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REVIEW

Placental contribution to nutritional programming of health and diseases: epigenetics and sexual dimorphism

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ABSTRACT

The recent and rapid worldwide increase in non-communicable diseases challenges the assumption that genetic factors are the primary contributors to such diseases. A new concept of the 'developmental origins of health and disease' (DOHaD) is at stake and therefore requires a paradigm shift. Maternal obesity and malnutrition predispose offspring to develop metabolic syndrome, a vicious cycle leading to transmission to subsequent generation(s), with differences in response and susceptibility according to the sex of the individual. The placenta is a programming agent of adult health and disease. Adaptations of placental phenotype in response to maternal diet and metabolic status alter fetal nutrient supply. This implies important epigenetic changes that are, however, still poorly documented in DOHaD studies, particularly concerning overnutrition. The aim of this review is to discuss the emerging knowledge on the relationships between the effect of maternal nutrition or metabolic status on placental function and the risk of diseases later in life, with a specific focus on epigenetic mechanisms and sexual dimorphism. Explaining the sex-specific causal variables and how males versus females respond and adapt to environmental perturbations should help physicians and patients to anticipate disease susceptibility.

KEY WORDS: DOHaD, Fetal programming, Placenta, Sex, Nutrition, Obesity, Maternal environment, Gestation

Introduction: developmental origins of health and disease

The recent and rapid worldwide increase in non-communicable diseases (NCDs) challenges the assumption that genetic factors are the primary contributors to such diseases (McAllister et al., 2009). The 'developmental origins of health and disease' (DOHaD) paradigm states that the environment during the periconception, gestation and lactation periods shapes the developing individuals, leading, in the case of a deleterious environment, to a predisposition to adult-onset diseases. This theory was popularised by D. J. Barker in the early 1990s (Barker, 1990) but the question had already been raised in earlier studies. In the 1960s and 1970s, different experiments on diverse mammalian species (rat, mouse, guinea pig, pig, etc.) showed that a reduction of maternal calorie or protein intake during pregnancy and lactation affects the growth capacity and cognitive ability of the offspring (reviewed in Roeder and Chow, 1972; McCance, 1976). Later, this concept was extended to phenotype in general, with a strong implication for quantitative traits in agriculture. An increasing number of studies pointed to the fact that maternal environment in cattle was an important parameter to fully express the genetically highly selected

potential of the animals (Wallace et al., 2010; Jammes et al., 2011; Tanghe et al., 2014).

Male and female susceptibility to NCDs is well described. It appears that in the DOHaD context, the same environmental exposure may affect the long-term health of the offspring with a discrepancy between males and females in terms of the timing, onset and severity of outcomes (Gabory et al., 2009; Waddell and McCarthy, 2012; Bale, 2011; van Abeelen et al., 2011), often with a long latency (Barker, 1992; Walker and Ho, 2012). Sexual dimorphism is therefore an important component of the DOHaD.

Among the mechanisms proposed to elucidate this programming, epigenetics is a likely candidate, explaining gene expression alterations that may persist in the long term. Epigenetic marks are fully remodelled during the developmental period and therefore exposure to adverse environments during critical developmental windows can trigger long-lasting influences on the epigenome of the differentiating cell (Attig et al., 2010). The resulting changes in epigenetic marks may alter cell fate decisions and the growth and development of tissues and organs, and subsequently be responsible for inadequate responses to later challenges such as an obesogenic environment in a sex-specific manner (Gabory et al., 2011). Placental development and function is another important feature of the DOHaD phenomenon. As the interface between mother and fetus, the placenta plays a key role in fetal growth and development and, as such, affects the fetal programming underlying subsequent vulnerability in adulthood (Godfrey, 2002; Thornburg et al., 2010; Gabory et al., 2013).

The aim of this review is to discuss the emerging knowledge on the sex-specific relationships between diverse environmental influences on placental functions and the risk of diseases later in life, with a specific focus on epigenetic mechanisms and sexual dimorphism. The literature is wide on environmental effects (pollutants, various stresses, etc.): we will focus specifically on the effects of nutrition.

The placenta and its role in intrauterine programming

The placenta controls fetal development and growth through the transport of nutrients, respiratory gases and waste, and hormone synthesis. Adverse maternal conditions have been demonstrated to affect placental morphology, blood flow, feto-maternal exchanges and endocrine function, which modify placental efficiency corresponding to the ratio of fetal to placental weight. The effects on placental function are closely linked to the stage of development, the type of insult and the sex of the conceptus. Nevertheless, the placenta is capable of adapting to its environment to optimise its functions and promote fetal survival. In the case of placental failure, fetal growth could be altered, leading to a higher risk of developing metabolic syndrome in adulthood. In this context, the placenta can be considered as a central actor of fetal programming (Myatt, 2006; Jones et al., 2007; Fowden et al., 2009).

