DIETARY FACTORS, EPIGENETIC MODIFICATIONS AND OBESITY OUTCOMES: PROGRESSES AND PERSPECTIVES

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ABSTRACT

Nutritional factors play a life-long role in human health. Indeed, there is growing evidence that one of the mechanisms by which nutrients and bioactive compounds affect metabolic traits is epigenetics. Complex interactions among food components and histone modifications, DNA methylation, non-coding RNA expression and chromatin remodeling factors lead to a dynamic regulation of gene expression that controls the cellular phenotype. Although perinatal period is the time of highest phenotypic plasticity, contributing largely to developmental programming, also during adulthood there is evidence about a nutritional influence on epigenetic regulation. Similarly to type 2 diabetes, hypertension, atherosclerosis and other metabolic disorders, obesity predisposition and weight loss outcomes have been repeatedly associated to changes in epigenetic patterns. Different non-nutritional risk factors that usually accompany obesity seem also to be involved in these epigenetic modifications, especially hyperglycemia, inflammation, hypoxia and oxidative stress. There are currently three major objectives in epigenetic research in relation to obesity: to search for epigenetic biomarkers to predict future health problems or detect the individuals at most risk, to understand the obesity-related environmental factors that could modulate gene expression by affecting epigenetic mechanisms, and to study novel therapeutic strategies based on nutritional or pharmacological agents that can modify epigenetic marks. At this level, the major tasks are: development of robust epigenetic biomarkers of weight regulation, description of those epigenetic marks more susceptible to be modified by dietary exposures, identification of the active ingredients (and the doses) that alter the epigenome, assessment of the real importance of other obesity-related factors on epigenetic regulation, determination of the period of life in which best results

are obtained, and understanding of the importance of the inheritance of these epigenetic marks.

Keywords: Histone modifications, DNA methylation, hyperglycemia, inflammation, oxidative stress, hypoxia, polyphenols, epigenetic biomarkers

Contents

1. Introduction

The prevalence of obesity is increasing in every country of the world and only in the United States there are now more than 100 million individuals with overweight or obesity (Lopez et al., 2012). The onset of obesity results from an imbalance between energy intake and expenditure, being this pandemic largely attributable to the high availability of foods with high caloric content coupled with the adoption of a sedentary lifestyle. The inter-individual differences have been often ascribed to genetic variants that affect hundreds of genes related to energy metabolism (Hinney et al., 2010), but in the last years other factors have been described to take part in the equation and contribute to increase (or decrease) the susceptibility to gain weight or to develop obesity-related complications, including cardiovascular diseases, type 2 diabetes, or steatohepatitis. Some of these alternative putative contributors that have been recently reviewed (McAllister et al., 2009) include infections, sleep debt, reduction in variability of ambient temperatures, endocrine disruptors, increasing maternal age, greater fecundity among people with higher adiposity, assortative mating, pharmaceutical iatrogenesis, intrauterine and intergenerational effects, and epigenetics. As the past decades have been accompanied with unprecedented transitions in our lifestyle, the study of these alternative contributors to obesity epidemics is being actively pursued (Symonds et al., 2011). Some of them are represented in figure 1.

Epigenetics, that has been defined as heritable changes in gene expression that cannot be explained by changes in DNA sequence (Christensen and Marsit, 2011), has been subject of special interest in the last years mainly because there is evidence on the impact of different nutrients, environmental compounds and metabolic situations on the epigenome, which may lead to the development of more personalized disease prevention and treatment strategies (Campion et al., 2010; Choi and Friso, 2010). As

epigenetic changes are modulated by environmental exposures, epigenetics is considered the interface between genetics and the environment. In this sense, only environmental (including nutritional) factors are able to explain the phenotypical and epigenetic differences reported in monozygotic twins, which increase over the years (Fraga et al., 2005). In fact, epigenetic mechanisms are good candidates for explaining the role of the diet and other environmental conditions of parents and grandparents, or the influence of perinatal dietary exposures as determinants of later health outcomes (Zeisel, 2009). These altered epigenetic marks are implicated in the aetiology of cancer, that is being extensively studied, but also of chronic non-communicable metabolic diseases like obesity, diabetes and cardiovascular diseases.

The main epigenetic mechanisms that regulate gene expression in placental mammals are 1) DNA methylation, predominantly in the fifth carbon in the cytosines that are followed by a guanine, 2) a variety of histone modifications that include methylation, acetylation, ubiquitination and sumoylation of lysine, phosphorylation of serine and threonine, and methylation of arginine, 3) other nuclear proteins that are critical for epigenetic gene regulation (such as chromatin remodeling complexes, effector proteins with various binding modules for different modifications, and insulator proteins), 4) genomic imprinting, by which the expression of a gene is limited to one of the two parental alleles, 5) non-coding RNAs including microRNAs (miRNAs), which can bind to and regulate multiple mRNAs, and 6) non-covalent mechanisms, such as physical alterations in nucleosomal positioning via nucleosome remodelers or replacement of canonical histone proteins with specialized histone variants such as H3.3 and H2A.Z (Kim JK et al., 2009; Sharma et al., 2010).

As described in figure 2, the most extensively studied epigenetic modifications in mammals are miRNAs, DNA methylation and covalent histone modifications

(Lavebratt et al., 2011). The last two mechanisms provide a stable gene silencing mechanism that plays an important role in regulating gene expression and chromatin architecture. Although these epigenetic marks are stable and tissue-specific, studies of identical twins have found an age-dependent progression of epigenetic change, with greater differences in global DNA methylation in older than in younger twins (Fraga et al., 2005). The plasticity, tissue-specific nature, and variability of epigenetic marks vary across individuals and not only aging but also other environmental exposures alter tissue-specific DNA methylation and could be implicated in the susceptibility to diseases like cancer, neurological problems or metabolic disorders (Chistensen and Marsit, 2009). The reversible nature of epigenetic aberrations has led to the emergence of new therapeutic epigenetic approaches that might be used to counter detrimental environmental exposure effects (Costello et al., 2009). In this sense, different molecules are being studied in cancer research due to their modulatory action on epigenetic processes, including azacitidine (Vidaza) and decitabine (Dacogen), two methyltransferase inhibitors that reduce DNA methylation, vorinostat or SAHA (Zolinza) and romidepsin (Istodax), which inhibit histone deacetylases. These molecules have been approved by the FDA for cancer treatment as they prolong cell survival and are less toxic than conventional chemotherapy (Boumber and Issa, 2011). In this sense, even better results have been obtained in cancer clinical trials when the treatment combines DNA methylation inhibitors and histone deacetylase inhibitors (Fandy et al., 2009; Stathis et al., 2011).

However, there are two important concerns regarding this therapeutic approach. The first one is the lack of specificity, as epigenetic modifications are used by the different tissues and cell lines and by both normal and impaired cells to regulate gene expression (Humeniuk et al., 2009). The other concern is related to the amount of change that must

be reverted. In cancer, it is usual to observe sharp changes between both normal and malignant cells, so that epigenetic drugs can be used in relatively high doses to reverse the abnormal methylation patterns and slow down or stop cancer cell growth (Alvarez et al., 2010). In relation to body weight, the reports that have analyzed differences in the percentage of methylation in cytosines of different genes have shown variations of about 10-20% at the most (Bouchard et al., 2010; Milagro et al., 2011). On paper, it seems difficult to modulate the epigenetic drug doses to revert such subtle differences, but the identification in the last years of several nutrients and food compounds that are able to slightly modify the epigenetic patterns of different cell lines and tissues are encouraging in order to look for functional foods that could help to combat or prevent metabolic diseases (vel Szic et al., 2010). Thus, the large differences in DNA methylation observed between human preadipocytes and mature adipocytes (Zhu 2012) suggest that epigenetics plays an important role in the process of adipocyte differentiation. Other epigenetic mechanisms that play an important role in adipogenesis are histone methylation and demethylation (Okamura et al., 2010), poly(ADP-ribosyl)ation (Erener et al., 2012), and acetylation and deacetylation (Kim SJ et al., 2011; Chatterjee et al., 2011). For example, the promoter of the PPARγ gene, a key transcriptional regulator of adipogenesis, is hypermethylated in 3T3-L1 preadipocytes, but is gradually demethylated upon induction of differentiation (Fujiki et al., 2009). Finally, the importance of epigenetic mechanisms in the regulation of energy balance is supported by the fact that 256 new candidate imprinted genes have been identified in the adult mouse hypothalamus, the key regulator of appetite and many endocrine functions (Gregg et al., 2010).

Obesity and its related complications have been repeatedly associated with epigenetic alterations. Thus, methylation changes in blood leukocyte DNA have been observed in

obese adolescents (Wang X et al., 2010), whereas methylation of TFAM gene promoter in peripheral white blood cells has been associated with insulin resistance in adolescents (Gemma et al., 2010). In type 2 diabetes, the large number of genes presenting differential methylation status in skeletal muscle from normal glucose-tolerant and diabetic subjects suggests that DNA methylation is an important contributor to the development of this pathology and associated complications (Barrès et al., 2009). In this sense, insulin gene expression is known to be regulated by cytosine methylation, and pathologic methylation patterns have been suggested to contribute to the development of diabetes (Kuroda et al., 2009). Some of the genes related to metabolic regulation that have been reported to be regulated by DNA methylation are shown in Table 1. Additionally, as obesity is a complex disease that usually appears as a polygenic condition affected by environmental factors (mainly unbalanced dietary patterns and physical inactivity), it is the combination of genotype and epigenotype that will best assist to reveal diet associations with susceptibility to obesity and other related disorders (Martinez et al., 2012).

In the current review, particular emphasis will be placed on the following aspects:

- identification of epigenetic marks that could be used as biomarkers in the diagnosis and prognosis of obesity and other non-communicable diseases. Some of these marks could be inherited from one or both carrier parents, and thus be detected early in life and, in some cases, steps might be taken to overcome them. Very interestingly, some epigenetic marks can be affected by environmental factors during *in utero* and perinatal stages, including maternal diet, uterine blood flow, lactation, and maternal nursing behavior (Hanley et al., 2010; Hanson et al., 2011a), so that they could be properly handled. Finally, others could be acquired during the process of aging due to metabolic, environmental and dietary factors that need to be thoroughly studied.

- presentation of some of the environmental factors related to obesity that could modulate gene expression by affecting epigenetic mechanisms. Some of these epigenetic modifications may have a causative role in the development of obesity-related complications and could also influence the response to anti-obesity treatments through diet and physical activity. It is of great interest to broaden the knowledge on the environmental factors that affect epigenetic mechanisms related to obesity in order to prevent unhealthy diets and attitudes and to personalize treatments, for which new *in vitro* and *in vivo* experiments must be designed and performed.

- suggestion of possible mechanisms (that must be deeply investigated) by which the obesity-prone epigenetic alterations could be neutralized by the use of drugs or, more interestingly, by favoring the intake of protective compounds being able to module the epigenetic processes.

2. Search for epigenetic biomarkers

Obesity biomarkers have a great potential to better characterize the obesity phenotype, which may be relevant for assessing the risk of diabetes and CVD beyond anthropometric and biochemical parameters or for personalizing weight loss plans. However, most current biomarkers have only modest predictive value, and there is a need to identify additional biomarkers from new biological pathways by employing the new platforms for profiling DNA, RNA, proteins and metabolites (May and Wang, 2008). In this sense, epigenetic marks, such as DNA methylation patterns and miRNAs transcription, are suitable for sensitive detection, are very stable phenomena in tissues and blood cells, and have a long history in oncology. Thus, methylated CpG islands of p16, Septin9, and MGMT are being used as predictive biomarkers for cancer diagnosis, classification, development, prognosis and chemosensitivity (Deng et al., 2010).

In obesity, the identification of epigenetic biomarkers that could help to detect at an early age those individuals more susceptible to later develop obesity or visceral adiposity may allow to prevent and personalize its progress and manifestations (Campion et al., 2009a). In a very interesting article, Feinberg et al. (2010) have identified four variably methylated regions (VMRs) that show covariation with body mass index and are located in or near genes implicated in regulating body weight or diabetes. Due to the long time that is needed to perform studies of prognostic biomarkers of obesity in humans, research has been focused on explaining individual differences in weight loss after an energy-restriction intervention (Campion et al., 2009b; Bouchard et al., 2010; Milagro et al., 2011; Cordero et al., 2011a). The most comprehensive studies have performed a DNA methylation array study comparing samples from responders and non-responders to a hypocaloric diet treatment followed by a validation with more individuals and employing a second technique, such as Sequenom EpiTYPER (Bouchard et al., 2010; Milagro et al., 2011). Other studies have used more focused approaches, such as direct bisulfite sequencing (Campion et al., 2009) or methylation-specific PCR (MSP) (Cordero et al., 2011a), and have found that several CpGs located in the promoters of leptin and TNF-α, when measured in blood cells, could be used as biomarkers of weight loss. In this sense, although it is of great interest to study the baseline epigenetic differences in obesity-related tissues, such as adipose or liver, peripheral blood mononuclear cells (or otherwise white cells) are being preferably used because of their less-invasive nature, low risk, and high diagnostic accuracy (Widschwendter et al., 2008). However, the study of specific DNA methylation in serum or whole blood should be encouraged as its economic cost is low, its availability is very easy and its prognostic significance has been demonstrated in different types of cancer, such as lung (Fujiwara et al., 2005) or gastric adenocarcinoma

(Al-Moundhri et al., 2010). The biological significance of whole-genome methylation appears irrelevant and usually lacks of prognostic value in metabolic diseases because a low sensibility. However, it has been reported as a dietary biomarker in the comparison of vegetarian vs omnivorous subjects (Geisel et al., 2005) and global DNA methylation was significantly higher in coronary artery disease patients than in controls, which was correlated with plasma homocysteine levels (Sharma et al., 2008). In this sense, global DNA methylation in peripheral blood leukocytes has been proposed as a biomarker for cardiovascular disease risk (Kim et al., 2010).

