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Nonstandard abbreviations:

APJ: apelin receptor

ARC: arcuate nucleus

AT-2R: angiotensin II receptor type-2

BAT: brown adipose tissue

DOX: Doxorubicin

ESR: electron spin resonance

GH: growth hormone

GH-RH: growth hormone-releasing hormone

GLUT: glucose transporters

GR: glucocorticoid receptor

HPA: hypothalamic-pituitary-adrenal axis

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

IκB: inhibitor of NF-κB

IL-1β: interleukin-1β

IL-6: interleukin-6

IR: insulin receptor

IRS-1: insulin receptor substate-1

JAK2: Janus tyrosine kinase 2

LPS: lipopolysaccharide

MMP: matrix metallo-proteinase

Mn-SOD: manganese-dependent superoxide dismutase

NFκ-B: nuclear factor κ-B

NPY: neuropeptide Y

Ob-Rb: leptin receptor type b

OF: overfed, overfeeding

PAI-1: plasminogen activator inhibitor-1

PAMP: pathogen associated molecular patterns

PI3K: phosphatidyl inositol 3-kinase

PIP_3: phosphatidyl inositol triphosphate

PNOF: postnatal overfeeding

PVN: paraventricular nucleus

RAS: renin-angiotensin system

RONS: reactive oxygen and nitrogen species

SOCS3: cytokine signaling-3

STAT3: signal transducer and activator of transcription 3

TLR: toll-like receptors

TNF-α: tumor necrosis factor-α

TNFR1: TNF-receptor-1

UCP-1: uncoupling protein-1

WAT: white adipose tissue

Abstract (449 words)

Numerous studies have demonstrated that early postnatal environment can influence body weight and energy homeostasis into adulthood. Over-nutrition during the perinatal period has been associated with susceptibility to overweight, obesity and related comorbidities. Rodents raised in small litters have been confirmed to be a useful experimental model for the study of consequences of early over-nutrition. These disturbances can lead to the development of short- and long-term modifications not only in body weight and food intake, but also of several biochemical and metabolic features.

The aim of this review is to summarize all the pathophysiological consequences of postnatal overfeeding (PNOF) in rodents.

Indeed, PNOF might be a tool of choice for the study of consequences of moderate overweight in youths and adults. At the central nervous system level, PNOF induces early disorganization and malprogramming of the hypothalamic system, inducing an acquired persisting central leptin and insulin resistance and an increase in orexigenic signals, as well as perturbations in hypothalamic neural circuits controlling reproductive function and growth. Concerning adipocytes, an increase in visceral white adipose tissue is observed in PNOF rodents, with increased lipogenic activity and inflammatory status, while brown adipose tissue shows reduced thermogenic activity. Glucose and insulin test tolerance tests suggest persistently reduced pancreatic glucose-responsiveness in PNOF rats and mice. Moreover, PNOF rodents frequently present disturbances in plasma lipids: elevated triglycerides(Plagemann, Harder et al. 1999; Boullu-Ciocca, Dutour et al. 2005; Kappeler, De Magalhaes Filho et al. 2009; Moreira, Teixeira Teixeira et al. 2009)(Plagemann, Harder et al. 1999; Boullu-Ciocca, Dutour et al. 2005; Kappeler, De Magalhaes Filho et al. 2009; Moreira, Teixeira Teixeira et al. 2009), free fatty acids, total and HDL cholesterol and elevated levels of plasma proteins. In adulthood, PNOF rats present elevated corticosterone secretion and significant changes in visceral adipose tissue glucocorticoids sensitivity. The increase in leptin levels in PNOF rodents is constantly reported in the literature, and can be related to the amount of fat mass; however, overfeeding induces central leptin resistance. PNOF also influences nephrogenesis and renal maturation, and the expression of renin and of the angiotensin II receptor type-2 (AT-2). Increased oxidative stress associated with PNOF 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 in PNOF rodents, and a rapid cardiac hypertrophy is observed at weaning; however, during maturation, an alteration of contractility and a fibrosis is observed. At the genetic level, myocardial genome expression is rapidly modified in overfed mice which may be responsible for some permanent changes in cardiomyocytes cytoskeleton structure and organization. Moreover, hearts of PNOF rodents are more sensitive to ischemia-reperfusion injury and to the cardiotoxicity of doxorubicin.

Altogether, these results suggest that the nutritional state in the immediate postnatal period should be taken into account since it may have an impact on cardiometabolic risk in adulthood, and especially in the context of transgenic mice that may be bred in small litters.

Keywords: Postnatal overfeeding – Early overnutrition – Programming – Leptin -

Introduction

Cardiovascular pathologies represent today not only one of the major causes of morbidity and mortality in Europe and North American continents, but their occurrence is also exponentially increasing in developing countries. Thus, the number of resulting deaths should reach more than 24 million in 2030, which represents one of the major public health concerns around the world. So-called "Western" lifestyle and its adaptation in other regions of the globe contribute to a world epidemic of metabolic syndrome and type 2 diabetes, both constituting major cardiovascular risk factors gathering features such as insulin-resistance, arterial hypertension, atherogenic dyslipidemia, overweight or central obesity.

In a comparable manner to that of cardiovascular diseases, the management of overweight and obesity is also one of the great challenges of this Century. The estimation of obesity and overweight prevalence among adults aged 20 to 70 years in the United States in 2007-2008 was 68% (Flegal, Carroll et al. 2010). Alarmingly, the prevalence of obesity in children almost tripled over the past 30 years (Inge, Garcia et al. 2004). Today, epidemiological studies on weight gain in populations provide alarming data justifying the interest for reinforcing either fundamental or clinical research in this field. It is indeed a crucial problem of public health since overweight and obesity represent a major risk factor for the development of chronic metabolic diseases that ultimately lead to cardiovascular disease (Malnick and Knobler 2006).

Aside from some genetic aspects, poor eating habits and a sedentary lifestyle are usually recognized as the main factors contributing to the current obesity epidemic. It is indeed very clear that a sort of "nutritional evolution", classically described as "Westernization", of alimentary and energetic behaviors leads to overweight, but also to cardio-metabolic and cancers diseases. However, the amplitude of the phenomenon indicates that other elements are likely to play a role, perhaps today underestimated, in the occurrence of this epidemic.

Historically, the epidemiological work conducted by Barker 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 the adulthood (Barker 1995). Indeed, in his work, he showed that newborns of low birth weight, due to a malnutrition status during pregnancy, were more prone to develop an adult phenotype of overweight and a greater sensitivity to the occurrence of cardio-metabolic disorders. This work was later corroborated by many other studies, conducted in humans or in experimentation animals, which have established the notion of “fetal programming”. Undeniably, it is now completely accepted that the nutritional or hormonal environment during embryonic and fetal periods plays an essential modulatory role, guiding the genome expression in one way or the other. However, in the immediate postnatal period, this genomic plasticity still remains, although perhaps at lower level. Indeed, in most mammals, the development of several organs is not complete at birth, and continues during the immediate postnatal suckling period (Kaung 1994; Grove and Smith 2003). Thus, environmental stimuli, of physical, mental or nutritional nature, can influence the offspring genomic expression, theoretically allowing improved adaptation to its environment. However, in the longer term, these changes may be inappropriate and even deleterious, since they predispose the adult organism to metabolic disorders. Metabolic mal-programming of vital regulatory pathways may however occur as a response to an altered nutritional

experience during the immediate postnatal life, a period that may be considered as a vulnerable time for permanent mal-programming of appetite and growth dynamics.