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Several studies have established an effect of environmental stimuli on placental morphology. For example, placental manipulations are practiced in sheep farming; pregnant ewes that were moved from rich to poor pastures at mid-gestation were undernourished. In response to maternal undernutrition, the placental area was prone to increase. At late gestation, if ewes were moved to rich pastures, the expanded placenta contributed to obtaining larger lambs at birth (McCrabb et al., 1991; McCrabb et al., 1992). In an equine model to study intrauterine programming according to maternal size and weight, embryo transfers between different breeds were undertaken to induce a restricted fetal growth. Transfer of Thoroughbred embryos into a pony uterus led to a growth retardation associated with a decrease of the microcotyledon surface density on the allantochorion compared with Thoroughbred embryos transferred into Thoroughbred mares (Allen et al., 2002).

Vasculogenesis and angiogenesis are key events for the development of the placenta. Placental vascularisation progresses throughout gestation and is controlled by both physical and chemical factors such as oxygen and growth factors (Charnock-Jones et al., 2004; Kaufmann et al., 2004). The maternal environment is able to modulate placental vascularisation and alters the transport of respiratory oxygen and nutrients. In ewes underfeeding during gestation, placental expression of vascular endothelial growth factor (VEGF) receptor transcripts was reduced by 50% on day 130 compared with ewes that received maintenance diet, while VEGF transcripts remained unchanged (Reynolds et al., 2005). Placental circulation through uterine and umbilical blood flow also contributes to the success of the pregnancy. In fact, abnormal blood flow was associated with intrauterine growth retardation (Kaponis et al., 2011). For example, overnourished adolescent sheep dams throughout pregnancy showed an increase of umbilical artery Doppler indices at mid-gestation, which preceded the reduction of fetal growth velocity (Carr et al., 2012).

The placenta is responsible for the transfer of substrates between maternal and fetal circulations. Substances have to cross several cellular layers, among them the trophoblast, through different transport mechanisms such as simple or facilitated diffusion or active transport, which depend on the type of components (Desforges and Sibley, 2010). Placental nutrient transfer capacity can be influenced by a wide range of factors such as placental surface area and thickness, the abundance of transporters, the gradient of concentrations between both maternal and fetal compartments, placental metabolism and utero-placental flows, themselves sensitive to environmental stimuli. For example, there is evidence that the expression and activity of system A, which is involved in neutral amino acid transport, is reduced in placentas from restricted fetal growth (Maïiendran et al., 1993; Jansson et al., 1998). Expression of glucose transporters is also affected by maternal metabolism. In the case of maternal food limitation during the last week of gestation in rats, the fetal growth restriction is associated with impairment of placental glucose transporter (GLUT-3) expression (Lesage et al., 2002).

The placenta is an endocrine organ, which secretes hormones in both maternal and fetal circulations. Some of them act on the maternal metabolism to facilitate glucose delivery to the fetus (progesterone, placental lactogen, placental variant of growth hormone). Another example is the insulin-like growth factor IGF-II, a cytokine, produced by the placenta, which is involved in feto-maternal exchanges specifically in the case of simple and facilitated diffusion of substrates (Sibley et al., 2004). Prostaglandins and corticotropin releasing hormone (CRH) have been described to control nutrient and oxygen supplies to the fetus by acting on fetal endocrine function, blood flow and myometrial contractility. Glucocorticoids are involved in organ development and maturation. Fetal exposure to glucocorticoids is closely linked to the maternal concentrations but also to the activity of 11-β-hydroxysteroid dehydrogenase type 2 (11βHSD2) expressed by the trophoblast. This enzyme converts the active glucocorticoids into inactive metabolites to protect the fetus against high concentrations of glucocorticoids. Moreover, inactivation of glucocorticoids is important to limit their effects on hormones that regulate nutrient supply (Fowden et al., 2008). These examples illustrate that hormones are able to modulate directly or indirectly the maternal and fetal metabolisms and nutrient transport in the placenta.

Maternal overnutrition/obesity and placental function

Obesity is defined as a body mass index (BMI) $\geq 30\ kg\ m^{-2}$. The prevalence of obesity has become a major health problem among women of childbearing age and reached 28.6% in the US population (National Center for Health Statistics, 2007).

During pregnancy, maternal obesity is associated with several outcomes for both mother and child: fetal growth abnormalities, macrosomia or intrauterine growth restriction, stillbirth, gestational diabetes mellitus (GDM), increased risk of caesarean section and pre-eclampsia. This maternal environment has profound long-term effects on the offspring, which are at greater risk of developing obesity and diabetes in childhood and adolescence (Catalano and Ehrenberg, 2006).

The metabolic diseases can take their origin in fetal adaptations to the adverse maternal environment. In the case of obese women, metabolic status during pregnancy is characterised by dyslipidaemia, hyperleptinaemia, hyperinsulinaemia and an exaggerated systemic inflammation (Ramsey and Glenn, 2002; Aye et al., 2014). Consequently, the feto-placental unit develops in an environment that predisposes the fetus to malprogramming.

