Epigenetics in Adipose Tissue, Obesity, Weight Loss, and Diabetes[1,2]

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ABSTRACT

Given the role that diet and other environmental factors play in the development of obesity and type 2 diabetes, the implication of different epigenetic processes is being investigated. Although it is well known that external factors can cause cell type-dependent epigenetic changes, including DNA methylation, histone tail modifications, and chromatin remodeling, the regulation of these processes, the magnitude of the changes and the cell types in which they occur, the individuals more predisposed, and the more crucial stages of life remain to be elucidated. There is evidence that obese and diabetic people have a pattern of epigenetic marks different from nonobese and nondiabetic individuals. The main long-term goals in this field are the identification and understanding of the role of epigenetic marks that could be used as early predictors of metabolic risk and the development of drugs or diet-related treatments able to delay these epigenetic changes and even reverse them. But weight gain and insulin resistance/diabetes are influenced not only by epigenetic factors; different epigenetic biomarkers have also been identified as early predictors of weight loss and the maintenance of body weight after weight loss. The characterization of all the factors that are able to modify the epigenetic signatures and the determination of their real importance are hindered by the following factors: the magnitude of change produced by dietary and environmental factors is small and cumulative; there are great differences among cell types; and there are many factors involved, including age, with multiple interactions between them. *Adv. Nutr. 5: 71–81, 2014.*

Introduction

Epigenetics can be defined as inheritable and reversible phenomena that affect gene expression without altering the underlying base pair sequence (1). Epigenomics is the study of genome-wide epigenetic modifications. Epigenetics was introduced as a theoretical framework seeking to understand putative undisclosed relations between genes and environmental settings (diet, inactivity, smoking, etc.) to generate a phenotype (2). Epigenetics can provide some insights to help understand genetic fetal programming, monozygotic twin differences, and chronic disease onset in adults, which interact with dietary intake and nutritional processes. Some epigenetic information might be inherited from one generation to the next. Although DNA methylation status is currently the most-studied epigenetic marker, there is increasing recognition that other modifications such as those of the histone code can modify the chromatin organization and folding (euchromatin vs. herochromatin) in such a way as to affect gene expression patterns (3). These mechanisms, together with other transcriptional regulatory events, ultimately regulate gene activity and expression during development and differentiation or in response to nutritional and environmental stimuli.

Important recent investigations have highlighted that chromatin modifications/accessibility mark important disease-relevant regulatory regions in the genome (4–6,8). Some of these studies suggest that common phenotypically associated single nucleotide polymorphisms (SNPs)[7], which are enriched for expression

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[7] Abbreviations used: AQP9, aquaporin 9; ATP10A, ATPase class V type 10A; *BHMT*, betaine-homocysteine *S*-methyltransferase; *CBS*, cystathionine β-synthase; C/EBP, CCAAT/enhancer-binding protein; CD44, CD44 molecule; CLOCK, clock circadian regulator; CTCF, 11-zinc finger protein; DMR, differentially methylated region; DNMT1, DNA methyltransferase 1; FABP4, fatty acid binding protein 4; *FASN*, fatty acid synthase; *FTO*, fat mass and obesity associated; *GNMT*, glycine N-methyltransferase; GR, glucocorticoid receptor; H, histone; HAT, histone acetyltransferase; HDAC, histone deacetylase; ICR/H19, H19 imprinting control region; *IGF2*, insulin-like growth factor 2; *LEP*, leptin; *MS*, methionine synthase; NDUFB6, NADH dehydrogenase (ubiquinone) 1 β subcomplex subunit 6; NOD, nonobese diabetic mouse; *NPY*, neuropeptide Y; *PEMT*, phosphatidylethanolamine *N*-methyltransferase; PER2, period circadian clock; *POMC*, proopiomelanocortin; *PRC2*, polycomb repressive complex 2; SNP, single nucleotide polymorphism; STZ, streptozotocin; TNF, tumor necrosis factor; WT1, Wilms tumor 1; ZDF, Zucker diabetic fatty rat.

quantitative trait loci, might act by altering gene regulatory regions (4). Whereas many expression quantitative trait loci and regulatory variants act universally, some of the most relevant to disease might have tissue-specific activity (9). In this sense, chromatin state differences between cell types are related to cell type-specific gene functions (5). The Encyclopedia of DNA Elements project (6) has systematically mapped regions of transcription, transcription factor association, chromatin structure, and histone modification within different cell types (including up to 12 histone modifications), which are allowing researchers to assign functional attributes to genomic regions. The study of Ernst et al. (5) also revealed that the levels of DNA methylation usually correlate with chromatin accessibility and that, because most of the disease-associated SNPs are either intronic or intergenic and show consistently higher overlap with Encyclopedia of DNA Elements annotations, it seems like the genome-wide association study-identified regions are the ideal place to look for such epigenetic signatures. The National Human Genome Research Institute Catalogue of Published genome-wide association study provides a quality-controlled, manually curated, literature-derived collection of all published GWA studies, which, as of 1 October 2013, included 1724 publications and 11,680 SNPs (7). One proposed mechanism of action of the SNPs is that they would affect the activity of enhancer elements regulating critical target genes. Thus, Maurano et al. (8) demonstrated that disease-associated variants systematically perturb transcription factor recognition sequences, frequently alter allelic chromatin states, and form regulatory networks in a tissue-specific way. For example, of the 67 SNPs for type 2 diabetes (implicating a total of 2776 H3K4me3 peaks) analyzed in this study (8), 14 (20.1%) were either highly specific for chromatin marks within the liver or pancreatic islets, 2 tissues having a key role in mediating glucose metabolism and insulin secretion.

