The role of nutrition on epigenetic modifications and their implications on health

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A B S T R A C T

Nutrition plays a key role in many aspects of health and dietary imbalances are major determinants of chronic diseases including cardiovascular disease, obesity, diabetes and cancer. Adequate nutrition is particularly essential during critical periods in early life (both pre- and postnatal). In this regard, there is extensive epidemiologic and experimental data showing that early sub-optimal nutrition can have health consequences several decades later.

The hypothesis that epigenetic mechanisms may link such nutritional imbalances with altered disease risk has been gaining acceptance over recent years. Epigenetics can be defined as the study of heritable changes in gene expression that do not involve alterations in the DNA sequence. Epigenetic marks include DNA methylation, histone modifications and a variety of non-coding RNAs. Strikingly, they are plastic and respond to environmental signals, including diet. Here we will review how dietary factors modulate the establishment and maintenance of epigenetic marks, thereby influencing gene expression and, hence, disease risk and health.

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"You may be an undigested bit of beef, a blot of mustard, a crumb of cheese, a fragment of underdone potato. There's more of gravy than of grave about you, whatever you are!"

A Christmas Carol, Charles Dickens

2012 marks the celebration of the Bicentennial of Charles Dickens. It is a good time to review his great narratives. They are full of elaborated descriptions of children growing under some sort of nutritional deprivation (Oliver Twist, David Copperfield) or even famine (Tiny Tim). Here we will review our current knowledge about the relationship between early malnutrition, later disease risk and how epigenetic mechanisms may link them.

1. Introduction: the rise of the field of Nutritional Epigenomics

Diet constitutes one of the major environmental factors that exert a profound effect on many aspects of health and disease risk. For example, in industrialized countries, excessive caloric intake is a major determinant of complex chronic diseases, such as obesity, type 2 diabetes, cardiovascular disease and even cancer. According to the World Health Organization these diseases account for more than half of the deaths worldwide and have a huge impact on national economies (World Health Organization, 2003). Conversely, in poor countries, malnutrition and undernutrition, especially during the perinatal period, increase not only neonatal mortality and perinatal morbidities but also the risk of chronic diseases during adulthood [1–3]. This association between perinatal nutrition and late-onset disease has been conceptualized into the Developmental Origins of Health and Disease Hypothesis (DOHaD, Box 1 ([16–26,28,29,32])). Finally, a paradigmatic scenario is illustrated by chronic caloric restriction (CR). It has been shown that moderate global caloric restriction is the most powerful way to increase lifespan in various model organisms from different taxa, such as yeasts, worms (Caenorhabditis elegans), insects (Drosophila melanogaster) or mammals (including mice, rats and monkeys) [4].

Here we will review the evidence that supports a role for dietary factors, including micro-nutrients, macro-nutrients, and non-nutrient dietary components, in mediating disease risk through epigenetic modifications. Special emphasis will be put on the role of dietary factors during early perinatal development in the context of DOHaD. We will focus primarily on the Metabolic Syndrome,
short in time (6 months), started and ended abruptly and therefore it is clearly circumscribed in time and place. Second, the population was ethnically homogeneous and without remarkable prior differences in dietary patterns. Likewise, food availability during rationing was largely unaffected by social class. Third, the official food rations were known, so that the number of calories available could be estimated by place and time of birth. Finally, and most importantly, long-term follow-up was possible, since the childhood and adult medical histories of the fetuses that survived could be traced through national population registers.

In sum, for all these reasons, the Dutch Cohort constitutes an “excellent” population for the study of developmental programming of adult disease. In accord, a huge number of critical reports have already been published and many more will certainly be published in the future. Also, the first reports describing an association between nutritional imbalances in utero and altered epigenetic marks during adulthood have been described in subjects from this cohort [102,103].

Epigenetics can be pragmatically defined as the study of stable inheritance of gene expression that occurs without modifications in the DNA sequence [10]. Epigenetic mechanisms in mammals include DNA methylation, histone modifications and, more recently, a variety of non-coding RNAs (key epigenetic concepts are summarized in Box 2) [11]. In the context of this review, it is relevant to state that epigenetic factors may be modulated by environmental cues, including nutrition, and thus provide a mechanism by which genomes integrate environmental signals into permanent changes of gene expression that may ultimately lead to health and disease risk [10–14]. This recognition has ignited the rapid growth of a novel field: Nutritional Epigenomics [15].

Box 2.

Epigenetics: Stable inheritance of gene expression that occurs without modifications in the DNA sequence. Epigenetic mechanisms include DNA methylation, histone modifications and, recently, a variety of non-coding RNAs.

DNA methylation: It is a covalent modification that consists on the addition of a methyl group at cytosines of the DNA template. In mammals, DNA methylation occurs primarily at CpG dinucleotides.

CpG islands are regions in the DNA with a disproportional high abundance of dinucleotides CpG. Typically CpG islands exist around promoter regions of the genes.

Very recently, other DNA modifications, like hydroxymethylation, have been identified. The impact of these modifications on programming of adult disease is currently unknown.

Histones: Histones are alkaline proteins found in eukaryotic cell nuclei that package the DNA into structural units called nucleosomes. They are the main protein components of chromatin, acting as spools around which DNA winds, and play a role in gene regulation.
Histone modifications: Covalent modifications of histone residues that can alter chromatin states and, thus, gene regulation. Histone modifications include a series of complex post-translational modifications including methylation (mono-, di-, and tri-methylation), acetylation, SUMOylation, biotinylation, phosphorylation, ubiquitination, and ADP-ribosylation.

Histone code: The histone code hypothesis suggests that specific histone modifications (or combination of modifications) may confer unique biological functions to regions of the genome where they associate. Given the fact that there exist 4 different histones and multiple types of modifications across the residues of the proteins, the combination of modifications is extremely high. This would result in a complex, locus-specific regulation of gene transcription.

Non-coding RNAs (ncRNA): They are functional RNAs that are not translated into proteins. Non-coding RNAs include transfer RNA (tRNA), ribosomal RNA (rRNA) and small nucleolar RNA (snoRNA). Recently, a series of new RNAs with regulatory activity have been added to the list: siRNA (small interfering RNA), miRNA (microRNA) and piRNA (piwi RNA).

Genome and Epigenome: The genome is the totality of the genetic information of a cell/organism that is contained in the DNA sequence. The epigenome consists of all chemical modifications of DNA and histones of a cell/organism that contribute to regulate gene expression independently of DNA sequence. One single genome may give rise to several epigenomes depending on environmental conditions, tissue specificity, developmental stages, etc. It is proposed that this relation (1 Genome/ n Epigenomes) constitutes the basis for fundamental biological issues such as pluripotency and cell differentiation, phenotypic variation, etc.

Metastable epiallele: It is an epiallele (an allele that can stably exist in more than one epigenetic state, resulting in different phenotypes) at which the epigenetic state can switch and establishment is a probabilistic event. Once established, the state is mitotically inherited.


This review is structured in order to address the following four key questions:

1. **WHEN** do dietary factors influence the epigenome, thus leading to long-term changes in gene expression? It is remarkable to note that current evidence linking diet to epigenetic modifications can be narrowed down to two specific scenarios: First, during "critical windows" of early development (specially during fetal development and/or early neonatal growth) and, second, in adult individuals, during "Dietary Transitions" (such as high fat feeding, caloric restriction, etc.) occurring over a relatively long period of time (Fig. 1). Therefore, before extensively reviewing most relevant examples linking nutrition and epigenetic modifications, we will summarize the concepts of "critical windows" and "homeostasis vs. chronic dietary transitions" (Section 2).

2. **WHAT** are the evidences linking diet and epigenetic modifications? Most relevant studies describing nutritional variation and epigenetically-associated metabolic phenotypes will be summarized in Section 3 (Tables 1–3).

3. **HOW** do dietary factors influence the epigenome? In other words, what are the mechanisms that link dietary factors and epigenetic modifications? Molecular mechanisms are reviewed in Section 4 (Figs. 2–4).

4. **WHY** is nutrition regulating gene expression through epigenetic modifications, particularly during specific stages of development or during the course of Dietary Transitions? As yet, this is an open question that generates an intense debate. In this last section we will comment on the current thinking relating the biological meaning of nutrition during development and its impact on long-term regulation of gene expression.

2. **WHEN** do dietary factors influence the epigenome?

Under what circumstances does nutrition induce epigenetic modifications? Epidemiologic and experimental evidences linking diet to epigenetic modifications can be narrowed down to two scenarios (Fig. 1): (1) First, during "critical windows" of development, including fetal and early neonatal growth. (2) Second, during "Dietary Transitions" occurring over a long period of time in adult individuals. Typical examples of these Dietary Transitions are chronic overfeeding, high fat feeding or chronic caloric restriction.

2.1. Critical windows of development

Developing organisms are under dynamic changes, and organ systems undergo rapid development characterized by cell proliferation/differentiation. Epigenetic mechanisms during early stages of development contribute to faithfully maintain undifferentiated stem-cells on one hand, and organogenesis on the other one [33,34]. Thus, early embryogenesis in mammals is the most critical period for the establishment of the epigenome. In particular, between fertilization and implantation, the embryo demethylates the genome widely [35–37]. Short after implantation, there is a wave of re-methylation that sets the epigenetic patterns for different cell types. Therefore, these periods constitute critical spatiotemporal windows of development during which the epigenetic marks are either partially erased or re-set. Failure to complete these programs in time might be irreversible and lead to permanent dysregulation of gene expression [15,38]. Importantly, this is a period especially vulnerable to environmental cues, such as nutrition, that can disrupt the correct establishment of epigenetic marks that, once established, remain highly stable. Arguably, this is the reason why nutritional challenges during early windows of development might have such long-term effects in the context of DOHaD.