Feto-placental weight

Maternal obesity can affect the feto-placental weight. In fact, in the Aberdeen Maternity and Neonatal Databank cohort including 37,482 women, placental weight and birth weight were highly correlated with increased maternal BMI, whereas placental efficiency decreased according to BMI (Wallace et al., 2012). Additionally, obese women have an increased risk of giving birth to a very low birth weight baby (McDonald et al., 2010). Finally, no alteration of fetal weight by maternal obesity was reported in another study, but it was associated with a decrease in placental efficiency (Dubé et al., 2012).

Biometry of the feto-placental unit has been obtained from different animal models of high maternal weight or obesity. Offspring from obese ewes increased their weight at mid-gestation whereas placentome weight was not altered (Zhu et al., 2009). In a mouse model, a high-fat diet administered before and throughout gestation resulted in fetal overgrowth associated with a normal placental weight (Jones et al., 2009). Mice fed a high-fat diet at mating had an increased body weight, while fetal weight was not changed at 15.5 days *post coitum* (Gallou-Kabani et al., 2010). In this last study, the diet affected placental weight and placental efficiency regardless of the sex of the fetuses. In rabbits, a high-fat diet before and throughout gestation induced high adiposity in the mothers, while their fetuses were of smaller weight, whereas placental efficiency remained unchanged (Tarrade et al., 2013). All these data demonstrate that variable results on fetal and placental weight have been reported, including a decrease or increase of weight or no effect, depending on species, diet composition and the time window of diet application.

Placental histology

Placental histology has been studied in the placenta of obese women. No placental maturity abnormalities, oedema, syncytial knots, fibrin deposition or chorangiosis have been shown. However, obese placentas displayed an increase in the muscularity of vessel walls in the villi (Roberts et al., 2011). In a non-human primate model where obesity was induced by a chronic high-fat diet, placentas showed an increase of infarction and chorionic villous calcification (Frias et al., 2011). Using transmission electron microscopy, an abnormal accumulation of lipid droplets has been described in placentas from rabbits fed a high-fat diet (Tarrade et al., 2013). In contrast, in placentas from mouse mothers fed a high-fat diet, no changes in the structure of the labyrinth and the spongiotrophoblast, or in the number and size of cells in the two layers were observed (Gabory et al., 2012).

Vascular function

In obese pregnant women, vascular function is impaired by impacting both endothelium and smooth muscle, which could explain the high risk of pre-eclampsia associated with obesity (Stewart et al., 2007), suggesting that placental vascularisation could be affected. In fact, chronic high-fat diet consumption before and throughout pregnancy reduced the uterine blood flow volume and the volume of blood flow on the fetal side of the placenta in primates (Frias et al., 2011). In a sheep model of overnutrition, the placenta demonstrated an impaired vascular development within the fetal cotyledon at an early stage (Redmer et al., 2009), which could explain the reduction of uterine and uteroplacental blood flow at mid- and late gestation (Wallace et al., 2002; Wallace et al., 2008). In another ewe model of obesity, a reduction of vascular density in cotyledonary tissue has been shown at mid-gestation (Zhu et al., 2009). Moreover, at this stage, cotyledonary arteriole diameters were greater in obese ewes, which could increase fetal growth, whereas cotyledonary arterial angiogenic factors from mid- to late gestation decreased in obese ewes, which could limit excessive placental vascular development (Ma et al., 2010).

Nutrient transport across the placenta

The transport of nutrients across the placenta depends on several mechanisms: the concentration gradients on the different placental sides, the expression and activity of transporters and the availability of substrates. Maternal obesity may affect placental transfer and nutrient availability. In obese women, placental transport and the metabolism of lipids have been described as being altered, which could contribute to the high adiposity in offspring during childhood (Dubé et al., 2012). Similar results from obese ewes have been published. High concentrations of cholesterol and triglycerides in both maternal and fetal blood increased placental fatty acid transporter in cotyledons at mid-gestation, which could explain fetal overgrowth and increased adiposity (Zhu et al., 2010b). In rodents, a maternal high-fat diet administered before and throughout pregnancy increased fetal growth and improved placental transport of glucose and neutral amino acids via an up-regulation of their transporters, GLUT-1 and SNAT-2, respectively (Jones et al., 2009). In contrast, a high-sugar and high-fat diet given throughout gestation reduced feto-placental growth at E16 but fetal weight was normalised at 19 days *post coitum*. Thus, the placenta adapts to its environment and increased glucose and amino acid transfer and expression of their transporters to promote fetal growth (Sferruzzi-Perri et al., 2013). Placental SNAT-4 activity, a major component of system A, was decreased in obese pregnant women, accompanied by maternal hyperleptinaemia and normal birth weight, and without

insulin resistance (Farley et al., 2010). Activation of insulin/IGF-I and mTOR signalling was also studied. These signalling pathways are increased in placentas from obese women (without gestational diabetes) and contribute to stimulate system A amino acid transporter activity in the setting of normal feto-placental weight (Jansson et al., 2013). Altogether, these data suggest that maternal obesity is able to modify nutrient transfer across the placenta and thus impact fetal growth.