Numerous studies have demonstrated that early postnatal environments can influence body weight and energy homeostasis into adulthood. Over-nutrition during the perinatal period has been associated with susceptibility to overweight, obesity and related comorbidities (Morrison, Friedman et al. 2008). Although infancy has not been the target of overweight/obesity prevention, several observations have shown that rapid weight gain in early stages may influence weight later in life as well as the future development of adult cardiovascular diseases (Stettler, Stallings et al. 2005; Morrison, Friedman et al. 2008).

In rodents, early over-nutrition can be induced by reducing litter size, as first demonstrated by Kennedy in 1957 (Kennedy 1957). In this context, the pioneering studies published by Plagemann in the 1990s (Dorner and Plagemann 1994; Heidel, Plagemann et al. 1999; Plagemann, Harder et al. 1999; Plagemann, Harder et al. 1999) showed in the rat that litter size reduction after birth allowed significant enhancement of dietary intakes due to a greater availability of breast milk in the critical immediate postnatal period. Therefore, rodents raised in small litters have been confirmed to be a useful experimental model for the study of consequences of early over-nutrition (figure 1). These disturbances can lead to the development of short- and long-term modifications not only in body weight and food intake, but also of several biochemical and metabolic features (Plagemann, Harder et al. 1999; Plagemann, Harder et al. 1999; Waterland and Garza 1999; Sivanandam, Sinaiko et al. 2006).

The aim of this review is to summarize all the pathophysiological consequences of postnatal overfeeding (PNOF) in rodents, rats at the first step but also mice.

1. Overweight or obesity?

Raising rat or mice pups in small litters allows reduced competition for milk during the suckling period, and therefore over-nourishment; the total intake of calories available for each pup being increased (Fiorotto, Burrin et al. 1991). Some studies have indeed observed that milk consumption in small litters was increased in rats (Cunha, Pereira et al. 2009; Moreira, Teixeira Teixeira et al. 2009) and that milk composition was enriched in lipids, especially in triglycerides (Sefcikova, Bujnakova et al. 2011). Due to this increase in milk consumption, rodents raised in small litters are overweighed at weaning, but levels may vary among strains, geographical origin and litter size from 56% to 10% in Wistar and Sprague Dawley rats, and nearly 30% in mouse (C57Bl/6 or Swiss).

In the post weaning period, postnatally overfed rodents maintain increased body weight gain throughout life. Depending on the studies, increase in body weight may considerably vary during maturity with some studies showing no permanent increase in body weight (Balonan and Sheng 2000; Velkoska, Cole et al. 2008) while the vast majority show a 10-25% increase in rat body weight at 12 months, whereas postnatally overfed mice are 20-30% heavier than control raised litters (Figure 1). One of the key elements of overweight in adult postnatally overfed rodents seems to be related to hyperphagia and persisting increase in food consumption into adulthood (Plagemann, Harder et al. 1999; Cunha, Pereira et al. 2009; Rodrigues, de Moura et al. 2011; Habbout, Delemasure et al. 2012), since limiting food intake to small litter rats after weaning inhibits weight gain and prevents fat cell proliferation (Bassett and Craig 1988).

Therefore, the very interesting element in postnatally overfed rodents is that they represent a model for the study of consequences of long-term moderate overweight, in opposition to usual models of genetic or diet-induced obese rodents. Indeed, several experimental models of obesity in rats and mice allowed considerable improvements in the understanding of mechanisms involved in the cardiovascular and metabolic consequences of this situation (Carroll, Voisey et al. 2004). Thus, some of these genetic models correspond to conditions where obesity is induced by deficiency in leptin signaling, either by the total lack of this adipocytokine (Zucker fa/fa rat, ob/ob mouse) or of 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 three times that of unaffected rodents, a feature characteristic of the highest grades of obesity if compared to humans. Unfortunately, monogenic forms of obesity in humans represent a very sporadic event, and are accompanied by severe metabolism abnormalities, with sometimes prematurely dramatic consequences. Another method to induce major overweight in experimental animal is the use of lipid or carbohydrate-enriched diets, which rapidly lead to major body weight gain, with sometimes the doubling of this of control. Likewise, the conditions of this diet-induced weight gain correspond to severe grades of obesity, probably poorly representative of conditions leading to simple overweight. Therefore, PNOF in rodents, for which adult body weight does not exceed 30% more than that this of controls, might be a tool of choice for the study of consequences of moderate overweight in youths and adults.

2. At the central nervous system level

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

The original experimentations of Andreas Plagemann (Plagemann, Heidrich et al. 1992; Plagemann, Harder et al. 1999) have focused on the alterations in the hypothalamic energy homeostasis in these rats. These primary observations showed a leptin and insulin resistance in the ARC (Davidowa and Plagemann 2000; Lopez, Seoane et al. 2005; Davidowa and Plagemann 2007), inhibition of neurons in the PVN and ventro-medial nucleus by NPY (Heidel, Plagemann et al. 1999; Velkoska, Cole et al. 2008), agouti-related protein (Li, Plagemann et al. 2002; Lopez, Seoane et al. 2005), cortico-trophin releasing factor and dopamine (Davidowa, Li et al. 2002), favoring feeding and reduced energy expenditure (Davidowa, Li et al. 2003). Morphometric studies showed that the number of NPY neurons in the ARC and that the number of galanin neurons in the PVN (Plagemann, Harder et al. 1999) were increased. Taken together, these observations strongly indicate a disorganization and malprogramming of the hypothalamic NPY system, induced by overfeeding during the critical period of early postnatal life, inducing an acquired persisting hypothalamic hypo-responsiveness in terms of a central resistance to leptin and insulin. The malprogramming and resistance of orexigenic as well as anorexigenic neurons in the hypothalamus might contribute to the occurrence of hyperphagia, overweight and hyper-insulinemia throughout later life (Plagemann 2006).

The hypothalamic neurons are not the only central cells affected by postnatal overnutrition. Indeed, microglia, the macrophages in the central nervous system, were found to be increased and activated in the ventromedial hypothalamic nuclei, but also in the cerebellum of adult rats exposed to neonatal overfeeding (Tapia-Gonzalez, Garcia-Segura et al. 2011). Since the levels of interleukin-6 in the hypothalamus and cerebellum were also significantly higher in these rats, it is postulated that this might represent a secondary response to systemic inflammation.

Recent studies suggest that alteration in neonatal nutrition can cause perturbations in hypothalamic neural circuits controlling reproductive function. Rat and mouse models of different litter size were used in order to investigate the effects of early postnatal overnutrition or under-nutrition on the maturation of hypothalamic circuits involved in reproductive function. Male rats from large litters, showing a lean phenotype, reached puberty later that those from control litters, had reduced plasma testosterone and elevated 17-beta-estradiol concentrations at puberty however, in postnatally overfed rats from small litters, the age of puberty was not affected (Smith and Spencer 2012). In female rats, the onset of puberty was significantly shortened for these rose in small litters, but was not delayed for the postnatally under-nourished ones. Conversely, neonatally undernourished mouse females displayed delayed puberty and defective development of axonal projections of the arcuate nucleus to the preoptic region, affecting the neuronal organization of neuronal projections containing

kisspeptin, a key neuropeptide involved in pubertal activation and fertility (Caron, Ciofi et al. 2012). In female mouse, PNOF also perturbed the development of neural projections, but this did not lead to alterations neither in kisspeptin projections, nor in onset of puberty. This study also showed that neonatally overnourished females had a 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 the reproductive function, along with lifelong functional perturbations.

3. At the adipose tissue level

As previously evoked, postnatally overfed rodents display early and long lasting increase in body weight, which is majorly associated with increased fat mass, as we observed in C57Bl/6 mice with the use of EchoMRI (ref), that demonstrated in 7-months-old mice a significant increase of body fat mass (+47%) at the expense of lean mass (-7%). Adipose tissue, a loose connective tissue composed of adipocytes, exists in two distinct forms: white adipose tissue (WAT) and brown adipose tissue (BAT).