Another multi-dimensional approach that tries to reveal the insights into how genetic and epigenetic factors may underlie their etiopathogenesis is the analysis of loss or gain of CpG dinucleotides (by CpG-creating SNPs). This mechanism has been argued to be a major driver in disease susceptibility, because it may lead to a genetically driven variation in DNA methylation and affect gene expression, as has been demonstrated for the fat mass and obesity associated (*FTO*) gene in obesity and type 2 diabetes (10). Another important report demonstrated that DNA methylation may influence genetic variation by measuring the incidence of genetic variations in methylation states in the human genome (11). This study found that the SNP rate significantly increased by ~50% if the neighboring CpG sites were methylated.

Recently, a number of studies are being carried out to understand the influence of different dietary compounds (macronutrients, micronutrients, phytochemicals, antioxidants, etc.) on the modification of these epigenetic marks and, consequently, on gene expression regulation and the probability to develop or prevent disease. Some of these nutritional factors are shown in **Table 1**.

Similarly to type 2 diabetes, hypertension, atherosclerosis and other metabolic disorders, predisposition to obesity, and weight loss have been associated with changes in epigenetic patterns. Besides nutritional factors, different non-nutritional risk factors that usually accompany obesity seem also to be involved in the epigenetic modifications affecting adipogenesis and insulin sensitivity, especially hyperglycemia, inflammation, endocrine disruptors, hypoxia, and oxidative stress (**Fig. 1**).

Two outstanding features of epigenetic processes are the ability for cellular memory transmission or transgenerational heritability as well as the involvement in spatial and temporal cell differentiation from totipotent cells. Therefore, epigenetics can provide some insights into unsolved mysteries such as cellular identity, stem cell plasticity, tissue regeneration, tumorigenesis, and ageing. Indeed, one of the challenges for investigators researching in the epigenetics field is to identify and characterize the epigenetic marks and those stimuli modulating the expression of some specific genes in pathways related to body weight homeostasis and energy balance such as adipogenesis, inflammation, appetite, insulin signaling, thermogenesis, and nutrient turnover. A bioinformatics analysis of the search for the CpG islands in the promoter regions of obesity-related genes has identified regions with a high density of CpGs in genes implicated in adipogenesis, such as human peroxisome proliferator-activated receptor gamma coactivator 1 (*PPARGC1*), small heterodimer partner (*NROB2*), glucocorticoid receptor

TABLE 1 Examples of nutritional factors having beneficial metabolic effects that are regulated by epigenetic mechanisms[1]

Nutritional factor	Metabolic disorder	Epigenetic mechanisms	Reference
Methyl donors			
Betaine	Insulin resistance, liver steatosis	Histone and DNA methylation	(13)
Choline	Liver steatosis	Histone and DNA methylation	(14)
Folate	Insulin resistance, adiposity	DNA methylation	(15)
Methionine	Insulin resistance, obesity	Histone and DNA methylation	(15)
Vitamin B-12	Insulin resistance, obesity	DNA methylation	(15)
Phytochemicals			
Curcumin	Inflammation, obesity	Histone acetylation, DNA methylation, and microRNA	(16)
Epigallocatechin 3-gallate	Obesity, insulin resistance, liver steatosis	Histone acetylation and DNA methylation	(17)
Genistein	Obesity	Histone acetylation and DNA methylation	(18)
Resveratrol	Obesity, liver steatosis	Histone acetylation	(19)
Sulforaphane	Adipocyte differentiation	Histone acetylation	(20)
Fatty acids			
Butyrate and other SCFAs	Insulin resistance, inflammation	Histone acetylation and propionylation	(21)

[1] Based on (12).