Inflammation

Maternal BMI is positively correlated with systemic inflammation including high levels of monocyte chemo-attractant protein-1 (MCP-1) and TNF-α (Aye et al., 2014), but also of IL-6 (Challier et al., 2008). Inflammation is not only systemic – it can also be located in the placenta. In fact, accumulation of macrophages in the stromal core of the villi and expression of the pro-inflammatory cytokines IL-1, IL-6 and TNF-α have been described (Challier et al., 2008). Macrophage infiltration was also reported in the intravillous part of the placenta from obese baboons (Farley et al., 2009). In contrast, in obese women without insulin resistance, no difference in the number of macrophages within the placental villi was observed (Roberts et al., 2011). An increase of pro-inflammatory cytokines has been demonstrated in the placenta of both non-human primates fed a chronic high-fat diet (Frias et al., 2011) and obese ewes at mid-gestation (Zhu et al., 2010a). Moreover, in obese ewes, activation of Toll-like receptor-4 (TRL-4) through free fatty acids from the maternal compartment could activate NF-κB and JNK inflammatory signalling pathways (Zhu et al., 2010a). Other inflammatory pathways were activated according to maternal BMI such as p38-MAPK and STAT-3 (Aye et al., 2014). All these data indicate an exaggerated local inflammatory response in the placenta. Interestingly, the proinflammatory cytokines such as IL-6 and TNF-α stimulated the activity of system A amino acid transporter in trophoblast cell cultures (Jones et al., 2009), but also trophoblast fatty acid accumulation through IL-6 (Lager et al., 2011). Consequently, placental inflammatory status could contribute to an excessive nutrient transfer.

Nutrition, placental function and sexual dimorphism

The long-term effects of the same environmental insult, such as maternal unbalanced nutrition or maternal stress, can have various phenotypic effects on male and female offspring (Bale, 2011; Aiken and Ozanne, 2013). This difference has led many researchers to target their efforts exclusively to one sex, especially to males. The physiological and molecular basis for the observed sexual dimorphism in fetal programming is not understood: which aspects of sex-specific differences in development lead to this differential susceptibility to the same programming environment? The placenta has long been considered as a 'sexless' organ. Because of its fetal origin, the placenta in fact bears the same genetic information of sex as the fetus: XX or XY, and can also be differentially sensitive to fetal hormones (Gabory et al., 2013). Sex differences in the rate of fetal growth have long been recognised, but the sex of the embryo affects the size of both the fetus and the placenta, together with the ability of the placenta to respond to adverse stimuli (Clifton, 2010). The differences may appear very early during development, as it was found that in the bovine blastocyst, one-third of the gene expression already showed sex differences (Bermejo-Alvarez et al., 2010), and in cattle, rodents and humans, the rate of cell division and early development is faster in XY than in XX embryos (Mittwoch, 1993). Therefore, in the extraembryonic lineages as well as in the embryo proper, sex differences appear before gonadal

differentiation and hormone secretion. In many species, male placentas are bigger than female placentas or have a different shape (Eriksson et al., 2010). It was established in mice that these differences are independent of androgen effects (Ishikawa et al., 2003).

The majority of studies linking the sexually dimorphic placental response to nutrition and metabolism concern poor maternal nutrition. The Dutch famine at the end of the 2nd World War induced a decreased in placental size and area and a disruption of the fetus-to-placenta ratio index (FPI). The effect on the placental area was more severe for boys than girls (Roseboom et al., 2011). Moreover, the health outcomes were also different: in boys, changes in placental shape were associated with the development of the hypertension later in life, which was not the case in girls (van Abeelen et al., 2011). In ewes, placental restriction, induced by a surgical reduction of the number of caroncules, was associated with small size at birth and the appearance of more features of metabolic syndrome in males than in females at adulthood (Owens et al., 2007).

Fewer studies have focused on the potentially deleterious effects of maternal overnutrition, obesity or metabolic disturbances on the future health of the offspring (Zambrano and Nathanielsz, 2013). Fructose consumption is associated with the obesity epidemic (Vickers et al., 2011). In a rat model, adding fructose in drinking water during the first 10 days of pregnancy induced an increased caloric intake and a hyperinsulinaemia at term. The placental weight was decreased only in females, while fetal weight was not affected in either sex (Vickers et al., 2011). In humans, it was postulated that male babies need more fatty acids than female babies during development. Maternal obesity leads to a decrease in oleic acid uptake in the placenta of boys and an increase in that of girls. The expression of the CD36 fatty acid transporter and the binding protein FABP5 was decreased in the placenta of boys and unaffected in that of girls (Brass et al., 2013). Rabbit females fed a lipid- and cholesterol-enriched diet developed high adiposity and dyslipidaemia and their offspring were overweight at adulthood, which was associated with hypertension (Picone et al., 2011). Interestingly, a specific sexually dimorphic response to maternal diet was observed in the placenta. The placentas of females presented an increased lipid storage compared with those of males, along with a differential gene expression between the two sexes. In contrast, fetuses displayed a dyslipidaemia that affected more males than females. Therefore, there was a physiological adaptation, with a relative protection of the female fetuses from developing dyslipidaemia (Tarrade et al., 2013). Finally, a recent study showed that maternal obesity in the mouse induced a reduction in placenta labyrinth thickness and cell proliferation. Moreover, inflammation in the placenta was increased in late gestation, with a sex-specific effect: placentas of males showed greater inflammation and macrophage activation than those of females (Kim et al., 2014).

