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Interplay of early life nutritional programming on obesity, inflammation and epigenetic outcomes.

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Abstract

The huge health burden accompanying obesity is not only attributable to inadequate dietary and sedentary lifestyle habits, since a predisposed genetic make-up and other putative determinants concerning easier weight gain and fat deposition have been reported. Thus, several investigations aiming to understand energy metabolism and body composition maintenance have been performed considering the participation of perinatal nutritional programming and epigenetic processes as well as inflammation phenomena. The Developmental Origins of Health and Disease (DOHaD) hypothesis and inheritance-oriented investigations concerning gene-nutrient interactions on energy homeostasis and metabolic cell functions have suggested that inflammation could be not only a co-morbidity of obesity, but also an etiological agent. There are several examples about the role of nutritional interventions in pregnancy and lactation, such as caloric deprivation, protein restriction and excess fat feeding, which determine a cluster of disorders affecting energy efficiency in the offspring as well as different metabolic pathways, which are mediated by epigenetics encompassing the chromatin information mainly encrypted by DNA methylation patterns, histone covalent modifications and ncRNA or miRNA. Indeed, epigenetic mechanisms may be boosted or impaired by dietary and environmental factors in the mother, intergenerationally or transiently transmitted, and could be involved in the obesity and inflammation susceptibility in the offspring. The aims currently pursued are the early identification of epigenetic biomarkers concerned in individual’s disease susceptibility and the description of protocols for tailored dietary treatments/advises to counterbalance adverse epigenomic events. These approaches will allow diagnosis and prognosis implementation and will facilitate therapeutic strategies in a personalized “epigenomically-modeled” manner to combat obesity and inflammation.
Introduction

The vast health problems associated with fat deposition resulting in obesity is not only a laziness or gluttony trouble associated to inadequate sedentary lifestyles and unbalanced dietary habits, since in addition to a more susceptible genetic make-up to easier gain weight and fat deposition a number of recognized scientific evidences have theorized about the roles of other putative determinants\(^1\). Thus, there are proofs that some other possible contributing factors to the obesity epidemic are microorganisms (infectobesity/microbioma), increased maternal age in human mothers, assortative mating plus a greater reproductive rates among couples with higher adiposity, sleep debits, hormonal and neurological disorders, undue weight gain associated to undesirable pharmacological side-effects, lesser variability of external weather conditions and different intergenerational or intrauterine/epigenetic mediated effects\(^2\).

In this context, it has been claimed that parental nutrition previous to conception, maternal perinatal feeding and early postnatal dietary intake are involved in the onset of some chronic conditions accompanied by inflammatory manifestations such as obesity, diabetes, hypertension, dyslipaemia, etc at the life cycle, which has laid down the foundations of the offspring metabolic encoding\(^3\). In addition to perinatal programming studies, several investigations aiming to understand energy metabolism and fuel utilization have been performed considering the interactions between genetics, inflammation phenomena and immunologically-related mediators\(^4\).

The term epigenetics was coined as a conceptual assumption for disentangling hither to undiscovered relationships between environmental settings and the genetic background to produce a given phenotypical outcome\(^5\). Thus, an early definition for epigenetics involved "the study of the mechanisms of temporal and spatial control of gene activity describing pathways different from those directly attributable to the underlying DNA sequence and with an influence on the adaptive reaction of an organism" overcoming the usual legacy assigned to DNA\(^6\). The "epigenetic code" encompass the chromatin information mainly encrypted by DNA involving methylation patterns in the nucleotide sequence, histone covalent modifications, miRNAs profiles and polycombs, which constitutes “the sum of the alterations to the chromatin template that collectively establish, modulate and propagate different patterns of gene expression and/or silencing from the same genome”\(^7\). Therefore, epigenetics can provide some creative insights to understand genetic foetal programming, monozygotic twin differences, transgenerational outcomes and the onset of chronic diseases with associated inflammatory features such as obesity in the adult, which is requiring newer and elegant experimental models with a focus on phenomena affecting gene expression but not linked to the nucleotide sequence\(^8\).
The Developmental Origins of Health and Disease (DOHaD) hypothesis and nutritional programming.

