatal wk 4. Importantly, electrophysiological studies (32) demonstrated that in young PNOF rats, neurons in the arcuate hypothalamic nucleus exhibit a reduced inhibitory response to leptin, suggesting that early overnutrition may similarly result in leptin resistance, a situation that may contribute to the long-term effects on body weight in this model. Indeed, Glavas et al. (45) showed that PNOF Swiss Webster mice exhibited early-onset leptin resistance that persisted into adulthood, despite being fed a healthy standard chow diet. Additionally, other factors associated with early postnatal overnutrition, for instance concurrent hyperinsulinemia, seem to play a pivotal role in the development of leptin resistance and life-long overweight in rats (56).

Concerning adiponectin, the results are less consensual. Previous studies performed in rats showed no difference between PNOF and normally fed groups at 4 and 6 mo (23,41), whereas Boullu-Ciocca et al. (40) showed a decrease in both circulating concentrations of adiponectin and mRNA expression in epididymal adipose tissue in 5-mo-old PNOF rats, a result that was confirmed by our laboratory (14). Adiponectin production is inversely proportional to whole body adipose mass and experimental studies suggest that it modulates the action of insulin and thus improves insulin sensitivity in peripheral tissues (57) and induces general cardioprotective effects (58).

PNOF Induces Oxidative Stress

Obesity and overweight are associated with increased oxidative stress not only in the bloodstream but also in myocardial tissue (59,60). In a study performed in rats in our group (14), we observed an increase in plasma hydroperoxides and a decrease in vitamin C concentrations, indicating exacerbated circulating oxidative stress. Further work performed in mice confirmed these initial data (43). Indeed, postnatal overnutrition in mice also induced an increase in oxidative stress in adult myocardial tissue, as evidenced by electron spin resonance spectroscopy (61,62). This increased nitro-oxidative stress in the myocardial

tissue of PNOF mice was a very original finding that confirmed the association between obesity and increased myocardial oxidative stress (60). We also observed a significant increase in the expression and activities of antioxidant manganese-dependent superoxide dismutase (SOD) and catalase in heart tissue homogenates, a paradoxical result at first sight, which could rather reflect the adaptive response to increased oxidative stress.

Recent findings concerning the liver of PNOF Wistar rats suggest a decrease in the activities of various antioxidant enzymes, such as catalase, SOD, and glutathione peroxidase (52), whereas Western blot analysis showed no differences in Cu/Zn SOD content. Additionally, markers of nitro-oxidative stress such as nitrates and malonaldehyde also increased in the liver and plasma. This imbalance between reduced antioxidant defenses and increased oxidative damage may predispose hepatocytes to injury and may trigger the development of insulin resistance.

PNOF Affects Kidney Function

Regarding renal development, whereas nephrogenesis is predominantly complete prenatally in mammals, renal maturation, consisting of increases in glomerular size and tubular length, continues postnatally. Some studies demonstrated that PNOF influenced nephrogenesis and renal maturation (63,64). Indeed, early PNOF is associated with impaired nephrogenesis, leading to an increase in the number of nephrons and decreased glomerular volume. These changes affect both young males and females but persist during aging only in males, inducing the development of proteinuria and glomerulosclerosis in the long term (64). The resulting low nephron endowment may contribute to an increased risk of cardiovascular and renal diseases in adulthood. In mature PNOF rats, a decrease in glomerular filtration rate, proteinuria, and glomerulosclerosis were observed. The expression of renin-angiotensin system components has also been explored in young PNOF rats, because this system is involved in the progression of renal disease. Indeed, the expression of renin and angiotensin II receptor type-2 were increased in the kidneys of young PNOF rats, whereas the expression of both PAI-1, usually regarded as a powerful fibrosis-promoting