Very few studies have investigated the sex-specific response to maternal diet at the transcriptomic level. In human successful full-term pregnancy, Sood and colleagues identified some gene sets that were correlated with the sex of the fetus in the placenta. A lot of these gene sets were located on the X (10/34 in females) and Y (3/7 in males) chromosomes but the majority were on autosomes. The genes that were more expressed in the placentas of females included genes implicated in immune regulation. This suggests that sex-specific placental function and gene expression might have a role in the differences between males and females observed in fetal development and physiology (Sood et al., 2006). This human study did not take into account maternal diet and this aspect was provided by animal model investigations. In a first model, female mice were

challenged with either a low-fat or a high-fat diet from week 5 for 30 weeks before breeding, inducing underweight and overweight, respectively. A striking sexual dimorphism was observed by microarray assay in the E12.5 placental gene expression: each diet led to a sex-specific response and more genes were deregulated in females than in males under both diets (Mao et al., 2010). The authors suggested that the increased female sensitivity to maternal diet might buffer the deleterious environment to protect the female fetuses, leading to a decreased negative programming impact in adulthood. In another mouse model, the females were fed a control or a high-fat diet from gestation days E0.5 to E15.5. Microarray experiments showed that the expression was sexually dimorphic under both diets and that the response to a maternal high-fat diet was sexually dimorphic. The differences were not only quantitative but also remarkably qualitative. The biological functions and networks of dysregulated genes clearly differed between the sexes: mainly cell signalling involving immune cells, and uptake and metabolism of amino acids for females, and development and function of the vascular system, and uptake and metabolism of glucose and fatty acids for males (Gabory et al., 2012). In this model, there was no evidence for a greater reactivity in female placentas to maternal high-fat diet, in terms of placental and fetal growth or the number of dysregulated genes. Finally, in a non-human primate model of nutrient restriction, food intake of the pregnant baboon female was reduced by 30% from day 30 of gestation to day 165 (close to term, day 183). Again, a sex-specific response was observed using microarray assay. Female placentas exhibited a highly coordinated response, including upregulation of genes related to programmed cell death and downregulation of genes associated with cell proliferation. The male placental transcriptome appeared less responsive in terms of the number of affected genes and pertinent pathways (Cox et al., 2013).

Altogether, when the sex of the embryo is taken into account, most human and animal studies show sexual dimorphism in placental function and the response to maternal diet. A dominant concept in the literature is that female and male fetuses have different growth strategies, leading to differential survival and pregnancy outcomes. The greater sensitivity of females to the maternal environment would lead to modest growth changes and finally a better adaptation to deleterious signals such as maternal asthma, and a restricted or high-fat diet. Male fetuses, in contrast, induce changes in minimum gene expression and biological processes, leading to poor adaptation to an adverse environment and a more divergent growth curve (Clifton, 2010; Eriksson et al., 2010; Cox et al., 2013). Other reports show no difference in reactivity between the sexes but clearly different biological functions (Gabory et al., 2012). In these cases, it is difficult to say whether one sex copes better than the other. Differences in adaptation between males and females may therefore be context, species and stage specific. The molecular mechanisms underlying this sexually dimorphic adaptive response are largely unknown and more detailed molecular analysis will be of great interest to explain the sex-specific adaptive phenotypes and pathologies in adulthood.

Epigenetics and nutrition

More and more studies are converging to propose that epigenetics may be one of the key molecular mechanisms underlying the developmental programming of the phenotype. Epigenetics refers to the field of science studying the heritable mechanisms regulating gene expression without changing the DNA sequence itself. There are two main mechanisms: covalent modification of the chromatin and non-coding RNA. The principal chromatin modifications are

epigenetic marks: DNA methylation, which takes place at cytosines, mostly in CpG dinucleotide configurations, and post-translational modifications including methylation and acetylation of the histone proteins forming the nucleosome. These modifications are set-up or removed by the enzymes of the epigenetic machinery, leading to a dynamic state of the chromatin. The combination of all epigenetic marks over the genome constitutes the epigenome. The epigenetic landscapes are transmitted through cell division, leading to a memory of the cell identity, which leads to the notion of their 'heritability'. A more detailed description of epigenetic marks is introduced by Daniel and Tollesfbol in this issue (Daniel and Tollesfbol, 2015).