WAT is generally believed to serve as a source of energy and, in rodents and humans, may be subdivided into subcutaneous and visceral (abdominal) fat. Visceral fat is composed of several adipose depots including mesenteric, epididymal, and peri-renal depots. This increase in visceral WAT is observed at weaning and later in postnatal overfed rats (Balonan and Sheng 2000; Boullu-Ciocca, Dutour et al. 2005; Velkoska, Cole et al. 2008; Cunha, Pereira et al. 2009; Rajia, Chen et al. 2010; Rodrigues, de Moura et al. 2011) and mice (Glavas, Kirigiti et al. 2010) and goes along with an increase in adipocyte surface (Boullu-Ciocca, Dutour et al. 2005; Boullu-Ciocca, Achard et al. 2008; Hou, Liu et al. 2011). However, subcutaneous fat mass is also increased in young but not adult overfed mice (Rodrigues, de Moura et al. 2011), 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 (Wiedmer, Klaus et al. 2002). At weaning and later, the higher fat contents in adipose tissue was associated with higher fatty acid synthase, an enzyme that catalyzes *de novo* synthesis of fatty acids, and lipogenic activities in retroperitoneal adipose tissue (Balonan and Sheng 2000). In epidymal WAT adipocytes isolated from OF rats, an impairment of insulin-stimulated glucose uptake was observed, and was associated with a significant decrease in glucose transporters GLUT-4 and GLUT-1, and of insulin receptor signaling pathway components such as insulin receptor substate-1 (IRS-1), phosphatidyl inositol 3-kinase (PI3K) expression and Akt serine kinase activity (Rodrigues, De Souza et al. 2007). Additionally, in mesenteric adipose tissue, the mRNA expression of interleukin (IL)-6, tumor necrosis factor (TNF)-α, TNF-receptor-1, resistin, plasminogen activator inhibitor-1 was increased in postnatally overfed rats (Boullu-Ciocca, Achard et al. 2008), revealing an inflammatory status of WAT.

In the rodent, one key structure involved in the regulation of body weight is the BAT, the major thermogenic structure. BAT is composed of primarily brown adipocytes that are characterized by multiple small lipid droplets, a large number of mitochondria, dense innervation by sympathetic nerves and abundant uncoupling protein-1 (UCP-1). Post-

weaning and 4-months old overfed rats showed reduced thermogenic activity and lower levels of UCP-1 mRNA (Velkoska, Cole et al. 2005; Xiao, Williams et al. 2007) and in 6-months old postnatally overfed mice, mRNA for UCP-1 was decreased in BAT (Glavas, Kirigiti et al. 2010). When exposed to acute cold, BAT from OF rats was less active and demonstrate reduced thermogenic responsiveness. At the molecular level, several transcriptional regulators (peroxisome proliferator-activated receptor-gamma, C/EBP-1) and lipases such as lipoprotein lipase and hormone-sensitive lipase showed reduced expression in the BAT, whereas the expression of the sympathetic β3-adrenergic receptor and the response to beta adrenergic agonist, isoproterenol, were decreased in OF rats. Clinical and experimental evidence show a close link between UCP-1 activity, β-adrenergic receptor response in BAT and metabolic rates in individuals, the deregulation of which may be involved in the development of overweight and obesity. In OF rats, excess weight gain during the early postnatal period led to permanent reprogramming of brown adipose tissue adaptive thermogenesis.

4. At the glucose/insulin homeostasis level

Results from numerous studies focused on the effects of early over-nutrition on adult insulin-glucose axis. Indeed, neonatally overfed rats and mice frequently display increased basal values of fasting glucose and plasma insulin, and alterations of glucose/insulin ratio (Plagemann, Harder et al. 1999; Balonan and Sheng 2000; Boullu-Ciocca, Dutour et al. 2005; Boullu-Ciocca, Achard et al. 2008; Cunha, Pereira et al. 2009; Kappeler, De Magalhaes Filho et al. 2009; Habbout, Delemasure et al. 2012). Glucose and insulin test tolerance tests suggested persistently reduced pancreatic glucose-responsiveness in OF rats (Plagemann, Harder et al. 1999; Balonan and Sheng 2000; Boullu-Ciocca, Achard et al. 2008; Cunha, Pereira et al. 2009; Hou, Liu et al. 2011) and mice (Kappeler, De Magalhaes Filho et al. 2009). These effects concerning the glucose-insulin balance seem to be at their highest level at weaning (Pereira, Moreira et al. 2006) but may decrease or disappear with maturation (Balonan and Sheng 2000; Waterland and Garza 2002; Rodrigues, de Moura et al. 2011). However, several studies failed to demonstrate any differences in glycaemia or insulinemia in postnatally overfed rodents (Lopez, Seoane et al. 2005; Rodrigues, De Souza et al. 2007; Xiao, Williams et al. 2007; Velkoska, Cole et al. 2008; Glavas, Kirigiti et al. 2010; Rajia, Chen et al. 2010). Interestingly, while litter manipulation had a significant effect in male rats, postnatally overfed females were found to be resistant to the occurrence of insulin hypo-responsiveness (Bassett and Craig 1988).

Some studies (Waterland and Garza 2002) showed defects in glucose-stimulated insulin secretion from isolated pancreatic islets, supporting the hypothesis that the endocrine pancreas contributes to primary metabolic imprinting in this model. In fact, insulin secretion in pancreatic islets beta-cells depends essentially on glucose uptake, a process that is driven by the glucose transporter GLUT-2. Paradoxically, OF rats exhibited elevated content in GLUT-2 in pancreatic islets (Cunha, Pereira et al. 2009), indicating that early postnatal overnutrition during a critical development period in life may induce permanent modifications in glucose-stimulated insulin secretion. Waterland *et al.* used microarrays to identify genes showing differential expression in rat isolated pancreatic islets. They observed an early differential gene expression in OF at weaning, some of these genes remaining differentially expressed later in life at 4 months (Waterland and Garza 2002). This data indicate that even

moderate overnutrition in the postnatal period may permanently alter endocrine pancreas gene expression, probably by influencing the epigenetic regulation of imprinted genes.

In the liver, recent studies have shown an impairment of insulin signaling in 6-months old rats that were raised in small litters. In fact, hepatic content of insulin-receptor-β, IRS-1, phospho-IRS-1, Akt1 and PI3-K were significantly decreased (Conceicao, Franco et al. 2012). This alteration of hepatic insulin signaling might then reinforce the alteration of glucose/insulin homeostasis.

5. At the plasma lipid and protein level

Postnatally overfed rodents, rats and mice, frequently present disturbances in plasma lipids: elevated triglycerides (Plagemann, Harder et al. 1999; Boullu-Ciocca, Dutour et al. 2005; Kappeler, De Magalhaes Filho et al. 2009; Moreira, Teixeira Teixeira et al. 2009), free fatty acids (Boullu-Ciocca, Dutour et al. 2005; Boullu-Ciocca, Achard et al. 2008; Kappeler, De Magalhaes Filho et al. 2009), total and HDL cholesterol (Boullu-Ciocca, Dutour et al. 2005; Kappeler, De Magalhaes Filho et al. 2009; Moreira, Teixeira Teixeira et al. 2009; Rodrigues, de Moura et al. 2011). However, depending on strains, litter size, age and geographical origin, the lipid perturbations are not systematically encountered in postnatally overfed rodents (Rodrigues, De Souza et al. 2007; Xiao, Williams et al. 2007; Rajia, Chen et al. 2010; Hou, Liu et al. 2011; Tapia-Gonzalez, Garcia-Segura et al. 2011)

Small litter rats presented higher levels of globulins in youth (post-weaning) and adult age (6 months), while circulating levels of albumin were higher at weaning and lower at adulthood (Rodrigues, de Moura et al. 2011). This increase in protein/albumin has also been described in 12-months-old postnatally overfed mice (Kappeler, De Magalhaes Filho et al. 2009). It is possible that an elevation of both globulin and albumin in post-weaned rodents, related to higher liver production, is a consequence of higher food intake during lactation. In the adulthood, the profile of lower albumin and higher globulin has been described in case of chronic inflammation, which is reported to be associated with obesity.