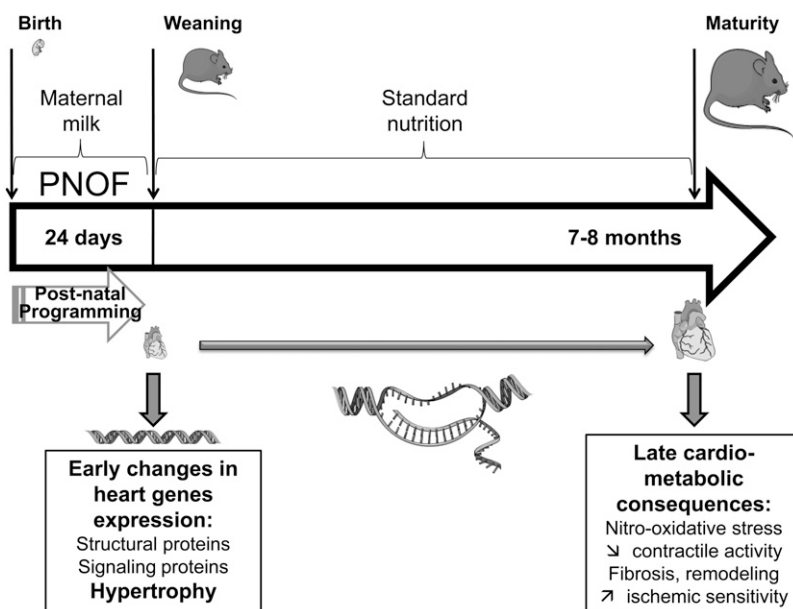
molecule, and matrix metallo-proteinase-9 decreased. This process might contribute to the abnormal programming of renal growth (65).

Related to these anomalies, renal inflammation as well as the expression and deposition of extracellular matrix molecules such as collagen I was increased (66). Furthermore, the expression of leptin, Ob-Rb, and the cytokines IL-6 and PAI-1 were upregulated. The phosphorylation of STAT3 and ERK1/2 in the kidney was decreased, indicating renal postreceptor leptin resistance. Moreover, the expression of SOCS-3 protein, a mediator of leptin resistance, was increased. These results demonstrate that early modifications in nutritional status may permanently affect renal function and induce profibrotic processes. They also provide evidence that peripheral leptin resistance is a potential underlying mechanism.

PNOF Induces Early and Late Modifications of Cardiovascular Function

Blood pressure levels. Hypertension is considered a major risk factor for CVDs, including stroke, myocardial infarction, and heart failure, and may cause chronic kidney disease. In rodents, obesity induced by a high-fat diet is associated with an increase in blood pressure (41). In slightly overweight adult PNOF rodents, an increase in diastolic and systolic arterial blood pressure has been observed (13,14,49,63,64), but this increase is usually minor (10–15 mm Hg) and sometimes nonsignificant (22,41,44,65,66). Several central and peripheral mediators, which are modified by PNOF, may contribute to the increase in blood pressure. Although the hypertensive actions of glucocorticoids are fully documented, leptin and NPY may also be important players. Indeed, leptin increases blood pressure not only via receptor-mediated activation of the sympathetic nervous system (67) but also by inhibiting NPY synthesis. Actually, NPY is involved in the control of energy intake and, at the nucleus of the solitary tract, can elicit the lowering of blood pressure and heart rate (68). However, the mechanism underlying the hypertensive effect of moderate overweight in rodents has not yet been elucidated. In our laboratory, we evaluated aortic and coronary reactivity in the thoracic aortas and isolated

FIGURE 3 Cardiac consequences of PNOF. After 24 d of increased milk consumption, myocardial genome expression was modified in PNOF mice through a postnatal programming effect that affected the expression of structural and signaling proteins and induced the development of hypertrophy. Late cardio-metabolic consequences appeared at maturity. These included myocardial oxidative stress, fibrosis, decreased contractility, and increased sensitivity to ischemia reperfusion injury. PNOF, postnatal(ly) overfeeding. Figures were produced using Servier Medical Art (www.servier.com).



perfused hearts harvested from PNOF Wistar rats. However, we were not able to demonstrate any differences in endothelium-dependent or -independent vasoreactivity between groups. In a very recent study performed in golden hamsters, endothelium-dependent microvascular function was assessed with acetylcholine in the cremaster muscle, and it was found that early postnatal overnutrition induced endothelial-dependent microvascular dysfunction in adulthood (69).

Hypertrophy and myocardial function. In Wistar rats, PNOF can induce cardiac hypertrophy at weaning, with a significant increase in ventricular wall thicknesses, shortening of left ventricular diameter, enlargement of cardiomyocyte area, and a decrease in coronary vessel density (19). If not compensated, this situation may lead to compromised myocardial vitality and impaired heart function. Several factors, such as biomechanical stretch or neuro-humoral mechanisms, may induce ventricular remodeling. Indeed, cardiomyocyte hypertrophy can be triggered by hormones, cytokines, and growth factor-signaling pathways, and, for instance, leptin or insulin may be considered either metabolic or growth factors for the heart (70,71). For example, in cardiomyocytes, insulin acts on its receptor through the recruitment of different IRSs, which will trigger PI3K phosphorylation, thus increasing the concentrations of phosphatidyl inositol triphosphate and activating phosphatidyl inositol triphosphate-dependent serine/threonine kinases. The long isoform of leptin receptors (Ob-Rb) is also present in the

heart and the signaling cascade of leptin in cells involves PI3K, JAK2, and STAT3. Interestingly, postweaning juvenile hearts from PNOF rats displayed activation of the insulin and leptin cascade, with increased concentrations of insulin receptor and Ob-Rb, PI3K, STAT3 and increased translocation of GLUT-4 (50). This higher sensitivity to insulin and leptin in PNOF juvenile rats might therefore induce a cardiac-adaptive response, resulting in improved glucose uptake and an increased supply of energy, but it may also induce a hypertrophic phenotype.