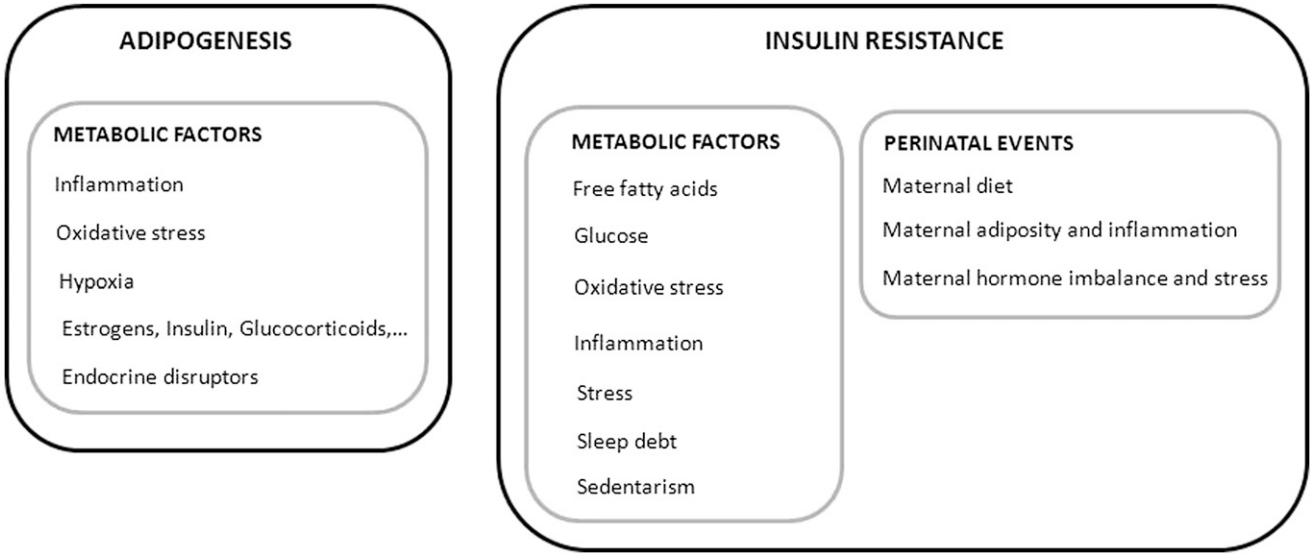

FIGURE 1 Metabolic and perinatal factors that have been related to the regulation of adipogenesis and insulin sensitivity and may act through epigenetic mechanisms.

(*NR3C1*), peroxisome proliferator-activated receptor gamma (*PPARG*), basic fibroblast growth factor (*FGF2*), phosphatase and tensin homolog (*PTEN*), cyclin-dependent kinase inhibitor 1A (*CDKN1A*), and estrogen receptor 1 (ESR1); in inflammation, such as suppressors of cytokine signaling 1 and 3 (*SOCS1/SOCS3*), leptin (*LEP*), and tumor necrosis factor (*TNF*)A; in apoptosis, such as caspase 9 (*CASP9*); and in intermediate metabolism and insulin signaling, such as cytochrome c oxidase subunit VIIa polypeptide 1 (*COX7A1*), lipoprotein lipase (*LPL*), hydroxysteroid (11-β) dehydrogenase 2 (*HSD11B2*), fatty acid binding protein 4 (*FABP4*), caveolin 1 (*CAV1*), and insulin-like growth factor binding protein-3 (*IGFBP3*) (22). These CpG islands are short, interspersed, CpG-rich DNA sequences that are generically equipped to influence local chromatin structure and function and simplify regulation of gene activity (23). In this sense, in the last years, many genes involved in the regulation of the main metabolic pathways have been reported, both in vivo and in vitro, to be affected by epigenetic regulation (**Table 2**).

In relation to epigenetic research in the fields of obesity and type 2 diabetes, there are currently 3 major objectives: to search for epigenetic biomarkers to predict future health problems or detect the individuals at greatest risk, to understand the obesity-related environmental factors that could modulate gene expression by affecting epigenetic mechanisms, and to envisage novel therapeutic strategies based on nutritional or pharmacological agents that can modify

TABLE 2 Examples of metabolic processes related to obesity and type 2 diabetes that are regulated by genes whose expression is controlled by epigenetic mechanisms

Metabolic process	Gene symbol	Common gene name	Epigenetic mechanism	Reference
Adipogenesis	*CEBPA*	CCAAT/enhancer binding protein (C/EBP) α	Histone acetylation and methylation	(24)
	PPARA	Peroxisome proliferator-activated receptor α	DNA methylation	(25)
Appetite regulation	*LEP*	Leptin	DNA methylation	(26)
	MC4R	Melanocortin 4 receptor	DNA methylation	(27)
	NPY	Neuropeptide Y	DNA methylation	(28)
	POMC	Proopiomelanocortin	DNA methylation and histone acetylation and methylation	(28)
Body weight homeostasis	*FTO*	Fat mass and obesity associated	DNA methylation	(29)
Glucose homeostasis	*ADIPOQ*	Adiponectin	DNA methylation and histone acetylation	(30)
	GLUT4	Insulin-responsive glucose transporter 4	DNA methylation and histone acetylation	(31)
	INS	Insulin	DNA methylation and histone acetylation	(32)
Hypoxia	*HIF1A*	Hypoxia inducible factor 1	DNA methylation and histone acetylation and methylation	(33)
Inflammation	*IFNG*	Interferon γ	DNA methylation	(34)
	TNF	Tumor necrosis factor α	DNA methylation	(35)
Lipid storage	*FASN*	Fatty acid synthase	DNA methylation	(36)
Stress	*NR3C1*	Glucocorticoid receptor	Histone acetylation	(37)
Thermogenesis	*UCP1*	Uncoupling protein 1	DNA methylation	(38)

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 that can alter the epigenome, assessment of the real importance of other obesity-related factors in epigenetic regulation, determination of the period of life in which best results are obtained, and understanding the importance of the inheritance of these epigenetic marks. In relation with the latter, epigenomic profiling of livers of offspring males whose fathers consumed a low-protein diet revealed numerous modest changes in cytosine methylation depending on the paternal diet, which could be related to changes in cholesterol and lipid metabolism in offspring (39).

The characterization of those individuals who at an early age could present changes in the methylation profile of specific genes could help to predict their susceptibility to later develop obesity, which may allow researchers to prevent and follow up on its progress as well as develop newer therapeutic approaches (40). The knowledge of the modifications of their methylation patterns as a result of different dietary factors, age, inflammation, or other physio-pathological events could be crucial to investigate the role of these mechanisms in the prevention, onset, and therapy of obesity and type 2 diabetes.