A striking example of the critical-window-concept arises from the Dutch Famine (Box 1) [39–41]. At the end of the Second World War, individuals from the Western Netherlands were exposed to acute undernutrition for a defined period of 4 months. The disease risk of the offspring’s of women who were pregnant during the Dutch Famine was different depending on if it was during the beginning, the middle or close to the end of gestation at the time of the famine. Individuals affected early in pregnancy have cardiovascular complications, including a pro-atherogenic lipid profile, and reduced cognitive functions [41–44]. Mid-gestational maternal undernutrition was associated with impaired kidney and lung function [41,45,46]. Lastly, individuals suffering starvation at the end of gestation had striking differences with regards to glucose tolerance at adult age, although this is a feature which is present in all groups at low levels [41,47]. Whether these differences are mediated, in part, by epigenetic
modifications remains unknown. But it is likely that (a) the time, (b) the intensity and (c) duration of an environmental factor may induce different epigenetic alterations in a tissue-dependent manner. At this point we lack a systematic survey describing the epigenomic modifications (and phenotypic effects) mediated by different dietary factors during specific well-controlled periods of development.

2.2. Dietary Transitions

Epigenetic variations are not only restricted to early windows of development but also may occur throughout an individual life-course (Figs. 1 and 2). Such epigenetic variations accumulate over a long period of time and may ultimately influence phenotypic outcomes (health and disease risk). This is clearly exemplified...
### Table 1
Summary of relevant studies showing effects of dietary conditions on DNA methylation in humans and model organisms.

<table>
<thead>
<tr>
<th>Dietary condition</th>
<th>Species</th>
<th>Period of dietary input</th>
<th>Tissue(s)</th>
<th>Methylation</th>
<th>Epigenetically regulated gene(s)</th>
<th>Observed phenotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>High fat diet</td>
<td>Mouse</td>
<td>Adult dietary transition</td>
<td>Brain (various regions)</td>
<td>↑</td>
<td>Oprm1 Th Dat</td>
<td>Dopaminergic (Th and Dat) and the opioid systems (Oprm1), which participate in the central regulation of food intake and the development of obesity, were altered.</td>
<td>[107,108]</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>Adult dietary transition</td>
<td>Islet cells</td>
<td>↓</td>
<td>Il13ra3</td>
<td>Progressive beta-cell dysfunction in islet cells from paternally high fat fed rats.</td>
<td>[111]</td>
</tr>
<tr>
<td></td>
<td>Mouse in utero</td>
<td></td>
<td>Brain</td>
<td>↓ ↓</td>
<td>Dat Mor Penk</td>
<td>Altered gene expression of dopamine and opioid-related genes may change behavioral preference for palatable foods and increase risk of obesity and obesity-related diseases.</td>
<td>[63]</td>
</tr>
<tr>
<td></td>
<td>Rat in utero</td>
<td></td>
<td>Liver</td>
<td>↓</td>
<td>Cdkn1a</td>
<td>Offspring from high fat fed dams developed hepatic steatosis and characteristics of non-alcoholic liver disease.</td>
<td>[89]</td>
</tr>
<tr>
<td>Neonatal overfeeding/overgrowth</td>
<td>Rat Neonatal</td>
<td></td>
<td>Hypothalamus</td>
<td>↑</td>
<td>Pmcr expr/leptin ratio</td>
<td>Early overfeeding resulted in a metabolic syndrome phenotype (obesity, hyperleptinemia, hyperinsulinemia, insulin resistance and diabetes).</td>
<td>[92]</td>
</tr>
<tr>
<td></td>
<td>Rat Neonatal</td>
<td></td>
<td>Hypothalamus</td>
<td>↑</td>
<td>Pmcr expr/leptin ratio</td>
<td>Same model than in [92]. Reduced insulin receptor expression leads to hypothalamic insulin resistance and predisposition to altered feeding behavior characteristic of this model.</td>
<td>[93]</td>
</tr>
<tr>
<td></td>
<td>Human Neonatal</td>
<td></td>
<td>Peripheral blood</td>
<td>↓</td>
<td>Tacstd2</td>
<td>Rapid postnatal growth is associated with increased childhood adiposity (9–15 years).</td>
<td>[189]</td>
</tr>
<tr>
<td>Low protein diet</td>
<td>Mouse Adult</td>
<td>Dietary transition—transgenerational effect</td>
<td>Liver</td>
<td>↑</td>
<td>PPARα</td>
<td>Increased hepatic cholesterol/lipid biosynthesis, increasing risk of fatty liver and steatosis.</td>
<td>[113]</td>
</tr>
<tr>
<td></td>
<td>Rat in utero</td>
<td></td>
<td>Global DNA hypermethylation</td>
<td>None (global analysis)</td>
<td>Low maternal protein availability during gestation results in glucose intolerance and hypertension in the adult.</td>
<td>[67]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rat in utero</td>
<td></td>
<td>Adrenal gland</td>
<td>↓</td>
<td>AT(1b)</td>
<td>Maternal low-protein diet resulted in the development of hypertension in the offspring.</td>
<td>[74,75]</td>
</tr>
<tr>
<td></td>
<td>Rat in utero</td>
<td></td>
<td>Hypothalamus</td>
<td>↑</td>
<td>Pmcr</td>
<td>Maternal low-protein nutrition can affect brain development and expression of orexigenic/anorexigenic genes.</td>
<td>[64]</td>
</tr>
<tr>
<td></td>
<td>Mouse in utero</td>
<td></td>
<td>Liver</td>
<td>↑</td>
<td>Lxrα</td>
<td>Protein restriction during pregnancy reduced Lxrα-dependent hepatic cholesterol biosynthesis.</td>
<td>[27]</td>
</tr>
<tr>
<td></td>
<td>Mouse in utero</td>
<td></td>
<td>Adipose tissue</td>
<td>↓</td>
<td>Lep</td>
<td>Offspring from mothers fed a low-protein diet showed increased food intake and increased adiposity. Reduced expression of Hnf4a contributes to beta-cell dysfunction and development of type 2 diabetes.</td>
<td>[76]</td>
</tr>
<tr>
<td></td>
<td>Rat in utero</td>
<td></td>
<td>Islet cell</td>
<td>↑</td>
<td>Hnf4a</td>
<td>Increased cytochrome c gene expression, may be involved in changed mitochondrial function</td>
<td>[206]</td>
</tr>
<tr>
<td></td>
<td>Pig in utero</td>
<td></td>
<td>Liver</td>
<td>↓</td>
<td>Somatic cytochrome c (CycS), PPARα GR</td>
<td>Altered expression of PPARα and the glucocorticoid receptor might contribute to altered carbohydrate/lipid homeostasis and hypertension, respectively.</td>
<td>[59,70,71]</td>
</tr>
<tr>
<td>Intrauterine malnutrition; placental artery ligation</td>
<td>Rat in utero</td>
<td></td>
<td>Liver</td>
<td>↑ ↓</td>
<td>Pdx1</td>
<td>Utero-placental insufficiency through bilateral artery ligation caused insulin resistance and diabetes in the adult.</td>
<td>[58]</td>
</tr>
<tr>
<td></td>
<td>Rat in utero</td>
<td></td>
<td>Islet cell</td>
<td>↑</td>
<td>Genome-wide HELP assay: 1400 loci differentially methylated (both hyper- and hypo-methylated).</td>
<td>Adult-onset type 2 diabetes. Diabetes was associated with progressive silencing of the transcription factor Pdx1. Same model as in [61]. Type 2 diabetes due, in part, to beta-cell dysfunction. Genome-wide DNA methylation analysis showed that alterations occurred near genes regulating processes such as vascularization, beta-cell proliferation, insulin secretion, and cell death.</td>
<td>[61]</td>
</tr>
<tr>
<td></td>
<td>Rat in utero</td>
<td></td>
<td>Genome-wide HELP assay: 1400 loci differentially methylated (both hyper- and hypo-methylated).</td>
<td>↑ ↓</td>
<td>validated loci are: Fgfr1</td>
<td></td>
<td>[66]</td>
</tr>
</tbody>
</table>
in studies with isogenic laboratory animals or monozygotic twins: In both conditions individuals are genetically identical. Yet, during aging one individual from the twin pair, or some individuals in a colony, shows phenotypic differences attributable to epigenetic factors. Examples include protein deficiency, hypercaloric diets, or caloric restriction, among others. This type of transitions may cause changes in gene expression, although potentially reversible, tend to be stable and contribute to the age-dependent increase of disease risk. The concept of Dietary Transitions, as it is used here, is better understood by contraposition to homeostasis. Homeostasis can be defined as the capacity of a system, generally a living organism, in maintaining a stable, constant set of parameters, such as nutrient levels, pH, temperature, etc. Homeostatic processes occur over a short period of time, ranging from seconds to days, and imply (many times) reversible changes in gene expression. A classic example is the homeostatic adaptation to feeding and fasting in mammals. During fasting glucagon is produced and activates the whole transcriptional program that regulates gluconeogenesis. This is characterized, in part, by a striking up-regulation of the expression of key gluconeogenic genes such as phosphoenolpyruvate carboxykinase (Pepck) or glucose-6-phosphatase (G6Pc). In feeding conditions, however, glucagon production is reduced and insulin represses gluconeogenesis, in part by inhibiting Pepck and G6Pase at the transcriptional level.

In contrast to homeostasis, during Dietary Transitions organisms are exposed over a prolonged period of time (ranging from weeks—months in rodents to years in humans) to a diet characterized by an excess or a deficiency in a particular set of nutritional factors. Examples include protein deficiency, hypercaloric diets, or caloric restriction, among others. This type of transitions may cause changes in gene expression, although potentially reversible, tend to be stable and contribute to the age-dependent increase of disease risk. The question now is to characterize the molecular mechanisms through which these types of dietary exposures may exert such long-term effects (Section 4).

3. WHAT are the evidences linking diet and epigenetic modifications?

Here we will review the most relevant studies linking variation in nutrition with epigenetic modifications. It is important to note that the most abundant and compelling evidence is based on studies relating early nutritional imbalances with later onset of chronic diseases in the context of DOHaD (Fig. 2). Whether this reflects a true biological scenario, meaning that early development is more sensitive to environmental cues than later stages of life, or an artifact, due to biased experimental designs aimed to search for epigenetic variation in early stages vs. later stages of life history, is not clearly established. Moreover, as these studies are generally
### Table 2
Summary of relevant studies showing effects of dietary conditions on histone modifications in humans and model organisms.

