Review

Marlene Remely*, Ana Laura de la Garza, Ulrich Magnet, Eva Aumueller and Alexander G. Haslberger

Obesity: epigenetic regulation – recent observations

DOI 10.1515/bmc-2015-0009
Received March 12, 2015; accepted May 5, 2015

Abstract: Genetic and environmental factors, especially nutrition and lifestyle, have been discussed in the literature for their relevance to epidemic obesity. Gene-environment interactions may need to be understood for an improved understanding of the causes of obesity, and epigenetic mechanisms are of special importance. Consequences of epigenetic mechanisms seem to be particularly important during certain periods of life: prenatal, postnatal and intergenerational, transgenerational inheritance are discussed with relevance to obesity. This review focuses on nutrients, diet and habits influencing intergenerational, transgenerational, prenatal and postnatal epigenetics; on evidence of epigenetic modifiers in adulthood; and on animal models for the study of obesity.

Keywords: adulthood; animal models; postnatal; prenatal; transgenerational.

Introduction

Obesity is a rapidly increasing epidemic disease worldwide. Nearly 39% of adults (aged 18 years and over) are overweight (BMI 25–30 kg/m^2) and 13% suffer from obesity (BMI >30 kg/m^2) and from comorbidities (1). At least 2.8 million people worldwide die each year as a result of being overweight or obese. High body weight increases blood pressure, cholesterol, triglycerides and insulin resistance, resulting in a higher risk for coronary heart disease, ischemic stroke and type 2 diabetes mellitus, as well as an increased risk for cancer (2). However, obesity is not only attributable to a genetic-driven imbalance between energy uptake and energy expenditure, although many genetic loci have been identified. Rather, an interplay between many systems is implicated, such as unbalanced energy uptake, gene mutations, an aberrant gut microbiota and epigenetics (3, 4). In recent years, many efforts have been undertaken to study epigenetic mechanisms and their influence on genes and their expression (5), as well as environmental factors, including nutrition and the developmental origins of obesity.

Epigenetic modifications are stable heritable patterns of gene expression occurring without changes in the DNA sequence. They include interacting components at the transcriptional level (DNA methylation and histone modifications) and at the posttranscriptional level – RNA interference (5–12) – which are modified by internal factors (e.g. inherited mutations, metabolic pathways, neuroendocrine balance, hormonal activities) and external factors (e.g. nutrients, bioactive food components, medication, tobacco, radiation, infectious organisms, stress) (7). The term ‘obesogens’, reviewed by Skinner et al. (2011), describes all the environmental factors and dietary, pharmaceutical and industrial compounds that have a potential role in the development of obesity or metabolic disease in the offspring by affecting the number of fat cells, or the size of fat cells, the hormones influencing appetite, satiety, food preferences and energy metabolism (13). Chemical pesticides in food and water, e.g. dicholorodiphenyldichloroethylene (14), bisphenol A (15), diethylstilbestrol (16), pharmaceuticals such as the diabetes drug Avandia® (rosigliatazone), are already linked to weight gain. Mechanisms of dietary factors modifying epigenetic marks are under study (17). In this context, nutrients and other food components, e.g. the soy phytoestrogen genistein (18) and

***Corresponding author: Marlene Remely,** Department of Nutritional Sciences, University of Vienna, Althanstraße 14, A-1090 Vienna, Austria, e-mail: marlene.remely@univie.ac.at
Ulrich Magnet, Eva Aumueller and Alexander G. Haslberger: Department of Nutritional Sciences, University of Vienna, Althanstraße 14, A-1090 Vienna, Austria
Ana Laura de la Garza: Faculty of Public Health and Nutrition, Autonomous University of Nuevo León, Monterrey, Nuevo León 64460, Mexico