The Developmental Origins of Health and Disease (DOHaD) hypothesis, which was initially based on epidemiological approaches, is now receiving steady back-up and boosted support through intensive genetically-founded experimental research in both cell or animal models and through human genome-related findings\(^9\).

The pioneer finding that an association between poor foetal and infant growth with a higher risk of suffering metabolic syndrome features in adulthood has allowed to sustain the thrifty phenotype hypothesis, which points out that early inadequate nutrition induces insulin resistance\(^10\). Additional studies conducted in other British Cohort\(^11\), in Danish subjects born between 1936 and 1983\(^12\), Dutch famine or Leningrad siege affected children\(^13\) have been consistent with the possibility of developmentally-mediated origin of obesity\(^14\).

Furthermore, the National Children Study (NCS), the Southampton’s women survey (SWS), the Viva project and the Behavioural Perinatology Research Program together with other more recently launched investigations such as the Avon Longitudinal Study of Parents and Children (ALSPAC) are contributing to identify critical processes underlying the interactions between retarded foetal growth and development with adiposity related outcomes and obesity features\(^15\).

Indeed, nutritional programming may explain the predisposition of some individuals to suffer non-communicable diseases in the adulthood linked to deprived in utero or infant development\(^16\). Inheritance-oriented investigations concerning gene-nutrient interactions on energy homeostasis processes and metabolic cell functions is extending to all clinically chronic relevant diseases such as diabetes and cardiovascular events as well as to obesity and associated inflammatory features\(^17, 18\). Also, some studies have identified that inflammation and transient infections could be not only a co-morbidity of obesity and diabetes\(^19\); but also an etiological agent\(^20, 21\).

The foetal or developmentally programmed genesis of adult sickness hypothesis settled that environmental factors and maternal lifestyles, particularly adverse nutritional disturbances, proceed in early life to drive the risks for the onset of metabolic diseases and excessive weight gain in later life stages\(^22\). Indeed, maternal nutrition can program gene expression patterns to the embryo that persist into adulthood and may contribute to the appearance of typical metabolic syndrome features such as hypertension, insulin resistance, hyperlipemia and abdominal obesity\(^23\). The parental conditions and lifestyles, which may involve maternal size/obesity, famine at perinatal periods, the use of nutritional supplements, alcohol or drug abuse as well the administration of therapeutical agents in this critical period may alter specific processes with an impact on embryonic, placental and foetal growth, organogenesis or regulatory set points for system functions affecting adiposity,
where inflammatory and immunologically mediated processes may be involved\textsuperscript{(24)}. Interestingly, some of these epigenetically mediated signals may be not permanent but transient, which is of interest not only for prevention, but also as a target for developing future therapeutic focus\textsuperscript{(25)}.

Unfavourable environmental cues coming from the mother such as psychological stress, infection, over- or undernutrition, smoking, neuroendocrine disruptors, trauma or diseases are signalled inputs negatively affecting the embryo, foetus or neonate\textsuperscript{(26)}. The adaptive responses may involve growth stunting or tissue remodelling with an impact on physiological functions and metabolism being the trade off and increased risk in later life\textsuperscript{(3)}. The characteristics of the programmed outcomes depend upon the insult or stimuli as well as on the timing of the exposure\textsuperscript{(27)}.

Understanding the maternal regulation of foetal development and programming involves a knowledge about the genome, baseline maternal body composition, dietary and metabolic status, utero-placental blood flow and substrate transfer that may condition the nutrient balance and foetal malnutrition by inducing hypoxemia or metabolic changes (cortisol, insulin, nutrient oxidation) and altering body composition in the newborns\textsuperscript{(28)}. In this context; the term developmental plasticity has been used to define the aptitude of a unique genotype to produce a variable phenotype in response to changing environmental circumstances, even though may be neither adaptive nor prognostic as stated by the developmental programming theory\textsuperscript{(29)}, but struggling for resources which may envisage or forecast future metabolic scenarios in an effort to tune gene expression to generate a phenotype best adapted to the predictable afterwards environment\textsuperscript{(30)}.