In the field of prognostic biomarkers of obesity, it is necessary to take advantage of prospective cohort studies that follow over time a group of individuals and that collect samples from an early age and compare the modifications with samples from the same subjects several years later. For this purpose, methylation microarrays or, in a near future, high-throughput sequencing are the tools of choice. In this sense, large-scale epigenome-wide association studies (EWASs) that evaluate hundreds of thousands of CpGs in thousands of individuals (the same model as GWAS) present novel opportunities for finding epigenetic marks that could be involved in the development of metabolic diseases (Rakyan et al., 2011a). The first systematic analysis of the temporal origins of disease-associated epigenetic variation for any human complex disease has been published for type 1 diabetes by following an EWAS strategy in discordant monozygotic twin pairs (Rakyan et al., 2011b). With a more modest approach, and using Sequenom MassARRAY, it has been recently reported that perinatal epigenetic analyses in umbilical cord tissue DNA may be used to identify individual vulnerability to later obesity at age 9 years (Godfrey et al., 2011). Similarly, altered DNA methylation of TACSTD2 gene was observed in preterm children when compared to

term born ones, which was associated with rapid postnatal growth and increased risk of adiposity at 9-15 years of age (Groom et al., 2012).

The other interesting point concerning the search for epigenetic biomarkers in obesity is the possibility of identifying those individuals that have more susceptibility to develop metabolic impairments with respect to those that are less prone. Similarly to prognostic biomarkers, prospective cohort studies that follow over time a group of individuals are necessary.

Finally, once that it is clear that obesity is associated to changes in DNA methylation in different gene promoters, such as in serotonin transporter gene, that has a critical role in regulating food intake, body weight and energy balance (Zhao et al., 2012), there is interest in finding diagnostic biomarkers that help to differentiate individuals that are developing a metabolic complication with respect to the rest of the population. For this purpose, studies comparing individuals suffering the complication in comparison with control subjects have been performed. Thus, an excess of differentially methylated sites in genomic regions of subjects with or without type 2 diabetes mellitus, including a CpG site in the first intron of the FTO gene has been described (Toperoff et al., 2012). FTO is the most important human obesity gene and its mRNA levels in the arcuate nucleus are regulated by feeding and fasting (Gerken et al., 2007). Interestingly, it encodes a 2-oxoglutarate-dependent nucleic acid demethylase and may have a potential role in DNA methylation regulation (Gerken et al., 2007). A similar approach has revealed that 187 genes were differentially methylated between diabetes patients with end stage renal disease and diabetes patients without nephropathy (Sapienza et al., 2011).

As a conclusion, epigenetic marks could be useful to personalize nutrition, to early detect those individuals with more risk to develop metabolic disorders or to better

respond to a treatment. However, a wider approach should be adopted, and epigenetic marks have to be studied at the same time that SNPs, miRNA expression and mRNAs in order to decipher the interactions among DNA sequence, epigenetics and gene expression, always taken into account the diet and other environmental factors.

3. Nutritional factors

In the last years, a number of studies have related different dietary patterns, nutrients and food components with epigenetic processes that regulate gene expression and may contribute to an increased susceptibility to develop obesity and other metabolic disorders (Table 2). They are analyzed more in detail in the following chapters.

3.1. Calorie restriction and low protein-diets

There are several epidemiological and animal studies linking suboptimal early nutrition and poor growth *in utero* with increased risk of hypercholesterolemia, hypertension, type 2 diabetes and obesity in adulthood (Martin-Gronert and Ozanne, 2010; Seki et al., 2012). In relation to the multiple mechanisms implicated, it is clear that epigenetics plays a clue role. Both calorie restriction and low protein diets are known to induce epigenetic modifications and metabolic alterations that persist in the adult. Thus, a moderate restriction of energy intake during the periconceptional period is considered a stressor and is accompanied by increased adrenal mass and an increased cortisol response to stress, but also by epigenetic modification such as decreased methylation in IGF2/H19 gene in the adrenal gland (Zhang et al., 2010). Energy restriction also induces changes in covalent histone modifications. Thus, chromatin immunoprecipitation assays of the GLUT4 promoter revealed that calorie restriction increases histone 4 acetylation in adipose tissue of mice fed a high-fed diet, which was not observed in mice that followed a treadmill exercise (Wheatley et al., 2011). The first

example of an association between periconceptional energy restriction and DNA methylation in humans comes from the deep analysis of people born during the Dutch Hunger Winter of 1944-1945, that shows persistent epigenetic differences associated with prenatal exposure to famine (Heijmans et al., 2008).

The article by Lillycrop et al. (2008), showing that a maternal protein-restricted diet persistently alters the methylation of specific cytosines in the hepatic PPARα promoter of the offspring, is a milestone in metabolic programming studies, establishing that methylation changes due to prenatal nutrition persist in adults. Subsequent studies have deepened into this concept. Thus, in liver, maternal low protein intake alters gene expression of Igf2 and H19 by regulating DNA methylation (Gong et al., 2010) and induces changes in histone acetylation and methylation that affect genes regulating the amino acid response pathway (Zhou and Pan, 2011). Maternal low protein diet also elevates cholesterol in the offspring and induces changes in liver histone methylation status (Sohi et al., 2011). In skeletal muscle, it increases acetylated histone 3 and acetylated histone 4 at the C/EBPβ (Zheng et al., 2011b) and GLUT4 promoter regions (Zheng et al., 2011c) but only in female pups, indicating a sex-dependent adaptation. These studies suggest that adequate weight control and nutrient supply during pregnancy and lactation are crucial in metabolic programming and could be responsible of health problems related to metabolic and neurological functions. Maybe, some of these epigenetic mechanisms could help to explain why the prevalence of obesity, diabetes and cardiovascular diseases is higher in low-income neighbourhoods and is alarmingly rising in the less developed parts of the world.

In adult humans, it has been reported that weight loss due to hypocaloric diets alter the methylation pattern of different genes in adipose tissue (Bouchard et al., 2010). In adult mice, the stress associated with caloric restriction seems to reprogram orexigenic

pathways and alter the reward circuitry in the brain by affecting epigenetic mechanisms (Pankevich et al., 2010). In these mice, caloric restriction promotes binge-eating, and this result opens the door to study whether the yo-yo effect that makes weight loss maintenance so difficult may lie, at least in part, in epigenetic mechanisms. However, it is difficult to assess whether these epigenetic effects are directly caused by calorie restriction or by weight loss. In this sense, caloric restriction is the most effective environmental manipulation for extending lifespan in various animal models and is potentially involved in delaying aging-related degenerative diseases in humans (Speakman and Mitchell, 2011). One of the mechanisms by which caloric restriction affects lifespan is epigenetics, for example by mediation of sirtuins (Li Y et al., 2011), but this is a challenge that requires better understanding.

3.2. Hypercaloric and high-fat diets

In response to high-fat diets (HFD), the medial hypothalamus changes the expression of neuropeptides regulating feeding and energy metabolism whereas adipocyte and liver gene expression is strongly affected. Different epigenetic processes could participate in this dysregulation. In adult rodents, it has been demonstrated that long-term HFD has an effect on the methylation status of important obesity-related genes such as leptin in adipose tissue (Milagro et al., 2009) or melanocortin receptor 4 in brain (Widiker et al., 2010), probably contributing to changes in gene expression and appetite regulation. On the other hand, whereas fasting decreases the number of acetylated histone H3- and H4-positive cells in the ventrolateral subdivision of the ventromedial hypothalamus, HFD during four weeks results in increased expression of histone deacetylases HDAC5 and HDAC8 (Funato et al., 2011). These studies strongly suggest that hypercaloric diets

alter the epigenetic mechanisms that regulate the expression of genes involved in the control of appetite and energy metabolism.

As gestation and lactation are the periods of life more susceptible to epigenetic modifications that will persist in the adulthood, a number of studies have been performed during the perinatal period, especially in rodents. Thus, it has been reported that maternal overfeeding with HFD in rats induce a markedly obese phenotype in the offspring completely independent of postnatal nutrition (Howie et al., 2009) and also nonalcoholic steatohepatitis, which has been explained by impaired hepatic mitochondrial metabolism and up-regulated hepatic lipogenesis (Bruce et al., 2009). The origin of other metabolic diseases in the adult can be also related with maternal overnutrition, such as hypertension and hyperlipidemia (Elahi et al., 2009) or insulin resistance and diabetes (Samuelsson et al., 2008), and maternal HFD is associated with changes in hypothalamic regulation of body weight and energy homeostasis by altering the expression of leptin receptor, proopiomelanocortin, and neuropeptide Y in the adult offspring (Page et al., 2009). Nevertheless, not all the studies arise to the same conclusion. There are examples in which HFD given during gestation and lactation protects, at least partially, the offspring from excessive weight gain through different hypothalamic mechanisms (Couvreur et al., 2011). So, more studies are needed to elucidate the intrinsic mechanism related with these effects, and complementary studies in humans should be envisaged. In fact, it has been postulated that it is not dietary fat but maternal adiposity which induces hyperleptinemia, insulin resistance and increased body weight in offspring that persists into adulthood (White et al., 2009). Accoding to this hypothesis, maternal hyperglycemia, hyperinsulinemia, hyperleptinemia and inflammation would be potential mediator candidates (Rooney and Ozanne, 2011).

These metabolic alterations in the offspring seem to be due, at least in part, by epigenetic modifications that persist during the adult life. Thus, neonatal overfeeding alters DNA methylation patterns of the hypothalamic promoter region of the main anorexigenic neuropeptide, proopiomelanocortin (POMC) (Plagemann et al., 2009). Maternal consumption of HFD can change the offspring's' epigenetic marks in brain and alter gene expression of genes related to the mesocorticolimbic reward circuitry (dopamine and opioids), inducing a preference for palatable foods rich in sucrose and fat (Vucetic et al., 2010). Interestingly, these epigenetic modifications persist across at least two generations of offspring (Dunn and Bale, 2009) and could contribute to the rising prevalence of obesity that is observed in most countries.

Although the intake of a hypercaloric diet is important, macronutrient distribution is also determinant in these epigenetic processes as has been demonstrated when adult rats were fed a pair-fed HFD and changes in fatty acid synthase promoter methylation were found in adipose tissue (Lomba et al., 2010). This kind of experiments is not easy to accomplish in humans, but it has been recently published that a dietary pattern characterized by a high intake of vegetables and fruits (called a "prudent" diet) may protect against the global DNA hypomethylation observed with a "Western" dietary pattern characterized by a high intake of meats, grains, dairy, oils, and potatoes (Zhang et al., 2011b). These results clearly demonstrate that obesity not only is the product of an energy imbalance, but that different epigenetic mechanisms are involved. These mechanisms would interact with age, the genetic background, dietary factors and other environmental influences to create interindividual differences in appetite regulation and obesity predisposition.

3.3 Methyl donors

Dietary methyl groups derive from foods containing methionine, serine, folate, biotin, and choline, that can transfer a methyl group to DNA and histones through S-adenosylmethionine (SAM) and influence the expression of many genes (Zeisel, 2009; McKay and Mathers, 2011; Park et al., 2012). The remethylation of methionine from homocysteine requires zinc, selenium and vitamins B6 and B12. For example, methionine can be formed in the liver from homocysteine using methyl groups from methyltetrahydrofolate, or using methyl groups from betaine that are derived from choline (Zeisel, 2009). Thus, when humans are deprived of choline, they use more methyltetrahydrofolate to remethylate homocysteine and increase dietary folate requirements, but, conversely, when they are deprived of folate, they use more choline and increase dietary choline requirement. However, not all the individuals develop similar health problems with similar methyl donor intake because other factors are involved. Thus, single nucleotide polymorphisms (SNP) in genes regulating choline and folate metabolism increase or decrease the risk of dietary methyl donor deficiency (Zeisel, 2009).