6. At the endocrine level

Hormones are essential environment-dependent organizers of the developing neuroendocrine-immune network, which regulate all the fundamental processes of life. As highlighted by Plagemann (Plagemann 2006), when present in non-physiological concentrations, induced by alteration in the intra-uterine or neonatal environment during critical periods of perinatal life, hormones can act as “endogenous functional teratogens”, leading to developmental disorders and diseases throughout life.

Concerning insulin, early postnatally overfed rats displayed not only hyper-insulinemia but also elevated intra-hypothalamic insulin concentrations during early post-natal life (Plagemann, Harder et al. 1999). Indeed, during fetal and early postnatal life, the saturable transport system for insulin in the blood-brain barrier is not yet mature, allowing increased insulin leakage from the circulation into the hypothalamus. However, in neonatally overfed rats, an acquired hypo-responsiveness to insulin in terms of central resistance to insulin was observed.

Several experimental evidences suggest that glucocorticoids are involved in the pathophysiology of abdominal obesity and its associated complications. Indeed, excess of glucocorticoids, when associated with hyperinsulinism, favors an increase of lipogenesis and a decrease in lipolysis, together with a stimulation of hepatic neo-glucogenesis and an inhibition of peripheral glucose utilization. In humans and in rodent models of obesity, changes in glucocorticoid signaling have been described. In juvenile rats, overfeeding induced an accelerated maturation of the hypothalamic-pituitary-adrenal (HPA) axis, together with an up-regulation of adipose tissue glucocorticoid receptor (GR) mRNA (Boullu-Ciocca, Dutour et al. 2005). In adulthood, neonatally overfed rats presented elevated basal and stress-induced corticosterone secretion (Boullu-Ciocca, Achard et al. 2008) and significant changes in visceral adipose tissue glucocorticoid signaling (Boullu-Ciocca, Dutour et al. 2005), with increases mRNA levels for GR and 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), an enzyme responsible for conversion of corticosterone in cortisol (Velkoska, Cole et al. 2005), increasing adipose tissue glucocorticoids sensitivity. It is strongly suggested that a primary alteration in the HPA axis occurs during the 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 the adulthood. In the liver, the activity and expression of 11β-HSD was also permanently increased in rats raised in small litters (Hou, Liu et al. 2011). In addition, the expressions of glucocorticoid inactivating enzymes, 5α-reductase type 1 and 5β-reductase, were 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.

Concerning the sympatho-adrenal activity, the turnover of norepinephrine in peripheral tissues such as brown adipose tissue, kidneys or heart can be increased by dietary enrichment with sucrose. However, in rats reared in small litters, this acceleration of NE turnover was either completely abolished or attenuated, showing that litter size alters the dietary activation of the sympathetic nervous system, presumably reflecting changes in central nervous system regulation (Young 2002).

During the postnatal development of postnatally overfed mice, coordinated alterations in somatotropic hormone axis regulation were observed, with a higher hypothalamic growth hormone (GH)-releasing hormone (RH) gene expression, increased pituitary GH release and elevated plasma insulin-growth factor-1 (Kappeler, De Magalhaes Filho et al. 2009). These alterations persisted throughout adulthood, highlighting the plasticity of the somatotropic axis, which can be oriented during the perinatal period by modifications in the nutritional environment.

7. At the adipocytokine level

It is well known that increase in central adiposity represents a significant risk factor for cardiovascular disease and may influence survival in subjects with coronary artery disease (Coutinho, Goel et al. 2011). Indeed, adipose tissue is able to secrete a plethora of peptides, named adipocytokine, which act in endocrine, paracrine or autocrine modes to influence a diverse array of biological functions, some of them being clearly involved in the development

of cardiovascular disease (Smith and Yellon 2011). Leptin is produced primarily by WAT and levels 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 induces also a variety of actions in the cardiovascular system. The increase in leptin levels in OF rats (Boullu-Ciocca, Dutour et al. 2005; Lopez, Seoane et al. 2005; Velkoska, Cole et al. 2005; Pereira, Moreira et al. 2006; Velkoska, Cole et al. 2008; Cunha, Pereira et al. 2009; Rodrigues, de Moura et al. 2011; Habbout, Delemasure et al. 2012) or mice (Kappeler, De Magalhaes Filho et al. 2009) is constantly reported in the literature, and can be explained by the fact that leptin levels are related to the amount of fat mass. Though OF rodents have increased circulating levels of leptin, previous studies suggested in rats that overfeeding may induce central leptin resistance, due to lower leptin type-b (long isoform) receptor (Ob-Rb) expression in hypothalamic nucleus (Schmidt, Fritz et al. 2001; Lopez, Seoane et al. 2005; Rajia, Chen et al. 2010). 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 activated STAT3 dimerizes and translocates to the nucleus stimulating gene transcription. The JAK2/STAT3 pathway stimulates suppressor of cytokine signaling-3 (SOCS3) transcription, a leptin-inducible inhibitor of leptin signaling pathway. PNOF induced lower JAK2 and phosphorylized-STAT3 and higher SOCS-3 expression in adult rat hypothalamus, indicating central leptin resistance (Rodrigues, de Moura et al. 2011). Several studies suggested that hyperleptinemia during a critical period of development may be a key signal for malprogramming of energy homeostasis. Whereas leptin is a critical trophic factor for the hypothalamus during development, inhibitory mechanisms mediating leptin effects on food intake are not fully functional until at least the fourth postnatal week. Importantly, electrophysiological studies (Davidowa and Plagemann 2000) demonstrated that in young OF rats, neurons in the arcuate hypothalamic nucleus exhibit a reduced inhibitory response to leptin, suggesting that early over-nutrition 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 (Glavas, Kirigiti et al. 2010) showed that OF Swiss Webster mice exhibit early-onset leptin resistance that persists into adulthood, despite being maintained on a healthy standard chow diet. Additionally, other factors associated with early postnatal overnutrition, for instance the concurrent hyper-insulinemia, seem to play a pivotal role for the development of leptin resistance and life-long overweight in rats (Schmidt, Fritz et al. 2001).

Concerning adiponectin, results are less consensual. Previous studies performed in rats showed no difference at 4 and 6 months between OF and NF groups (Velkoska, Cole et al. 2005; Rodrigues, de Moura et al. 2011), while Boullu-Ciocca et al. showed a decrease in both circulating levels of adiponectin and mRNA expression in epididymal adipose tissue in 5-months old OF rats (Boullu-Ciocca, Achard et al. 2008), a result that was confirmed by our laboratory (Habbout, Delemasure et al. 2012). 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 (Matsuzawa 2006) and induces general cardioprotective effects (Gualillo, Gonzalez-Juanatey et al. 2007).