A number of mammal’s models have been developed to examine the potential processes and mechanisms involved in perinatal programming that depends on nutrition\textsuperscript{(31)}. The phenomena and manifestations ascribed to early nutritional programming have been explained through different mechanisms such as the involvement of the adipose tissue, the participation of different hormones and endocrine systems, enzymes, transcription factors and signalling mediators (glucocorticoids, insulin, PPAR family, adipokines,..), the regulation of specific nutrient related metabolic pathways (lipogenesis, glyconeogenesis,…), the control of neural networks affecting the appetite system (specific orexigenic/anorexigenic neuropeptides and the HPA axis) and the up/down feedback concerning the gene expression machinery and epigenetic marks\textsuperscript{(32, 33)}.

The question if later obesity is in utero or early induced after birth has been repeatedly addressed and researched\textsuperscript{(34, 35)}. Thus, it seems that glucose, insulin and leptin coming from the mother’s blood or taken from the breast milk is of relevance for the persistent programming of food intake control at least in the rodent\textsuperscript{(36)} as well as on obesity related peptides and hormones such as insulin\textsuperscript{(37)}. Furthermore, hormonal and metabolic signals acting during the perinatal period could alter the structure and functions concerning the fat-brain axis or adipogenic genes in the adipose tissue, that
regulates the energy balance during later life\textsuperscript{(38)}. Alternatively, adverse intrauterine exposures may produce long-term changes in mRNA levels leading to a thrifty phenotype with changes affecting liver, muscle and renal anatomy and physiology\textsuperscript{(14)} as well as longlasting changes in mitochondria that can be associated to obesity and insulin resistance in later life\textsuperscript{(39)}. Other mechanism that have been investigated in different animal models in order to clarify the role of the perinatal feeding on tissue structure have revealed an important impact of the maternal diet on proliferation and differentiation processes in the pancreas and in the brain involving an overexposure to glucocorticoids mediated by a reduced activity of the placental 11-beta hydrosteroid dehydrogenase\textsuperscript{(33)}. Also, a maternal obese condition influences foetal growth and body composition with implications in the future offspring health depending on the genetic background, the intrauterine metabolic environment and the generated maternal metabolites\textsuperscript{(22)}. Thus, a mild maternal overnutrition led to increased adiposity, glucose intolerance and altered brain appetite regulators in offspring\textsuperscript{(40)}, while food-deprived dams may transfer to the offspring patterns of increased hepatic gluconeogenesis, enhanced release and impaired oxidation of fatty acids from adipocytes, resistance to ketosis and changes in glucose uptake mediated for an increased insulin receptor expression\textsuperscript{(10)}. On the other hand, intrauterine growth restriction due to an induced perinatal impaired uteroplacental function or nutrient deficiency have been linked to lower leptin, normal or lower adiponectin and higher ghrelin as well as visfatin levels, while contradictory results have been reported concerning apelin/resistin and other pro-inflammatory markers such as TNF-alfa and IL-6\textsuperscript{(41)}. Interestingly, maternal perinatal undernutrition attenuates T-Cell function in adult male rat offspring\textsuperscript{(42)}.

Finally epigenetic marks affecting a number of genes regulating energy metabolism, adipogenesis or inflammatory processes are providing new clues to understand the relationships between nutritional programming and obesity in the adulthood\textsuperscript{(24, 43)}.

Despite the general acceptance of the DOHaD hypothesis, the terms of such proposal has not been always demonstrated in epidemiological surveys, while that some inconsistent results have been reported in animal models\textsuperscript{(26)}, a systematic error in interpreting experimental data and a publication bias due to missing information is not rule out.