Recently, we observed that early postnatal overnutrition led to modifications of basal cardiovascular parameters in adult mice (Fig. 3). These included an enlargement of ventricular diameters and impaired left ventricular ejection fraction (43). To date, none of these modifications has been reported in overweight mice.

Fibrosis. Collagen deposition is commonly used to determine cardiac fibrosis and we and others were able to demonstrate elevated left ventricle total collagen in adulthood in 4-mo-old (22) and 6-mo-old rats (14) (Fig. 3). Histological studies showed higher collagen density in the left and right ventricles of adult PNOF rats and mice. Additionally, we observed an increase in both matrix metallo-proteinase-2 expression and activity in the ventricular tissue of PNOF rodent hearts, accounting for an alteration of the architectural organization (43). Several environmental factors such as oxidative stress or increased leptin levels might be involved in the occurrence of this remodeling

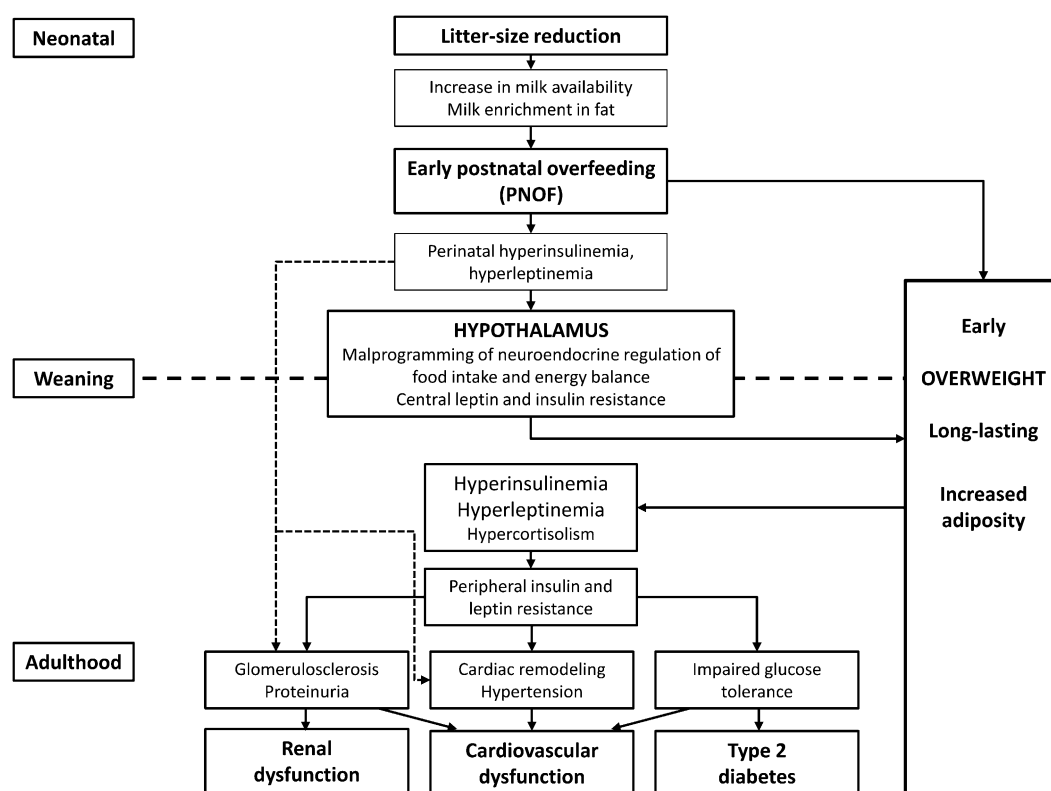


FIGURE 4 Schematic diagram representing the step-by-step alterations induced by PNOF. In the immediate postnatal period, litter size reduction leads to increased availability of milk, allowing higher consumption of a milk of higher fat content and therefore to PNOF. Due to this increase in milk consumption, rodents raised in small litters are overweight at weaning. PNOF rodents develop hyperinsulinemia and hyperleptinemia in early life and these are responsible for hypothalamic malprogramming of the neuroendocrine-mediated regulation of food intake and energy balance and for central leptin and insulin resistance. Rodents develop permanent, long-lasting overweight and increased adiposity, leading to elevated concentrations of insulin, leptin, and glucocorticoids in adulthood and peripheral insulin and leptin resistance. Together with early modifications of organ gene expression and organization, these mechanisms are responsible for a predisposition to renal and cardiometabolic dysfunction. PNOF, postnatal(ly) overfeeding.