Apelin is an adipocytokine that is rapidly gaining in significance (Castan-Laurell, Dray et al. 2011). Apelin and its G-protein coupled receptor, APJ, are expressed in various tissues and play roles in fluid balance, satiety and immune function. Apelin is present in plasma at levels ranging between 0.2 and 1.5 ng/mL. We reported recently (ref) that, despite no changes in

apelin circulating levels in young 24-days-old or adult 7-months-old postnatally overfed mice, local changes in this adipocytokine may occur in specific organs such as the myocardium. Indeed, we observed that the mRNA expressions of both apelin and of its receptor were increased in young, but not adult, mice that were overnourished in the immediate postnatal period. In the cardiovascular system, apelin and APJ are abundantly expressed in the heart, APJ being predominantly expressed in cardiomyocytes (Kleinz, Skepper et al. 2005) whilst apelin was shown to be present in endocardium endothelial cells (Kleinz and Davenport 2004). Indeed, cardiovascular function has been reported to be mediated, in part, by the apelin/APJ system. For instance, apelin increases endothelium-dependent vasodilation (Tatemoto, Takayama et al. 2001) and was found to exhibit powerful inotropic activity (Berry, Pirolli et al. 2004). Additionally, apelin may play an important role in cardiomyocyte specification and heart development (Scott, Masri et al. 2007). Therefore, we speculate that an initial modification of apelin receptor signaling in early postnatal life may have permanently impacted the development of the myocardium, a situation that may have ultimately impaired myocardial function in physiological conditions, but also after an acute pathologic situation such as ischemia-reperfusion injury.

8. At the renal level

Regarding renal development, whereas nephrogenesis is predominantly completed prenatally in mammals, renal maturation proceeds postnatally and consists of increment of glomerular size together with tubular length. Some studies demonstrated that postnatal over-nutrition influenced nephrogenesis and renal maturation (Boubred, Buffat et al. 2007; Boubred, Daniel et al. 2009). Indeed, early PNOF is associated with enhanced nephrogenesis, leading to an increase in the number of nephrons and decreased glomerular volume. These changes affect both young males and females and persist, but only in males, during aging inducing long-term development of proteinuria and glomerulosclerosis (Boubred, Daniel et al. 2009). The consecutive low nephron endowment may contribute to an increased risk for cardiovascular and renal diseases in the adulthood. Hyperleptinemia contributes to renal damage not only due to its profibrotic effect but also as a stimulator of the sympathetic nervous system and leptin-mediated sympathetic stimulation is thought to play a pivotal role in obesity-related nephropathy. In mature postnatally overfed rats, a decrease in glomerular filtration rate, proteinuria and glomerulosclerosis were observed. The expression of renin-angiotensin system (RAS) components has also been explored in young overfed rats, since this system is involved in the progression of renal disease. Indeed, the expression of renin and of the angiotensin II receptor type-2 (AT-2) were increased in kidneys of young overfed rats, whereas plasminogen activator inhibitor (PAI)-1, usually regarded as a powerful fibrosis-promoting molecule, and matrix metallo-proteinase (MMP)-9 expression were decreased, a process that might contribute to abnormal programming of renal growth (Yim, Ha et al. 2012).

In link with these anomalies, renal inflammation and expression as well as deposition of extracellular matrix molecules such as collagen I were increased (Alcazar, Boehler et al. 2012). Furthermore, expression of leptin, its receptor (Ob-Rb) and the cytokines interleukin-6 (IL-6) and PAI-1 were up-regulated. The phosphorylation of STAT3 and ERK1/2 in the kidney was decreased, indicating renal post-receptor leptin resistance. Moreover, SOCS-3 protein expression, a mediator of leptin resistance, was elevated. These results demonstrate that

early modifications in nutritional status may permanently affect renal function and induce profibrotic processes, but also provide evidence of a peripheral leptin resistance as a potential underlying mechanism.

9. At the intestinal microbiota level

Accumulating data suggest that the intestinal microbiota, a collection of microorganisms inhabiting the mammalian gastrointestinal tract, has underestimated consequences on host metabolism. Indeed, animal models of obesity show significant changes in the composition and metabolic function of gut microbiota, affecting nutrient acquisition and energy regulation (DiBaise, Zhang et al. 2008). Moreover, lipopolysaccharide derived from the intestinal microbiota may act as a triggering factor linking inflammation to high-fat diet-induced metabolic syndrome. In this field, postnatal over-nutrition was also found to be responsible for changes in gut microbiota and alkaline phosphatase activity (Sefcikova, Bujnakova et al. 2011).

10. At the pulmonary level

The effects of overweight and obesity on the respiratory tract are under-appreciated; however, a growing body of evidence indicates an elevated incidence of asthma in overweighed and obese individuals. For instance, several studies showed that body mass index was associated with an increased risk of developing airway hyper-responsiveness, a reliable marker of asthma. Additionally, the severity of asthma is increased with obesity, a situation known to create systemic low-grade inflammation, probably through an enhancement of airways inflammation. In 5-months old PNOF mice, an enhanced response to metacholine was observed, but also a substantial pulmonary inflammation, as reflected by macrophages infiltration around bronchi and interstitium (Ye, Huang et al. 2012). Moreover, lung mRNA expressions of TNF-α and TGF-β1 were increased, while collagen deposition around the bronchi was also observed in these mice. These results suggest that neonatal overfeeding could increase pulmonary disease susceptibility through enhancement of lung inflammation; which in turn may lead to airway hyper-responsiveness and fibrotic remodeling.

11. At the nitro-oxidative stress level

Obesity and overweight are associated with increased oxidative stress not only in the bloodstream, but also in myocardial tissue (Laight, Desai et al. 1999; Vincent, Powers et al. 1999). In fact, nutrient overload in the metabolic pathways leads to an increased electron flux through mitochondrial electron transfer chain. The consequent electron leak from respiratory complex I and III of electron transfer chain leads to an increased production of ROS from the mitochondria, such as superoxide and hydrogen peroxide. In a study performed in rats in our group (Habbout, Delemasure et al. 2012), we observed an increase in plasma hydroperoxides and a decrease in vitamin C levels indicating enhanced circulating oxidative stress. Further work performed in mice confirmed these initial data (ref). Indeed, postnatal over-nutrition in mice also induced an increase in oxidative stress levels in the adult myocardial tissue, as evidenced by electron spin resonance (ESR) spectroscopy

investigations. ESR is one of the few techniques which allow the direct measurement of free radical species; however most of biological reactive oxygen and nitrogen species (RONS) of interest are too short-lived to be measured directly with ESR spectroscopy. Hydroxylamine spin-probes such as CMH can be oxidized by superoxide anion or peroxynitrite, giving rise to a more stable long-lived ESR-detectable $CP^{\bullet}$ nitroxide, allowing detection of these unstable species in cell cultures or isolated organs (Oudot, Martin et al. 2006; Delemasure, Sicard et al. 2007). This observation of increased nitro-oxidative stress in myocardial tissue of postnatally OF mice was a very original finding, confirming the association between obesity and increased myocardial oxidative stress (Vincent, Powers et al. 1999). We also observed a significant increase in the antioxidant manganese-dependent superoxide dismutase (Mn-SOD) and catalase expression and activities in heart tissue homogenates, a paradoxical result at first sight, which could rather reflect the adaptive response to increased oxidative stress.

In the liver of postnatally overfed Wistar rats, recent findings suggest a decrease of some antioxidant enzyme activities, such as catalase, SOD and glutathione peroxidase (Conceicao, Franco et al. 2012) while Western blot analysis showed no differences in Cu/Zn SOD content. Additionally, markers of nitro-oxidative stress such as nitrates and malonedialdehyde were also increased in the liver and plasma. This imbalance between reduced antioxidant defense and increased oxidative damage may predispose hepatocytes to injury and be a trigger in the development of insulin resistance.