<table>
<thead>
<tr>
<th>Dietary condition</th>
<th>Species</th>
<th>Period of dietary input</th>
<th>Tissue</th>
<th>Histone modification(s)</th>
<th>Epigenetically regulated gene(s)</th>
<th>Observed (or associated) phenotype (health and disease)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>High fat diet (HFD)</td>
<td>Japanese macaques</td>
<td>In utero</td>
<td>Liver</td>
<td>↑Acetylation (H3K14)</td>
<td>Correlations between hepatic H3 and gene expression are absent or subtle (P &gt; 0.05).</td>
<td>Maternal high fat feeding increased fetal liver triglyceride accumulation. Likewise, hepatic histology correlated with non-alcoholic liver disease.</td>
<td>[91]</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>Adult dietary transition: from weaning to age &gt; 18 weeks</td>
<td>Brain</td>
<td>↑Acetylation (H3K9)</td>
<td>p16INK4a and p21Cip1</td>
<td>Chronic high fat diet resulted in altered food behavior (preference for sucrose diets) and obesity in the offspring.</td>
<td>[107]</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>Adult dietary transition: HF diet containing 45% Kcal from fat for 13 weeks</td>
<td>Liver</td>
<td>↑Acetylation (H3, H4)</td>
<td>Pck1</td>
<td>Obesity prone rats fed a high fat diet showed activation of the cellular senescence pathway (p16INK4a and p21Cip1), which was associated with hepatic steatosis.</td>
<td>[109]</td>
</tr>
<tr>
<td>Mouse</td>
<td>Mouse</td>
<td>Three consecutive generations (F0, F1, and F2)</td>
<td>Liver</td>
<td>↑Acetylation (H4)</td>
<td>LXRα and ERO1-a</td>
<td>The male offspring of the F2 generation (derived from both grand-maternal and maternal obesity) were highly susceptible to developing obesity and hepatic steatosis.</td>
<td>[110]</td>
</tr>
<tr>
<td>Pig</td>
<td>Pig</td>
<td>Gestation and lactation</td>
<td>Skeletal muscle</td>
<td>↑Acetylation (H3)</td>
<td>Mstn</td>
<td>Maternal low protein diet influences myostatin gene expression at weaning and finishing stages influencing muscle mass, and potentially insulin sensitivity, in the offspring.</td>
<td>[197]</td>
</tr>
<tr>
<td>Rat</td>
<td>Rat</td>
<td>Pregnancy and lactation</td>
<td>Liver</td>
<td>↑Acetylation (H3)</td>
<td>Cyp7a1</td>
<td>Body weight and liver growth were impaired in the male offspring. Likewise, circulating and hepatic cholesterol levels were increased in the adult offspring.</td>
<td>[82]</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>In utero</td>
<td>Skeletal muscle</td>
<td>↑Acetylation (H3, H4)</td>
<td>C/EBPβ</td>
<td>Low protein availability during gestation altered amino acid and energy homeostasis in skeletal muscle and fat deposition during muscle development in the offspring.</td>
<td>[198]</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>In utero</td>
<td>Liver</td>
<td>↑Acetylation (H3, H4, and H3K9)</td>
<td>GR</td>
<td>Increased hepatic expression of the glucocorticoid receptor in the offspring contributed to glucose intolerance and increased hepatic glucose production.</td>
<td>[70]</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>In utero</td>
<td>Liver</td>
<td>↑Acetylation (H4)</td>
<td>Asns; Atf3</td>
<td>Maternal low protein diet programmed the amino acid response pathway in the liver of the offspring. These alterations might potentially lead to liver dysfunction, including defective glucose homeostasis.</td>
<td>[199]</td>
</tr>
<tr>
<td>Mouse</td>
<td>Rat</td>
<td>In utero</td>
<td>Liver</td>
<td>↑Acetylation (H3)</td>
<td>Global H3 hyperacetylation in livers from P0 and P21 rat offspring.</td>
<td>Uteroplacental insufficiency (UPI) leads to increased risk of insulin resistance, hypertriglyceridemia, hyperglycemia and overt diabetes in the adult rat offspring.</td>
<td>[58]</td>
</tr>
<tr>
<td>Pig</td>
<td>Rat</td>
<td>In utero</td>
<td>Liver</td>
<td>↑Acetylation (H3K9, H3K14 and H3K18)</td>
<td>PGC1a and CPT1a</td>
<td>Same model as in [58]; changes in PGC1a and CPT1a may contribute to hepatic metabolic dysfunction.</td>
<td>[200]</td>
</tr>
<tr>
<td>Rat</td>
<td>Rat</td>
<td>In utero</td>
<td>Brain</td>
<td>↑Acetylation (H3K9Ac and H3K14Ac)</td>
<td>Global histone modifications (no specific loci are described)</td>
<td>UPI caused permanent changes chromatin structure of the hippocampus and the periventricular white matter of the offspring. These alterations might be associated to poor neurodevelopmental outcomes.</td>
<td>[68]</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>In utero</td>
<td>Liver</td>
<td>↑Acetylation (H3K9Ac and H3K14Ac)</td>
<td>Dusp5</td>
<td>Same model as in [58]; Dusp5 is a phosphatase that dephosphorylates Erk1 and 2, which in turn increases serine phosphorylation of IRS. IRS serine-phosphorylation contributes to hepatic insulin resistance.</td>
<td>[80]</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>In utero</td>
<td>Hippocampus</td>
<td>GR</td>
<td>Same model as [58,68]; intrauterine growth restricted rats showed</td>
<td>Same model as [58,68]; intrauterine growth restricted rats showed</td>
<td>[84]</td>
</tr>
</tbody>
</table>
conducted from the clinical perspective, with pathologies as readout, we currently do not know whether we miss the advantageous, evolutionary beneficial effects of epigenetic adaptations because of this biased view.

Recent articles have reviewed some aspects covered in this section [56,57]. Therefore, we have kept it short and summarized most experimental data in Tables 1–3.

3.1. Nutrition during early development: epigenetics and DOHaD

3.1.1. Animal models

The association between dietary changes during specific windows of development and epigenomic modifications has been reported in several animal models (Tables 1–3) [27,58–68]. They constitute an excellent tool to understand how particular nutritional regimens or specific dietary factors may influence the epigenome. The most widely studied nutritional challenges include protein deficiency, global caloric restriction, high fat feeding and excessive neonatal food intake. A special chapter is constituted by protein deficiency, global caloric restriction, high fat feeding and excessive neonatal food intake. A special chapter is constituted by

3.1.1.1. Protein malnutrition. Protein restriction is frequently used as a model for maternal malnutrition. Often, diets of 18% casein (control) and 9% casein (restricted) are compared, but sometimes other percentages of protein are used or restricted diets are compared to chow. This should be kept in mind when comparing different studies. Feeding a low protein diet to pregnant rats resulted in global DNA hypermethylation in livers from the offspring [67]. This was among the first studies showing a link between nutritional imbalances during intrauterine development and epigenetic modifications. More recent studies have also confirmed that maternal low-protein feeding during gestation may also result in locus-specific changes in DNA methylation (Fig. 2, Tables 1–3). More importantly, these changes remain stable until adulthood, thus providing a molecular basis for DOHaD. Reported genes (or loci) include the glucocorticoid receptor (GR), peroxisome proliferator-activated receptor alpha (PPARα) and liver X receptor-alpha (LXrα) in liver [27,59,69–73]; the hepatocyte nuclear factor-4-alpha (Hnf4α) in islet cells [65]; the AT(1b) angiotensin receptor in adrenal gland [74,75]; the orexigenic/anorexigenic genes neuropetide Y (Npy) and pro-opiomelanocortin C (Pomc) in hypothalamus [64]; and the leptin gene (Lep) in adipose tissue [76]. Importantly, in the previous examples, changes in DNA methylation correlate with altered gene expression. Therefore, such nutritionally-induced changes in DNA methylation may explain, at least in part, metabolic dysfunction in the adult. Hence, altered expression of GR, PPARα and LXrα may explain altered lipid metabolism and hepatic steatosis which in turn contributes to hepatic insulin resistance. Dysregulated expression of Hnf4α in islet cells may lead to beta-cell dysfunction and type 2 diabetes. Finally, Npy, Pomc and Lep regulate appetite in rodents. Therefore, aberrant expression of these genes may alter feeding behavior and explain the development of obesity and obesity-related diseases including insulin resistance and diabetes. In sum, there is now sufficient evidence to support that maternal protein malnutrition may induce permanent alterations in gene expression through epigenetic modifications. These alterations can contribute, in part, to the development of obesity, insulin resistance and type 2 diabetes in the adult.

3.1.1.2. Global caloric restriction: placental artery ligation. Global caloric restriction is another frequently used model for maternal malnutrition. Caloric restriction in animal models has been accomplished by either placental artery ligation or by global caloric restriction.

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**Table 2 (continued)**

<table>
<thead>
<tr>
<th>Dietary condition</th>
<th>Species</th>
<th>Period of dietary input</th>
<th>Tissue</th>
<th>Histone modification(s)</th>
<th>Epigenetically regulated gene(s)</th>
<th>Observed (or associated) phenotype (health and disease)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Increased expression of hippocampal glucocorticoid receptor, which is an important regulator of the hypothalamic-pituitary–adrenal axis. Intrauterine growth restriction altered PPARα expression, causing altered lung alveologenesis and postnatal lung disease in the male offspring. Intrauterine growth restriction resulted in adult-onset type 2 diabetes. Adult diabetes was associated with progressive silencing of the transcription factor Pdx1, which is critical for beta-cell function and development</td>
<td>[86]</td>
</tr>
<tr>
<td>Rat</td>
<td>In utero</td>
<td>Lung</td>
<td></td>
<td>[Acetylation (H3K9)]</td>
<td></td>
<td>50% caloric restriction during the last week of gestation represses skeletal muscle Glut4 expression in the adult rat offspring. 50% caloric restriction during gestation decreased H3K4Me2 and increased H3K4Me3 in the IGF1 locus.</td>
<td>[60]</td>
</tr>
<tr>
<td>Rat</td>
<td>In utero</td>
<td>Intrauterine growth restriction (50% caloric restriction)</td>
<td></td>
<td>[Acetylation (H3K14)]</td>
<td>Glut-4</td>
<td></td>
<td>[81]</td>
</tr>
<tr>
<td>Rat</td>
<td>In utero</td>
<td>Liver</td>
<td></td>
<td>[Methylation (H3K4Me2)]</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Bilateral placental artery ligation in rats has been widely used as a model of reduced nutrient and oxygen availability for the fetus [77,78]. This surgical procedure may both induce genome-wide DNA hypomethylation in fetal livers [58] and affect the histone code at specific loci in the offspring (Tables 1 and 2) [58,60–62,68]. For example, in utero undernutrition in rats reduces expression of the homeobox 1 transcription factor (Pdx1) in islet cells [61]; the dual specificity phosphatase 5 (Dusp5) [80] and cholesterol 7alpha-hydroxylase (Cyp7a1) [82] in liver; dual specific phosphatase 5 (Dusp5) and the glucocorticoid receptor (GR) genes in hippocampus [83,84]; 11beta-hydroxysteroid dehydrogenase type 2 (Hsd11b2) in kidney [85]; and the peroxisome proliferator-activated receptor gamma (PPARγ) in lungs [86].