Epigenetics, because of its dynamic nature, can be sensitive to the environment and there is an obvious link between nutrition, energy metabolism and epigenetic processes (Delage and Dashwood, 2008; Choi and Friso, 2010; Gabory et al., 2011; Lillycrop and Burdge, 2012; Kaelin and McKnight, 2013; Vanhees et al., 2014). Briefly, nutrients and their metabolites can be direct substrates of the epigenetic machinery enzymes that appose the epigenetic marks. *S*-Adenosine methionine (SAM) is the donor of -CH$_3$ (methyl) groups for DNA and histone methylation. SAM is part of the one-carbon metabolism involving the folate cycle. This involves different dietary micronutrients, such as vitamin B9 (folate) but also vitamins B2, B6 and B12, methionine, betaine, choline or zinc (Anderson et al., 2012). Acetyl-CoA is the source of -COCH$_3$ for the histone acetylation reaction. It is a very important metabolic hub and its biogenesis can be obtained by many different pathways, particularly in glucose, fatty acid and amino acid metabolism. Nutrients and their metabolites can also be direct activators or inhibitors of the epigenetic machinery enzymes. Finally, nutrients and their metabolites can be substrates for membrane and nuclear receptors, thus leading to the local modification of the chromatin on the target gene sequences via different cell signalling pathways (Gabory et al., 2011).

There are two main periods of major chromatin remodelling: gametogenesis, to reset the epigenetic pattern from the primary germ cell into gamete cell identity, and conceptus development, to erase the gamete epigenetic identity towards totipotent and then cell type-specific information. Therefore, these periods are particular time windows during which the sensitivity of the epigenetic programme to the environment is important: a perturbation of the erasure and resetting of the epigenetic patterns can have long-lasting consequences on the development and function of the tissues and therefore long-term effects on phenotype and diseases (Attig et al., 2010).

Epigenetics, placental function and nutrition
From the first step of trophoblast lineage differentiation, there is an asymmetry in the apposition of the epigenetic marks compared with the embryonic lineage. These differences appear to persist throughout embryonic development (Nelissen et al., 2011; Rugg-Gunn, 2012). Global DNA methylation is lower in the trophectoderm than in the inner cell mass of the blastocyst, and this lower methylation is retained in the extra-embryonic tissue compared with the somatic tissue (Santos et al., 2002). However, this does not mean that DNA methylation is not a factor contributing to placental function. It is indeed required for placental formation and function, as mice mutants of DNA methyltransferases (*Dnmt*) show important placental defects. Histone modifications also present distribution asymmetry between the two first lineages in the blastocyst and this pattern is conserved throughout gestation. The trophectoderm blastomeres appear to be depleted in H3K27 methylation (methylation is apposed on the lysine residue in position 27 of histone H3), H3K4 and H4K16 acetylation marks compared

with the inner cell mass at the global level. Another important mark might be H3K9 methylation, as the Kmt1a (Suv39h1) methyltransferase has a role in trophectoderm cell fate identity maintenance *in vitro* and *in vivo*. Histone modifications are crucial for establishment of the trophoblast lineage, maintenance of this extra-embryonic identity and placental function (reviewed in Rugg-Gunn, 2012).

Another noteworthy epigenetic feature in the placenta is genomic imprinting. This is an epigenetic mechanism leading to a parent-of-origin-specific gene expression. The apposition of specific epigenetic marks in the two separate germ lines confers a memory of this origin on a region called the imprinting centre that leads to a maternal- or paternal-specific expression of the imprinted genes. For example, the *H19/Igf2* locus is methylated during spermatogenesis and protected from DNA methylation during oogenesis, leading to the expression of *H19* on the maternal allele and *Igf2* on the paternal allele (Gabory et al., 2010). Genomic imprinting is present in most, if not all, somatic and extra-embryonic tissues but its importance in placental development and function is highlighted by two facts. First, its emergence during evolution parallels the emergence of viviparity (Renfree et al., 2013). Second, a substantial number of genes are imprinted only in the placenta and exhibit a classic bi-allelic expression in somatic tissues (Wagschal and Feil, 2006). The epigenetic mechanisms governing the monoallelic expression of these genes in the placenta might be different from those in somatic tissue, with greater importance for histone modifications in the extra-embryonic lineages than in the proper embryo (Lewis et al., 2004; Umlauf et al., 2004). The specific feature of genomic imprinting and its particularities in the placenta led to the hypothesis that it might 'buffer' the environmental impact and imprinted genes appeared to be good candidates to mediate nutrition-linked transgenerational effects on growth (Pembrey, 1996), and hence to participate in the DOHaD process. On the one hand, imprinted genes may not be more susceptible to alteration related to maternal nutrition and metabolic states. They have been particularly studied because the regulatory sequences and epigenetic mechanisms implicated in the regulation of their expression are well described. The whole genome is affected. But on the other hand, their expression is finely tuned by epigenetic marks and, consequently, a small change in these marks may be transmitted to the next cellular generations and have a real impact on gene expression and tissue function. Therefore, these genes might not be specific targets of nutrition but sentinels of a nutritional and metabolic programming.

Because of the role of the placenta and of epigenetic processes in fetal programming, and given the placenta's unique position at the materno-fetal interface, a growing number of studies are examining this organ and these processes either as biomarkers for early perturbation linked with adult-onset diseases or from a fundamental mechanistic point of view. As the impact of exposure to toxicants (cigarette smoking, toxic metals, etc.) or maternal stress on DNA methylation of specific candidate genes or on the whole DNA methylome, as well as on microRNA expression, has been reported in this issue (Marsit, 2015) and elsewhere (Maccani et al., 2010; Bale, 2011; Lee and Ding, 2012; Monk et al., 2012; Suter and Aagaard, 2012), we will focus on the very few publications about maternal nutrition and metabolism.