**Animal models and epigenetic regulation**

There is a number of experimental interventions in animals about the role of nutrition in pregnancy and lactation such as caloric deprivation, protein restriction and excess fat feeding\textsuperscript{(33)}. Such investigations have proven a cluster of disorders affecting energy efficiency as well as the impairment of different metabolic pathways and adverse predisposition for suffering cardiovascular
diseases, glucose intolerance and obesity on the offspring\(^8\), and unfavourable inflammatory interactions\(^{44}\).

A loss of diurnal variation in heart rate and blood pressure in adulthood has resulted from maternal undernutrition followed by postnatal overnutrition in rodents, while hyperphagia resulting from perturbed development of the hypothalamic circuitry devoted to food intake control may contribute to overweight and developmental changes in fat cell precursors\(^{45}\). On the other hand, maternal obesity has an effect on pancreatic beta cells inducing a higher risk of diabetes\(^{46}\). The mitochondrial DNA content of the liver and skeletal muscle were reduced in fetal and early postnatal undernourished animals even when balanced nutrition was provided after weaning, which were accompanied by a decrease in mitochondrial DNA-encoded gene expression indicating that poor nutrition in early life causes long-lasting changes in mitochondria that may contribute to the development of insulin resistance in later life\(^{39}\). The adverse effects of a in utero low protein dietary intake have been associated with diabetes\(^{47}\), increased systolic blood pressure\(^{48}\), altered glucose tolerance\(^{48}\), hyperisulemia and reduced insulin signalling protein expression\(^{49}\). Also, a reduced maternal protein consumption during pregnancy and lactation has window of exposure and sex-specific effects on offspring growth, adiposity, appetite, glucose utilization and circulating leptin\(^{50}\). A programming of hepatic insulin-sensitive enzymes in the offspring of rat dams fed a protein-restricted diet was found, where glucokinase activity decreased (approximately 50%), whereas phosphoenoilpyruvate carboxykinase (PEPCK) activity increased (approximately 100%) with parallel changes in gene expression in both enzymes\(^{51}\). Indeed, the understanding of genetic and epigenetic contributions to human nutrition and health is contributing to translate basic biology into clinical applications\(^{1,52}\) concerning perinatal nutrition and disease in the adulthood.

Three genomic targets have been involved in the modulation of the gene expression changes: epigenetic marks at the promoter regions of some epiobesigenes, transposable elements that lie adjacent to genes with metastable epialleles and the regulation of imprinted genes\(^{53}\). In this context, epigenetic studies are contributing to unravel some putatively hidden phenomena that are not being explained by the accomplishment of the Human Genome Project in relation to obesity\(^{54}\). In the last years, different examples of dynamical changes in DNA methylation patterns, histone covalent modifications and the involvement of non coding RNAs due to the restriction or supplementation with different nutrients have been reported\(^{4,24}\) as well as related to obesity\(^{55}\). Thus, the methylation pattern of the leptin promoter in adipocytes is affected by a high fat intake in rats following an inverse trend to body weight changes\(^{56}\), while weight gain induced by an isocaloric pair-fed high fat diet produced a nutriepigenetic outcome on FASN and NDUFB6 gene promoters\(^{57}\).
Interestingly, maternal supraphysiological methyl group (folate, cobalamine, choline and betaine) or genistein supply throughout pregnancy modified DNA methylation of some key metabolic genes, with implications in adiposity\(^{58, 59}\), while protein restriction of pregnant rats induced DNA hypomethylation in the glucocorticoid receptor and peroxisomal proliferator-activated receptor-\(\alpha\) genes in the liver of the newborns, which was prevented by folic acid supplementation\(^{60}\). Also, it has been found that a chronic high-fat diet in fathers epigenetically programs \(\beta\)-cell dysfunction in female rat offspring\(^{61}\). These findings are in agreement with previous observations suggesting that transgenerational epigenetic inheritance may be sex dependent for specific traits\(^{62, 63}\).