Similar to what we have described for the low protein diet, some of the previously described genes can contribute to different aspects of the metabolic syndrome. For example, Pdx1 is a key transcription factor that regulates beta-cell differentiation. Hence, altered Pdx1 expression may lead to beta-cell dysfunction and diabetes. On the other hand, Dusp5 is a protein from MAPK-signaling pathway that can modulate insulin signaling. Thus, altered expression of Dusp5 may induce tissue-specific insulin resistance that can ultimately contribute to whole body insulin resistance and diabetes. In sum, all these data clearly establish that in rodent models altered gestational nutrition may induce chromatin remodeling at metabolically relevant loci, through changing histone marks.

To finish, we would like to notice a recent report from Nüskén and colleagues [79]. They have compared surgical uterine artery ligation with protein restriction in rats and found striking differences in the resulting phenotype [79]. Therefore, these acute and severe surgical interventions cannot be completely compared with any dietary regimen. It constitutes, though, a valuable model to understand developmental programming of the offspring in response to placental dysfunction/placental insufficiency which causes reduced nutrient and oxygen availability to the fetus.

### 3.1.1.3. Global caloric restriction: nutritional deprivation

In rats, 50% global caloric restriction during the last week of gestation resulted in reduced expression of the glucose transporter 4 (Glut4) in skeletal muscle from the offspring [60]. This alteration is mediated by specific changes of histone modifications (H3K14 deacetylation and increased H3K9 di-methylation). Glut4 is a landmark protein that allows insulin-stimulated glucose uptake into peripheral tissues. Therefore, altered epigenetic regulation of glut4 may contribute to the development of insulin resistance and diabetes in this rat model. In a similar rat model, 50% caloric restriction decreased the abundance of H3K4Me2 at the IGF1 locus of liver from the newborn offspring. This epigenetic modification alters

### Table 3
Summary of relevant studies showing effects of dietary conditions on micro-RNA expression in humans and model organisms.

<table>
<thead>
<tr>
<th>Dietary factor</th>
<th>Species, timing</th>
<th>Tissue(s)</th>
<th>Observed phenotype (miRNAs modulated)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal high fat feeding</td>
<td>Mouse (in utero)</td>
<td>Liver</td>
<td>Maternal high fat feeding prior to conception, during gestation and lactation changed the expression of 23 miRNAs (from 579 miRNAs present in a microarray) in livers from the adult offspring. Strikingly, methyl-CpG binding protein 2 was the common predicted target for several of the identified miRNAs (miR-709, -let7a, -122, -194 and -26a).</td>
<td>[177]</td>
</tr>
<tr>
<td>High fat feeding supplemented with linoleic acid</td>
<td>Mouse (adult)</td>
<td>White adipose tissue (WAT)</td>
<td>Expression of miR-103, miR-107 (lipid metabolisms) and miR-103, miR-107 (altered in obesity) changed in response to the treatment with conjugated linolenic acid, currently used to induce fat loss.</td>
<td>[179]</td>
</tr>
<tr>
<td>Biotin</td>
<td>Human (in vitro)</td>
<td>Primary human cells</td>
<td>Physiological concentrations of biotin increased miR-539 abundance in a dose-dependent manner, miR-539 regulates holocarboxylase synthetase, which catalyzes the covalent binding of biotin to carboxylases and histones.</td>
<td>[202]</td>
</tr>
<tr>
<td>Polyphenols from yaupon holly leaves (quercetin and kaempferol 3-rutinoside)</td>
<td>Human (in vitro); Mouse</td>
<td>Human colon cells;</td>
<td>Flavonol-rich fractions extracted from yaupon holly leaves exert anti-inflammatory properties in both human and mouse cells: 1. Quercetin and kaempferol 3-rutinoside up-regulated miR-146a in human colon cancer cells, which is a negative regulator of the pro-inflammatory factor NF-kB. 2. Quercetin treatment in mouse macrophages down-regulated the pro-inflammatory miR-155.</td>
<td>[180,203,204]</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Human; Mouse</td>
<td>Colon biopsies and caco-2 cells; fetal brain</td>
<td>Ethanol induced expression of miR-212, which causes gut leakiness, a key factor in human alcoholic liver disease; prenatal ethanol exposure changed expression of several miRNAs in fetal brain from mice (miR-10a, 10b, 9, 145, 30a, 152, 200a, 496, 296, 30e-5p, 362, 339, 29c, 154). miR-10 up-regulation mediated, in part, Hoxal down-regulation. Co incubation with folate reverted these effects.</td>
<td>[178,205]</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>Rat (Dietary Transitions)</td>
<td>Liver</td>
<td>Vitamin E-deficient diet (6 months) caused a down-regulation of miR-122a and miR-125b, which contribute to regulate lipid metabolism and cancer-inflammation, respectively.</td>
<td>[181]</td>
</tr>
<tr>
<td>Starvation</td>
<td>Rat</td>
<td>Liver</td>
<td>Mild starvation (12 h) increased hepatic levels of miR-451, –122a, –29b, Insig1, which in turn inhibits Srebp1 production, is a predicted target of miR-29.</td>
<td>[183]</td>
</tr>
</tbody>
</table>
IGF1 expression and contributes to post-natal catch-up growth and subsequent risk of diabetes in the adult [62,81].

Moderate caloric restriction (30%) to pregnant non-human primates (Baboon) decreased methylation in fetal kidney during early stages of gestation, whereas it increased DNA methylation by the end of gestation [87]. Likewise, DNA methylation was also increased in the frontal cortex during late gestational stages [87]. In a follow-up study, expression of the glucogenogenic enzyme

Fig. 2. Summary of the loci that show altered expression in association with an epigenetic modification. Results are grouped by dietary intervention, type of epigenetic event and window of intervention. Data included in this figure is derived from Tables 1–3, including humans and model organisms.

IGF1 expression and contributes to post-natal catch-up growth and subsequent risk of diabetes in the adult [62,81].

Fig. 3. Intracellular signals that translate nutrition into epigenetically-mediated metabolic phenotypes. A, diet, through not completely known mechanisms depicted by the black box, alters the epigenome. B, intracellular second-messengers synthesized in response to extracellular nutritional/energetic states and that are able to modulate the epigenome. C, the production of the second-messengers depends, directly or indirectly, from the synthesis of ATP (or the ATP/ADP ratio), which in turn is determined by the energetic state of the cell. ATP acts as a cofactor or it is necessary to fully activate the enzymes that catalyze the synthesis of NAD, FAD, α-KG and SAM. NAD (nicotinamine adenine dinucleotide), FAD (flavin adenine dinucleotide), α-KG (α-ketoglutarate), SAM (S-adenosyl methionine), ATP (adenosine triphosphate). 1: Class III histone deacetylase (sirtuins); 2: LSD1-containing domain histone demethylase; 3: jumonjiC-containing domain histone demethylase; 4: DNA methyl transferase; 5: histone methyl transferase; 6: nicotinamide/nicotinic acid mononucleotide adenylyltransferase; 7: riboflavin kinase and FAD synthase; 8: α-ketoglutarate dehydrogenase; 9: S-adenosyl methionine transferase.
phosphoenolpyruvate carboxykinase 1 (PCK1) was increased in the fetal liver [88]. Strikingly, up-regulation of this gene occurred in association with the hypomethylation of the PCK1 promoter. These data support that moderate maternal nutrient reduction in non-human primates causes organ-specific and gestational age-specific changes in DNA methylation. These changes may have long-term effects on fetal organ development [87] and be causative for metabolic dysfunction later in life [88].
3.1.1.4. High fat diet. In utero malnutrition (by either protein or global caloric restriction) is not the only experimental model by which maternal diet influences offspring epigenome during development. Hence, two recent reports showed that maternal high fat diet may alter DNA methylation and gene expression in the offspring. First, maternal high fat feeding during gestation altered methylation and gene expression of dopamine and opioid related genes in the brain from the offspring [63]. This change may influence behavioral preference for palatable foods, thereby increasing obesity and obesity-associated risk for metabolic syndrome. The second report demonstrated that offspring from mothers fed a high fat diet showed reduced methylation, and increased expression, of the cyclin-dependent kinase inhibitor 1A (Cdkn1a) during neonatal liver development [89]. This alteration is responsible for changing hepatic proliferation and liver size, two aspects that are compatible with the development of a fatty liver phenotype [90].

Interestingly, maternal high fat feeding also altered the epigenome of the developing offspring of the Japanese macaque [91]. Consumption of a high fat diet during gestation increased fetal liver triglyceride content and led to non-alcoholic fatty liver disease. These phenotypic adaptations occurred in association with increased histone acetylation at H3K14 and H3K18. Next, by chromatin immunoprecipitation assays, the authors were able to identify locus-specific H3 candidate genes, such as DnaJ (Hsp40) homolog, subfamily A, member 2 (DNAJA2) or glutamic pyruvate transaminase 2 (GPT2). It is currently unknown whether these two genes may contribute to the accumulation of lipids and the development of fatty liver. It will be certainly interesting to further explore the potential implication of these genes on liver metabolism.