The importance of maternal methyl donor intake on changing the germ-line epigenetic state was first reported by Cropley et al. (2006) for the Avy agouti allele. A seminal article by Waterland et al. (2008) subsequently reported that methyl donor supplementation induces DNA hypermethylation during development and prevents transgenerational amplification of obesity in agouti viable yellow (Avy) mice. After that, many studies have analyzed the epigenetic effects of a diet deficient in methyl donors or with methyl donor supplementation during pregnancy in different metabolic diseases, including altered behavior in offspring (i.e, anxiety) through permanent changes in hippocampal DNA methylation (Konycheva et al., 2011). However, until now, few studies have associated perinatal methyl donors with long-term changes in

body weight and metabolism. In sheep, low periconceptional supply of vitamin B12 and folate has been associated to fatter adult offspring, insulin-resistance and elevated blood pressure, which was accompanied by widespread epigenetic alterations to DNA methylation (Sinclair et al., 2007). In rats, perinatal methyl donor deficiency induces modest changes in the insulin axis of the fetus (Maloney et al., 2009) and programs glucose homeostasis in adult male but not female offspring (Maloney et al., 2011). Few studies have been performed in humans. In this sense, in India, a combination of high folate and low vitamin B12 concentrations in maternal diet was correlated with more risk to develop insulin resistance and obesity in the offspring (Yajnik et al., 2008) whereas in Nepal folate maternal supplementation was observed to reduce the risk of metabolic syndrome in the children (Stewart et al., 2009). Hence, more long-term intervention studies are needed to understand the benefits and risks of methyl donor doses in relation to obesity development because nutrient supplementation during pregnancy is widespread in the effort to combat anemia and other micronutrient deficiencies.

Although methyl donor supply during the perinatal period is determinant for developing metabolic disturbances, also the intake in adulthood seems to be crucial. Thus, in adult mice, a methyl deficient diet results in the progressive accumulation of morphological changes in the liver similar to human non-alcoholic steatohepatitis that is accompanied by prominent epigenetic abnormalities, such as aberrant histone modifications and loss of genomic cytosine methylation, especially at major and minor satellites (Pogribny et al., 2009). In patients with nonalcoholic fatty liver disease, choline deficiency is associated with increased fibrosis (Guerrerio et al., 2012).

Interestingly, it has been demonstrated that the fetal epigenetic programming is reversible in adult life by methyl donor supplementation. Thus, maternal programming

of stress responses is reversed by the central infusion of methionine (Weaver et al., 2005), whereas methyl donor supplementation prevents HFD-induced non-alcoholic fatty liver (Cordero et al., 2011b). However, we are far from being able to recommend supplementation or personalized intake of folate and other methyl donor because genotype (i.e., MTHFR 677C→T variant, CC vs. TT) seems to affect DNA methylation levels (Crider et al., 2011), and because aberrant changes in DNA methylation due to diet may lead to the development of age-associated diseases, including cancer (Kim KC et al., 2009). Thus, further research in animal models and human intervention studies are needed to accurately define the relationship between supraphysiological intake of methyl donors and chronic diseases, and much care must be taken when recommending these compounds during pregnancy. In this sense, some recent studies have focused on beneficial effects of betaine supplementation in adipose tissue dysfunction and insulin resistance (Wang Z et al., 2010), and folate supplementation in obese subjects with type 2 diabetes (Gargari et al., 2011).

3.4 Fatty acid and amino acids

Many nutrients act as regulators of DNA methylation and histone modifications either by directly inhibiting enzymes that catalyze the processes, or by altering the availability of substrates necessary for the enzymatic reactions (Choi and Friso, 2010).

Although one of the most widely used models of diet-induced obesity in animals is the intake of a high-fat diet, and some works have analysed the epigenetic modifications induced by this dietary approach in rodents (Lomba et al., 2010), it is still unknown the capacity of the different types of fatty acids to induce epigenetic modifications. Few studies have evidenced the role of n-3 and n-6 PUFA on DNA methylation, although there are examples concerning effects of eicosapentaenoic (Ceccarelli et al., 2011),

docosahexaenoic (Kulkarni et al., 2011) and arachidonic (Kiec-Wilk et al., 2011) fatty acids on DNA methylation. It has been also reported that monounsaturated fatty acids can modulate other epigenetic mechanisms such as histone acetylation (Ku et al., 2011). Thus, more studies must be designed as it is well known that the dietary fatty acid composition is one of the main factors in the development of obesity and metabolic syndrome; particularly in relation to the beneficial effects associated to high long-chain n-3 PUFA intake, whose anti-inflammatory properties are being studied to reduce obesity-related low-grade inflammatory condition (Calder et al., 2011).

One special case is butyrate, a short chain fatty acid produced during gut flora-mediated fermentation of dietary fiber sources that is rapidly absorbed and is the major energy source for colonocytes. This molecule induces high rates of *in vitro* apoptosis and cell cycle arrest presumably related to its strong HDAC inhibitory activity (Dashwood and Ho, 2007). As obesity is associated with important changes in gut microbiota composition, bacteria-produced butyrate could be one of the factors that links both phenomena through epigenetic mechanisms. Other short chain fatty acids produced by the gastrointestinal microbiota have been also associated with epigenetic changes that should be thoroughly analyzed. Thus, acetate, that enters the peripheral circulation to be metabolized by peripheral tissues, increases brain histone acetylation and inhibits histone deacetylase activity and expression (Soliman and Rosenberger, 2011), whereas propionate, that is the major donor molecule for histone propionylation, is involved in histone H3 lysine 23 propionylation in different cell lines (Liu et al., 2009).

The amino acid that seems to play a major role in epigenetic mechanisms is methionine, the main source of methyl groups in biomethylation reactions and the key regulator of the one-carbon metabolism pathway (McKay and Mathers, 2011). However, the metabolism of other amino acids (serine, glycine and histidine) also plays an important

role in provision of methyl donors for DNA and histone methylation (Wang et al., 2012). However, neither methionine nor other amino acids are considered dietary factors that could affect the predisposition to suffer from obesity. Alternatively, changes in the circulating levels of several essential amino acids, particularly methionine, cysteine, tyrosine, phenylalanine and branched-chain amino acids, are apparently linked with obesity and insulin resistance and even occurs before the onset of type 2 diabetes (Adams, 2011).

3.4. Minerals and vitamins

A recent study performed in cord blood samples from offspring of Gambian women has reported that periconceptional maternal micronutrient supplementation with a supplement of 14 minerals and vitamins affect fetal gene promoter methylation that, in some cases, persist in infants at 9 months, with clear differences in patterns between the sexes (Khulan et al., 2012). Similar changes have been observed at imprinted loci in the same experiment (Cooper et al., 2012). However, this strategy might not be appropriate for preventing obesity because, in rats, multivitamin supplementation during pregnancy has been associated with increased food intake and obesity development in the offspring when fed a high-fat diet, which has been hypothesized to be mediated by epigenetic mechanisms (Szeto et al., 2009). As multivitamin supplementation is being used increasingly in modern societies, more studies must be designed to evaluate this issue.

Different minerals have been associated with changes in epigenetic mechanisms regulating gene expression, such as selenium and zinc, which intervene in the regulation of DNMT activity and play a key role in the one-carbon pathway and in the activation of most HDACs (Ho et al., 2011). High levels of various inorganic and organic forms of heavy metals have been also related to epigenetic effects, including chromium, arsenite,

lead, cadmium, copper and nickel (Cheng et al., 2012). Magnesium is other element that is able to modify epigenetic marks. Thus, magnesium deficiency in pregnant rats induces metabolic complications in the offspring by altering methylation of specific cytosines in the hepatic hydroxysteroid dehydrogenase-2 promoter (Takaya et al., 2011). However, for the moment, few minerals have been associated to increased risk of developing obesity or insulin resistance.

Magnesium intake has been repeatedly associated with obesity. Thus, low magnesium status has been associated with numerous pathological conditions characterized by chronic inflammatory stress, such as atherosclerosis, hypertension, osteoporosis, diabetes, and obesity (Nielsen, 2010). In animals there are numerous examples. Thus, maternal and postnatal magnesium status is important in the long-term programming of body adiposity and insulin secretion in rat offspring (Venu et al., 2008). However, in humans there are divergent conclusions about the effect of magnesium deficiency in obesity development. Even so, some intervention studies have found that oral magnesium supplementation improves insulin sensitivity in non-diabetic subjects with insulin resistance and in diabetic patients (Guerrero-Romero et al., 2004).

Chromium is other element that has been related to obesity development. Thus, maternal chromium restriction significantly increases body weight and adiposity in rats probably by increasing stress and altering lipid metabolism in the offspring (Padmavathi et al., 2010), whereas chromium treatment is associated with a reduction in liver triglyceride levels, endoplasmic reticulum stress and insulin resistance in ob/ob obese mice (Sreejayan et al., 2008). However, despite the numerous studies performed in humans, there is no evidence that high-dose chromium treatment is effective in obese patients with type 2 diabetes (Kleefstra et al., 2006) and the involvement of epigenetic modifications has not been analyzed until now. An adventurous hypothesis postulate

that, as chromium is an important modulator of insulin and glucose metabolism, increased exposure to chromium in preconceptional and fetal stage could lead to altered epigenetic control and altered endocrine and metabolic functioning (Ghambir and Phadke, 2011).

Calcium is the mineral that seems to be more related to weight regulation (Zemel, 2005). Thus, a systematic review of randomized clinical trials suggests that calcium supplementation generates small but statistically significant weight loss in overweight and obese individuals (Onakpoya et al., 2011). However, calcium has not been associated but indirectly with epigenetic modifications (Bocheva and Boyadjieva, 2011) and many more studies are necessary to discard that calcium could be a protective factor for obesity by acting through epigenetic mechanisms.

Among micronutrients, vitamin C, vitamin E and carotenoids are being studied in obesity and related disorders because they are able to decrease the circulating concentrations of inflammatory and oxidative markers (Calder et al., 2011). Thus, there are studies that report a reduction in body weight and adiposity in cafeteria diet-induced obese rats when supplemented with ascorbic acid (Campion et al., 2008). Although more studies should be conducted, all these molecules are able to induce epigenetic changes. Thus, ascorbate promotes widespread DNA demethylation in human embryonic stem cells (Chung et al., 2010), whereas retinol alters histone phosphorylation levels in rat Sertoli cells (Moreira et al., 2000). Vitamin E, lipoic acid and conjugated linoleic acids (CLA) require deeper analysis, although molecular modeling studies of all of them suggest a putative role as HDAC inhibitors (Dashwood and Ho, 2007). Nevertheless, it is necessary to design new *in vitro* and *in vivo* experiments with these antioxidants and other micronutrients to identify their epigenetic mechanisms of action. In summary, dietary supplementation with functional fatty acids,

amino acids, vitamins and minerals might be useful as epigenetic therapies for metabolic disorders, although much work is needed with *in vitro* and animal models to understand the basic mechanisms of the specific nutrients.

3.5. Polyphenols and other plant compounds

Given the responsiveness of epigenetic marks to dietary factors, there are numerous studies that try to identify new plant compounds with effects on the epigenetic molecular processes in order to apply them in obesity prevention and treatment (Chaudhary et al., 2012). In a near future, we could talk about "epigenetic foods" that will be a kind of functional foods that contain bioactive compounds with capacity to modulate miRNA expression, DNA methylation or histone modifications. As in other issues related to epigenetics, this concept has been primarily developed in relation to cancer prevention, and several plant components are being studied in relation to apoptosis, cell cycle regulation, differentiation, inflammation, angiogenesis, and metastasis, as well as in stress response (Ross et al., 2011). In a similar way, understanding the epigenetic effects of dietary botanicals may provide insight on prevention strategies to reduce obesity prevalence and prevent its comorbidities.

Polyphenols and other plant compounds are good candidates because they are considered potential therapeutic agents that can be used to prevent or treat obesity-mediated inflammation and oxidative stress, as well as other metabolic disease-related health problems such as type 2 diabetes, atherosclerosis and hypertension (Fraga et al., 2010). Among the bioactive compounds of plant origin that mediate epigenetic modifications there are agents like genistein (soybean), resveratrol (grapes), curcumin (turmeric), tea catechins (green tea), and sulforaphane (cruciferous vegetables) that have

been considered in cancer prevention and therapy (Meeran et al., 2010; Li and Tollefsbol, 2010).