In relation to the Developmental Origins of Health and Disease hypothesis (41), there are several examples of the role of nutritional interventions in pregnancy and lactation, such as energy deprivation, protein and micronutrient restriction, and high-fat diet, which determine a cluster of disorders affecting energy efficiency and different metabolic pathways in the offspring. Some of these events seem to be mediated by epigenetic processes encompassing the chromatin information encrypted by DNA methylation patterns, histone covalent modifications, and non-coding RNA or microRNA. Thus, epigenetic mechanisms may be boosted or impaired by dietary and environmental factors in the mother, intergenerationally or transiently transmitted, and involved in the obesity and inflammation susceptibility in the offspring (42). For example, in a study of sheep that suffered moderate maternal malnutrition, a decrease in the methylation of proopiomelanocortin (*POMC*) and glucocorticoid receptor (*GR*) gene promoters was found in the fetal hypothalamus, which potentially could lead to long-term energy balance deregulation. These changes were associated with decreased DNA methyltransferase activity and altered histone methylation and acetylation (43). In humans, data from the people exposed to the 1944–1945 Dutch famine when they were between age 0 and 21 y evidenced that a short period of moderate or severe undernutrition during postnatal development increases type 2 diabetes risk in adulthood (44).

Besides early in life, there are also examples of diet-induced epigenetic changes in adulthood due to the restriction or supplementation with different nutrients. In this sense, methyl donors have been linked to DNA methylation in a dose-dependent manner and seem to play a role in liver steatosis by affecting fatty acid synthase (*FASN*) methylation (45). Also, high-fat or -sugar intake and situations of excessive body weight in rodents are associated with changes in DNA methylation patterns, affecting the promoter region of different genes involved in energy homeostasis and obesity such as *LEP* (46), NADH dehydrogenase (ubiquinone) 1 β subcomplex subunit 6 (*NDUFB6*), and *FASN* (36). On the other hand, epigenetic biomarkers are being identified in humans to predict weight loss and body weight maintenance after weight loss, including *LEP* and *TNF* (47,48), aquaporin 9 (*AQP9*) (49), ATPase class V type 10A (*ATP10A*), Wilms tumor 1 (*WT1*), and CD44 molecule (*CD44*) (50).

Current Status of Knowledge

Adipose tissue/obesity and epigenetics. Alterations in the pattern of DNA methylation that occur in utero are capable of inducing changes in gene expression that contribute to the development of obesity by increasing adipose tissue growth and expansion. Understanding maternal dietary influences that program an embryonic and fetal environment that promotes obesogenic epigenetic changes is vital in reducing obesity risk. Studies have suggested that intrauterine under-nutrition during development epigenetically programs the offspring for survival in a nutrient-poor postnatal environment, thus resulting in postnatal catch-up growth and consequential development of obesity (51,52). Postnatal catch-up growth and obesity risk may be most likely to be expressed when offspring who have experienced protein under-nutrition are exposed to an energy-rich diet. Several adipose tissue growth-stimulating auto-, para-, and endocrine hormonal factors can induce adipose tissue expansion. Among them, insulin-like growth factor 2 (*IGF2*), which is one of the best known epigenetically imprinted genes, is associated with greater body weight (53,54) and obesity (55,56). One possible mechanism for the increase in adipose tissue *IGF2* expression is increased methylation of differentially methylated regions (DMRs) in the H19 imprinting control region (ICR/H19). Thus, studies on the *IGF2/H19* locus showed that hypomethylated CpG islands in the 5′ region of the *H19* gene serve as critical binding sites for the 11-zinc finger protein CTCF (57). Other studies have also shown that both mutated *ICR*/*H19* DMR (58) and hypermethylation of the *ICR*/*H19* DMR region inhibit binding of CTCF proteins (57). Inhibition of CTCF proteins to the ICR/H19 DMR region allows the distal enhancer to interact with the *IGF2* promoter to activate *IGF2* gene transcription (59). Importantly, activation of *IGF2* gene transcription is regulated via maternal diets such as low dietary protein and folate through increased methylation of *ICR*/*H19* DMR (60–62). Another potential mechanism for greater *IGF2* expression is acetylation of histone 4 (H4) at lysines 16 and 8 (63), whereas polycomb repressive complex 2 (PRC2) is recruited through the interaction of CTCF with Suz12 PRC2 subunit, leading to allele-specific methylation at lysine 27 of histone H3 (H3-K27) and suppression of the maternal *IGF2* promoters (64). The independent contributions of alterations in histone acetylation and methylation

for adipose tissue *IGF2* gene expression have not yet been addressed. Results from a recent study demonstrated that prenatal low-protein and postnatal high-fat intake result in adipose tissue catch-up growth through alterations in expression of *IGF2* gene and *IGF2/H19* locus CpG island methylation in the same tissue (65). These studies corroborate that histone methylation can result in different transcriptional consequences depending upon the residue affected. For example, H3 lysine 4 methylation is associated with enhancers and promoters, H3 lysine 36 methylation is associated with transcription, and H3 lysine 27 methylation is associated with repressive chromatin (66).