12. At the cardiovascular level

Hypertension is considered as a major risk factor for cardiovascular diseases including stroke, myocardial infarction, heart failure, and may cause chronic kidney disease. In rodents, obesity induced by high-fat diet is associated with an increase in blood pressure (Velkoska, Cole et al. 2005). In slightly overweighed adult rodents which had been raised in small litters, an increase in diastolic and systolic arterial blood pressure has been observed (Plagemann, Harder et al. 1999; Boubred, Buffat et al. 2007; Boubred, Daniel et al. 2009; Kappeler, De Magalhaes Filho et al. 2009; Habbout, Delemasure et al. 2012) but this increase is usually minor, 10-15 mmHg, and sometimes non-significant (Velkoska, Cole et al. 2005; Velkoska, Cole et al. 2008; Rajia, Chen et al. 2010; Alcazar, Boehler et al. 2012; Yim, Ha et al. 2012). Several central and peripheral mediators, which are modified during PNOF, may participate to the increase in blood pressure. While the hypertensive actions of glucocorticoids are fully documented, leptin and neuropeptide Y may also be important players. Indeed, leptin increases blood pressure via receptor mediated activation of sympathetic nerve activity (Dunbar and Lu 1999), but also by inhibiting NPY synthesis. Actually, NPY is not only involved in the control of energy intake, but also, at the nucleus of solitary tract, can elicit lowering of blood pressure and heart rate (Michel and Rascher 1995). However, the underlying mechanism of such a hypertensive effect of moderate overweight in rodents has not yet been elucidated. In our Laboratory, we have evaluated the aortic and coronary reactivity in thoracic aortas and isolated perfused hearts harvested from postnatally overfed Wistar rats. However, we were not able to demonstrate any differences in endothelium-dependent (perfusion of bradykinin) or -independent (perfusion of sodium nitroprussiate) vasoreactivity between groups. Therefore, the origin of this increase in blood pressure still remains to be understood. In a very recent study performed in golden hamsters,

the endothelium-dependent microvascular function was assessed with acetylcholine in the cremaster muscle, and it was observed that early postnatal overnutrition induced endothelial-dependent microvascular dysfunction in adulthood (Leite, Kraemer-Aguiar et al. 2012).

Over-nutrition in the early life can induce rapid cardiac hypertrophy at weaning in Wistar rats, with significant increase in ventricular walls thicknesses, shortening of left ventricular diameter, enlargement in cardiomyocyte area and decrease in coronary vessels density (Moreira, Teixeira Teixeira et al. 2009). If not compensated, this situation may lead to compromised myocardial vitality and impaired heart function. Several factors may induce ventricular remodeling, such as biomechanical stretch or neuro-humoral mechanisms. Indeed, cardiomyocyte hypertrophy can be triggered by hormones, cytokines and growth factors signaling pathways, and for instance, leptin or insulin may be considered either as metabolic or as growth factors for the heart (Belke, Betuing et al. 2002; Xu, Chen et al. 2004). For instance, in cardiomyocytes, insulin acts on its receptor (IR) through the recruitment of different insulin receptor substrates (IRS), which will trigger PI3K phosphorylation, increasing the level of inositol triphosphate (PIP_3) and activating PIP_3-dependent serine/threonine kinases. The activation of this specific pathway results in the translocation of the glucose transporter GLUT-4 from intracellular sites to the cell membrane, leading to increased glucose uptake. The long isoform of leptin receptors (Ob-Rb) is also present in the heart, and the signaling cascade of leptin in cells involves PI3K, Janus tyrosine kinase 2 (JAK2) and signal transducer and activator of transcription 3 (STAT3). Interestingly, post-weaning juvenile hearts from overfed rats displayed an activation of the insulin and leptin cascade, with increased levels of IR and Ob-Rb, PI3K, STAT3 and increased translocation of GLUT-4 (Pereira, Moreira et al. 2006). This higher sensitivity to insulin and leptin might therefore induce a cardiac adaptive response, resulting in improved glucose uptake, increased supply of energy but may also induce a hypertrophic phenotype in overfed juvenile rats.

Recently, we observed that early postnatal over-nutrition led to modifications of basal cardiovascular parameters in adult mice, such as an enlargement of ventricular diameters and impairment in left ventricular ejection fraction (ref). To date, none of these modifications had been reported for overweighed mice.

Collagen deposition is commonly used to determine cardiac fibrosis, and we and others were able to demonstrate elevation of left ventricle total collagen in adulthood, in 4 months (Velkoska, Cole et al. 2008) and 6 months old rats (Habbout, Delemasure et al. 2012). Histological studies showed a higher collagen density (+54%) in the left and right ventricles of adult overfed rat hearts, but also in C57/Bl6 adult mice. The analysis of matrix metallo-proteinase type-2 (MMP-2) expression by quantitative RT-PCR and activity by gelatin zymography in both ventricles showed an increase in both MMP-2 expression and activity in the ventricular tissue of overfed rodent's hearts, accounting for an alteration of the architectural organization (ref). Several environmental factors such as oxidative stress or increased leptin levels might be involved in the occurrence of this remodeling phenomenon. Indeed, studies have highlighted the role of leptin and insulin in the elongation of cardiac myocytes (Abe, Ono et al. 2007), ventricular hypertrophy (Fredersdorf, Thumann et al. 2004; Perego, Pizzocri et al. 2005), and heart failure (Schulze, Kratzsch et al. 2003). Additionally, these changes in myocardial organization might be the late consequences of the early modifications observed in gene expression of some isoforms of actin, myosin, collagen,

dystrophin and other structural proteins that may have impacted definitively the intracellular and organ structure.

13. At the genetic level

A recent study performed in our Laboratory (ref) afforded new original data putting in evidence that nutritional changes in the immediate postnatal life, such as overnutrition, may influence the expression of several genes involved in heart 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). It is noteworthy that these changes were obtained in pups that were randomly assigned to either small or large litters, and that changes might not be related to genetic differences due to distinct ascendants. Therefore, in only 24 days of increased milk consumption, myocardial genome expression was modified in overfed mice a situation that may have induced some permanent changes in cardiomyocytes cytoskeleton structure and in myocardial cardiomyocyte organization. Indeed, microarrays were processed in whole heart ventricles, which contain several types of cells such as endothelial, vascular smooth muscle and fibroblastic cells in addition to cardiomyocytes. Thus, additional data are now necessary to confirm at the mRNA and protein levels the changes detected by microarrays, but also to determine which cell type is concerned by these modifications.

Therefore, perinatally acquired microstructural and epigenetic modifications in regulatory systems of metabolism and body weight seem to be critical, leading to a cardiometabolic risk-prone phenotype throughout life.

14. Pathologic challenges

High-fat diet

While maintained under standard chow diet, some of the central and metabolic abnormalities observed after postnatal overnutrition have little detrimental consequences for body weight and glucose homeostasis. However, in response to a metabolic challenge such as a high fat diet, postnatally overfed mice demonstrated transient hyperphagia and rapid development of obesity, insulin resistance and hepato-steatosis (Glavas, Kirigiti et al. 2010). These findings demonstrate that early postnatal nutrition can permanently reprogram energy homeostasis and sensitize adult animals to the damaging effects of diets enriched in fat.

Sucrose-enriched diet

High carbohydrate consumption is generally associated with an increase in weight and with obesity-associated complications, and a diet with high-levels of sucrose, which is of 50% fructose and 50% glucose, has varying effects on metabolic parameters. Most of the responses to a sucrose-enriched diet, i.e. increase in total energy intake, adiposity, leptin, adiponectin, acylated ghrelin levels, were accentuated in neonatally over-nourished rats, which also had increased insulin and triglyceride levels (Fuente-Martin, Garcia-Caceres et al. 2012). These results indicate that neonatal overnutrition may increase the detrimental response to a diet rich in sucrose later in life.