It is interesting to remark that the number of reports linking maternal high fat feeding with late onset disease is lower as compared to those linking caloric deprivation or protein restriction. We believe that this is just a methodological bias, because the initial paradigm described in the DOHaD was maternal caloric deprivation, starting already with Barker’s focus on low birth weight. We expect that the number of studies focusing on maternal high fat feeding (or overnutrition, as a general idea) will grow over the next years. These studies will be extremely relevant because in Westernized societies the prevalence of maternal obesity (and maternal overnutrition) during gestation is increasing alarmingly.

To conclude, experimental data demonstrate that developmental programming of adult disease occurs at both sides of the spectrum, due to either caloric deprivation or nutritional excess. Although the specific mechanisms leading to adult disease in both situation will be likely different, the current evidences support that the epigenome might be a common molecular link between them.

3.1.1.5. Neonatal overfeeding. Nutritional effects on the epigenome are not limited to the intrauterine life, but extend to early neonatal period (Figs. 1 and 2). Thus, neonatal overfeeding in rats increased methylation of the promoter of the hypothalamic anorexigenic factor proopiomelanocortin, Pomc [92]. Permanent down-regulation of Pomc augments food intake, promotes obesity and may provide a mechanism to explain, in part, metabolic syndrome in this model [92]. Likewise, in a follow-up study, neonatal overnutrition increased mean methylation of the insulin receptor promoter in the hypothalamus [93]. This alteration might additionally contribute to induce hypothalamic insulin resistance, thus contributing to the development of metabolic syndrome. To finish, neonatal overfeeding in the mouse also provoked permanent modifications in DNA methylation in the liver from adult individuals, as assessed by CpG island microarrays (Pentinat & Jiménez-Chillaron, unpublished results). 91 loci were differentially methylated (49% hypermethylated, 51% hypomethylated). Cluster analysis demonstrated enrichment on developmentally-related genes (Wnt signaling pathway). Whether altered expression of Wnt proteins may mediate hepatic metabolic dysfunction remains to be determined.

To conclude this part, the data summarized above demonstrate that nutrition during early stages of development can induce permanent changes in gene expression of somatic cells through epigenetic modifications. The three important points that we would like to highlight are: (1) maternal malnutrition influences the epigenome of the fetus. (2) Some of the epigenetic marks established during early development remain stable until adulthood. (3) Perinatal malnutrition causes both global and locus-specific epigenetic modifications.

3.1.2. The agouti mouse model

The agouti viable yellow mouse (Avy) is a well established animal model for fetal programming studies and often used as a key example for the importance of epigenetic modifications [13,94–97]. The Avy allele resulted from the transposition of a murine retrotransposon upstream of the agouti gene. Although agouti is normally expressed only in hair follicles, its expression in other cells is regulated by methylation of this locus. Thus, agouti offspring varies in agouti expression depending on developmental methyl group availability. The agouti signaling molecule both induces yellow pigmentation and antagonizes the satiety signaling cascade (at the melanocortin 4 receptor in the hypothalamus). This results in variably yellow fur and susceptibility to obesity by hyperphagia in correlation to the level of DNA methylation. This clearly shows the direct link between nutrition, epigenetics, and the resulting phenotype.

Therefore, besides regulatory pathways involved in regulation of metabolism (like GR, Pomc and LXR, summarized above and in Table 1), several other genomic loci have been identified as being especially vulnerable to epigenetic modifications. The agouti viable yellow mouse and the axin fused mouse are the most prominent examples [98–100], but recently the first human examples have been described [101]. These loci are suitable proof-of-principle candidates for measuring changes in DNA methylation following dietary challenges. However, it should be noted that it is currently not clear whether this phenomenon is universal or may be only limited to some exceptional loci.

3.1.3. Human evidences

As noted previously, the Dutch hunger winter was a period late during World War 2 when the Western part of the Netherlands was blocked from food transports for 4 months (Box 1). There is plenty of data on health outcome available from the abovementioned cohorts, linking fetal environment (particularly nutrition) and postnatal health [39–41]. Very recently, the links between famine and epigenetic markers in adults have been examined. In an elegant study, Heijmans and colleagues isolated DNA from white blood cells of individuals being peri-conceptionally affected by famine [102]. They were among the first to demonstrate that the insulin-like growth factor 2 (IGF2) locus was less methylated in the famine group when compared to matched controls [102]. In a subsequent study they extended their analysis to more genes and examined sex-specific effects. DNA methylation in the famine-exposed group was increased for GNAS antisense RNA 1 (GNASAS), maternally expressed 3 (MEG3), interleukin 10 (IL10), ATP-binding cassette, sub-family A, member 1 (ABCA1) and leptin (LEP), while it was decreased for INS-IGF2 readthrough (INSIGF) [103]. Interestingly, they found that at least some of the epigenetic changes observed were sex specific. Until now, a detailed analysis of the putative physiological consequences of these findings is missing. However, it
is tempting to speculate that methylation changes in promoters of genes such as LEP (involved in satiety regulation) and ABCA1 (involved in cholesterol transport and HDL formation) may link early nutrition to adult metabolic disease. To finish, it is remarkable that in both studies differences in DNA methylation were apparent more than 60 years after birth. It remains to be determined whether this type of alterations are already present at birth and maintained throughout life, or appeared secondarily in response to progressive metabolic dysfunction. Here, careful physiological studies have to follow in future.

Seminal studies from the Dutch cohort have been followed by a series of reports: A recent study by Waterland and colleagues extended our knowledge of nutritional influences during gestation on the epigenome to seasonal changes in nutrition [101]. The authors examined DNA methylation in individuals from rural Gambia. There, nutrition during the rainy season is largely different from nutrition during the dry season. The rainy season is characterized by reduced nutrient availability whereas the dry season is characterized by high nutrient availability. The authors reported that several putative metastable epialleles (Box 2) were differentially methylated (BOLA3, FLJ20433, PAX8, SLITRK1, ZFYVE28). These loci are stochastically methylated early during development and in mice reflect nutritional influences. Here, this phenomenon could be demonstrated for the first time in humans. Importantly, the authors also examined the methylation of other loci which have been previously been identified as targets of differential methylation (e.g., LINE1, GNASAS, IL10) and failed to demonstrate any nutritional influences. This may indicate that the duration and severity of the malnutrition has a pronounced effect on the establishment of epigenetic effects.

The key question is what the relevance of these changes in metastable epialleles for human disease is. On one hand, it is not known whether they can influence adult metabolism in any way. They might be useful, though, as biomarkers of early nutrition. They can be a good tool to determine whether an individual has developed under nutritional stress or not. This information might be extremely useful in order to enroll positive individuals into specific programs aimed to prevent late onset metabolic dysfunction.

To finish, a set of very recent studies have determined patterns of DNA methylation in cells from cord blood [104–106]. For example, Einstein and colleagues analyzed global patterns of DNA methylation in hematopoietic stem cells (CD34+) from cord blood in intrauterine growth restriction and control babies by microarray analysis [104]. Bioinformatic analysis yielded that a small subset of 56 loci showed significant differences in methylation between groups. These genes were involved in processes critical for stem cell function (cell cycle, cellular maintenance). Strikingly, the diabetes-related gene hepatoctye nuclear factor 4, alpha (HNF4A) appeared among these differentially methylated loci. It remains unclear whether these changes will remain stable into adulthood and therefore contribute to diabetes risk (or chronic disease risk in general) later in life. In this regard, the authors suggest that epigenetic modifications in multipotent progenitor cells (such as the CD34+ cells analyzed in this study) might influence chronic diseases later in life as the cell population expands over time and induce functional changes during tissue differentiation and maturation. While very attractive, this hypothesis deserves further investigation. In any case, these types of studies are extremely important because of the potential use of DNA methylation at birth as an early marker of future disease risk [104–106].

In another recent set of studies, DNA methylation of several candidates was assessed in cord blood from two independent populations of children with normal birth weights [105,106]. Strikingly, the authors show that the methylation of retinoid X receptor alpha (RXRa) and endothelial nitric oxide synthase (eNOS) at birth correlated with adiposity by age 9 years [105]. In addition, in a follow-up study, DNA methylation of the promoter region of eNOS also correlated with bone mineral density at age 9 years [106]. Thus, these studies constitute the first proof of principle to show that DNA methylation at birth might be a powerful molecular marker (of early nutrition) for later risk of disease (adiposity, bone density). Additional data from other cohorts will validate this concept and additional follow-up studies to define whether these changes in methylation persist well into adulthood.

3.2. Adult nutrition during “Dietary Transitions”

As previously mentioned, epigenetic variations are not only restricted to early windows of development and may also occur throughout an individual life-course. However, the amount of data linking adult dietary interventions with epigenetic modifications is much more limited than that for dietary interventions during early development (Figs. 1 and 2), and it is yet unknown whether this is a bias or truly shows differential biological responses to different developmental stages. Regardless, as we will discuss here, dietary factors may influence the epigenome in adult individuals (Tables 1–3). Taking into account the available data, nutrition may induce epigenetic modifications in adults when it fulfills at least these two conditions: First, dietary interventions take place over a long period of time and, second, there is a transition from the previous to a novel type of diet. This is clearly exemplified in numerous animal models: from chow diet-to-high fat diet, from chow diet containing normal protein content-to-chow diet containing low protein content, from ad lib feeding-to-caloric restriction (CR), etc.

3.2.1. Chronic high fat feeding

Chronic high fat diet in mice (from weaning until 20 weeks of age) altered patterns of DNA methylation within the promoter regions of the genes encoding tyroxine hydroxylase, the dopamine transporter and the µ-opioid receptor in the brain [107,108]. These genes are part of the neurotransmitter systems that participate in the regulation of food intake. Thus, these epigenetically-induced alterations can contribute to the development of obesity and obesity-related diseases occurring later in life. In another rat model, high fat feeding in obese prone rats for 13 weeks resulted in increased transcription of p16INK4a and p21Cip1 in the liver [109]. These changes, which might contribute to liver disease, occur in response to modifications in the histones residing in the regulatory and coding regions of both genes.