Increases in adipose tissue mass and obesity occur via enlargement of existing fat cells (hypertrophy), increased proliferation (hyperplasia), and increased rate of differentiation of adipocytes from preadipocytes (67). Reciprocal regulation of adipocyte hyperplasia by adipogenic transcription factors and chromatin remodeling enzymes has been demonstrated. For example, a well-known adipogenic transcription factor, CCAAT/enhancer-binding protein (C/EBP) α promoter, is hypermethylated in differentiated 3T3-L1 adipocytes, whereas methylation of promoter regions of another key adipogenic transcription factor, PPARγ, decreases during differentiation of 3T3-L1 cells (68). Because chromatin remodeling enzymes such as histone deacetylases (HDACs) and histone acetyltransferases (HATs) are involved in cell differentiation, a study showed that HDAC9 inhibits adipocyte differentiation (69). Further evidence of HDAC9 involvement is confirmed by the fact that overexpression of HDAC9 in 3T3-L1 preadipocytes suppressed adipogenic differentiation, whereas HDAC9 gene knockout mice had accelerated adipogenic differentiation (69). Adipogenic process regulation is mediated via a complex interaction of several factors. Actually, during the adipocyte differentiation, HDAC9 is downregulated, resulting in dissociation from the rest of the transcription factor complex while binding of other transcription factors, such as p300, HAT to the promoter region of adipogenic transcription factor C/EBPα (69). In general, promoters of the adipogenic genes such as *LEP, LPL*, *PPAR*γ, and *FABP4* are hypomethylated in undifferentiated adipocyte precursor cells; however, DNA methylation patterns were found not to be related to adipogenic gene expression (70). Another study suggested that the amount of histone3 k27 acetylation was associated with transcription factor binding to lipogenic gene promoters (71). This study also provided high-resolution views of chromatin remodeling during cell differentiation and allowed the identification of thousands of putative preadipocyte- and adipocyte-specific, *cis*-regulatory elements based on dynamic chromatin signatures.

Apart from histone modifications, changes in DNA methylation have also been observed in the early determination of adipose precursor cells and during adipogenesis (72). Thus, the promoter regions of the late adipogenic genes insulin-responsive glucose transporter 4 (*GLUT4*) and *LEP* display demethylation during adipogenesis, which corresponds with the expression of these genes in mature adipocytes (31,73). These findings open the door to the manipulation of epigenetic marks in preadipocytes by using drugs, hormones, or nutritional factors to inhibit the adipogenic process. For example, the treatment of 3T3-L1 preadipocytes with azacytidine inhibits its differentiation in a stage-dependent manner (74).

Epigenetics of obesity and weight loss. An excessive fat accumulation resulting in overweight or obesity situations constitutes a serious health issue worldwide affecting an increasing number of people. This adverse metabolic condition should be attributed to not only gluttony or sloth conditions involving unbalanced dietary habits or sedentary behaviors but also to interactions with genetics/epigenetics and other pathophysiological factors (75). Hence, as suggested by McAllister et al. (76), additional putative contributors to the global epidemic of obesity include the participation of microorganisms (infectobesity), greater than ever fecundity among parents with elevated adipose tissue reserves and the continuous rise in the age for the first pregnancy, selective mating for social reasons within individuals with overweight status, food overconsumption associated with insomnia, neuro-endocrine disturbances involving appetite and energy homeostasis disruptions, undesired pharmacological side effects, adverse climate and environmental influences affecting energy expenditure, as well as intrauterine and trans-generational effects, including epigenetic phenomena. Understanding the role of such potential causal agents of obesity is helping to generate effective strategies for the prevention and treatment of this global epidemic, where epigenetics may play an important role.

The aims currently pursued are the early identification of epigenetic biomarkers concerned in an individual's disease susceptibility and the description of protocols for tailored dietary treatments or advice to counterbalance adverse epigenomic events (12). These approaches will allow diagnosis and prognosis implementation and facilitate therapeutic strategies in a personalized manner to combat obesity and associated comorbidities, such as inflammation, insulin resistance, dyslipemia, etc. In this sense, identification of those individuals presenting changes in DNA methylation profiles, certain histone modifications, or other epigenetically related processes could help to predict their susceptibility to gain or lose weight and be used in the prevention of excessive fat deposition, the prediction of the most appropriate weight reduction plan, and the implementation of newer therapeutic approaches (2).

Indeed, it is becoming evident that inter-individual differences concerning the outcomes of nutrition-related chronic diseases such as diabetes and obesity depend on not only the dietary intake and genetic background but also on the inherited epigenome and different nutritional influences (during the intrauterine or the adult periods) that modify the epigenetic marks and are able to affect gene expression. These processes include DNA methylation, covalent histone modifications, and chromatin remodeling (3). Epigenetic marks are envisaged to have applications not only as predictors of obesity but also as prognostic markers of weight loss.

Body weight homeostasis is regulated through complex metabolic mechanisms that depend on not only exogenous factors, such as dietary intake and physical activity, but also on orchestrated internal processes involving neuroendocrine and nutritional pathways strictly controlled by genetic and epigenetic machineries (75). Obesity-associated adipose tissue enlargement is characterized by an enhanced proinflammatory status and an exacerbated secretion of adipokines (i.e., leptin) and cytokines (i.e., TNFα), where epigenetic regulation of gene expression has emerged as a potentially important determinant.

Thus, research was carried out to analyze whether epigenetic regulation of human *TNF* promoter by cytosine methylation could be involved in the predisposition to lose weight after following a balanced hypocaloric diet (47). Obese men with successful weight loss (>5% of initial body weight) had lower levels of total *TNF* promoter methylation, especially in the positions −170 and −120 bp in peripheral blood mononuclear cells. Baseline TNFα circulating concentrations were positively associated with total promoter methylation and methylation at position −245 bp. Therefore, it was hypothesized that *TNF* promoter methylation could be a good biomarker predicting the diet-induced weight loss, which constituted a first step toward personalized nutrition based on epigenetic criteria. In a subsequent study, Cordero et al. (48) described that both *TNF* and *LEP* methylation levels in the adipose tissue could also be used as epigenetic biomarkers to predict the response to a low-calorie diet. Thus, at baseline, women with a better response to the dietary intervention ($\geq$5% of initial body weight) had lower promoter methylation levels in both genes than those in the nonresponder group.