Tea catechins, the most abundant of which is (–)-epigallocatechin-3-gallate (EGCG), are a group of flavonoids considered as one of the most promising treatments for metabolic syndrome. Thus, EGCG treatment in mice significantly reduces body weight gain, blood glucose levels and insulin resistance, decreases liver damage and liver triglyceride levels, and attenuates plasma cholesterol and inflammatory cytokines such as MCP-1, CRP or IL-6 (Chen et al., 2011). In obese KK-ay mice EGCG decreases glucose level and increases glucose tolerance in animals and, as in 3T3-L1 adipocytes, reduces ROS content (Yan et al., 2012). Some of these effects are surely mediated by epigenetic mechanisms as it is well known that EGCG is able to inhibit DNA methylation in different human cancer cell lines (Fang et al., 2003). One of the mechanisms of EGCG appears to be the direct inhibition of DNA methyltransferases (DNMT) by interaction with the catalytic site of the DNMT1 molecule (Fang et al., 2003; Li and Tollefsbol, 2010), but it also acts by inhibiting histone acetyltransferase activity (Choi et al., 2009).

Genistein is a flavonoid occurring in soybean that can act as an endocrine-disrupting substance playing a role in the etiology of obesity. However, at certain doses and dependent of sex and age, it could also inhibit adipogenesis *in vitro* in a similar way to female hormone estrogen (Dang, 2009). A seminal study of Dolinoy et al. (2006) showed that maternal genistein supplementation in mice during early embryonic development shift the coat color of heterozygous yellow agouti (Avy/a) offspring, which was significantly associated with increased methylation in a retrotransposon upstream of the transcription start site of the Agouti gene. In this model, genistein decreased ectopic Agouti expression and protected offspring from obesity in adulthood

by altering the epigenome. Similarly, in nonhuman primates, soy protein and isoflavones improve body weight, insulin sensitivity and lipid profiles and modify the DNA methylation patterns in liver and muscle (Howard et al., 2011).

Curcumin, a polyphenol occurring in turmeric with potent anti-inflammatory activity, is one of the natural compounds that have more potential as antiobesity treatment since, *in vitro*, is able to suppress 3T3-L1 differentiation and cause apoptosis (Ejaz et al., 2009). In HFD-fed mice, curcumin supplementation reduces body weight gain and adiposity probably by increasing oxidation and by decreasing fatty acid esterification in adipose tissue (Ejaz et al., 2009). In HFD-fed rats, the beneficial effect of curcumin is mediated by attenuating lipogenic gene expression in the liver and the inflammatory response in the adipose tissue (Shao et al., 2012). However, although curcumin is able to modulate histone deacetylases and acetyltransferases, DNA methyltransferase I, and miRNAs (Reuter et al., 2011), until now no studies have analyzed the involvement of curcumin-induced epigenetic mechanisms in obesity models. An interesting precedent is the study of Yun et al., (Yun et al., 2011), in which curcumin decreased hyperglycemia-induced cytokine production in monocytes via epigenetic changes involving NFκB, including a decrease of HAT activity, p300 level and acetylated CBP/p300 gene expression, and the activation of HDAC2.

Resveratrol is a stilbenoid with potent free radical scavenger properties that shows beneficial effects on type 2 diabetes and cardiovascular diseases, sharing some healthy actions with caloric restriction (Camins et al., 2010). Among other effects, this compound decreases hepatic steatosis severity in rats (Bujanda et al., 2008) and exerts an inhibitory effect on insulin secretion (Szkudelski, 2007). Although antiobesity actions of resveratrol have been extensively described in isolated adipocytes and in mice, including increased lipolysis and reduced lipogenesis in mature adipocytes,

decreased adipogenesis and viability in maturing preadipocytes or protection from HFD-induced weight gain in mice (Baile et al., 2011), for the moment there are no big intervention trials showing similar effects in humans. Apart from its strong antioxidant activity, resveratrol is a potent activator of sirtuin 1, a (NAD(+))-dependent histone deacetylase that modulates gene expression in all the tissues (Camins et al., 2010). However, more information is needed about resveratrol bioavailability and mechanisms of action because some nutraceutical formulations containing resveratrol are widely available in the market.

Different organosulfur compounds have been reported to exert anticarcinogenic effects probably acting through the inhibition of histone deacetylase activity (Nian et al., 2009). The most interesting natural molecules are sulforaphane, an isothiocyanate found in cruciferous vegetables that shows beneficial properties on human colon, prostate, and breast cancer cells, and garlic organosulfur compounds, such as diallyl disulfide, that are metabolized to allyl mercaptan and induce histone hyperacetylation in human colon cancer cells (Nian et al., 2009). Although no reports have demonstrated the obesity protective effects of sulforaphane in animal models, this isothiocyanate is effective in inhibiting adipocyte differentiation in culture through cell cycle arrest (Choi et al., 2012) and, in relation to diabetes, reverses the oxygen intermediate production and inflammatory damage in experimental diabetic neuropathy and nephropathy (Negi et al., 2011; Zheng et al., 2011a). On the other hand, although the mechanisms are not well known, garlic has been successfully used to reduce HFD-induced obesity in rodents (Lee et al., 2011). One of the potential causes could lie in the effects of diallyl trisulfide, which inhibits the differentiation of 3T3-L1 preadipocytes through ERK activation (Lii et al., 2011). However, it has also been reported that diallyl disulfide accelerates 3T3-L1 preadipocyte differentiation because of its inhibition of histone deacetylase activity (Lee

et al., 2007). Thus, more studies must be conducted to investigate the role of organosulfur compounds on adipogenesis and body weight regulation as well as the implication of epigenetic mechanisms in the process.

Other bioactive compounds have been reported to inhibit DNMTs or modify histone acetylation, including lycopene, garcinol, luteolin, butein, apigenin, silymarin, rosmarinic acid, anacardic acid or baicalein (Campion et al., 2010; Meeran et al., 2010; Park et al., 2012). Some of them could have some benefits in the treatment or prevention of metabolic disorders. Moreover, there is currently great interest in identifying new plant compounds with epigenetic properties that could be applied as therapeutic agents in obesity (Chaudhary et al., 2012). So, numerous plant extracts and compounds are being screened for modulating histone modifying enzymes and DNMTs.

The low bioavailability of most polyphenolic compounds and the impossibility of exactly determining the amounts included in the foods makes difficult to know the doses and the real effects of dietary polyphenols on DNA methylation in humans. So, more long-term studies testing the effects of different amounts of purified compounds, both in animals (periconceptually or in adults) and in humans, are needed. Also, the design of new compounds potentiating the stability and bioavailability of the natural molecules is an important aim in this topic. Finally, it seems likely that synergistic interactions may take place between different phytochemicals and nutrients, so that combinations must be developed to find stronger effects than individualized treatments. On the other hand, the combination of plant compounds and synthetic drugs might be a new therapeutic approach whose study should be encouraged.

4. Epigenetic drugs

Drugs targeting key enzymes regulating histone modifications and DNA methylation (HATs, HDACs and DNMTs) are being extensively studied as anticancer agents. In the last years, as several epigenetic mechanisms have been reported to control adipogenic differentiation and influence energy metabolism (Kim SJ et al., 2011; Chatterjee et al., 2011), new interest has been generated about the possible use of epigenetic drugs in the treatment of obesity and other metabolic diseases. For example, HDAC9 is differentially expressed in subcutaneous and omental adipocytes and functions as a negative regulator of adipogenesis (Chatterjee et al., 2011), although in the same experiment HDAC inhibitor trichostatin A failed to suppress adipogenic differentiation of human subcutaneous preadipocytes.

Ligation of uterine arteries reduces uteroplacental blood flow and is used to generate a model of intrauterine growth retardation (Park et al., 2008). This model has been proposed to alter gene regulation patterns due to epigenetic modifications and has been linked to the onset of metabolic disease in adulthood, particularly type 2 diabetes in rats. Deacetylation of histones H3 and H4 and methylation changes in histone 3 lysine 4 (H3K4) and histone 3 lysine 9 (H3K9) have been observed in this model. Very interestingly, these epigenetic changes could be reversed by HDAC inhibition during the neonatal period (Park et al., 2008).

The repressive actions of the jumonji domain containing 2C/lysine demethylase 4 C (JMJD2C/KDM4C) on PPARγ transcriptional activation and in preadipocyte differentiation have been recently described. Interestingly, trichostatin A, a HDAC inhibitor, was able to reduce the repressive effect of JMJD2C, and also decreased the fat storage capacity of the differentiating 3T3-L1 adipocytes (Lizcano et al., 2011).

In relation to diabetes, several studies have highlighted the beneficial effects of different drugs that act through epigenetic mechanisms on restoring insulin secretion (i.e. 3,5-

disubstituted isoxazoles increasing increased the activity of the histone acetyl transferase p300 through an ERK1/2-dependent mechanism) (Dioum et al., 2011), or on the control of pancreatic β-and δ-cell differentiation (i.e. mutant mice for HDAC4, -5, and -9) (Lenoir et al., 2011). In other interesting approach, expression of endogenous GLUT4 mRNA in preadipocytes was increased when the levels of class II HDACs in the nuclear compartment of 3T3-L1 preadipocytes were reduced using two experimental strategies. First, when preadipocytes were treated with phenylephrine, an α-adrenergic receptor agonist that drove HDACs out of the nuclear compartment, and secondly when class II HDAC concentrations were reduced using siRNA knockdown (Weems and Olson, 2011). The oral HDAC inhibitor ITF2357, that reduces production of pro-inflammatory cytokines *in vitro* and systemic inflammation *in vivo*, favors β-cell survival during inflammatory conditions in mice with streptozotocin-induced hyperglycemia and *in vitro* (Lewis et al., 2011). In this sense, HDAC inhibitors have shown promising anti-inflammatory properties in different animal and cellular models of inflammatory diseases and regulate the activity of a wide range of nonhistone proteins including NFκB (Christensen et al., 2011). Moreover, inhibition of various HDACs (HDAC1, -2, -4 and -5) appears to be a promising novel therapeutic principle to correct the insulin-resistant state, as HDACs play a regulatory role in physiological insulin signaling by increasing GLUT4 translocation and augmenting basal and insulin-induced glucose uptake in skeletal muscle (Takigawa-Imamura et al., 2003), but also by promoting differentiation of embryonic stem cells into insulin-producing cells (Tarayamma et al., 2006).

However, there is a lack of clinical studies and much concern must be given to the secondary effects that these epigenetic drugs could cause in the different tissues.

5. Obesity-related physiological factors

5.1. Inflammation

An increasing number of experimental and epidemiological evidences link complex diseases, such as obesity, type 2 diabetes, cardiovascular diseases, asthma, allergy, and several types of cancer, with chronic inflammation. In the case of obesity and type 2 diabetes, all major risk factors (overnutrition, low dietary fiber, sedentary lifestyle, stress, sleep deprivation and depression) have been found to induce local or systemic low-grade inflammation (Solinas and Karin, 2010). In fact, it has been suggested that chronic low-grade inflammation eventually leads to overt diabetes when counter-regulatory circuits to inflammation and metabolic stress are compromised because of genetic or epigenetic predisposition (Kolb and Mandrup-Poulsen, 2010).

The inflammatory response is very complex and requires the precise control of many functional mechanisms operating at different levels, such as the presence of different types of immune cells in the tissue, the responsiveness of different signaling pathways to the inflammatory stimuli and also the regulation of gene expression by epigenetic modifications (Medzhitov and Hong, 2009). Recent therapeutic interventions using HDAC and DNMT inhibitors (Altucci and Stunnenberg, 2009) as well as increasing data demonstrating the effect of certain anti-inflammatory dietary elements on DNA methylation or chromatin remodeling (vel Szic et al., 2010) highlight the connection between inflammation, non-communicable diseases and epigenetics.

5.1.1. Participation of immune cells

Immune cell specific mechanisms operate at the level of different tissues and include regulation of their recruitment and activation. Acute inflammation is mediated by granulocytes or polymorphonuclear leucocytes, while chronic inflammation is mediated by mononuclear cells such as monocytes and macrophages that produce pro-

inflammatory cytokines, ROS and NOS. These cells can be further stimulated to maintain inflammation through the action of an adaptive cascade involving T, B and dendritic cells. (vel Szic et al., 2010). As a consequence, in chronic inflammatory diseases there is a distortion in the extent of the inflammatory response, in the sense that it cannot be reset again. Genetic and non-genetic (environmental or epigenetic) factors are behind these differences (Bäckdahla et al., 2009). For instance, Th1 cells have been associated with chronic inflammatory diseases and activate macrophages, while Th2 have the opposite effect and induce antibody production. A specific Th lineage that produces IL17 (Th17) has been also pointed as a strong mediator of inflammation and development of chronic inflammatory diseases. Th17 differentiation is dependent on epigenetic modifications like the histone acetylation and H3K4me3 of the IL17 promoter (Akimzhanov et al., 2007).

5.1.2. Transcription Factors

Transcription factors (NFkB, IRF, CREB) constitutively expressed in many cell types are responsible for the integration of signals from different pathways and are in charge of the primary response to inflammation, triggering the initial phase of gene induction. Other factors require de novo synthesis following inflammatory stimulation (C/EBPd) or are expressed in a cell type or differentiation specific manner (Runx, PU.1, IRF8, C/EBP) establishing a particular pattern of gene expression by epigenetic mechanisms such as chromatin remodeling. All these transcription factors do not act independently but function coordinately to control the inflammatory transcriptional response, defining clusters of genes that are regulated in a coordinate manner (Medzhitov and Hong, 2009; Ramirez-Carrozi et al., 2009). In chronic tissue-specific inflammatory diseases there is a downregulation of the anti-inflammatory responses together with the upregulation of the

proinflammatory ones exerted at specific sites by transcription factors and another DNA binding proteins.