Gestational diabetes mellitus and obesity
The incidence of women with gestational diabetes mellitus (GDM) and obesity is increasing. It is therefore essential to understand how these factors can impact development and predispose people to metabolic disorders in the long term.

Placenta DNA methylation at the global level was measured by the luminometric methylation assay (LUMA) in an American cohort of pregnancies complicated by GDM, pre-eclampsia or obesity (Nomura et al., 2014). This technique measures methylation of CG sites in the CCGG position at the global genome level. Placental methylation levels were lower in patients with GDM or pre-eclampsia while they were higher in patients with obesity. The differences were modest (−4.35% for GDM, N=8 versus 24 controls, and +2.46% for obesity, N=18 versus 32 controls) but as it is global methylation, which reflects CG-rich region methylation such as CpG islands or gene promoters, it can be considered as a substantial difference throughout the genome at large. This implies that maternal metabolism during pregnancy can impact whole-genome epigenetic marks (Nomura et al., 2014) but this does not allow comprehensive interpretation of the programming of adult diseases. With that goal, gene-specific methylation studies have been carried out.

A Canadian team has studied placental methylation of candidate genes in a cohort of women with impaired glucose tolerance [IGT, as defined by a 2 h post-75 g oral glucose tolerance test (OGTT), glycaemia ≥7.8 nmol l^{-1}], which is a clinical standard of GDM (Ruchat et al., 2013a). While they could not find a statistical link between IGT and term placenta DNA methylation, they did find a significant correlation between 2 h post-OGTT glycaemia and DNA methylation at the *LEPTIN*, *ADIPONECTIN* and *ABCA1* genes. For the *IGF1R* and *IGFBP3* genes, DNA methylation levels were lower in IGT placentas and correlated with the 2 h post-OGTT glycaemia (−4.3 and −2.5 percentage points for *IGF1R* and *IGFBP3*, respectively, N=102 normal glucose tolerance and 28 IGT placentas). The *LEPTIN* gene promoter methylation was also measured in the Rhode Island Child Health Study (Lesseur et al., 2014). Global promoter methylation was higher in the placenta of obese mothers and this association was mediated by GDM (+2.5 percentage points difference between GDM patients, N=47, and controls, N=432). Interestingly, *LEPTIN* promoter methylation is higher in the placentas of males than females (+2.3 percentage point difference). A German team studied DNA methylation of 16 candidate regions in women with diet- or insulin-treated GDM (El Hajj et al., 2013). They found a decrease in placental DNA methylation in four genes and two repeated regions. Significantly, *MEST* gene DNA methylation was downregulated by 7 percentage points in GDM (N=80 GDM patients and 83 insulin-dependent GDM patients and N=57 controls). The authors also observed a significant decrease in blood *MEST* methylation (3 percentage points, N=37 obese patients and 37 controls) in adults with morbid obesity, supporting the idea that intrauterine exposure to GDM may have effects on the epigenome of the offspring throughout life.

In the Canadian cohort, DNA methylation was also measured at 485,000 CpG sites, using the Infinium HumanMethylation450 BeadChips platform (Ruchat et al., 2013b). CpGs in 3271 genes were found to be affected by maternal GDM. Interestingly, these genes were principally associated with pathways implicated in metabolic diseases (cardiovascular diseases and metabolic disorders are the top pathways in the bioinformatical studies). Moreover, in almost 10% of these genes, the methylation levels were correlated with newborn weight, which is itself a marker of predisposition to adult metabolic diseases.

Taken together, these data support the implication of placental epigenetic adaptations in genes involved in fetal growth, and energy, glucose and lipid metabolism in response to maternal glucose concentration and GDM. We can discuss the accuracy of these epigenetic studies, regarding the small differences that were found of between 2.3 and 7 percentage points. Methylation is an on or off

feature and therefore the percentage of methylation more or less reflects the percentage of cells in which the sequence is methylated in the tissue. Thus 2.3–7% of the placental cells are affected by GDM or obesity: is it biologically relevant? If the difference is diluted in the whole tissue, the effects might be restrained or absent. However, if the differences were restricted to a small number of cells with a specific role, the effects would be substantial. Expression studies linking the methylation difference to expression change would answer this question but this is unfortunately not systematically investigated. We can also be interested in these differences not from a mechanistic point of view but as biomarkers. The variations that are described are statistical, not systematic, and inter-individual variation is quite important. Finally, large prospective studies will be needed to prove that such placental epigenetic changes are indeed associated with changes in disease susceptibility in adulthood.