phenomenon. Indeed, studies have highlighted the role of leptin and insulin in the elongation of cardiac myocytes (72), ventricular hypertrophy (73,74), and heart failure (75). Additionally, these changes in myocardial organization might be the late consequences of the early modifications observed in the gene expression of some isoforms of actin, myosin, collagen, dystrophin, and other structural proteins that may have a definitive impact on the intracellular and organ structure.

Early changes in cardiac gene expression. A recent study performed in our laboratory (43) provided new original data showing that nutritional changes, such as overnutrition, in immediate postnatal life may influence the expression of several genes involved in the heart's structural organization (collagens, myosin, actin, dystrophin, dynein), metabolism (insulin-growth factors), vasoreactivity (endothelin-1 and receptor), cell signaling/communication, and oxidative stress (glutathione *S*-transferase) (Fig. 3). After only 24 d of increased milk consumption, myocardial genome expression was modified in PNOF mice, a situation that may have induced permanent changes in the cytoskeleton structure and organization of cardiomyocytes.

Therefore, perinatally acquired microstructural and epigenetic modifications in systems that regulate metabolism and body weight seem to be critical in that they lead to a cardiometabolic risk-prone phenotype throughout life.

PNOF increases susceptibility to myocardial ischemia reperfusion injury. Myocardial ischemia results from the severely impaired supply of oxygen and nutrients to the heart's territory, which leads to irreversible injury. Reperfusion of the ischemic myocardium is essential to rescue tissue, limit infarct size, and reduce mortality. Paradoxically, however, the return of blood flow can also result in additional cardiac damage and complications, referred to as reperfusion injury. In hearts isolated from PNOF mice or rats, baseline cardiac parameters were similar to those in control animals. However, after 30 min of ischemia followed by reperfusion, the recovery of preischemic cardiac output was impaired in hearts from PNOF rodents, and the total amount of lactate dehydrogenase, a marker of cell injury, released in the coronary effluent during the period of reperfusion was found to be significantly higher (14). In hearts from PNOF mice, the recovery of coronary flow and contractility parameters were also significantly impaired after ischemia. In addition, the evaluation of still-viable and necrotic zones after 2 h of reperfusion showed a greater area of infarction increase (+74%) in PNOF mouse hearts than in controls (43). These results suggest that PNOF induced a greater susceptibility of the heart to ischemia-induced injury, resulting in more severe cell damage. We speculate that, in stressful conditions such as ischemia-reperfusion, which generates a massive release of reactive oxygen and nitrogen species (76,77), the metabolic alterations induced by PNOF, which are rather benign in normal conditions, render the myocardium more susceptible to injury.

In conclusion, our review emphasizes that overnutrition during the immediate postnatal period in rodents leads to early changes in hypothalamic circuits controlling energy homeostasis, which in the short-term affect circulating concentrations of hormones, the function of several organs, and gene expression. These early changes may permanently modify structural organization and metabolism (Fig. 4) and may induce later alterations in function and structure, which are very probably involved in the higher susceptibility of these rodents to pathological challenges. Altogether, the results collected here suggest that the nutritional state in the immediate postnatal period

should be taken into account, because it may have an impact on the cardiometabolic and renal risk in adulthood and especially in the context of transgenic mice that may be bred in small litters.

Indeed, a cautionary note should therefore be made concerning the use of transgenic animals to evaluate the potential role of certain key protein genes and their impact. Indeed, due to difficulties in breeding, transgenic mice are frequently raised in small litters and, compared with their wild-type control counterparts, raised in normal (large) litters. In view of the present data, caution should be exercised when transgenic mice are compared with wild-type mice by making sure that the mice were raised in litters of equal numbers. Otherwise, some of the negative impacts of inactivating or upregulating a gene in transgenic mice could only be due to the long-term cardiometabolic consequences of nutritional differences during the critical postnatal period.

Finally, it is necessary to understand the biological mechanisms underlying the early but permanent modifications induced by PNOF to determine their importance for human health and design potential interventions to prevent or treat their adverse consequences.

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