monosodium glutamate (19), are among the factors that have been associated with epigenetic modifications in obesity. Most of the epigenetic modifying enzymes require nutrients or their metabolites as substrates or cofactors, thus dietary composition and bioactive nutrients are of current research interest. In addition, intergenerational, transgenerational and prenatal effects must be taken into account. Caloric restriction of parents and also grandparents is already known to increase the risk of metabolic disorders in offspring reaching adulthood (20). Whereas, a high-fat diet either strongly influences epigenetic modifications to induce metabolic stress, resulting in obesity, insulin resistance, diabetes and cardiovascular disease (21). Thus, previous generations are suggested to increase the risk of obesity or type 2 diabetes for their offspring through genetic and/or epigenetic mechanisms. Although there is no possibility to modify the genetic basis, we can change our epigenome: e.g. a healthy diet and exercise are suggested as sufficient to change the epigenome (22). More information is available on the evidence of specific nutrients (e.g. folate, butyrate, secondary plant metabolites) in obesity-mediated inflammation and oxidative stress, as well as other metabolic syndrome-related diseases including type 2 diabetes, atherosclerosis and hypertension (23). Methyl donors like folate and SAM (S-Adenosylmethionin) alter the methylation of the DNA and histones. Curcumin, genistein, epigallocatechin gallate (EGCG), resveratrol and equol inhibit DNMT (DNA methyltransferase) activity (24–27). Dietary components such as butyrate, sulforaphane, resveratrol and diallyl sulfide inhibit histone deacetylases (HDAC) and curcumin inhibits histone acetyltransferase (HAT) activities (7). However, a precise breakdown of these activities is not possible as the effects depend on the nutrients' structure, nutrient interaction and interaction with other lifestyle factors (7). In addition, epigenetic patterns occur as a result of developmental and regenerative processes, with tissue specificity reflecting the temporary gene expression (28). The epigenetic pattern is the result of the interdependent interactions of methylation, miRNAs and histone modifications (29). Methylation in a promoter or other regulatory region of a gene is usually associated with repressed gene transcription (29) by blocking transcription factor binding (30), whereas DNA demethylation (hypomethylation) is suggested to induce gene activation. In the case of histone acetylation combined with an open chromatin structure and transcriptional activation, a high level of acetylation combined with rapid deacetylation has been indicated as an important factor (31).

In the present review we focus on nutrients, diets and habits influencing intergenerational, transgenerational, prenatal and postnatal epigenetics (Table 1); on evidence of epigenetic modifiers in adulthood (Table 2); and on animal models for the study of obesity, as the understanding of epigenetic patterns in metabolic syndrome might provide invaluable evidence for prevention, diagnosis and development of future therapeutic interventions.

Evidence for intergenerational and transgenerational inheritance

Epigenetic modifications are already accepted to have a critical role in the regulation of gene expression, but in general a deletion and re-establishment in each generation has been suggested. After fertilization, the global DNA methylation is thought to be reduced to ±10% (32); the occurrence of histone modifications is unknown. These phenomena make the offspring susceptible to parents' or even grandparents' behaviors, extending their responsibility over generations (33). The grandmother's nutrition might be recurring to influence cardiovascular disease, type 2 diabetes, or hypertension in adulthood of the offspring because of transmission *in utero*, although little is known about imprint establishment or deletion down the male line (34). However, most of the studies only include the F0–F2 generation, but not the F3 generation, although the term 'transgenerational inheritance' refers to the F3 generation and up as the F1 and F2 generations are exposed *in utero* of the F0 generation as a fetus or as the germline of a fetus. Thus, the F1 and the F2 generation are suggested to be described as 'intergenerational epigenetic inheritance' and only the F3 generation and up shall be considered as 'transgenerational inheritance' (Figure 1) (35).

The most prominent human study was of the Dutch famine of 1944, in which starvation in one generation affected the health of their grandchildren. Painter et al. (2008) did not observe intergenerational inheritance after prenatal exposure to famine on metabolic disease rate nor on birth weight. This F1 generation was associated with higher F2 neonatal adiposity and poor health in adulthood (20). Data collected in the Överkalix parish in northern Sweden in 1890, 1905 and 1920 and continuing until death or 1995 are one of the first picking up the epigenetic hypothesis, suggesting that overeating during the child's slow growth period (before prepubertal peak in growth velocity) was an important factor in the offspring's risk of death from cardiovascular diseases and diabetes. Reduced dietary availability during the father's slow growth period

Table 1: Influencing factors and their metabolic effects on obesity.