Very recently, an interesting study explored the effect of continuous high fat feeding for three generations on the development of fatty liver in the mouse offspring [110]. At 4–6 weeks of age, C57BL/6 females (F0) were fed with a diet containing 60% Kcal of fat. This high-fat feeding was continued for two more generations, F1 and F2. After this nutritional intervention, the authors report that obesity occurred earlier and became more severe in F2 male offspring that in F1 and F0 mice. Likewise, F2 offspring also developed the highest degree of hepatic steatosis. Hepatic steatosis in F2 mice was accompanied by a transgenerational trend to up-regulate lipogenic genes, including fatty acid synthase (Fasn), stearoyl–coenzyme A desaturase 1 (Scd1), sterol regulatory element binding protein-1 (Srebp), liver X nuclear receptor alpha (Lxra), liver X nuclear receptor beta (Lxrb) or the endoplasmic reticulum oxidation 1 (Ero-1α). Strikingly, Lxra and Ero1α expression are explained, in part, by reduced relative protein levels of H3K9Me2 and H3K27Me3 binding to their promoter regions. Thus, the authors conclude that the effects described in F2 male offspring
are “presumably consequence of transregenerational accumulation of epigenetic modifications leading to accumulation of lipogenesis in the liver” [110]. In sum, a sustained dietary change for three generations leads to progressive accumulation of epigenetic modifications that may modulate metabolic phenotypes. To note, the effects described in F2 male mice are actually a combination of long dietary interventions, plus the nutritional impact received during development. It will be important to design appropriate experiments to dissect the relative contribution of developmental vs. adult nutrition on the development of fatty liver.

Interestingly, effects of high fat feeding may induce transgenerational (epigenetic) consequences: chronic high fat diet (during 10 weeks, from age 4 weeks) in male Sprague–Dawley rats programmed beta-cell dysfunction in their female offspring, which has not been exposed to high fat diet during its development [111]. Beta-cell dysfunction was characterized by altered expression of genes involved in Calcium-, MAPK- and Wnt-signaling pathways. This alteration may be attributed, in part, to changes in DNA methylation. This is exemplified by the interleukin 13 receptor alpha-2 gene (IL13ra2), which shows the highest fold change in expression in concordance with hypomethylation of its regulatory region. These authors argue that this is an example of non-genetic, intergenerational transmission of metabolic dysfunction through the paternal lineage. Since males only contribute to their offspring through the information contained in the sperm, it is pointed out that nutritional variations may influence the epigenome not only in somatic cells but also in cells from the germ line. Next, these modifications should remain after the reprogramming of the epigenome during the processes of meiosis and first post-zygotic divisions and inherited into the next generation offspring. While extremely plausible, direct evidence that this is actually happening in germ cells from this model is not experimentally provided [112] and alternative explanations might occur: For example, it might be possible that reported epigenetic alterations occurring in the rat offspring are not inherited from the father, but develop secondarily to the pre-diabetic phenotype that develops in response to the beta-cell dysfunction. Undoubtedly, an accurate analysis of the epigenome of germ cells and sperm will be necessary to ascertain that nutritional imbalances, such as high fat diet, may induce heritable epigenetic modifications in mammals.

3.2.2. Low protein diet
Transgenerational effects have also been shown in C57/BL6 male mice fed a low protein diet from weaning to age 9–12 weeks [113]. Offspring of males fed a low protein diet showed elevated hepatic expression of genes involved in cholesterol and lipid metabolism. Likewise, paternal low protein diet induced numerous changes of DNA methylation, as assessed by microarray analysis, in livers from the offspring. Among positive loci, an enhancer of the lipid regulatory protein PPARα was identified [113]. The authors conclude, as in the previous study, that paternal nutrition may program the epigenome of the germ line that, in turn, might be inherited and influence offspring disease risk, such as lipid–cholesterol metabolism. Again, a direct molecular link has not been shown yet, since the sperm epigenome from low protein fed male mice appeared normal [114]. Thus, the identification of the environmentally-induced epigenetic marks that are transmitted to the offspring will be a matter of intense research over the next years.

3.2.3. Diets containing methyl-supplements
A recently published work explored the contribution of a sustained dietary change on the epigenome of isogenic mice over the course of six generations [115]. The authors fed founder mice with methyl-supplements from 2 weeks prior of mating and maintained this diet over 6 generations. They report that such sustained diet increased DNA methylation variation in liver from the isogenic C57/BL6 mice. This study concludes that epigenetic modifications (DNA methylation) are stochastic in nature, and occur in both controls and nutritionally-treated mice. But, methyl-supplemented mice show a greater variability on positive differentially methylated loci. Again, as previously described by Li et al. [110], the accumulated variation in DNA methylation observed in mice offspring from the sixth generation results from combining inherited- and nutritionally-induced-epigenetic variation.

3.2.4. Caloric restriction (CR)
The effects of chronic caloric restriction have deserved special attention to the scientific community since it is, by far, the most powerful mechanism to extend lifespan in many animal models such as yeast, C. elegans, Drosophila and mammals (mouse, rat, and monkeys) [116–118]. It is important to note that CR not only increases maximal lifespan but also delays onset of chronic age-related diseases, including cardiovascular disease, type 2 diabetes, degenerative diseases and cancer in both nonhuman primates and humans [118–122]. Thus, as stated in the title of this review, CR constitutes an example where dietary interventions influence health, as opposed to disease risk. A number of recent reviews have covered the potential role of nutrition involved in aging and longevity through epigenetic mechanisms [123–128]. In this section we will just summarize the main aspects.

CR may exert its beneficial effects on aging-related degenerative diseases through multiple mechanisms, including (1) reduction of oxidative stress and (2) modulation of metabolic pathways through the endocrine system (insulin/IGF1 signaling) [4,129]. More recently, chromatin remodeling has been included as an additional key mechanism in mediating lifespan extension through CR [52,124]. In this regard, early evidences have shown that aging is associated with global DNA hypomethylation, in conjunction with hypermethylation of specific promoter regions, such as cyclin-dependent kinase inhibitor 2A (p16), Harvey rat sarcoma virus oncogene (H-Ras), runt-related transcription factor (RUNX), or retinoic acid receptor responder (tazarotene induced) 1 (TIG1) [130–135]. Likewise, global DNA hypomethylation has been observed in many different age-related diseases, including cancer, atherosclerosis or neurodegenerative diseases [136,137]. Global DNA hypomethylation and multiple changes in the histone code result in loss of chromatin integrity [138]. There is now emerging data to support that CR mediates its beneficial effects by modulating chromatin function and increasing genomic stability through reversing DNA methylation and increasing global histone deacetylases activity [124]. Thus, it has been shown that CR may reverse aberrant DNA methylation in specific loci, such as H-ras in rats, or p16 and RUNX3 in human samples, but not global hypomethylation associated to the process of aging [139]. Likewise, CR may also reverse aberrant locus-specific DNA methylation in age-related disorders such as obesity. Accordingly, short-term CR on obese people may change DNA methylation is specific loci including ATPase, class V, type 10a (ATP10a), Wilms tumor 1 (WT1) or tumor necrosis factor α (TNFα) [140–143]. It has been proposed that these changes might be useful as indicators of diet-induced weight loss responders vs. non-responders. To finish, CR influences expression of specific genes associated to age-related diseases (p16/WDHD1, cancer) and senescence (Human Telomerase Reverse Transcriptase, hTERT) through modulating the enrichment binding of HDAC1 to their promoter regions [144,145].

3.2.5. CR and sirtuins
Recent experimental data suggests that CR mediates its effects through the activation of the members of the Class III of histone deacetylases (HDAC), also known as the sirtuin family. Sirtuins are
NAD+ dependent HDAC (see section 4) that have been linked to regulation of CR-mediated lifespan [55]. Among mammalian sir-tuins, sirtuin 1 (SirT1) is best characterized and has been one of the key players translating CR into biological responses in mammals [146,147]. SirT1 is activated in response to CR and increases lifespan in most model organisms [148]. The role of other sir-tuins in mammals in CR-mediated increased lifespan is not clearly established [55]. SirT1 acts as a metabolic sensor and its activation in response to CR mediates a series of metabolic adaptations compatible with aging retardation: (1) Increased stress resistance by regulating the tumor protein p53 and the forkhead box O gene (FOXO); (2) inhibition of lipogenesis and regulation of mitochondrial function and glucose homeostasis [53]. Importantly, the beneficial effects of SirT1 are mediated through directly deacetylating target proteins, such as stress-dependent transcription factors (FOXO, NF-kB, p53), transcription factors involved in regulation of metabolism, including liver X receptor (LXR), glucocorticoid receptor (GR), peroxisome proliferator activated receptor gamma coactivator 1 alpha (PGC1α) and liver kinase B1 (LKB1), or cell growth-proliferation Target of rapamycin (TOR) [149–153]. On the other hand, SirT1 regulates multiple functions through coordination of heterochromatin formation deacetylation of H4K16Ac and H3K9Ac residues [124].

To finish, given the promising role of sir-tuins (or at least SirT1 in mammals) in mediating lifespan, the search for activators of sir-tuins has been a very active field. In this regard, a component of grape and red wine, resveratrol, has been shown to be a potent activator of SirT1 in vitro and in vivo [55,142]. Although it is not completely clear whether the effects of resveratrol in vivo are SirT1-dependent or independent, its identification opens the possibility of searching for molecules in the diet that might mimic, in part, the beneficial effects of CR. Accordingly, two chemical activators of SirT1 (SRT1720 and SRT2183) have protective effects against age-related effects on metabolic dysfunction [154].

4. HOW do nutrients modify the epigenome? MECHANISMS

So far, along this review we have described that nutrition may induce epigenetic modifications in mammals. But, how do dietary components bring about epigenetic modifications? This question is visually depicted by the black box in the Fig. 3A. Over the last few years, the molecular mechanisms that translate nutritional variation into epigenetic modifications have started to emerge. We will describe the main findings below.

4.1. Nutrition factors and DNA methylation

There are now mounting evidences supporting that nutrients may modify the pattern of DNA methylation, either at the global scale or at locus-specific sites (Table 1). It has been proposed that nutrition influences patterns of DNA methylation in three possible ways (Fig. 4A,B): First, by providing directly the substrates necessary for proper DNA methylation. Second, by providing the co-factors that modulate the enzymatic activity of DNA methyltransferases (DNMTs) which catalyze the incorporation of methyl-groups into DNA. Three, by altering the activity of the enzymes that regulate the methionine cycle (also known as one-carbon cycle) which in turn provide the bioavailability of methyl-groups. Obviously, all 3 mechanisms are not mutually incompatible and may operate together in time. Evidence that supports these three mechanisms is reviewed in more detail below.