Response to immune challenge with lipopolysaccharide

Fever is a crucial component of the body's response to an immune challenge, modifying cytokine expression and creating a suboptimal environment for pathogen growth and survival. Pathogen associated molecular patterns (PAMP) on lipopolysaccharide (LPS), a component of gram negative bacteria cell membranes, is recognized by toll-like receptors (TLR). The recognition of LPS by TLR4 is followed by the synthesis of pro-inflammatory cytokines such as TNF-α and IL-1β via the phosphorylation of inhibitor (I)κB and subsequent translocation of NFκ-B to the immune cell nucleus where it initiates the cytokine transcription. Pro-inflammatory cytokines then stimulate the production of prostaglandin, which stimulate heat conservation and production, thus fever, at the ventromedial pre-optic area. Clarke *et al.* recently demonstrated that febrile response to lipopolysaccharide (LPS) was exacerbated in adult male and female Wistar rats overfed during the postnatal period (Clarke, Stefanidis et al. 2011), with a higher peak body temperature, an enhanced pro-inflammatory cytokine profile, elevated HPA axis activation and a prolonged corsticosterone response. The expression of TLR-2 and 4 in adipose tissue was also elevated and IκB phosphorylation was increased, exacerbating NF-κB activation. Additionally, the expression of macrophage markers was increased in inguinal WAT. Such an exacerbated response to LPS was also seen in adult rats made obese by acute high-fat diet, illustrating that this susceptibility is likely due to the elevation in adiposity itself than the early life programming.

Myocardial ischemia reperfusion injury

Myocardial ischemia results from severe impairment of oxygen and nutriments supply in a heart's territory, which leads to irreversible injury. Reperfusing the ischemic myocardium is an absolute requisite to rescue the 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. A growing body of evidence suggests that RONS can mediate myocardial tissue injury during ischemia but to a greater extent during reperfusion.

In hearts isolated from postnatally overfed mice or rats, baseline cardiac parameters are not modified. However, after 30 min of ischemia, followed by reperfusion, the recovery of pre-ischemic cardiac output was impaired in hearts from PNOF rats, and the total amount of lactate deshydrogenase, a marker of cell injury, released in the coronary effluent during the period of reperfusion, was found to be significantly higher (Habbout, Delemasure et al. 2012). In OF mice's hearts, the recovery of coronary flow and contractility parameters were also significantly impaired after ischemia and, in addition, the evaluation of still viable and necrotic zones after 2 hours of reperfusion showed a major increase (+74%) in infarcted areas (ref). These results suggest that PNOF induced a greater susceptibility of the heart to ischemia-induced injury, inducing more severe cell damage. We speculate that, in a stressful condition such as ischemia-reperfusion injury, which generates a massive release of RONS (Vergely, Maupoil et al. 1998; Vergely, Tabard et al. 2001), the metabolic alterations induced by OF, which are more or less inconsequent in basal conditions, renders the myocardium more susceptible to injury.

Doxorubicin-induced cardiotoxicity

Doxorubicin (DOX), an anthracycline commonly used anticancer drug, is known to cause serious and potentially life-threatening cardiotoxicity, leading to dose-dependent congestive heart failure. Multiple mechanisms of DOX-induced cardiac cellular injuring have been proposed. Oxidative stress is believed to be an important pathway in the cardiac side-effects of anthracycline therapy and related to the production of RONS during the intracellular metabolism of the quinone. Unpublished data from our Laboratory suggest that PNOF mice are more sensitive to the doxorubicin-induced cardiotoxicity, showing higher deterioration of left ventricular function, higher enlargement of ventricular diameters, but also increase in heart tissue oxidative stress markers. This study reinforces the concept that hearts from adult mice that underwent PNOF are more susceptible to pathologic challenges related to nitro-oxidative stress.

15. PNOF in genetically modified rodents

Zucker rats

Zucker rats are genetically deficient for the leptin receptor gene (fa), and homozygous (fa/fa) individuals are obese and exhibit hyperphagia, hyperinsulinemia, and hyperlipidemia. If heterozygous Zucker rats (Fa/fa), are reared in small litters, they develop at weaning a 60% higher fat content and correspondingly higher leptin levels that pups of the same genotype

reared in normal litters (Schmidt, Schoelch et al. 2000). While “wild type” (Fa/Fa) pups reared in small litters were prevented from access adiposity by preventive treatment with leptin (100 pmol/g/day) during the suckling period, heterozygous (Fa/fa) pups were leptin resistant and developed a similarly high body fat content as their saline-treated littermates. Taken together, these results suggest a strong interaction of early PNOF with a defined genetic lesion in the leptin receptor, as well as with undefined strain-related differences in the genetic background.

Conclusion

To conclude, our review emphasizes that over-nutrition during the immediate postnatal period in mice leads to early changes in several organ functions and gene expression that may permanently modify their structural organization and metabolism, inducing late-coming alterations of function and structure which are very likely to be involved in the higher susceptibility to pathologic challenges. Altogether, the results collected here suggest that the nutritional state in the immediate postnatal period should be taken into account since it may have an impact on the cardiometabolic 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 pointed concerning the use of transgenic animals in order to evaluate the potential role of certain key proteins genes and their impact. Indeed, due to difficulties in breeding, transgenic mice are frequently raised in small litters, and compared to their wild-type control mates, raised in normal (large) litters. In view of the present data, caution should be done to compare transgenic and wild-type mice originating from litters of equal numbers. Otherwise, some of the negative impacts of inactivating or up-regulating a gene in transgenic mice could only be due to the long-term cardio-metabolic consequences of nutritional differences during the critical postnatal period.

Finally, understanding the biologic mechanisms underlying PNOF early-induced permanent modifications is a necessary step towards determining their significance for human health and designing potential interventions to prevent or treat their adverse consequences.

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Figure Legends:

Figure 1: Postnatal overfeeding (PNOF) model by litter-size reduction in rats and mice.
Control litters of 10-12 pups per dam are considered as normal-fed (NF) rodents, while small litters (SL) of 3-4 pups induce PNOF. At weaning, depending on strains, PNOF rodents weight 10% to 56% heavier in Wistar and Sprague Dawley rats, and nearly 30% heavier in mouse than control NL littermates. In the post weaning period, postnatally overfed rodents maintain increased body weight gain throughout life.

Figure 2: Pathophysiological consequences of PNOF in rodents.
Hypothalamic nuclei: arcuate (ARC), para-ventricular (PVN), ventro-median (VM)
Orexigenic neuropeptides: galanin, neuropeptide Y (NPY), pro-opio-melanocortin (POMC)
Adipose tissue: white adipose tissue (WAT) and brown adipose tissue (BAT)
Fatty-acid synthase (FAS); lipoprotein lipase (LPL); hormone-sensitive lipase (HSL); glucose transporter-1 and -4 (GLUT-1 and GLUT-4); uncoupling protein1 (UCP-1); triglycerides (TG), interleukin-6 (IL-6), tumor necrosis factor-α (TNFα); plasminogen activator inhibitor-1 (PAI-1); glucocorticoid receptor (GR), 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1); angiotensin II receptor type-2 (AT-2R); superoxide dismutase (SOD); catalase (CAT); malonedialdehyde (MDA); blood pressure (BP); left ventricular ejection fraction (LVEF)