Influencing factors	Model	Metabolic effect	References
Transgenerational/intergenerational inheritance			
Prenatal famine	Human	F1 generation no metabolic effects F2 generation neonatal adiposity, poor health in adulthood	(30)
Excessive food intake of paternal grandfather during slow growth period	Human	Higher diabetes mortality shortened life-span	(31)
F0 over-nutrition/F1 and F2 no intervention	Mouse	Peripheral glucose tolerance in F2	(32)
F0 high-fat/F1 and F2 no intervention	Mouse	Impaired gluceose tolerance and increased body weight in F2 and F3	(33)
F0 high-fat paternal diet/F1 and F2 no intervention	Mouse	Impaired epigenome of sperm in F2	(34)
F0 high-fat die/F1 and F2 no intervention	Rat	Higher cardiovascular risk in F1 not in F2	(35)
F0 low-protein diet/F1 and F2 no intervention	Mouse	Increased hepatic cholesterol/lipid biosynthesis in F2	(36)
F0 low-protein diet in females/F1 and F2 no intervention	Rat	Impaired glucose and insulin metabolism in F2	(37)
F0 low-protein diet/F1 and F2 no intervention	Rat	Impaired glucose and insulin metabolism in F2	(38)
F0 low-protein diet/F1 and F2 no intervention	Rat	Impaired glucose tolereance, birthweight in F2	(39)
F0 low-protein diet/F1 and F2 no intervention	Mouse	Impaired glucose and insulin metabolism, pancreatic islet mass, adiposity in F2	(42)
F0 low-protein diet/F1 and F2 no intervention	Mouse	Impaired glucose tolerance, adiposity in F2	(40)
F0 and F1 high-energy diet/F2 no intervention	Rat	Methylation status in F2	(50)
F0 low-protein diet/F1 and F2 no intervnetion	Rat	Cardiovascular effects in F2	(43)
F0 maternal caloric restriction/F1 and F2 no intervention	Rat	Impaired glucose/insulin metabolism in F2	(46)
F0 maternal caloric restriction/F1 and F2 no intervention	Rat	Decreased beta cell mass in the F1 generation as well as the F2 generation	(45)
F0 potent endocrine disruptors (vinclozolin and methoxychlor; fungicides and pesticides)	Rat	Alterations of DNA methylation patterns in the male gametes persisting in the F3 generation	(51)
Prenatal/postnatal evidences			
Mothers supplemented with methionine, choline, folic acid and vitamin B12	Agouti mice	Obese phenotype in their offspring	(87–89)
Maternal supplementation with genistein	Agouti mice	Protective against obesity in offspring	(90)
Decreased competition for energy during pregnancy	Humans	Increase of infant obesity	(91)
Diabetes mellitus during gestation	Humans	Hypermetylation of the IGF2 promoter region more susceptible to obesity and metabolic syndrome	(92)
Increase in maternal energy-dense diet	Mouse	Increased expression and decreased DNA methylation (DAT, MOR) and PENK in offspring correlating with an increase in obesity	(93)
Maternal malnourishment	Humans	Reduction in methylation in IGF2R in girls and GTL2-2 in boys	(94, 95)
Poor growth *in utero*	Humans	Develop metabolic disease and obesity	(28)
Prenatal exposure to PAH	Humans	Obesity in the offspring, increased gene expression of PPAR γ, C/EBP α, Cox2, FAS and adiponectin and lower DNA methylation of PPAR γ	(96)
Supplementation of vitamin B12 and folate during pregnancy	Sheep	Increase in body weight and insulin resistance in adult offspring	(97)
Breastfeeding	Rats	Protective against obesity	(98)

Table 1 (continued)