Following these seminal studies, 3 articles have examined DNA methylation patterns of high and low responders to a hypocaloric diet by microarray. Bouchard et al. (77) described significant DNA methylation differences at 35 loci in abdominal subcutaneous adipose tissue biopsy samples between the high and low responders before dieting. Some of the genes, such as potassium voltage-gated channel, shaker-related subfamily, member 3 (*KCNA3*), insulinoma-associated 1 (*INSM1*), nuclear factor I/X (*NFIX*), V-ets avian erythroblastosis virus E26 oncogene homolog 1 (*ETS*), and GLIS family zinc finger 3 (*GLIS3*), are involved in weight control and insulin secretion. On the other hand, in peripheral blood mononuclear cells, Milagro el al. (50) found baseline DNA methylation differences in the *ATP10A* and *CD44* genes depending on the weight-loss outcome. A similar approach was used to explore differential DNA methylation patterns between high and low responders to a multidisciplinary weight loss intervention in overweight or obese adolescents within the EVASYON (Development, implementation and evaluation of the efficacy of a therapeutic programme for adolescents with OW/OB: integral education on nutrition and physical activity) study (49). After validation, 5 regions located in or near *AQP9*, dual specificity phosphatase 22 (*DUSP22*), homeodomain interacting protein kinase 3 (*HIPK3*), troponin T type 1 (*TNNT1*), and troponin I type 3 (*TNNI3*) genes had differential methylation levels between high and low responders to the intervention. Moreover, a calculated methylation score was significantly associated with changes in weight, BMI-SDS, and body fat mass loss after the treatment. These methylation changes may help to better understand the weight loss response in obese adolescents.

On the other hand, the circadian clock system instructs 24-h rhythmicity on gene expression in essentially all cells, including adipocytes, and epigenetic mechanisms may participate in this regulation. In a recent study (78), differences between normal-weight and overweight/obese participants were found in the methylation status of different CpG sites at clock circadian regulator (CLOCK) (CpGs 1, 5–6, 8, and 11–14) and, with lower significance, aryl hydrocarbon receptor nuclear translocator-like (BMAL1) (CpGs 6–7, 8, 15, and 16–17) in white blood cells. Moreover, the methylation levels of *CLOCK* CpG 1 and period circadian clock 2 (PER2) CpGs 2–3 and 25 before a 16-wk weight reduction program correlated with the magnitude of weight loss. This study demonstrates an association between methylation status of CpG sites located in clock genes and obesity, metabolic syndrome, and weight loss. The authors hypothesized that the methylation status of different CpG sites in *CLOCK* and *PER2* could be used as biomarkers of weight loss success, particularly *CLOCK* CPGs 5–6.

Recent research has reported for the first time that DNA methylation could serve as a biomarker to predict weight regain after an energy restriction program. In this study (28), lower methylation levels of *POMC* CpG sites +136 and +138 bp in leukocytes were associated with success in weight loss maintenance, whereas lower total methylation levels in the neuropeptide Y (*NPY*) gene promoter were associated with higher risk of weight regain 32 wk after dieting stopped in men who followed an 8-wk nutritional intervention. Because both genes are involved in appetite regulation and might be implicated in the weight regain process, this research opens the door to use leukocytes as biomarkers of the less available hypothalamus cells.

Several investigations have shown that the reversibility of the epigenetic marks that are altered by unbalanced dietary patterns and metabolic diseases is a slow process. A study in humans found that the intake of a high-fat diet, even acutely, induced changes in the methylation of 6508 genes in skeletal muscle, with a maximum change in methylation of 13% (79). These variations were only partially and not significantly reversed after 6–8 wk of a normocaloric diet. In rats, a high-fat sucrose diet intake for 20 wk hypermethylated several CpG sites in the *LEP* gene promoter (6,7,29,30) and hypomethylated CpG 15 in visceral adipocytes (26). The shift to a chow diet (and the subsequent weight loss) reverted high-fat sucrose diet-induced DNA methylation changes of these CpG sites. Moreover, the change of the dietary pattern hypomethylated a CpG site of sterol regulatory element binding transcription factor 1 (*SREBF1*) and hypermethylated other CpGs on peroxisome proliferator-activated receptor gamma, coactivator 1-α (*PPARGC1A*) and *FASN* promoter. These studies shed light on the reversibility of phenotypical and epigenetic changes induced by the intake of an obesogenic diet. A similar result was observed after Roux-en-Y gastric by-pass–induced weight loss;

the expression and methylation of the majority of the genes were normalized to levels observed in the normal-weight, healthy participants (80).

Epigenetics and diabetes. Both type 1 and type 2 diabetes have a detrimental impact on millions of individuals, along with the concomitant complications associated with disease progression. It was recently recognized that epigenetics plays a role in diabetes both in terms of its development and progression as well as its complications. Methylation of DNA and histones represents an epigenetic mechanism to regulate gene expression. This section will focus on the relation between DNA methylation and disease progression for both type 1 and type 2 diabetes.

There have been a number of reports demonstrating that hyperglycemia results in epigenetic modification of histones and subsequent alterations in the expression of genes in cell culture, animal, and human models (81–85). Moreover, these changes may be persistent and remain even following the restoration of normal circulating glucose concentrations.