For an efficient interaction with DNA, transcription factors need to cooperate with chromatin remodeling factors (DNMTs, histone methylases and demethylases, HATS and HDACs etc.). Multiple post-translational modifications (methylation, acetylation, phosphorylation, ribosylation, sumoylation, ubiquitination) of these proteins and cofactors allow a very dynamic interaction among them in order to integrate epigenetically a variety of different environmental inputs that will determine the adjusted expression of inflammatory genes (Perissi et al., 2010; Dong et al., 2008). Some primary response inflammatory loci may not require chromatin remodeling activity for activation, since their promoters contain CpG islands that fall into constitutively active regions assembled in unstable nucleosomes. Others, on the contrary, fall in heterochromatic regions and bear fairly stable nucleosomes, requiring the participation of chromatin remodeling complexes for active transcription (Ramirez-Carrozzi et al., 2009).

NFκB is a key factor of the proinflammatory immune response. It regulates the expression of hundreds of genes including many mediators that amplify the inflammatory response aggravating the disease status (Olefsky, 2009). Different members of the NFκB family work in concert with other transcription enhancer factors such as CREB, C/EBP, AP1, NFAT, SP1, STAT etc. (O'Dea and Hofmann, 2010) modulating a tailored response with the participation of HATs and HDACs (Werner et al., 2005) which can finally regulate the epigenetic modification not only of the NFκB target genes (Vanden Berghe et al., 2006).

5.1.3. Inflammation, oxidative stress and hypoxia

In inflammatory processes, the release of reactive oxygen and nitrogen species (ROS and RNS) mainly by neutrophils and macrophages leads to oxidative stress and tissue damage, which can inhibit HDACs thereby activating silenced genes. Likewise, ROS production also leads to the activation of signaling cascades such as ERKs, which in turn induce NFκB immune response provoking changes in histone acetylation/deacetylation that finally result in altered transcription initiation (Adler et al., 1999). An increase in proinflammatory cytokines that contributes to the chronicity comes as a consequence of these ROS mediated mechanisms.

Inflammation can induce a certain level of hypoxia which in turn can reinforce the inflammatory state essentially by the HIF-1 capacity of attracting macrophages that would increase the array of proinflammatory mediators (MIF, TNF, IL-6, IL-10) (Shah et al., 2008). HIF-1 can also affect histone code through HDAC2 inhibition (Charron et al., 2009) and the direct induction of H3K9 demethylases expression (Pollard et al., 2008). This would favor a less compacted state that will make DNA more susceptible to damaging agents generated during chronic inflammation. At the same time a possible epigenetic mechanism is depicted whereby HIF-1-mediated induction of histone demethylases would favor the removal of H3K9 methyl marks, the loosening of chromatin compaction and the exposure to transcription of otherwise silent genes (Brigati et al., 2010). This increase of demethylases activity could rapidly spread and boost the number of derepressed genes (Talbert and Henikoff, 2006).

5.1.4. Environmental influence

Environmental conditions are also crucial for understanding the epigenetics of inflammation, where a whole array of molecular influences could take part in the modification of the histone code. This emphasizes the importance of diet and lifestyle in the shaping of a changing epigenome. Furthermore, increasing evidence indicates that

these environmental influences are also critical transgenerationally impacting gametogenesis and embryogenesis.

As an example, after a transient glucose level increase NFκB transcriptional activity is enhanced and maintained high through H3K9 demethylation of its promoter (El Osta et al., 2008; Brasacchio et al., 2009). On the other hand, lifestyle factors known to promote type 2 diabetes are associated with a local and/or systemic inflammatory reaction. Likewise, high glucose levels increase mitochondrial and respiratory activity and give rise to enhanced release of superoxide anions (El Osta et al., 2008). This oxidative stress induces the expression of pro-inflammatory cytokines, such as IL-6 and TNF-α, through signaling cascades involving JNK, MAP-kinases, and transcription factors, such as NFκB and AP-1 (Kempf et al., 2007). The cell response varies depending on the tissue affected and may include ectopic fat storage, mitochondrial dysfunction and endoplasmic reticulum stress, but the inflammatory response is usually self-limiting. However with increased exposure to pro-inflammatory challenges, the counter-regulatory anti-inflammatory feedback inhibition mechanisms may start to fail and chronic inflammation ensues in those genetically predisposed ending up in diabetes. In this regard, a dysfunction of neural regulatory systems such as leptin resistance, which is also associated to type 2 diabetes, has been closely linked with local inflammation (Velloso et al., 2008).

Accordingly, persons with type 2 diabetes, metabolic syndrome or obesity exhibit more pronounced and longer-lasting inflammatory responses to a challenge than metabolically normal controls in most studies. The endoplasmic reticulum is involved in most metabolic routes and has become a key cellular structure in the adaptive response to nutritional changes, such as those accompanying obesity (hyperglycemia, fatty acid overload). When the challenge overcomes endoplasmic reticulum response capacity, the

unfolded protein response comes into place involving many inflammation and stress signaling pathways related with chronic inflammatory diseases (Hotamisligil 2010; Mandl et al., 2009). Some of the deleterious effects of endoplasmic reticulum have been restored by the treatment with HDAC inhibitors, such as trichostatin A (Kimura et al. 2012).

Apart from high glucose concentrations, excess of saturated fatty acids induce oxidative stress with NAD(P)H oxidase activation, ROS production and endoplasmic reticulum stress, which lead to transcriptional activation of proinflammatory cytokines (IL-1, IL-6, TNF-α) through the activation of NFκB, AP-1 or CREB (Schenk et al., 2008; Milansky et al., 2009). TLR4 receptor mediates the pro-inflammatory effects of saturated fatty acids and also of high glucose concentrations, both of which might result in a sustained chronic inflammatory process without a balanced regulatory feedback (Milansky et al., 2009).

5.1.5. Epigenetic modifications

The epigenetic code involved in gene expression regulation is modified during chronic inflammation. Thus, agents produced by oxidative stress, such as ROS and RNS, cause loss of DNA methylation after mitosis, since DNMT1 does not recognize oxidized methyl groups, whereas halogen derivatives produced by inflammatory processes mimic cytosine methylation producing a gain of methylation by DNMT1 (Valinluck and Sowers, 2007). In a complex regulatory network, the expression of cytokines, such as TNF-α, is tightly regulated by epigenetic mechanisms (Sullivan et al., 2007), but cytokines, such as IL-6, regulate other genes and processes by inducing histone modifications and DNA methylation (Wehbe et al., 2006).

Additionally, the administration of a range of HDAC inhibitors has been reported to suppress the expression of proinflammatory cytokines in experimental models of

inflammatory diseases (Lin et al., 2007). In this sense, chronic inflammation seems to promote a transcriptional repressive state since the HDAC inhibitors block enzymes that silence transcription. Inhibition of both HDACs and DNMT1 are of therapeutic benefit in chronic inflammation and both types of enzymes integrate a transcriptional repression complex interacting through the methyl binding protein MeCP2 (Jones et al., 1998). In another report studying histone methylation inhibition during the inflammatory response induced by LPS, it was demonstrated that the H3K27me3 demethylase (Jmjd3) is directly regulated by NFκB (De Santa et al., 2007). Essentially, tissue-specific chronic inflammation is characterized by an epigenetic state with enrichment of hypo-acetylated histones and hyper-methylated CpGs that contribute to a persistent inflammatory response.

5.1.6. Future perspectives

A better understanding of the epigenetic consequences of chronic inflammation in metabolic diseases will open the possibility for prevention but also for therapeutic actions, since the reversible nature of these processes foresees a great potential for the treatment, stabilization and even recovery of these disorders. In obese persons at risk of developing type 2 diabetes, besides implementing a healthy lifestyle and calorie restriction, a logical approach would be to aim at re-establishing the mechanisms underlying the self-limiting of inflammatory responses (Kolb and Mandrup-Poulsen, 2010). The possibility of modulating the methylation status of critical inflammatory genes by dietary interventions with a variety of different natural products is receiving much attention, as a mean to protect or ameliorate inflammatory diseases. However there is concern about the possibility that the use of inhibitors or activators of epigenetic enzymes (DNMT, histone methylases, histone demethylases, HAT, HDAC) would lack enough specificity, especially in long term treatments.

It will be also necessary to search for novel epigenetic targets aiming at the selective modulation of the inflammatory signaling network in the affected organs, in order to broaden the possibilities of anti-inflammatory therapeutic intervention. In this regard microRNAs and long ncRNAs are arising as very promising candidates especially because they show a clear target sequence specificity that could represent the direct connection between the genome and the epigenome.

5.2. Oxidative stress

Excessive oxidative stress results from an imbalance between tissue oxidants (free radicals or reactive oxygen species (ROS)) and antioxidants that is usually exacerbated in the different tissues of obese subjects, including plasma (Urakawa et al., 2003), liver (Milagro et al., 2006) and adipose tissue (Furukawa et al., 2004). This oxidative stress triggers the development of insulin resistance and is responsible of some of the comorbidities that accompany obesity (Urakawa et al., 2003, Vincent and Taylor, 2006), including the inhibition of preadipocyte differentiation that may contribute to age-associated adipose tissue dysfunction (Findeisen et al., 2011). In addition, animal studies show that oxidative stress in adipose tissue contributes to dysregulation of adipokine production via PPARγ-responsive element in the adiponectin gene promoter and to obesity (Furukawa et al., 2004; Houstis et al., 2006). The main strategies that have been implemented in the reduction of obesity-related oxidative stress are weight loss and antioxidant supplementation. Weight loss reduces enhanced oxidative stress and different methods, such as physical activity, caloric restriction, and surgical intervention, have been proposed to combat free radicals and reduce adipose tissue (Vincent and Taylor, 2006). Regarding antioxidant supplementation, Yeon et al. (2011) have shown that the plasma total antioxidant capacity significantly increases as the

quartile value of dietary fiber and antioxidant vitamins, such as vitamin A, β-carotene, vitamin C, and vitamin E increases, whereas 8-OHdG level is negatively associated with vitamin A and β-carotene intake. Both mechanisms (weight loss and dietary antioxidants) have been reported to induce changes in epigenetic marks (Bouchard et al., 2010; Chung et al., 2010).

Oxidative DNA damage may induce DNA structure and base modifications, such as deletions, strand breakage, and chromosomal rearrangements (Valko et al., 2006). Replacement of guanine to 8-hydroxy 2'-deoxy-guanosine (8-OHdG), one of the major DNA oxidative damage by-products, substantially diminishes the binding of methyl-CpG binding proteins (important to manage site-specific chromatin organization and transcriptional repression) and results in heritable epigenetic changes (Valinluck et al., 2004). In addition, some DNA lesions have been shown to interfere with the ability of DNA to function as a substrate for the DNA methyl transferases (DNMTs), resulting in global hypomethylation (Zhang et al., 2007). Thus, increased DNA damage and chromosomal degradation could be associated with alterations of either hypermethylation or hypomethylation of the DNA (Lim et al., 2008). There are studies reporting that ROS interact with histone deacetylases and DNA methyltransferases and can stimulate the methylation of specific promoters, such as E-cadherin, a gene involved in cell adhesion (Lim et al., 2008), or the extracellular superoxide dismutase SOD3 (Zelko et al., 2011).

With these encouraging precedents, more research is needed in the field of epigenetics and antioxidant compounds in relation to obesity and type 2 diabetes, including the study of the interactions with other nutritional factors and the genetic background.

5.3. Hypoxia

Obesity is characterized by hypertrophy and hyperplasia of adipocytes (Spalding et al., 2008). This excessive fat deposition in adipose tissue is limited, being the diffusion limit of oxygen considered one of the main reasons. Hypoxia occurs when oxygen availability does not match the demand of the surrounding tissue, resulting in decreased oxygen tension. Hypoxic stress plays a pivotal role in normal human development and physiology, including embryogenesis and wound repair, and has been well studied for its importance in the pathogenesis of several human diseases, including heart disease, stroke, diabetes, and cancer (Gore et al., 2010). In adipose tissue of obese mice there is evidence for hypoxia, which elicits dysregulated production of adipocytokines and downregulates adiponectin expression by mediation of endoplasmic reticulum stress-dependent transcriptional and -independent posttranscriptional mechanisms (Hosogai et al., 2007). Therefore, local hypoxia in adipose tissue may provide cellular mechanisms for chronic inflammation, macrophage infiltration, adiponectin reduction, leptin elevation, adipocyte death, endoplasmic reticulum stress and mitochondrial dysfunction in obesity. The concept suggests that inhibition of adipogenesis and triglyceride synthesis by hypoxia may be a new mechanism for elevated free fatty acids in the circulation in obesity (Hosogai et al., 2007; Woods et al., 2009).