Intrauterine calorie restriction

Intrauterine calorie restriction is associated with growth retardation in newborn human and animal models. In rodents, it has been associated with various features of metabolic syndrome in offspring, such as glucose intolerance, increased fat mass and hypercholesterolaemia. Using RNA-sequencing and reduced representation bisulphite sequencing (RRBS) techniques, DNA methylation and gene expression were studied in the placenta of calorie-restricted (CR) mice (Chen et al., 2013). At the global level, CR placentas were hypomethylated and this feature was interestingly more pronounced in placentas of males than females. The changes in DNA methylation in response to maternal CR had a strong sex-specific component. Firstly, differentially methylated regions between CR and control placentas are not the same in placentas of males and females. Secondly, the networks associated with the differentially methylated genes were also different: linked with lipid metabolism, nervous system development and developmental disorders in females and linked with cell and organ morphology in males. Finally, a candidate gene approach identified imprinted genes and microRNA coding gene with differential methylation (Chen et al., 2013). For further studies in this field, it would be necessary to investigate how placental histone marks are affected by maternal caloric restriction.

Effect of a high-fat diet

In a mouse model, pregnant females were fed a high-fat diet, from 0.5 to 15.5 days *post coitum*, the middle of the fetal period. We compared the gene expression patterns of 20 imprinted genes and analysed DNA methylation in the placentas from male and female fetuses (Gallou-Kabani et al., 2010). A high-fat diet during gestation triggers the deregulation of imprinted genes, among which the *Igf2r* cluster, which plays an important role in the control of many cellular, metabolic and physiological functions potentially involved in adaptation and/or evolution, was particularly significant. We analysed the DNA methylation on the imprinting control region of this locus and observed sex- and diet-specific differential methylation of CpGs in two subregions. While we cannot conclude there is a direct link between the observed differences in DNA methylation and gene expression, bioinformatic analysis suggested that the differentially methylated CpGs lie within recognition elements or binding sites for transcription factors and factors involved in chromatin remodelling (Gallou-Kabani et al., 2010). Using transcriptomic datas from the same placentas, we observed a downregulation of three important epigenetic modifiers in the placentas of fetuses from mothers fed a high-fat diet. *Kmt1a* and *Kmt1b* (*Suv39h1/h2*) are H3K9 methyltransferases (Gabory et al.,

2012). Interestingly, *Dnmt3l*, a cofactor of the Dnmt3 enzymes, was downregulated specifically in placentas of females but not males. Placental global DNA methylation, as assessed by the LUMA technique, was also downregulated in females fed a high-fat diet compared with those fed a control diet.

Finally, we observed that the expression of the *Kdm5c* and *Kdm5d* genes, which encode the Jarid1c and Jarid1d H3K4 demethylases, were sexually dimorphic (Gabory et al., 2012). These genes are paralogues on the X- and Y-chromosomes. The *Kdm5c* gene is on the X-chromosome and escapes X-chromosome inactivation. We indeed found that it was more expressed in placentas of females than males, regardless the maternal diet. *Kdm5d* is Y-linked and therefore only expressed in placentas of males. In our model, we found that the *Kdm5d* gene expression in males was not able to compensate for the expression of *Kdm5c*, as the expression, studied by RT-qPCR with primers recognising both *Kdm5c/5d* transcripts, was still higher in females than in males (Gabory et al., 2013). Whether the two proteins have the same function or can be associated with different partners, therefore targeting different regions on the genome, is unclear. The epigenetic enzymes could therefore mark the epigenome in a sex-specific manner, at both the quantitative and qualitative levels (Xu et al., 2002).

Conclusions

Altogether, the data presented in this review converge on the fact that adaptation in placental phenotype in response to maternal diet and metabolic status (GDM, obesity, undernutrition, etc.) alters fetal nutrient supply, leading to a higher susceptibility to develop metabolic disorders under adverse adult life conditions, such as an obesogenic diet and/or morbidity.

Sexual dimorphism is another very important aspect of the DOHaD process that is increasingly being taken into account. It is becoming clear that the placenta reacts differently to the same environment depending on the sex of the fetus. The sex specificity of the adult-onset phenotype is therefore already partly shaped *in utero* and the placenta is at stake in this sex-specific feature. Finally, the fetal programming processes and the sex specificities imply important epigenetic mechanisms that are, however, still poorly documented. Effort should be concentrated not only on the observation of sex-specific features but also, and more importantly, on the understanding of the underlying mechanisms. A possible process might be that the sex-specific epigenome, apposed by epigenetic enzymes encoded by genes on the X-/Y-chromosome, such as *Kdm5c/5d*, *Utx (Kdm6a)/Uty*, *MeCP2*, would be differentially affected by maternal diet and metabolism leading to male and female divergence in programming. Epigenetic mechanisms, such as DNA methylation, multiple histone modifications and non-coding RNAs, governing placental differentiation and function, and therefore the fetal growth and phenotype, could contribute in this programming process. Maternal metabolic status can impact epigenetic mark apposition and memory, which could lead to an altered placental gene expression and function with direct consequences for fetal tissue development, and thus potentially have an impact on adult offspring phenotype.

Explaining the sex-specific causal variables and how males versus females respond and adapt to environmental perturbations should help physicians and patients anticipate disease susceptibility.

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