In addition to changes in methylation patterns, epigenetic transfer may involve histone modifications and microRNA mediated mechanisms\(^{64}\). Thus, an energy-dense maternal diet driving to obesity epigenetically impairs fetal chromatin structure in primates via covalent modifications on histones\(^{65}\), while a role for microRNA in the alternative expression of IGF-2 in fetal livers from high-fat fed dams has been reported\(^{66, 67}\).

All these data and experiments strongly suggest that epigenetic mechanisms may be boosted or impaired by dietary and environmental factors in the gestating mother and could be involved in obesity susceptibility in the offspring\(^{9, 35}\).

**Obesity, inflammation and epigenetics**

Inflammation is a protective complex biological response mounted by tissues to combat injurious stimuli in order to maintain cell homeostasis\(^{68}\), which include host defence, tissue remodelling and metabolic changes and involve multiple mechanisms such as the contribution of immune cells (recruitment and activation of leukocytes, granulocytes, monocytes, B-\(T\) lymphocytes and dendritic cells), the involvement of different mediators (interleukins, TNF-alfa, leptin, adipokynes….) or the regulation of signalling pathways (insulin, glucose, lipids…) and eventually the epigenetic regulation of the expression of some related genes\(^{4, 69}\). Indeed, many important risk factors for obesity (overnutrition, low dietary fibre intake, sedentary lifestyles, sleep debts, neuroendocrine status or genetic make-up) have been found to be implicated in local or low-grade systemic inflammation\(^{19}\). Thus, an excessive adiposity has been seen either as a cause or as a consequence of chronic inflammatory disorders and epimutations\(^{20, 23}\). Interestingly, inflammatory signaling as mediator of epigenetic modulation in tissue-specific chronic inflammation has been identified\(^{70}\).

One of the challenges for investigators researching in the epigenomics field is identifying and characterizing the epigenetic marks and those stimuli modulating the expression of some specific genes (epiobesogenes) in pathways involving obesity/body weight homeostasis and energy balance.
processes such as adipogenesis, inflammation, appetite, insulin signalling, thermogenesis or macronutrient turnover\(^{(24)}\). Indeed, a bioinformatics analysis of promoter regions for the search of epigenetic biomarkers of obesity, have identified affected methylation patterns on several obesity-related genes such as FGF2, PTEN, CDKN1A and ESR1, implicated in adipogenesis, SOCS1/SOCS3, in inflammation, and COX7A1 LPL, CAV1 and IGFBP3, in intermediate metabolism and insulin signalling\(^{(71)}\). The characterization of those individuals that at an early age could present changes in the methylation profiles of specific genes could help to predict their susceptibility to later develop obesity, which may allow to prevent and follow-up its progress, as well as to research and develop newer therapeutic approaches. Thus, from approximately 760 human genes under putative epigenetic regulation (http://www.ncbi.nlm.nih.gov/sites/entrez), about 20% of them, defined as epiobesigenic genes, could be associated to obesity\(^{(72)}\). The knowledge of the modification of their methylation patterns due to different dietary factors, age, inflammation or some of the physiological aspects surrounding overweightness, could be crucial to investigate the role of these mechanisms in the prevention, onset and therapy of obesity as well the reversibility/stability of the epigenetic code and the involvement of specific enzymes (methylases, acetylases…) is of interest\(^{(54)}\).

Signal-specific inflammatory mechanisms epigenetically-mediated may operate through transcription factors (NFkB family), kinases (IKK-related kinases, MSK, PKA, PI3k, AKT…), the endoplasmic reticulum (calcium), biochemical activation of DNA methyltransferases (DMT, DNMT) and histone-modifier enzymes (HDAC/HAT, HMT, SIRT…), changes in cellular pools of acetylCoA, NAD or methyl donors, which are sensible to oxidative stress, hyper- or hypoglycemia, fatty acids load, and be activated or inhibited by overnutrition\(^{(67},^{73})\). Furthermore, in addition to epigenetic processes involving methylation marks, histone modifications, also noncoding RNAs are susceptible to dynamic inflammatory control\(^{(4)}\).