Hypoxia in general decreases global transcriptional activity, in part, through epigenetic regulation. Thus, a global decrease in H3K9 acetylation is a possible consequence of hypoxia-induced HDAC upregulation (Johnson et al., 2008). In fact, the H3K9 acetylation reduction occurs by the increase G9a histone methyltransferase expression, which raises global H3K9 methylation, a repressive histone mark (Chen et al., 2006). Changes in DNA methylation have been also associated with hypoxic conditions, including loss of global methylation in colorectal and melanoma cancer cell lines

(Shahrzad et al., 2007). A recent example by Nanduri et al. (2012) describes that intermittent hypoxia exposure in neonatal rats causes an exaggerated response to hypoxia associated with increased oxidative stress. In this animal model, hypermethylation of a single CpG dinucleotide close to the transcription start site of superoxide dismutase 2 seems to regulate SOD2 gene expression. Although these findings are preliminary, the observed epigenetic modifications occurred in transcribed genomic regions in obesity by hypoxia.

A key regulator of the adaptive response to alterations in oxygen tension is hypoxia-inducible factor-1 (HIF1), a transcription factor that accumulates during hypoxia and increases the expression of a wide variety of genes that stimulate erythropoiesis, angiogenesis, and glycolysis (Singh et al., 2012). This transcription factor may influence gene expression at the level of histone modifications. Thus, HIF-1α binds to specific recognition sites in the genes encoding the jumonji family histone demethylases JMJD1A and JMJD2B and induces their expression in hypoxic conditions (Beyer et al., 2008). Genomic DNA methylation is also regulated by activation of HIF-1α through the up-regulation of methionine adenosyltransferase II alpha (MAT2a) in hepatoma cells under hypoxic conditions (Liu et al., 2011). This study revealed a positive correlation between HIF-1α and MAT2a promoter activity expression in Hep3B cells after hypoxic treatment, and the use of siRNA to knockdown HIF-1α prevented the expression of MAT2a. Furthermore, ChIP analysis detected a significant increase in the binding of HIF-1α to the MAT2a promoter within hypoxic Hep3B cells. On the other hand, histone deacetylase inhibitors have been employed to disrupt the function of hypoxia-inducible factors in cancer and chronic ischemic disorders (Chen and Sang, 2011). For the moment, no results have been reported in the treatment of metabolic disorders.

Both hypoxia and hyperoxia have been proposed as an alternative treatment for weight loss treatment (Quintero et al., 2010). For example, when studying the reasons for weight loss at high altitudes, higher metabolic rate and reduced food intake have been described, although the physiological mechanisms remain unclear (Lippl et al., 2010). The implementation of these potential therapies are subject to different genetic and environmental factors that must be clarified, including oxygen concentration, time of exposure, acclimation time, physical activity and dietary nutrient composition, some of which probably act by epigenetic mechanisms.

5.4. Hyperglycemia

Obese individuals are at a significantly greater risk for hyperglycemia than those who are nonobese, irrespective of their age (Wakabayashi et al., 2012). In fact, obesity is considered an important contributor to the impairment of insulin signaling in the peripheral tissues and type 2 diabetes development. In this sense, a recent article has evidenced the role of epigenetics in the pathogenesis of type 2 diabetes, finding 276 CpG loci affiliated to 254 gene promoters displaying significant differential DNA methylation in diabetic pancreatic islets (Volkmar et al., 2012).

Glucose is considered one of the main metabolic factors that epigenetically regulate gene expression. Thus, glucose modulates the transcription of the insulin gene by hyperacetylation of histone H4 (Mosley and Ozcan, 2003). In fact, the histone acetyltransferase p300 is recruited to the insulin promoter only at high concentrations of glucose via its interaction with the beta-cell-specific transcription factor Pdx-1 (Mosley et al., 2004). Another interesting report has shown that glucose regulates acetylation and methylation of various histone residues at the gene promoter of L-type pyruvate kinase, the rate-limiting step of the glycolytic pathway (Burke et al., 2009).

In the last years, several studies have analyzed the relationship between poor glycemic control in diabetic patients and epigenetic alterations that could be involved in the comorbidities associated to this disease. Thus, poor glycemic control mediates post-translational modifications to the H3 histone tail and, by using ChIP-seq and CpG-seq, it has been shown that hyperglycemia-mediated induction of genes associated with endothelial dysfunction occurs through modulation of acetylated H3K9/K14 and is inversely correlated with methyl-CpG content (Pirola et al., 2011).

Transient episodes of hyperglycemia can induce changes in gene expression that are dependent on histone tails modification and these changes persist after return to normoglycemia (Tonna et al., 2010). Some of these changes have been found in the promoter of the nuclear factor κB (NFκB) subunit p65 in aortic endothelial cells both *in vitro* and in nondiabetic mice, and are a cause of long-lasting increase in p65 gene expression (El-Osta et al., 2008). The phenomenon by which diabetes complications persist and progress after glycemic recovery is achieved is called metabolic memory. Interestingly, a recent report has demonstrated that metabolic memory is heritable and its transmission correlates with hyperglycemia-induced DNA hypomethylation and aberrant gene expression in streptozotocin-induced hyperglycemic zebrafish (Olsen et al., 2012). The daughter tissue, which was never exposed to hyperglycemia but was derived from tissue that was, does not exhibited higher levels of advanced glycation endproducts (AGEs) and oxidative stress, but hyperglycemia-induced global DNA hypomethylation persisted and was correlated with aberrant gene expression for a subset of loci in this daughter tissue (Olsen et al., 2012). To summarize, environmental factors, including the diet and episodes of transient hyperglycemia, contribute to the etiology and the microvascular and macrovascular complications of diabetes mellitus by

generating long-lasting epigenetic modifications that lead to changes in chromatin structure and gene expression (Pirola et al., 2010).

According to this hypothesis, obese individuals are more prone to suffer episodes of transient hyperglycemia. Even if these individuals return to normal glycemic control, hyperglycemia may induce long lasting effects by producing alterations in epigenetic processes and marks that facilitate the development and progression of metabolic complications (Siebel et al., 2010). Obviously, although it has not yet been studied in depth, genetic sequence, hormones, oxidative stress, inflammation and other environmental factors should play a role in the regulation of metabolic memory. Hence, two top priority objectives in this field are: 1) to early detect those individuals with more hyperglycemia-induced epigenetic alterations in order to prevent the future development of diabetic complications; and 2) to find new therapeutic agents that could slow down or stop the degenerative process by epigenetic mechanisms.

5.5. Circadian rhythms and sleep

Sleep duration has declined from 8-9 h per night to 7 h or less in the last 50 years, largely as a consequence of voluntary sleep restriction (McAllister et al., 2009). Sleep debt has been associated with decreased insulin response and glucose effectiveness, impaired glucose regulation by reduced lipolytic effects and increased sympathetic nervous system activity (Spiegel et al., 2009). During sleep restriction, plasma leptin levels are decreased, whereas ghrelin, cortisol and orexin secretion is increased, which has been associated with an increase of food intake and obesity incidence (Bo et al., 2011).

Apart from sleep deprivation, bright light exposure at night, shift work and nocturnal eating are frequent situations in obesity (Garaulet et al., 2010) that could influence the

methylation levels of clock genes and other epigenetic mechanisms. Thus, shiftwork exposure induces alterations in blood DNA methylation, including gene-specific methylation of IFN-γ and TNF-α promoters (Bollati et al., 2010). Melatonin, the major hormonal regulator of circadian rhythm, is able to modulate histone acetylation and DNA methylation (Korkmaz et al., 2009) and may influence diabetes by inhibiting insulin secretion, but also by protecting pancreatic β-cells from reactive oxygen species (Peschke and Mühlbauer, 2010). Hence, the involvement of epigenetic mechanisms in the melatonin-dependent synchronization of circadian insulin secretion to day/night changes must be envisaged.

Rhythmic changes in histone acetylation at circadian clock genes regulate the temporal modulation of gene expression by modifying chromatin. In this sense, a seminal study of Alenghat et al. (Alenghat et al., 2008) suggested that circadian regulation of metabolism is critical for normal energy balance. In this article, genetic disruption of the interaction between the histone deacetylase 3 (HDAC3) and its activator nuclear receptor corepressor 1 (Ncor1) altered the oscillatory patterns of several metabolic genes and resulted in leaner mice with more insulin-sensitivity owing to increased energy expenditure.

CLOCK gene is the first essential component of the mammalian clock. It is a histone acetyltransferase that contributes to the time-dependent regulation of circadian physiology by acetylating its own partner BMAL1 (Hirayama et al., 2007). In this sense, alterations in clock gene methylation have been detected in different diseases like Parkinson (Lin et al., 2012), whereas DNA hypermethylation-associated loss of BMAL1, which has been reported in leukemia/lymphoma cells, prevents the recruitment of its natural partner, CLOCK, to their common targets, further enhancing the perturbed circadian rhythm of the cancer cells (Taniguchi et al., 2009). Very

importantly, a maternal diet rich in fat modulates *in utero* the acetylation of fetal histone H3 at lysine 14 (H3K14ac) in NPAS2, a paralog of the CLOCK transcription factor, affecting thus the peripheral circadian system of the fetus (Suter et al., 2011). This result invites investigation on the different factors (nutritional or environmental) that could affect the epigenetic regulation of clock genes in various organs, both in fetal and adult life. Apart from animal studies, the epigenome of people working in shifts, at night or with irregular work hours, or the comparison of people with morning or evening preference could be of interest in the development of personalized nutrition for combat obesity and its comorbidities.

5.6. Ambient temperature

Indoor heating during cold seasons and air conditioning during warm seasons may contribute to rising obesity because the body expends less energy in temperature ranges associated with climate-controlled settings (Bo et al., 2011). Brown adipose tissue (BAT) is an important contributor to heat production in response to cold, and obese individuals seem to have less BAT mass and activity than normal weight subjects (Ravussin and Galgani, 2011). In this sense, it has been reported that mice knockout for the histone H3k9-specific demethylase Jhdma2a have reduced amount of BAT and increased body weight after 8 weeks of age (Inagaki et al., 2009; Tateishi et al., 2009). They are also more susceptible to cold-induced hypothermia and less able to oxidize fat primarily due to a disruption of β-adrenergic stimulation. The expression of UCP1, the main protein regulating thermogenesis in BAT, seems to be mediated by epigenetic mechanisms. Thus, demethylation of the UCP1 promoter by 5-aza-deoxycytidine increases UCP1 expression in adipose cell lines while methylation of UCP1 promoter-reporter constructs decreases the expression, which demonstrates a role for CpG

methylation in the control of UCP1 transcription. Unfortunately, no differences in UCP1 promoter methylation have been found when comparing BAT of mice exposed or not to a cold environment (Shore et al., 2010). The authors suggest that increasing the expression of UCP1 by epigenetic mechanisms could be a potential treatment for obesity. It is also likely that the emergence of cold-induced brown adipocytes in mouse white adipose tissue is determined predominantly by white to brown adipocyte transdifferentiation, which could involve epigenetic mechanisms mediated or not by β3-adrenoceptors (Barbatelli et al., 2010).

5.7. Psychological stress

In modern societies, mental and psychosocial stress has become a typical trait that involves chronic activation of the neuroendocrine systems, which has been linked to the increased prevalence of obesity (Siervo et al., 2009). For instance, in a recent report, major life events and vital exhaustion have been associated with weight gain and risk of obesity (Iversen et al., 2012).

Although few epigenetics studies have been performed in adult population, maternal and early life stress have been extensively analyzed and, in all cases, strong epigenetic modifications have been found in the offspring that, in many cases, remain until adult life. In this sense, it is clear that maternal stressors, including hypoxia and protein or calorie restriction, result in cellular and hormonal damage and affect epigenetic responses in the placenta, compromising placenta functions and leading to long-term consequences for fetal development (Gheorghe et al., 2010). Perinatal undernutrition is one of these stressors. This nutritional stress has been reported to remove methyls at CpGs located in leptin promoter that persists throughout life and is associated with lower levels of leptin mRNA and an imbalance between food intake and energy

expenditure in adults (Jousse et al., 2011). Maternal constraint is other of these stressors. First-born children experience a greater degree of maternal constraint during gestation and, as adults, they often show higher adiposity and visceral obesity than their younger siblings (Reynolds et al., 2010). Epigenetic processes could partially explain these data because they are involved in matching fetal growth to mother's complexion, body composition and lifestyle (Hanson et al., 2011b).