Influencing factors	Model	Metabolic effect	References
Animal models			
(+) Genistein	Cynomolgus monkeys fed a high-fat diet	Insulin sensitivity in liver and muscle	(99)
(-) Methyl donors	Gestation and lactation diet in rats	Impairment of fatty acid oxidation	(100)
(+) Methyl donors	High-fat sucrose fed rats	Prevention of fatty liver disease	(101)
(+) Betaine	Mice fed a high-fat diet	Hepatoprotective effect in non-alcoholic fatty liver disease	(102)
(+) Grapefruit extract rich in flavonoids	Db/db mice	Ameliorate inflammation associated to obesity	(103)
(+) Linoleic acid	Adult mice fed high-fat diet	Changes in response to the treatment to induce fat loss	(104)
(+) Flavonoid-rich extract from yaupon holly leaves	Mice	Down-regulation of pro-inflammatory miR-155	(105)
(-) Vitamin E	Rat	Down-regulation of miR-122a implicated in lipid metabolism	(106)
(+/-) Curcumin	High-fat diet fed C57BL/6J mice	Inflammation, body weight	(108)
(+) Resveratrol	High-fat diet-induced weight gain in mice	Body weight	(109)
Human intervention studies			

lowered the risk for cardiovascular diseases. Excessive food intake of the paternal grandfather during their slow growth period induced higher diabetes prevalence and shortened the life-span, whereas food scarcity extended the life-span (36).

In mice an impaired glucose tolerance in the F1 and F2 generation has been shown to be associated with the paternal over-nutrition in the F0 generation (37). Paternal exposed F0 generation to a high-fat diet also induced the epigenome of sperm via increased acetylation and differential microRNA content in further generations (38). A low-protein diet in the F0 generation also transmitted changes in DNA methylation in liver cell loci (e.g. involved in lipid metabolism) via the paternal line (39).

Due to transmission via the maternal line, a high-fat diet showed adverse effects in the F2 generation compared to F1, namely higher cardiovascular risk (40). F1 males exposed to maternal high-fat diet in the F0 generation transmit impaired glucose tolerance to the F3 generation (41). Under-nutrition in the maternal line decreased beta cell mass in the F1 as well as the F2 generation (42) and showed impaired glucose/insulin metabolism (43). In comparison a female protein restriction showed effects on glucose and insulin metabolism (44–46), adiposity (46), DNA methylation in the liver (47), pancreatic islet mass (48) and cardiovascular effects (49, 50).

Studies of transgenerational inheritance in the F3 generation are variable, with conflicting results as well as difficulties in interpretation when further interventions are applied to F1 generations. An increased insulin/glucose ratio persisted in the F3 generation due to protein restriction during pregnancy even though a decreased insulin/glucose ratio compared to controls was observed in the F1 generation (51). Protein restriction in the F0 generation, with normal diet due to generations F1–F3, produced an F3 generation with reduced basal insulin levels and decreased pancreatic beta cell mass (48). Others showed only effects from the F0–F2 generation with no persistence in F3 (50–52). In contrast, experiments on rats with persistent high-fat diet exposure over three generations showed epigenetic changes in all exposed generations. However, a reprogramming in each generation cannot be excluded (53).

Exposure of the F0 generation to potent endocrine disruptors (vinclozolin and methoxychlor; fungicides and pesticides) induced alterations of DNA methylation patterns in the male gametes persisting in the F3 generation (54). Little evidence exists about transgenerational effects of histone modifications (55) or of miRNAs. Additionally, the majority of environmental factors and exposures interact with somatic cells and tissues and thus are critical for

Table 2: Epigenetic and metabolic effects in human studies.

Tissue	Target	Effect	References
Whole blood subcutaneous adipose tissue skin	485,000 CpGs	Positive correlation between HIF3A methylation and BMI	(76)
Whole blood	485,000 CpGs	More variance in methylation in obese Prediction of obesity status in another cohort	(77)
Lymphocytes	4.5 mill. CpGs 11y apart	PM20D1, MMP9, PRKG1, RFC5 correlated with BMI at both time points	(79)
Whole blood	Alu element	U-shaped methylation distribution with lowest methylation at BMI 25–30 kg/m^2	(80)
Blood mononuclear cells	TNF-α	Methylation as predictive marker for weight loss response in caloric restriction	(81)
Whole blood	27,578 CpGs	AQP9, DUSP22, HIPK3, TNNT1, TNNI3 showed different methylation between high and low responder to weight loss before the intervention	(82)
Blood mononuclear cells	27,578 CpGs	1034 differentially methylated CpG-sites between high and low weight loss responder before intervention 15 CpGs differentially methylated after intervention ATP10A and CD44 as possible biomarker	(83)
Whole blood	450,000 CpGs	Statistical difference between obese and lean reduced after gastric bypass 51 promoters differentially methylated before and after surgery	(84)
Plasma	754 miRNAs	18 miRNAs different expressed in lean and obese 14 miRNAs changed after surgery	(85)
Subcutaneous and intra-abdominal adipose tissue	1884 miRNAs	miR-125a-3p different expressed in obese women and men Higher expression correlated with insulin-signalling related genes	(88)