An emerging field in diabetes involves the numerous reports that both type 1 and type 2 diabetes alter the normal metabolism of methyl donors, folate, homocysteine, and choline. Using a streptozotocin (STZ)-induced rat model, it was shown that an acute type 1 diabetic state leads to hepatic induction of glycine *N*-methyltransferase (*GNMT*), phosphatidylethanolamine *N*-methyltransferase (*PEMT*), betaine-homocysteine *S*-methyltransferase (*BHMT*), and cystathionine β-synthase (*CBS*), whereas the activity of folate-dependent methionine synthase (*MS*) was significantly diminished (86–90) (**Fig. 2**). Likewise, treatment of rats or hepatic cell lines with glucocorticoids (e.g., dexamethasone) also induced the expression of *GNMT* and *CBS* (87,89,90). Insulin administration has been shown to prevent these alterations in both rats and cell lines (87,89,91,92), indicating that these metabolic alterations were likely due to a lack of insulin and or elevated counter-regulatory hormones and thus are diabetes specific and not secondary to the use of a chemically mediated model. Consistent observations have also been reported for type 2 diabetes using the Zucker diabetic fatty (ZDF) rat (93), demonstrating that insulin resistance produces a similar effect. Most importantly, these findings using rodent models closely reflect what has been reported in type 1 and type 2 human studies (94,95).

Homocysteine imbalance (i.e., hyperhomocysteinemia) is an important biomarker for a number of pathologies (96) and also may reflect aberrant changes in methyl group metabolism that can affect epigenetic control via methylation. Hypohomocysteinemia as a result of enhanced homocysteine catabolism in the liver has been a consistent finding in acute studies using animal models of diabetes; however, human diabetic studies have reported a condition of hyperhomocysteinemia (95). This discrepancy has been addressed by the demonstration that as diabetes progresses, renal dysfunction ensues and the ability to catabolize homocysteine becomes compromised, resulting in a state of hyperhomocysteinemia (97). A human kinetic study demonstrated that transmethylation, homocysteine trans-sulfuration, and clearance of homocysteine were significantly reduced in diabetics with nephropathy (98).

Taken together, these characteristics of altered methyl group metabolism as a function of diabetes suggest that the methylation of DNA, a key epigenetic process to control gene expression, may be compromised. The strongest data

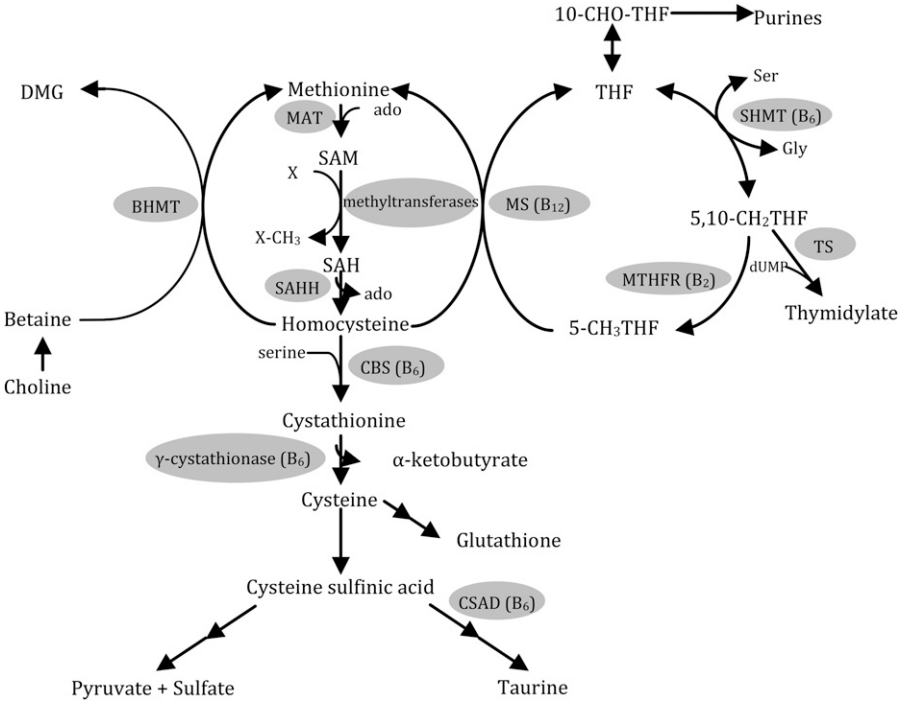

FIGURE 2 Hepatic methyl group metabolism. The X denotes a methyl acceptor substrate for SAM, such as glycine, DNA, or histones. Thus, the key SAM-dependent methyltransferases for this review are GNMT, DNMTs, and histone methyltransferases. Ado, adenosine; BHMT, betaine-homocysteine *S*-methyltransferase; CBS, cystathionine β-synthase; 10-CHO-THF, 10-formyl tetrahydrofolate; CSAD, cysteinesulfinic acid decarboxylase; DMG, dimethylglycine; DNMT1, DNA methyltransferase 1; GNMT, glycine N-methyltransferase; MAT, methionine adenosyltransferase; MS, methionine synthase; MTHFR, 5,10-methylene-THF reductase; SAH, *S*-adenosylhomocysteine; SAHH, SAH hydrolase; SAM, *S*-adenosylmethionine; SHMT, serine hydroxymethyltransferase; THF, tetrahydrofolate; TS, thymidylate synthase.