Stress at early ages is also clearly associated to hormonal alterations and epigenetic modifications. Thus, differences in histone acetylation and in DNA methylation of glucocorticoid receptor in the hippocampus have been found when comparing the offspring of rat mothers that showed high and low levels of pup licking and grooming and arched-back nursing (Weaver et al., 2004). These differences emerged over the first week of life but persisted into adulthood. In humans, maternal touch and feed are considered also critical regulators of behavioral and stress responses in the offspring (Walker et al., 2010), although its importance as an obesity risk factor and the alteration of epigenetic processes have never been analyzed. However, it has been observed that childhood abuse, neglect and loss induce alterations of the epigenetic mechanisms (McGowan et al., 2009). They are major risk factor for developing depressive disorders later in life (Heim and Binder, 2012) and could be related with epigenetic mechanisms and chronic alterations in cortisol secretion in children that could affect body composition and be causative factors of early-onset obesity, metabolic syndrome, and type 2 diabetes (Pervanidou and Chrousos, 2011).

Although epidemiological data suggest that psychological stress could act also as a causative factor of obesity in adults, there are many confusing factors that must be carefully studied and the etiological mechanisms remain unclear (Tamashiro et al., 2011). However, there are suggestive precedents indicating that mice living in an

enriched environment with complex physical and social stimulation leads not only to improved cognitive and metabolic health, but also to a switch of white to brown fat phenotype and the expression of genes involved in thermogenesis associated with resistance to diet-induced obesity (Cao et al., 2011). Epigenetics could be behind some of these observations but new *ad hoc* studies must be carried out. Moreover, as maturation rates and the extent of plasticity across development are highly variable for different brain regions and for the different tissues, timing of the stressor events is a critical factor and it is important to identify the most sensitive periods (Heim et al., 2012).

5.8. Socioeconomic status

In countries with a higher gross domestic product, but not in most developing countries, lower income inequality is one of the strongest determinants to develop overweight, obesity and cardiovascular diseases (Jones-Smith et al., 2011). It has been usually explained by decreased physical activity, unhealthy behaviors and cultural and social barriers (Voorhees et al., 2009) but, in the last years, psychological and sociological biological determinants have been reported to be associated with epigenetic mechanisms. Thus, in the pSoBid cohort, which is characterized by an extreme socio-economic and health gradient, global DNA hypomethylation was observed in the most socio-economically deprived subjects and was also associated with biomarkers of cardiovascular disease and inflammation (McGuinness et al., 2012). Also, microarray analyses carried out in males of the British Birth Cohort Study show that adult blood DNA methylation profiles is associated with disadvantaged socio-economic status, especially with that of childhood (Borghol et al., 2011). These results strongly support the hypothesis that low early-life social class is a key factor in enduring differences in

adult expression of genes critical for human health by leaving their mark on the epigenome. Anyway, more studies are needed in order to know the specific factors with the most impact in epigenetic changes induced by the socioeconomic status, with perinatal and early life nutrition and stress being some of the most likely candidates.

5.9. Hormonal and neuropeptide imbalance

The onset of obesity and insulin resistance is sometimes associated with hormonal imbalances. Also, fat distribution in visceral and subcutaneous depots is regulated by the secretion of different hormones including cortisol, insulin, adiponectin, leptin, estrogens, dihydrotestosterone and growth hormone (Mattson and Olsson, 2007). Although the direct effects of these hormones on fat deposition and insulin signaling are well established, some of them have been reported to induce epigenetic modifications. For instance, whereas 17β-estradiol hypermethylates O6-methylguanidine-DNA in lung cancer cells (Lai et al., 2009), insulin induces global hypomethylation in HepG2 liver cells in culture (Chiang et al., 2009) and reduces the methylation level at H3R17, downregulating PEPCK and G6Pase, the rate-determining enzymes of gluconeogenesis (Hall et al., 2007). Leptin, a key gene in energy balance regulation, is hypomethylated in whole blood of humans being born small for gestational age due to prenatal growth restriction early in pregnancy (Tobi et al., 2011). Glucocorticoids, which affect food intake and abdominal obesity, cause local DNA demethylation of the tyrosine aminotransferase gene around a glucocorticoid response element that appears to contribute to the memorization of long-term effects of glucocorticoids in neonatal brains (Thomassin et al., 2001). According to these encouraging data, more emphasis must be made in the study of the epigenetic alterations due to transient or chronic hormonal imbalances, which are so common in obesity and related comorbidities, as well as in the

knowledge of the epigenetic mechanisms that regulate the secretion of hormones as a result of dietary and environmental factors.

Appetite regulation involves a complex interplay of hunger and satiety signals that is based in the hypothalamus. Some of these signals consist of orexigenic and anorexigenic neuropeptides, such as POMC, MC4R, NPY and CART, some of which have been reported to be regulated epigenetically. Thus, periconceptional undernutrition induces proopiomelanocortin (POMC) promoter hypomethylation in the hypothalamus of sheep (Stevens et al., 2010), and is also associated with reduced DNA methyltransferase activity and altered histone methylation and acetylation (Begum et al., 2012). On the other hand, when suffering early overfeeding, the hypothalamic POMC promoter becomes hypermethylated (Plagemann et al., 2009). Neuropeptide Y (NPY), one of the main appetite-inducing factors, was one of the ten CpG sites that were hypermethylated in mucosa from colorectal cancer patients when compared to normal mucosa (Kim YH et al., 2011), and is also frequently hypermethylated in hepatocellular carcinoma tissue samples (Shin et al., 2010). In this sense, it is crucial to understand the epigenetic mechanisms involved in the regulation of these genes in the hypothalamus in response to different hormonal, dietary and environmental factors in order to understand physiological and pathological aspects of appetite regulation that could provide potential targets for the treatment of obesity. In particular, more emphasis must be made in maternal nutrition, which modifies, in some cases through epigenetic mechanisms, metabolic or endocrine signals acting on genes encoding neuropeptides.

5.10. Endocrine disruptors and toxics

Environmental endocrine disruptors are synthetic chemicals that resemble natural hormones and are known to cause epigenetic perturbations and profound effects on

development and fertility (Kang et al., 2011). Endocrine disrupting chemicals like tributyltin interfere with the adipose tissue biology, endocrine hormone systems and hypothalamic-pituitary-adrenal axis and deregulate the homeostatic mechanisms that control weight (Grün and Blumberg, 2010). There is evidence that early life exposure (pre- or early postnatally) to endocrine-disrupting chemicals may be a risk factor for obesity and related metabolic diseases later in life (Miyawaki et al., 2007). Polluting chemical substances which may potentially induce obesity in humans are organotins (TBT, TPT) and phthalates, which act as PPARγ activators, but also diethylsylbestrol (DES), genistein and bisphenol A, three substances mainly acting upon estrogen receptors (Newbold, 2010). During this period of life, and more specifically during embryonic gonadal sex determination, chemicals can exert effects at very low levels of exposure and may involve epigenetic events that could be transgenerationally transmitted (Anway et al., 2005). Hence, some research projects are studying the effects of these chemicals on obesity development, such as OBELIX (Legler et al., 2011), and will include epigenetic analyses in both animal models and children.

There are examples in which vinclozolin, an endocrine disruptor with antiandrogenic effects, or bisphenol A modulate the monoallelic expression of imprinted genes (Kang et al., 2011), and can alter the male germ-line DNA methylation, transmitting thus transgenerational adult onset disease (Anway and Skinner, 2008). In some cases, these alterations have been observed in the sperm of F3 generation rats whose F0 generation mother was exposed to vinclozolin (Guerrero-Bosagna et al., 2010). In this sense, endocrine disruptors act by inducing epigenetic alterations in estrogen responsive elements (ERE) sensitivity to estrogens (Bromer et al., 2010). As it is well established that early exposure to bisphenol A leads to adult obesity (Rubin, 2011), and that this compound induces profound alterations in DNA methylation and other epigenetic

processes, it is necessary to design new perinatal and transgenerational experiments in rodents that could determine the targets and periods of life that are more sensitive to these endocrine disruptors, as well as the doses.

Even in the adult age, toxins are able to alter the epigenome. In this sense, particulate air pollution has been associated with increased cardiovascular events, and lower blood DNA methylation has been found in processes related to cardiovascular morbidity (Baccarelli et al., 2010). Lower blood DNA methylation has been also found after exposure to traffic particles (Baccarelli et al., 2009) and after prolonged exposure to black carbon and SO_4 particles (Madrigano et al., 2011). Other toxics associated with carcinogenesis, including asbestos, alcohol, radiation and arsenic, are known to act also by epigenetic mechanisms (Christensen and Marsit, 2011). However, as their importance in obesity pandemics is not well known, more studies in humans are necessary. One example is the result of a longitudinal analysis of the SUN cohort showing that recent ex-smokers and active smokers both experienced significantly greater weight gains than never-smokers (Basterra-Gortari et al., 2010). In this sense, exposure to cigarette smoke condensate induces changes in histone modifications in epithelial cells and immortalized bronchial epithelial cells (Liu et al., 2010), suggesting that epigenetics could play a role in smoking-cessation-induced weight gain.

5.11. Infections

In the last years, ten adipogenic pathogens have been associated with obesity in humans and in animals, including several viruses such as adenovirus 36 (Pasarica and Dhurandhar, 2007). However, although inflammation has been proposed as one of the triggering factors, the detailed cellular mechanism is unclear (Na and Nam, 2012). In this sense, it has been proposed that viral DNA insertion into established mammalian

genomes is able to induce *de novo* DNA methylation of the integrate but also alterations of methylation patterns across the recipient genome (Doerfler, 2011). Although many efforts must be done to expand our knowledge on infectobesity, its real contribution to obesity pandemics is still unknown.

On the other hand, among the numerous pathways and processes that are altered by obesity, impairment of the immune response is an important one (Marti et al., 2001). Thus, it has been hypothesized that altered immune cell metabolism and epigenetic modifications could influence the immune response to infectious disease in the obese host (Karlsson and Beck, 2010), and be responsible of the increased susceptibility to infection with a number of different pathogens. However, there are few reports observing the effect of nutritional factors on the epigenetic modification of immune cells and further studies are needed.

5.12. Microbiome

The microbiome plays a key role in a wide range of host-related processes, including obesity. This concept was demonstrated in an elegant experiment by Turnbaugh et al. (2006). They colonized with microbiota of genetically obese mice the gut of germ-free mice, and these mice significantly increased total body fat when compared with germ-free counterparts colonized with microbiota from genetically lean mice. Low-grade inflammation due to metabolic endotoxemia is one of the candidates to link the gut microbiota with obesity, given that high levels of lipopolysaccharide and enhanced intestinal permeability have been found in obese and diabetic mice (Cani and Delzenne, 2011). In this sense, lipopolysaccharide, a major component of the outer membrane of Gram-negative bacteria with potent inflammatory properties, induces *de novo* cytosine methylation in mouse embryonic fibroblasts (Tatemichi et al., 2008). On the other hand,

lesions induced by lipopolysaccharide are attenuated with a pretreatment with butyrate, which has been attributed to an effect on inflammatory cytokine production (TNF-α, IL-1β and nitric oxide) and NFκB activation (Ni et al., 2010).

Although the effects of microbiome are found in the whole organism, colonic mucosa is the interface between the gut microbiota and the mammalian host. A new and exciting field in obesity research is the study of the epigenomic modifications associated with alterations in mucosal microbial composition (Kellermayer et al., 2011). For example, probiotics are being widely used in the prevention or treatment of chronic inflammatory conditions and are related with the production of short-chain fatty acids that regulate epigenetic processes (Licciardi et al., 2010). The few experiments performed in humans show that prebiotic, probiotic, and symbiotic dietary supplementation induce changes in fecal microbiota but did not significantly alter mucosal DNA methylation (Worthley et al., 2009).

6. Physical activity

Physical activity is one of the major links between the hormonal modulators of energy intake and output. Alongside with resting metabolic rate, it is a major contributor to maintain energy balance. For preventing obesity development, acute low- and moderate-intensity exercise is usually recommended because it causes hormonal changes that facilitate lipolytic activity, probably by mediation of hormones (such as catecholamines, cortisol, insulin, growth hormone, and thyroid hormones), cytokines and oxidative stress (McMurray and Hackney, 2005).

It is clear that physical activity confers many health benefits, and some of them may occur by alterations in epigenetic landscapes. Thus, Zhang et al. (2011a) have reported that, although the differences are small, individuals with high physical activity (30

min/day) have a significantly higher level of global genomic DNA methylation in LINE-1 in peripheral blood than those with low physical activity (10 min/day). Unraveling the complexities of the histone code before and after exercise will undoubtedly reinforce the importance of skeletal muscle plasticity in health and disease. Thus, the class II HDACs 4, 5, 7, and 9 are known to play a key role in skeletal muscle development and adaptation and have been implicated in exercise adaptations (McGee and Hargreaves, 2011). As an example, after 60 minutes of exercise, changes have been observed in histone modifications associated with chromatin remodeling and transcriptional activation in human skeletal muscle (McGee et al., 2009). In rats, a single session of treadmill exercise reduces HDAC activity, increases HAT activity and increases the HAT/HDAC balance in rat hippocampus, suggesting that part of the neuroprotective effects of exercise could be mediated by epigenetic mechanisms (Elsner et al., 2011).