4.1.1. Methyl-donors from diet

S-Adenosyl-methionine (SAM) is the universal methyl-donor for methyltransferases, including both DNA methyltransferases and protein methyltransferases [155] (Fig. 4A). SAM is synthesized in the methionine cycle from several precursors present in the diet, including methionine, folate, choline, betaine and vitamins B2, B6 and B12 (Fig. 4B) (reviewed in [12,56]). All of them enter at different sites in the methionine pathway and contribute to the net synthesis of SAM. Therefore, it has been proposed that reduced availability of methyl donors will result in low SAM synthesis and global DNA hypomethylation. Conversely, increased availability of methyl donors will result in the opposite effect.

Accordingly, it has been shown that diets deficient in methyl donors (no folate, no choline and very low methionine) result in global DNA hypomethylation in rodents [156,157,190–192]. Likewise, low protein diets may result in reduced availability of the methionine precursor homocysteine and lead to DNA hypomethylation [158]. Conversely, maternal diet supplemented with methyl donors increases DNA methylation in specific loci [99,100,159,194]. Whether high methyl-donor intake also results in global DNA hypermethylation remains as yet undetermined.

Although the previous data support the idea that changes in DNA methylation are mediated, in part, through the provision of methyl-donors from diet, recent studies have pointed out to a more complex scenario: First, global methylation profiling, by means of specific microarrays, has shown that in mice, low protein or 50% global malnutrition during gestation leads to both hypermethylation and hypomethylation at specific loci in the offspring [27] (Martinez and Jimenez–Chillaron, unpublished data). Also, human studies have shown that exposure to maternal folic acid supplementation before or during pregnancy decreased methylation levels at the differential methylation region of H19, which is a negative regulator of IGF2 [195]. Likewise, in utero undernutrition in humans resulted in both hypo- and hyper-methylation of different specific loci [101–103]. Although it is not reported whether the amount of methionine (and methyl-donors) is reduced in these specific studies, it is commonly accepted that maternal undernutrition correlates with reduced methyl-donor availability. Thus, an accurate measurement of these precursors will be extremely helpful to understand the role of methyl-donors on the establishment of methyl-DNA. In sum, these and other forthcoming articles point out that there is not a simple correlation between methyl donor concentration and DNA methylation. Hence, other mechanisms might contribute, together with the availability of methyl donors, to set patterns of DNA methylation in cells.

4.1.2. DNMT activity

DNA methyltransferases require SAM as a cofactor for their full activation (Fig. 4B). As we have outlined in the previous section, methyl donors from the diet may contribute to modulate DNMT activity by changing the intracellular concentration of SAM. In addition, dietary polyphenols, such as epigallocatechin 3-gallate (EGCG), found in green tea, or genistein, present in soybean, are able to inhibit DNMT, at least in mice [95,161,193]. Importantly, in one study the authors confirm that genistein does not seem to exert its effects on DNA methylation through the one-carbon cycle because both SAM and S-adenosyl-homocysteine concentrations remained unaltered [161].

The clinical interest that arises from these types of studies is that it is potentially feasible to modulate patterns of DNA methylation by increasing the availability of polyphenols through dietary supplementation. It is questioned, though, whether consumption of these polyphenols from beverages and diets may have any effect on DNA methylation in humans, because they are present at a very low concentrations in a normal diet [162]. As yet, experimental data is lacking to show that this type of supplementation will influence DNA methylation with no side undesired toxic effects. Therefore,
more studies are needed in order to fully establish its viability as a dietary supplement with therapeutic effects.

4.1.3. Activity of enzymes from the methionine cycle

Vitamins B6 and B12 are cofactors involved in the regulation of the catalytic activity of enzymes from the folate cycle, thus determining SAM bioavailability (Fig. 4A,B). Specifically, vitamin B6 regulates the activity of serine hydroxymethyl-transferase (SHMT) favoring the conversion of folic acid into 5,10-methylene THF. Vitamin B12 is a cofactor of the 5-methyltetrahydrofolate-homocysteine methyltransferase (MTR) that catalyzes the conversion of homocysteine (Hcy) into methionine, the direct precursor of SAM. Therefore, bioavailability of these cofactors may influence DNA methylation by modifying the activity of the one-carbon cycle and the production of SAM [12].

Thus, it is conceivable that supplementing diets with these vitamins will contribute to the maintenance or establishment of DNA methyl marks. An indirect proof of principle is provided by the effects induced by excessive ethanol consumption: High ethanol consumption inhibits the availability of vitamins B6 and B12, thus interfering with the production of SAM and appropriate DNA methylation, through the folate/methionine cycles [163].

4.2. Nutrition factors and histone modifications

Histones may undergo a series of post-translational modifications, including methylation, acetylation, SUMOylation, ubiquitination, phosphorylation, or ADP ribosylation, which alter their activity and, therefore, chromatin states (reviewed elsewhere in this special issue of Biochimie). There is evidence supporting that nutritional factors may influence some histone modifications.

4.2.1. Nutrition and histone methylation

We have described multiple examples where nutrition changes patterns of histone methylation (Table 2). Similar to their role in DNA methylation described in the previous Section 4.1, dietary methyl donors may contribute to change patterns of histone methylation through the provision of SAM, produced through the one-carbon cycle (Fig. 4A,B). Histone methylation is a function of the opposing activities of histone methyltransferases (HMTs) and histone demethylases (HDMs). SAM is a cofactor necessary to fully activate HMTs (Fig. 4B). Therefore, dietary methyl donors may modulate levels of histone methylation through the regulation of HMT activity. On the other hand, the activity of histone demethylases may be modulated by metabolic cofactors produced during the metabolism of high-energy nutrients (carbohydrates, proteins or fat). There are two types of HDMs: The LSD1-containing domain demethylases and the JumonjiC (JmjC) domain containing demethylases [162]. Each type of HDM requires a different coenzyme: the LSD1-containing domain HDM uses flavin adenine dinucleotide (FAD) as a cofactor, whereas the JmjC-containing domain HDM requires α-ketoglutarate (α-KG) [164,165]. Therefore, as we will discuss later, it is proposed that extracellular nutrient availability will influence histone methylation through metabolism of energy-containing molecules and production of extracellular nutrient availability will thus change pattern of histone methylation through this proposed mechanism.

4.2.2. Nutrition and histone acetylation

Histone acetylation depends on the opposing activities of histone deacetylases (HDAC) and histone acetyltransferases (HAT). Many studies show that several nutrients are able to modify the activity of Histone Deacetylases (HDAC) (Table 2). There exist three classes of histone deacetylases (I, II, III). Classes I and II HDAC are inhibited by short-chain carboxylic acids and polyphenols, whereas Class III HDACs, also known as sirtuins, require nicotinamide adenine dinucleotide (NAD+) as a cofactor.

4.2.2.1. HDAC I and II. It is long-known that butyrate, a short-chain carboxylic acid (C4) produced by bacterial carbohydrate fermentation in the intestinal lumen, is a potent inhibitor of Classes I and II HDAC, thus leading to histone hyperacetylation in vitro and in vivo [166–168]. A series of studies have linked the production of intestinal butyrate with transcriptional regulation mediated by changes in histone acetylation and colon cancer risk. Whether this also applies for metabolic dysfunction is still controversial and needs further evaluation. It has been proposed that diet composition will result in different concentration of butyrate that will lead to a gradient of histone acetylation. This mechanism may theoretically link nutrition, the bacterial flora and epigenetic regulation.

To note, butyrate is not the only fatty acid in mediating changes in histone acetylation: Indeed, acetate (C2), propionate (C3), valerate (C5) and caproate (C6) may also induce hyperacetylation of histones, but to a lesser extent than butyrate (C4) [167]. In addition to carboxylic acids, other dietary compounds, including iso-thiocyanates and allyl sulfides present in cruciferous plants and garlic respectively, may modulate histone acetylation, through modulation of HDAC and/or HAT activities [169,170].

4.2.2.2. HDAC III (sirtuins). Special attention has recently been received by the Class III of histone deacetylases, also known as sirtuins because they can mediate, in part, the beneficial effects of caloric restriction on lifespan [123,171]. The role of nutritional regulation on Class III HDAC has been recently reviewed [55]. Sirtuins use NAD+ as cofactor to deacetyl target proteins [172], which is synthesized from amino acids. Thus, hypercaloric diets give rise to a low NAD+/NADH ratio and, consequently, low sirtuin activity. Conversely, caloric restriction results in a high NAD+/NADH ratio, thus increasing sirtuin 1 activity. Therefore, it has been proposed that sirtuins can mediate nutritional-dependent chromatin states, through its capacity to sense cellular energy state, based on the NAD/NADH ratio [124].

To finish, natural dietary polyphenols may influence histone acetylation through modulating the activity of HDAC or HAT. Thus, it has been shown that SirT1 activity may be modulated by a natural polyphenol, resveratrol, that is particularly abundant in red grapes (and red wine) [173]. Likewise, dietary polyphenols from green tea may act as histone acetyl transferases inhibitors (HAT) [174–176]. Given this relationship it is tempting to suggest that dietary compounds may influence, at least in part, gene expression through modulation of HDAC–HAT activity and resulting in histone hyper- or hypo-acetylation.

4.2.3. Nutrition and other histone modifications

At this point it is not known whether dietary factors may influence other histone marks. It is plausible through, that this might be the case given the fact that nutrients have a wide range of implications in the cell. Nevertheless, the impact of specific dietary components on histone modifications other than methylation or acetylation, thus influencing gene expression and phenotype, remains to be fully characterized.

4.3. Nutrition factors and non-coding RNAs

Recently, non-coding RNAs have extended the list of molecular mechanisms with epigenetic regulatory potential [11]. One of the
most widely studied non-coding RNA is the microRNA (miRNA). As reviewed in this special issue of Biochimie, miRNAs are a large family of small non-coding RNAs (20–22 nucleotides long). They can regulate expression of up to 30% of the human genome, primarily through post-transcriptional targeting of mRNA. Recent evidences support that a wide range of nutrients, including fat feeding, protein, alcohol, vitamin E, hormones and a number of polyphenols may alter expression of specific miRNAs [177–183,202–205] (Table 3).