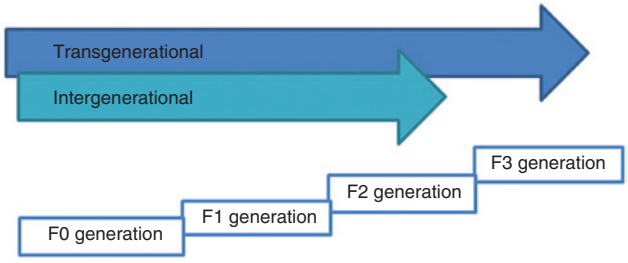

Figure 1: Deviation of epigenetic inheritance over generations into the terms intergenerational and trans generational.

adult-onset diseases rather than for their offspring. Only the maternal transgenerational epigenetic inheritance includes effects of the intrauterine environment, of the age of pregnancy, somatic epigenetics and mitochondrial programming (35). For the further research it is of interest to acquire knowledge about the exact mechanism of epigenetic inheritance and to identify molecular patterns that are either transmitted or deleted between the generations. However, the epigenetic memory might fill the gap of missing genetic heritability for complex diseases such as obesity, schizophrenia, but also longevity.

Evidence of prenatal epigenetic influences

It is well known that the prenatal period is crucial in the establishment of the epigenome (21, 56–58). One of the most critical processes during pregnancy is the establishment of imprinted genes. Caloric availability as well as metabolic status of the mother during this period can influence the programming of imprinted genes and the development of metabolic disease and obesity in the offspring (59). The earlier discussed risk factors such as parental obesity or malnourishment as well as obesogen exposure have compelling connections to changes in the epigenetic environment of the embryo leading to an increased risk for obesity and metabolic disease in the offspring (57).

The *agouti* viable yellow mouse (Avy) model, in which coat color variation is correlated with epigenetic patterns and whose heavily overweight phenotype facilitates diabetes development, has been one of the first models employed for investigating the impacts of nutritional and environmental influences on the fetal epigenome. The Avy genotype is characterized by the

presence of a transposon upstream of the *agouti* gene promoter that predisposes hyperphagia in obese mice. In this model, expression and DNA methylation of the *agouti* gene are correlated with the coat color when supplementing the mother during pregnancy with a pro-methylation cocktail (methionine, choline, folic acid and vitamin B_{12}). The administration of these substances increases the methylation of the upstream IAP (intracisternal A-particle) transposable element, which in turn leads to the reactivation of the *agouti* gene altering the coat color and suppressing an obese phenotype in their offspring (60–62). Dolinoy et al. (2006) have shown that maternal supplementation with genistein (250 mg/kg) during pregnancy protected offspring against obesity in agouti mice (27). Mouse models have shown that the increase in maternal energy-dense diet (high-fat, high-sugar) leads to increased expression and hypomethylation in the promoter region of central signaling molecules such as dopamine reuptake transporter (*DAT*), μ-opioid receptor (*MOR*), preproenkephalin (*PENK*) and *leptin* in the offspring, correlating with an increase in obesity (55). A study on rats has also shown that HFD during the pregnancy causes changes in the DNA methylation in the imprinted gene *agouti* related protein homolog (AgRP) in the hypothalamic arcuate nucleus (ARC), which plays a role in the regulation of energy balance leading to an increased obesity risk (63). Furthermore the development of gestational diabetes mellitus contributes to increased insulin in the embryo in response to increased intrauterine glucose levels. This induces hypermethylation of the insulin-like growth factor 2 (*IGF2*) promoter region leading to down-regulation of this gene and impaired insulin secretion, making the offspring more susceptible to obesity and metabolic syndrome (64).