supporting this possibility are the induction of *GNMT* and *CBS* during diabetes (88,89), thereby irreversibly disposing methyl groups and depleting the cell of homocysteine for remethylation, respectively. The impact of both of these diabetes-specific changes on methyl group metabolism was examined as a function of diabetes progression in STZ-treated type 1 diabetic rats (99). The induction of *GNMT* was clearly evident in hepatic tissue and remained elevated throughout the 8-wk experimental period of type 1 diabetes, whereas a similar increase of GNMT activity and abundance in kidney tissue was transient. For *CBS* expression, hepatic tissue exhibited a consistent increase in its expression, whereas expression was diminished in renal tissue. Most importantly, the hypomethylation of DNA in the liver was evident in the early stages of type 1 diabetes and reached significance by 8 wk. Moreover, the expression of DNA methyltransferase 1 (*DNMT1*) was also elevated, indicating a potential compensatory response related to the lack of sufficient methyl groups for DNA methylation. Alterations in the renal methylation of DNA were not observed, likely owing to the lack of sustained induction of *GNMT* and retention of homocysteine balance via decreased CBS expression. All of these findings, including hypomethylation of DNA, were abrogated by insulin treatment, indicating that these metabolic perturbations were diabetes specific and not the result of STZ treatment to mediate type 1 diabetes (91). In addition to a chemically mediated model of type 1 diabetes, similar findings have been reported using the nonobese diabetic mouse, a genetic model of type 1 diabetes (100).

Similar findings were reported for type 2 diabetes progression using the ZDF rat as a model. As mentioned earlier, induction of *GNMT*, *CBS*, *PEMT*, and *BHMT* appears to be a very consistent finding in both type 1 and type 2 animal models (86–93). However, for type 2 diabetes in the ZDF rat, hepatic DNA was hypermethylated in striking contrast to the findings reported for type 1 diabetes. *DNMT1* expression was also elevated; however, other nuclear proteins involved in the methylation of DNA were not altered (101).

Epigenetic marks are tissue specific and could specifically target the organs that play a role in the pathogenesis of the disease and its complications. Thus, Trynka et al. (4) reported that H3K4me3 is significantly associated with type 2 diabetes susceptibility regions specifically in pancreatic islets. Therefore, it is important to perform multi-factorial analyses of genetic, epigenetic, and transcriptomic data in different cell types in order to reveal the pathological role of distinct epigenetic marks in the different genes and diseases.

In summary, much remains to be understood regarding the relation between diabetes and epigenetics, in particular the alterations in DNA methylation that have been reported as a function of type 1 and type 2 diabetes progression. Clearly, understanding the impact of these alterations and the specific influence they have on gene expression in specific tissues is the most urgent goal of this research. From a mechanistic standpoint, it will be important to understand the tissue-specific signaling involved in diabetes and DNA methylation and determine the differences reported between type 1 and type 2 diabetes. Subsequently, this new knowledge may be used in future dietary and therapeutic recommendations regarding the control of diabetes and its complications. For example, numerous recent reports have demonstrated that dietary resistant starch or probiotics have an important beneficial impact on diabetes and obesity (102,103), suggesting that intervention strategies that target the gut microbiota represent a viable approach for disease prevention or treatment.

Epigenetic phenomena seem to be involved in the onset and development of metabolic diseases such as obesity and type 2 diabetes, because they have the ability to change gene expression. Besides a genetic predisposition, there are differences in the susceptibility to suffer these diseases, or in the success in weight loss treatments, that could be explained at least in part by epigenetic differences between individuals. These differences can be inherited or acquired during the lifetime, especially in utero and at early ages, and environmental factors such as diet seem to play a key role in the modulation of the epigenetic marks (DNA methylation and covalent histone modifications). Due to the reversibility of some of these marks, epigenetics is now considered an attractive field of nutritional intervention (3). Some bioactive food compounds such as polyphenols, isothiocyanates, SCFAs, vitamins, and some minerals may induce beneficial effects because of their ability to modulate epigenetic processes. In this sense, many in vitro and in vivo studies have corroborated these outcomes.

The knowledge of nutritional epigenetics is still scarce, because it is difficult to delineate the precise effects of the different environmental factors, including the bioactive food compounds, on each epigenetic modulation and cell type as well as their associations with physiological and pathological processes. The regulation of these processes, the magnitude of the changes and the cell types in which they occur, the individuals more predisposed, and the more crucial stages of life remain to be elucidated. The main objectives for the future are the identification of epigenetic marks that could be used as early predictors of metabolic risk and the development of drugs or diet-related treatments able to delay these epigenetic changes and even reverse them. Although the technology to afford this task (sequencing and microarrays) is available, there are huge difficulties that must be overcome. There are many factors involved, including age, with multiple interactions between them, each epigenetic phenomenon also interacts with the others, there are great differences among cell types, and the magnitude of change produced by dietary and environmental factors is small and cumulative, therefore making it difficult to seek the origin with retrospective studies.

On the other hand, the discovery of new genetic and epigenetic biomarkers is expected to lead to customized diets for obesity and diabetes prevention or to personalized treatments. In this way, large studies with careful design followed by replication from independent groups are needed to find these biomarkers. For example, the use of microarray-based

assays for methylation are now allowing epigenome-wide association studies, which identify differentially methylated sites associated with disease without taking into account the genotype (104).

Acknowledgments

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