The concept of epigenetic regulation is gradually being recognized as an important factor in inflammatory related events such as obesity, diabetes and cardiovascular diseases\(^{(74)}\). Gene silencing in severe systemic inflammation have been associated to the reprogramming of acute pro-inflammatory genes, the intervention of the NFkB and compartmentalization of the epigenetic process\(^{(75)}\). In this context, the impact of inflammation on global DNA methylation has been demonstrated in chronic kidney disease, while an epigenetic regulation of high-glucose induced proinflammatory cytokine production in monocytes have been described for the polyphenol curcumin involving NFkB\(^{(76)}\). On the other hand, histone deacetylase inhibitors (HDACi) are emerging as possible epigenetic modulators of gene expression controlling the inflammatory response in some circumstances\(^{(77)}\). Also, an increased expression of DNA methyltransferase3a in
obese adipose tissue has been reported\(^{(78)}\), while the methylation of polycomb target genes may be mediated by inflammation\(^{(79)}\). Furthermore, redox modulation of chromatin remodeling may have an effect on histone acetylation/deacetylation modulating the expression of pro-inflammatory genes\(^{(80)}\). Finally, enhanced levels of micro RNA125b are associated to increased to specific inflammatory gene expression on db/db mice\(^{(81)}\). A relevant locus-specific DNA methylation affecting inflammatory processes have been reported for at least the following genes: leptin (LEP), superoxide dismutase (SOD), glucocorticoid receptor (GR), peroxisome proliferator-activated receptor (PPAR), tumor necrosis factor-alfa (TNF-a), endothelial and inducible nitric oxide synthase (eNOS/iNOS) and hypoxia-inducible factor (HIF), whose epigenetic machinery understanding will contribute to the inflammation management and associated disorders such as obesity\(^{(4)}\).

Obesity-associated adipose tissue enlargement is often associated to an elevated secretion of proinflammatory adipokines such as leptin and cytokines such as TNF-alpha, whose epigenetic regulation has emerged as a potentially important gene expression determinant\(^{(82)}\). Thus, at baseline, obese women with better response to an energy restricted dietary intervention designed to induce weight loss showed lower promoter methylation levels of leptin and TNF-alpha than the non-responder group, which suggest that leptin and TNF-alpha methylation levels could be used as epigenetic biomarkers concerning the response to a low-calorie diet. Indeed, the methylation profile could help to predict the susceptibility to weight loss as well as some comorbidities such as hypertension or type 2 diabetes\(^{(83, 84)}\). Additional investigations concerning the interactions between obesity and inflammation under a epigenetic perspective have allowed to identify different CpG sites from WT1 and ATP10A genes, which were significantly modified as a result of an hypocaloric-diet-induced weight loss in humans by altering DNA methylation status of these specific genes indirectly related with inflammatory processes, suggesting that baseline DNA methylation patterns may be used as a prognostic epigenetic markers that could help to predict weight loss\(^{(85)}\).

Summing up, there is growing evidence suggesting that interindividual differences in obesity susceptibility depend not only in the DNA sequence (genetics) but also on epigenetic factors affecting gene expression such as DNA methylation, covalent histone modifications, chromatin folding and the regulatory actions of miRNAs and polycombs complexes, in which inflammatory phenomena may be involved. Thus, epigenetics is providing novel insights on cellular identity, stem cell flexibility, tissue regeneration, tumorigenesis, and aging and to understand monozygotic twin differences and interestingly the onset of chronic diseases in the adult such as obesity. The following aims are presently pursued in the ground of obesity and epigenomics: the early identification of epigenetic biomarkers concerned in individual’s disease susceptibility and the
description of weight lowering protocols for tailored dietary treatments/advises to avoid/neutralize likely adverse epigenomic events. Other questions that remain to be answered are to understand the regulation of epigenomic phenomena, the period(s) for intervention, the key nutritional factors and doses, which will allow diagnosis and prognosis implementation and will facilitate Preventive/curative strategies in a personalized “epigenomically” based manner to combat obesity.

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