Other contributors to the obesity epidemic are obesogens that influence the establishment of an epigenetic equilibrium during the pregnancy. Some of these have already been well studied: TBT (tributyltin hydride) is implicated in the disruption of endocrine signaling and leads to an increased adipocyte differentiation, especially in the mesenchymal stem cell compartment through epigenetic imprinting *in vitro* and an increase in adipose tissue mass in a mouse model (56). Mice treated with high levels of phytoestrogens during pregnancy gave birth to offspring more susceptible to estrogen exposure upon which they showed sex-specific changes in the methylation of the *Acta1* promoter region (65). Another obesogen is BPA (bisphenol A), which in pregnant *agouti* mice decreases CpG methylation on an IAP retrotransposon upstream of the *Agouti* gene in the offspring. This effect could be reversed upon supplementation with methyl donors or a phytoestrogen to the diet (66). Supplementation of vitamin B12 and folate in physiological amounts in sheep during gestation leads to an increase in body weight and insulin resistance in adult offspring and this is closely associated with an increase in genome wide DNA methylation, most notably in male subjects (67).

Infants have an innate sense of satiation and hunger; thus aberrations during the embryogenesis are suggested as crucial in the development of childhood obesity and also to play a role in the development of adult obesity and metabolic disease (68). This can be explained through an increase of caloric availability, decreasing the competition for energy during pregnancy and in turn favoring the development of adipocytes and β-cells over other cell types, leading to increased birth weight and infant obesity. This tendency for heightened birth weight is further increased due to the rise in frequency of cesarean sections, which eliminates the evolutionary disadvantage of oversized newborns during birth (58). DNA methylation has been established as one of the best epigenetic marker for the prediction of the development of obesity. In particular, aberrations in the early establishment of imprinted genes seem to predict quite accurately the development of metabolic syndrome in the offspring of obese parents (69). On the one hand there is a clear link between maternal obesity and its effects on childhood obesity, but on the other hand malnourishment of the mother during the pregnancy also favors obesity (70). One of the best-studied examples of the effects of malnutrition during gestation on the offspring is the Dutch Hunger Winter. This study investigated the events during the famine of 1944–1945 in the Netherlands and showed a compelling link between decreased caloric intake and the subsequent increase in metabolic disease and obesity in the following generation (71). A more recent example is a study of the effects of decreased nutrient intake during a famine in Gambia (72). It suggests a correlation between the reduction of caloric intake during preconception and the phenotype of the offspring. This malnourishment leads to a reduction in methylation in two imprinted genes in a sex-specific manner, namely the insulin-like growth factor 2 receptor (*IGF2R*) in female offspring and the Gon-Two Like (TRP subfamily) (*GTL2-2*) in male offspring. These two genes have important roles in the regulation of blood glucose levels and in growth, respectively (73). This link between maternal caloric intake and child development can be explained by the 'thrifty phenotype hypothesis', which states that there is a connection between poor growth *in utero* and in infancy and an increased risk to develop metabolic disease and obesity later in life if metabolic homeostasis is disrupted due to high caloric abundance (33). Studies

in humans on exposure to obesogenic substances showed an increased obesity risk. One example is prenatal exposure to polycyclic aromatic hydrocarbon (PAH), which increased the incidence of obesity in the offspring. It also increased the expression of peroxisome proliferative activated gamma (*PPAR* γ), CCAAT/enhancer binding protein alpha (*C/EBP*α), cytochrome C oxidase assembly factor (*Cox2*), Fas cell surface death receptor (*FAS*) and *adiponectin* and resulted in lower DNA methylation of *PPAR* γ (74). In addition, BPA was found to increase the expression of *FABP4* and *CD36*, which play an important role in lipid metabolism while at the same time downregulating *PCSK*, a gene influencing insulin production indicating that the exposure to BPA deregulates the metabolism, which increases the risk to develop metabolic syndrome later on in life (75).