Figure 1

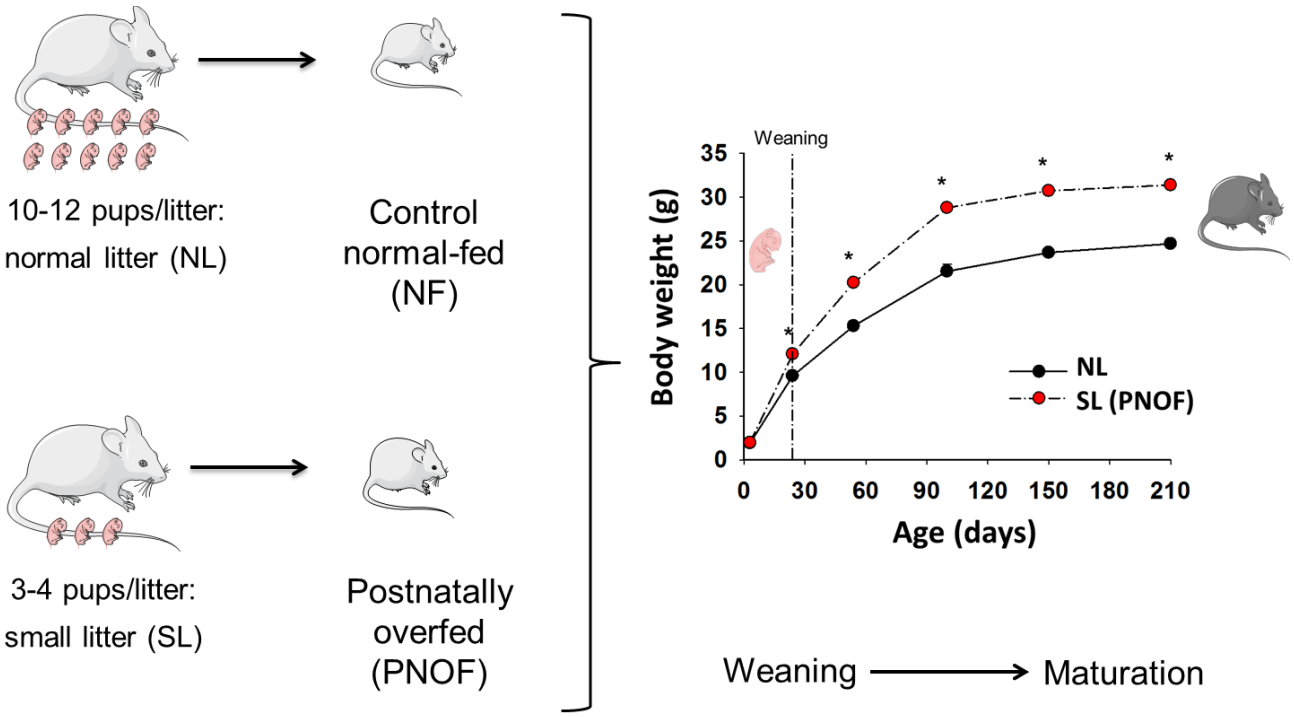
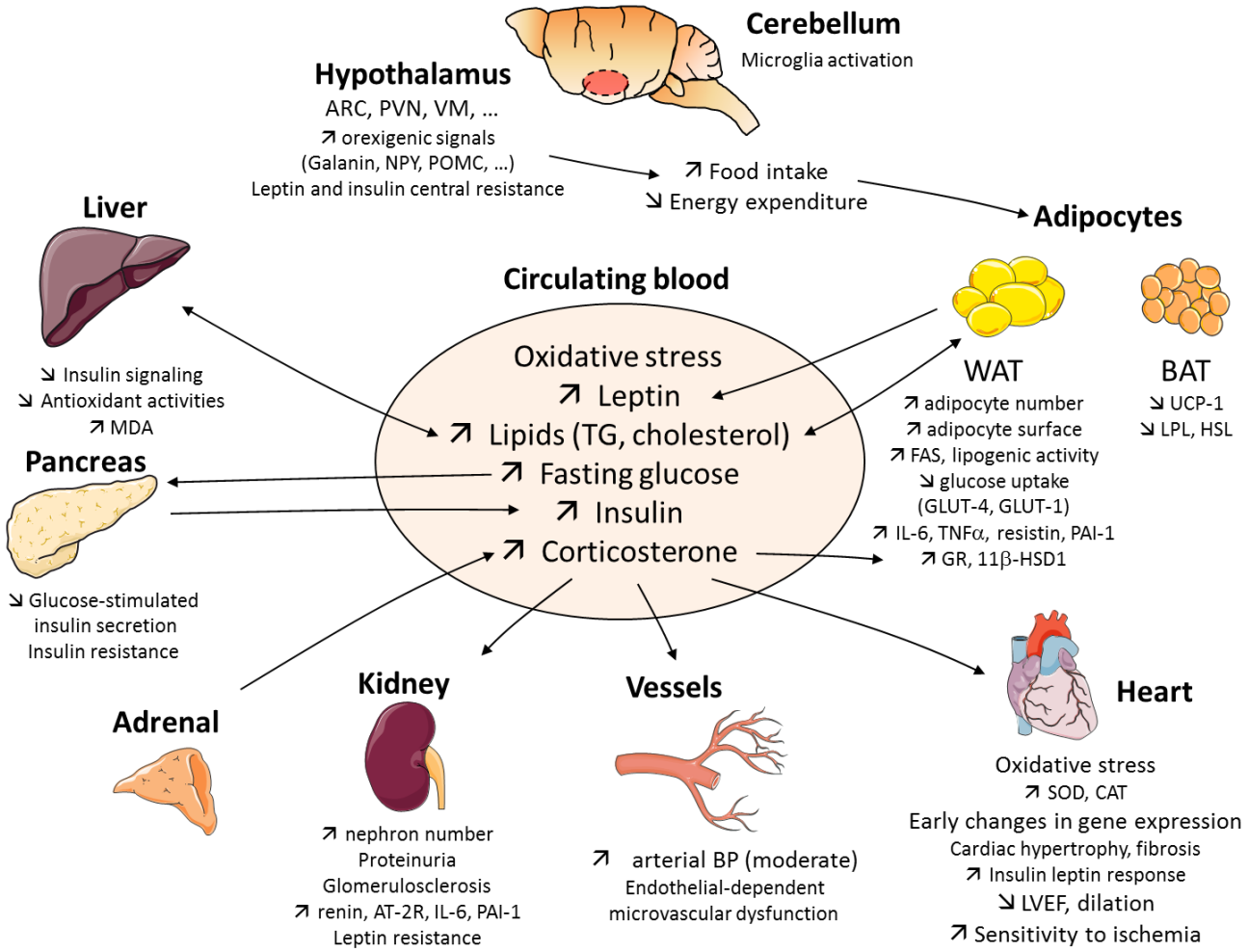

10-12 pups/litter:
normal litter (NL)
Control
normal-fed
(NF)
3-4 pups/litter:
small litter (SL)
Postnatally
overfed
(PNOF)
Weaning
Body weight (g)
Age (days)
NL
SL (PNOF)
Weaning
Maturation

Figure 2


Cerebellum
Microglia activation
Hypothalamus
ARC, PVN, VM, …
↗ orexigenic signals
(Galanin, NPY, POMC, …)
Leptin and insulin central resistance
↗ Food intake
↘ Energy expenditure
Liver
↘ Insulin signaling
↘ Antioxidant activities
↗ MDA
Adipocytes
Circulating blood
Oxidative stress
↗ Leptin
↗ Lipids (TG, cholesterol)
↗ Fasting glucose
↗ Insulin
↗ Corticosterone
WAT
↗ adipocyte number
↗ adipocyte surface
↗ FAS, lipogenic activity
↘ glucose uptake
(GLUT-4, GLUT-1)
↗ IL-6, TNFα, resistin, PAI-1
↗ GR, 11β-HSD1
BAT
↘ UCP-1
↘ LPL, HSL
Pancreas
↘ Glucose-stimulated
insulin secretion
Insulin resistance
Adrenal
Kidney
↗ nephron number
Proteinuria
Glomerulosclerosis
↗ renin, AT-2R, IL-6, PAI-1
Leptin resistance
Vessels
↗ arterial BP (moderate)
Endothelial-dependent
microvascular dysfunction
Heart
Oxidative stress
↗ SOD, CAT
Early changes in gene expression
Cardiac hypertrophy, fibrosis
↗ Insulin leptin response
↘ LVEF, dilation
↗ Sensitivity to ischemia