As physical activity reduces the risk of breast cancer and other malignancies, several studies about the epigenetic modulation of gene expression due to physical activity have been carried out in oncology. For instance, physical activities for at least one year are inversely related to promoter hypermethylation of the tumor suppressor gene APC in breast cancer (Coyle et al., 2007). These studies in cancer suggest that exercise induces epigenetic modifications that could be behind its health beneficial effects. However, the mechanisms by which physical activity induces these modifications are far from being elucidated, with oxidative stress, changes in nutrient supply to the cells, inflammation or hormonal stimuli as some of the most likely contributors.

As fetal period is more sensitive to epigenetic-induced alterations that could influence adult health status, much interest has arisen in the study of exercise during pregnancy. Thus, regular aerobic exercise during pregnancy seems to lead to a small reduction in

offspring birth weight when compared with the offspring of nontraining women, which could be explained by maternal hormonal alterations, the regulation of nutrient availability for fetal growth through placenta, and epigenetic processes (Hopkins et al., 2010). However, these effects on offspring growth were not associated with changes in maternal insulin sensitivity (during a healthy pregnancy) and no effects in offspring body composition were found (Hopkins et al., 2011). Anyway, the authors hypothesized that the reduction of offspring body weight may lead to a long-term reduction in the risk for obesity.

7. Perinatal events (gestation and lactation)

Established DNA methylation patterns are not immutable and can be modified during our lifetime by the environment (Kim KC et al., 2009; Martinez et al., 2012). However, it is well established that organ-specific DNA methylation patterns are established through epigenetic reprogramming during the fetal period and this is the period in which dietary and environmental factors play a major role. Indeed, a persistent change in early gene transcription may alter behavior and organ functions and be involved in the susceptibility and the age of onset of different metabolic disorders due to the *in utero* insult (Heerwagen et al., 2010). This is in relation with the concept of developmental plasticity, that enables an organism to adjust its phenotype to the environmental cues (Gluckman et al., 2009). A mismatch between the phenotypic outcome of adaptive plasticity and the current environment increases susceptibility to metabolic diseases and is the basis of the Developmental Origins of Health and Disease (DOHaD) hypothesis. This hypothesis postulate that unbalanced nutrition *in utero* and during infancy plays an important causative role in noncommunicable diseases, including diabetes,

cardiovascular disease, allergy, some forms of cancer, cognitive decline, and affective disorders (Hanson and Gluckman, 2011).

Although the whole perinatal period in which fetal development takes place is susceptible to epigenetic modifications, there is a period in which the epigenome seems to be more sensitive to environmental factors. After the fertilization methylation of specific sites is erased, and after the implantation methylation is restored by the gastrulation stage (Campion et al., 2009). Thus, at implantation, the DNA methylation patterns of individual promoters dynamically change and a study has identified by genome-wide analysis that *de novo* methylation occurs during implantation in 476 of 691 promoters that are methylated in the E9.5 embryo (Borgel et al., 2010). However, it is also true that DNA methylation patterns established around implantation can be modified by further methylation or demethylation during subsequent stages of organogenesis as a result of environment factors that are not yet well known. An illustrative example is the effect of the phytoestrogen genistein, which has been described to mediate global changes in DNA methylation levels during mouse embryonic stem cell differentiation in the post gastrulation period but before germ layer differentiation (Sato et al., 2011). Genistein-induced epigenetic alterations seems to share important similarities with those found in intrauterine growth retardation neonates (Einstein et al., 2010), but the magnitude is markedly lower than the differences found between tissues or in cancer. As previously described, perinatal undernutrition (Jousse et al., 2011) or overnutrition (Plagemann et al., 2009), the intake of particular nutrients like methyl donors (Waterland et al., 2008) or PUFA (Kiec-Wilk et al., 2011), or the exposure to endocrine disruptors like bisphenol A (Rubin et al., 2011) are crucial in the *in utero* modulation of the epigenetic marks (DNA methylation or histone modifications), and more efforts must be made to unravel the epigenetic mechanisms

involved in the pathogenesis of obesity and metabolic syndrome, including the doses of each compound, the interactions with the genotype and the period or length of the exposure. One interesting example of possible application of this research is the demonstration that leptin administration during the suckling period has protective effects against later obesity probably by inducing changes in promoter methylation of the hypothalamic POMC gene (Palou et al., 2011).

These perinatal events are important in defining the epigenetic marks that will persist until the adult age. Some of these epigenetic marks could be used as early prognostic markers of disease to identify those individuals with more risk to develop a metabolic disorder. Thus, a recent report has evidenced that the methylation of different gene promoters (RXRA, eNOS) at birth is associated with child's later adiposity (Godfrey et al., 2011), suggesting that prenatal developmental may alter the epigenetic regulation of key components of metabolic disease risk and that a perinatal epigenetic analysis might identify subjects at high risk of developing later obesity. Another interesting result has been recently found when comparing cord blood global Long Interspersed Nucleotide Element-1 (LINE-1) methylation among newborns with low and high birth weight as well as among prematurely born infants, which might contribute to the increased risk of chronic diseases among individuals with these characteristics (Michels et al., 2011). Similarly, lower methylation of LINE-1 repetitive elements has been associated to higher risk for incident ischemic heart disease, stroke and total mortality (Baccarelli et al., 2010).

8. Inheritance of epigenetic traits

Apart from the higher availability of energy-dense foods, one of the hypothesis that try to explain the rising prevalence of obesity and hepatic steatosis is a feed-forward circle

that may exacerbate these disorders throughout multiple generations. This hypothesis was audaciously postulated by McAllister et al. (2009), and, beyond genetics, they tried to explain it because obesity in women is associated with lower socioeconomic status, which has been linked to producing more offspring, and because there exists an assortative mating based on spousal resemblance in many physical characteristics, including body weight (Katzmarzyk et al., 1999). However, the intricate mechanisms are very difficult to demonstrate empirically in humans.

In animals, it has been demonstrated that long-term exposure to a methyl donor rich diet for six generations results in a large number of loci exhibiting epigenetic variability, suggesting that some of the induced changes are heritable (Li CC et al., 2011). The offspring of HFD-fed mice are more susceptible to develop hepatic steatosis when weaned on the same HFD than on normal chow (Li J et al., 2011). The metabolic effects of maternal high-fat diet exposure on body length and insulin insensitivity persist also across at least two generations of offspring (Dunn et al., 2009). This may probably be explained due to transgenerational accumulation of epigenetic modifications leading to up-regulation of lipogenesis and endoplasmic reticulum stress in the liver. These epigenetic alterations are transmitted not only by the mother but also by the father, as demonstrated by Ng et al. (Ng et al., 2010), who observed that chronic HFD consumption in rat fathers induced increased body weight and adiposity, impaired glucose tolerance and insulin resistance in the female offspring. Other studies in rodents have evidenced the transgenerational progression of metabolic phenotypes through the male lineage, supporting thus a potential role for epigenetic mechanisms as a consequence of broad programming events at imprinted loci (Pertinat et al., 2010; Dunn and Bale, 2011), and changes in PPARα cytosine methylation due to paternal low-protein diet (Carone et al., 2010).

In 1987 the hypothesis of the inheritance of epigenetic defects was postulated (Holliday, 1987), breaking away from the idea of inheritance as an exclusive matter of Mendelian genetics. It stated that epigenetic defects in germline cells can be usually repaired by recombination at meiosis but that some are transmitted to offspring. Since them, many evidences have been accumulated in the defence of this audacious postulate but only in the last few years there is more widespread acceptance of the theory. Although the molecular basis of this inheritance remains unclear, recent evidence points towards diffusible factors, in particular RNA, rather than DNA methylation or chromatin (Daxinger and Whitelaw, 2012). It seems that there are two crucial moments in which epigenetic information undergoes extensive reprogramming and becomes erased and re-established: during gametogenesis and again in early embryogenesis, two events characterized by extensive changes in DNA methylation and chromatin modifications (Feng et al., 2010). However, there are some epigenetic marks that escape both incidences of reprogramming and are able to persist in the somatic cells of the individual. The knowledge of the mechanisms that participate in the protamine-, methylation-, histone- and small RNA-mediated inheritance will be of great importance in the understanding of the transgenerational inheritance of chronic non-communicable disease risk.

9. Summary and future perspectives

The state of the art in obesity research is that most of the cases of obesity are due to a combination of energy imbalance (higher energy intake than expenditure) with inherited genetic factors that, in conjunction with other hormonal and environmental signals, determines the individual predisposition to develop obesity and associated complications. However, emerging evidence suggests that one of the links between the

environmental factors and the higher predisposition to develop obesity and metabolic syndrome is epigenetics. To date, there are evidences that many nutritional factors could act by modulating DNA methylation or histone modifications and some of them might be used in obesity therapy due, at least in part, to their epigenetic mechanisms (Fig. 3). This outcome is more evident in relation to the methyl donors (folate, methionine, choline and vitamins B6 and B12), especially when maternal diet is supplemented. Methyl donors are critical during fetal development, when they alter DNA methylation and influence neural precursor cell proliferation and correct brain development. The personalization of methyl donor necessities during pregnancy due to the genetic background is one of the most important tasks for the next few years. But also in the adult population there are differences in the health outcomes of methyl donor deficiency (fatty liver, insulin resistance) due to the common genetic variants.

Other food components that have a promising future as epigenetic therapeutic agents against obesity and related disorders are polyphenols and organosulfur compounds. They have elicited very positive effects in cancer prevention and treatment, and many of them have been associated to lower risk of obesity, inflammation and oxidative stress. A careful investigation on the epigenetic properties of other nutrients, such as several fatty acids (particularly PUFA), minerals and vitamins, could open the door to new therapeutic approaches to combat obesity and its associated disorders.

Another important research line concerns the applications of drugs targeting HATs, HDACs, or DNMTs as a novel therapy for diabetes. However, although *in vitro* and animal studies show encouraging results in different aspects (i.e. HDAC inhibitors enhance β-cell differentiation and survival and insulin signaling), a careful evaluation of the potential secondary effects is mandatory. The use of these drugs in obesity must reinforce even more the study of the secondary effects.

Well designed and controlled *in vitro* and animal studies must be designed to understand the epigenetic mechanisms that modulate adipocyte differentiation, hepatic steatosis, insulin signaling, appetite regulation and insulin secretion, as well as important bioenergetic pathways, such as lipolysis, fat oxidation and glucose uptake and oxidation. In these studies, the epigenetic effects of inflammatory cytokines, reactive oxygen species, hypoxic conditions, high glucose levels, hormonal imbalance, endocrine disruptors and stress must be elucidated.

Large, prospective studies are needed to understand whether changes in obesity risk factors, including the diet, physical exercise, stress and other environmental and metabolic factors, are associated with alterations in DNA methylation patterns or histone covalent modifications. Special emphasis must be laid on the effects of maternal environmental conditions on epigenetic regulation of gene expression and the long-term metabolic effects in the offspring. It is important to understand the perinatal epigenetic programming of the lifetime function of fundamental regulatory systems to successfully prevent diet-related metabolic diseases.

On the other hand, microarray-based or sequencing approaches will be of great value for the identification of epigenetic biomarkers that can be used for early diagnosis of obesity, diabetes and cardiovascular diseases, and, in conjunction with other factors, for the design of further personalized therapeutic approaches (Fig. 4). As a major objective for the next 10 to 20 years, epigenetic knowledge should interact with genetic information, transcriptomic, proteomic and metabolomic data, and environmental variables to set the bases of a personalized treatment of metabolic diseases. The inheritance of these epigenetic marks is another issue that should be envisaged.

To conclude, three ideas must be highlighted: 1) epigenetic mechanisms are involved in the onset and development of obesity and related metabolic dysfunctions; 2) the

epigenetic marks could be useful in the prognosis and early diagnosis of obesity and related comorbidities; and 3) nutritional or pharmacological agents might be used as novel therapeutic strategies due to their ability to modulate the epigenetic processes, particularly during the perinatal period. In the next years, the research on obesity-related epigenetic mechanisms might help to prevent and control excessive fat deposition and insulin resistance by designing a personalized weight reduction plan.

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Figure 1. Interactions among the different factors that are involved in the onset and development of obesity.

Figure 2. Major epigenetic mechanisms involved in gene expression regulation.

Figure 3.

Metabolic, dietary and environmental factors that have been related to the regulation of adipogenesis and insulin sensitivity and may act by epigenetic mechanisms.

Figure 4. Epigenetics as an important factor in personalized nutrition.

Table 1. Some examples of key genes involving metabolic processes whose expression is controled by epigenetic mechanisms.

Table 2. Some examples of metabolic effects attributed to nutritional factors acting through epigenetic processes.