Specifically, maternal high fat feeding during gestation and lactation changed the expression of 23 miRNAs in liver from the offspring [177]. Likewise, maternal exposure to ethanol also changed the expression of several miRNAs in the fetal brain from the offspring [178]. At this point, it remains undetermined whether these altered patterns of miRNA expression contribute to increase adult disease risk. Given its wide regulatory capacity it is highly plausible that some of the altered miRNAs may contribute to the development of unhealthy phenotypes later in life.

Likewise, adult Dietary Transitions also contribute to alter either global or specific expression of miRNAs. Thus, supplementing linoleic acid for 4–9 weeks to high fat fed mice changed the expression of lipid/obesity specific miRNAs in white adipose tissue (miR-130, −107) [179]. On the other hand, polyphenols from yaupon holly leaves, such as quercetin, down-regulated the pro-inflammatory miR-155 in mouse macrophages [180]. In addition, vitamin E deficiency in rats (6 months) caused a down-regulation of miR-122a and miR-125b, which contribute to regulate lipid metabolism and inflammation, respectively [181].

At the molecular level, it is not well characterized the way nutrition modulates miRNA abundance. But it is proposed that it can be achieved through transcriptional regulation, via RNA-Pol II, in a similar fashion than miRNAs [179].

**4.4. Nutrition: physiological and pharmacological regulation of the epigenome**

In this section we will discuss how dietary nutritional factors on one hand and non-nutrient dietary compounds on the other one might have clinical relevance. Nutritional factors may play a role as physiological regulators of the epigenome whereas non-nutrients might be relevant as pharmacological modulators of the epigenome (Fig. 5).

4.4.1. Dietary nutritional factors: physiological regulation of the epigenome

Nutrients can be subdivided arbitrarily into two categories: macronutrients and micronutrients. Macronutrients include carbohydrates, protein and fat. Macronutrients are metabolized in the cell, giving rise to a number of intracellular signals, including SAM, FAD, α-ketoglutarate or NAD⁺, that, in turn, influence the establishment of epigenetic marks (DNA methylation, histone methylation and histone acetylation) (Fig. 3B). Therefore, these cofactors can be considered as intracellular signals that convey extracellular nutritional status into epigenetically-derived metabolic responses (phenotypic variation).

It has been recently proposed that ATP might be a potential signal that integrates the energy contained in high-energy macronutrients [162] onto the biosynthesis of these specific coenzymes SAM, FAD and α-KG (Figs. 3C, 4C and 5). Specifically, ATP is required for the activity of the enzyme S-adenosyl methionine transferase (MAT), which in turn converts methionine into SAM. Likewise, FAD, required for the LSD1-containing domain histone demethylases, depends on ATP for it synthesis, α-ketoglutarate, the coenzyme for the JmjC class of HDMs, is produced in the TCA cycle from glutamate, through the catalytic action of α-ketoglutarate dehydrogenase (α-KGDH). Strikingly, ATP regulates levels of α-ketoglutarate through inhibition of α-KGDH activity. To finish, intracellular NAD⁺ level also fluctuate in response to extracellular macronutrient availability. During periods of feast ATP/ADP ratios are high and there is net conversion of NAD to NADH (and low sirtuin activation). In contrast, during fasting, or periods of caloric restriction, intracellular concentrations of NAD are high and consequently increased sirtuin activity [55]. In sum, ATP might be a common link between nutrition (i.e. the energetic state of the cell) and the generation of the multiple second-messengers that induce physiological appropriate epigenetic adaptations to intracellular energetic conditions.

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**Fig. 5.** Epigenetic modifications mediated by dietary compounds: macronutrients, micronutrients and non-nutritional dietary factors.
In the context of this review, we have included the group methyl donors in the class of micronutrients because they are present at a very low concentration in a regular diet. Methyl donors are metabolized through the folate and methionine cycles in order to produce SAM. To note, SAM production through the one-carbon cycle requires ATP. So, although methyl donors may influence SAM content in a substrate concentration-dependent manner, ATP (possibly produced from macronutrient metabolism) is also required in order to provide SAM for DNA and protein methylation. This suggests that micronutrients (or at least methyl donors) at physiological levels do not largely influence the epigenome if additional signals from diet (ATP) are missing. Whether superphysiological pharmacological doses may induce epigenetic modifications independently from ATP (or any other dietary-derived signal) clearly needs further investigation.

4.4.2. Dietary non-nutritional factors: “pharmacological” regulation of the epigenome

Non-nutrient (i.e. non-metabolized) dietary factors may also induce changes in the epigenome (Fig. 5). The main difference with nutrients is that they do not require its own metabolism/oxidation to generate additional signals and messengers that can indeed modify epigenetic marks. Therefore, its influence on the epigenome will largely depend on its bioavailability characterized by its extracellular concentration, its transport into cells and stability. Despite moulting proof to support that these dietary factors, primarily polyphenols, may induce epigenetic modifications, the question is whether they are physiologically relevant in humans on a normal diet. Generally, these non-nutrient dietary factors are present at very low concentrations and, although may potentially influence the epigenome, their relevance might be limited. In contrast, diets deficient in one or more polyphenols or diets and beverages containing them at pharmacologic levels might have an impact on the epigenome. Nevertheless, more studies are currently needed in order to fully establish their relevance on health and disease.

To conclude, diet can remodel chromatin as a function of (1) intracellular energy status and (2) bioavailability of non-nutrient dietary coenzymes.

This distinction leads to the following implications: first, nutrients (both micro- and macro-nutrients) are the main metabolic substrates that influence the epigenome. Therefore, nutritional imbalances, specially occurring during sensitive periods of growth or during chronic Dietary Transitions, may permanently change patterns of gene expression through modifying the epigenome. Second, non-nutrient dietary factors, when present in physiological concentrations, should not largely influence the establishment of epigenetic marks. In contrast, diets deficient in one or more polyphenols or at pharmacologic levels might have an impact. This point is of great relevance because it opens a window for the development of pharmacological or nutritional interventions aimed to modify metabolic phenotypes by influencing, at least in part, the epigenome.

5. Final considerations: WHY does nutrition regulate gene expression through epigenetic mechanisms?

The aforementioned studies convincingly show that nutrition during critical periods of development induces epigenetic changes in a variety of organs and thus permanently influences the physiology of the individual. Similarly, long-term dietary interventions (Dietary Transitions) can induce epigenetic changes at least in animal models. Therefore, it is not under debate that nutrition is influencing the epigenome, but why. In this section, we note the questions that we believe need to be addressed urgently.

In this regard, it has been proposed that the nutritionally-derived epigenetic changes induced during the perinatal development in mammals may be a preparation for the environment-to-be-expected. This is now discussed as the Predictive Adaptive Response Hypothesis [30,31,184]. It states that developing individuals sense their environment as a prediction of the environmental conditions that a foetus/neonate will eventually encounter during adulthood. Thus, such developing individual will adopt physiological and anatomical modifications that will be advantageous in a predicted environmental condition. The epigenome is the substrate where the environment may induce such long-term permanent changes. Thus, the fetal/neonatal epigenome is modified as a reaction to maternal environment to prepare the offspring for future environmental clues after birth and therefore increase its evolutionary fitness. Although this hypothesis is compelling for the survival of small, short lived mammals, its significance in humans needs further debate.

Accordingly, we note that, until now, it is not clear whether epigenetic modifications upon maternal malnutrition are a pathological side effect of the shortage in nutrients, or a coordinated response to environmental challenges. It has been proposed that the former is true, mainly based on the fact that maternal undernutrition in animal studies led to hypomethylation of several gene promoters [59]. However, untargeted genome-wide studies have shown that the number of hypermethylated genes under these conditions is comparable to that of hypomethylated genes [27,87,88]. Moreover, pioneering studies on protein restriction have even shown global hypermethylation [67]. Similarly, also the Dutch Famine studies revealed both hypomethylation and hypermethylation loci in individuals perinatally undernourished [103]. This together, points to a directed response rather than a simple shortage of methyl donors.

5.1. Final considerations

So far, the DOHaD ideas are merely of academic value, but they might have a strong practical impact in the near future. Many of the epigenetic modifications induced by nutrition can be assessed in peripheral blood, as exemplified by the Dutch Famine data [102,103]. Therefore, they may be useful as additional biomarkers to retrospectively determine nutrient deficiency and prospectively to define individuals at risk for metabolic diseases. This is linked to a potential application of nutrients as epigenetic modifiers: opposite to the fetal situation, the newborn is easily reachable by nutritional manipulations. This means that early (baby) nutrition may in future be used to epigenetically program an individual which is less susceptible to chronic disease at adult age. This could even be combined with the aforementioned use of epigenetic biomarkers. However, this use clearly needs not only further scientific but also ethical consideration which goes far beyond the focus of this review.

More and more studies now focus on the influence of the gut microbiota for human health, especially with regards to the development of obesity. It has been demonstrated in mouse models that germ-free mice consume more food but accumulate less body fat than conventional, non-germ free mice [185]. Colonization of germ-free mice by conventional murine bacterial flora induces hepatic lipogenesis in the host. Moreover, germ-free mice are protected from high-fat diet induced obesity. Lastly, it has been demonstrated that the colonic flora of obese mice (ob/ob) varies from that of lean control mice; when transferred to germ-free mice, the recipients of the ob/ob-derived flora showed a significantly higher fat gain [186]. Interestingly, the bacterial flora produces large quantities of specific metabolites. It has been proposed that...
these metabolites directly influence gene expression in the host and, hence, influence metabolic rates [187,188]. It is important to note that these substances include several epigenetic modifiers (folate, butyrate) which in large quantities reach the intestinal epithelium, epithelial stem cells and, via the portal system, the liver. The epigenetic properties of folate and butyrate have extensively been discussed above. In our opinion future research needs to investigate to what extent these bacterial metabolites influence the epigenome of the host.

In summary, it has been known for a long time that the composition of the diet, and adequate amount, are a pre-requisite for a healthy life. Charles Dickens, with whom we started this review, already knew about healthy infant nutrition. Data from the last two decades now prove that this needs to be extended to even the fetal period. Thus the old saying you are what you eat is too simple and we should start considering that in addition you are what your parents ate.

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