Evidence of epigenetic modifiers in humans

A growing number of studies have investigated DNA methylation in different DNA regions associated not only with obesity but also with weight loss interventions. A recent study published by Dick et al. (2014) investigated 485,000 CpG-sites with the Infinium Human Methylation 450 array (Illumina, USA) (76). Whole blood samples of 479 individuals and two replication cohorts with a total of 2128 participants were analyzed. Analysis between BMI and the methylation state of the investigated CpG-sites showed positive correlations in all three cohorts between three CpG-sites in the first intron of the hypoxia-inducible factor 3α (HIF3A)-gene and the BMI of the study participants. Although the increased methylation state might only be a consequence of the increased BMI rather than a cause, or it is possible that other confounding factors might influence the BMI and the methylation state of the identified CpG-sites. A comparison of obese and lean study groups is missing (76).

A study performed with the same array platform by (77) compared differentially methylated CpG-sites (based on mean statistics) and differentially variable CpG-sites (based on variance statistics) in blood samples of 48 obese and lean youths. They found that the CpG methylation in obese participants was more variable than in the lean controls. Furthermore, they showed that the differentially variable CpG-sites as well as the differentially methylated CpG-sites could predict the obesity status of another sample set (78). Feinberg et al. (2010) analyzed 4.5 million CpG-sites in whole blood of 74 individuals at two time points 11 years apart. Four CpG-sites were identified that correlate with BMI in the same strength and direction at both sampling time points. These methylation sites were in or near the genes peptidase M20 domain containing 1 (*PM20D1*), matrix metallopeptidase 9 (*MMP9*), cGMP dependent protein kinase type 1 (*PRKG1*) and replication factor C subunit 5 (*RFC5*). In total, they identified variably methylated regions in or near 13 genes that correlated with the BMI at both investigated time points and many of which have been described to be involved in obesity or diabetes in earlier studies (79).

Yeon Kyung Na et al. (2014) investigated the methylation status of the Alu elements as a marker for global methylation in blood of 244 Korean women. A U-shaped distribution of methylation levels has been identified, with overweight women (BMI 25–30 kg/m^2) having the lowest methylation levels and lean (BMI<25 kg/m^2) and obese (BMI>30 kg/m^2) individuals showing higher methylation levels. These interesting results need further investigation to elucidate the underlying mechanisms. The authors also draw comparison with the U-shaped association between BMI and overall mortality, which is lowest in a BMI range between 22 and 26 kg/m^2 and a biphasic dose-response-model for toxins but they did not suggest an explanation for their result. They excluded cofounders such as age, smoking and alcohol consumption but maybe effects of physical activity or inflammatory processes are responsible for the U-shaped methylation curve (80).

Despite differences in the methylation level of CpG-sites between overweight or obese and lean individuals, several studies published in recent years have investigated the changes of methylation during weight reduction. The results of these studies showed either methylation changes occurring during the weight loss or differences in the baseline methylation levels between individuals who successfully lose weight and those who do not. Campion et al. (2009) suggested the methylation of distinct CpG-sites in the promoter region of the tumor necrosis factor alpha (*TNF-*α) gene as a predictive biomarker for weight loss response in a caloric restricted diet (81). Moleres et al. (2013) investigated blood samples of 107 overweight individuals undergoing a 10-week weight loss intervention. Differences in the methylation level of five regions between low and high responders before the start of the intervention were identified. These CpG-sites were located near or in the genes aquaporin 9 (*AQP9*), dual specificity phosphatase 22 (*DUSP22*), homeodomain interacting protein kinase 3 (*HIPK3*), troponin T type 1 (*TNNT1*) and troponin I type 3 (*TNNI3*) (82). Another study indicated 1034 differentially

methylated CpG-sites between high and low responders to weight loss before the start of the intervention. After 8 weeks of caloric restriction the methylation level of 15 CpG-sites was still significantly different. Detailed analyses revealed CpG-sites on the genes ATPase class V type 10A (*ATP10A*) and CD44 as a possible biomarker for weight loss prediction (83). A small number of studies have focused on changes in DNA methylation during weight loss after bariatric surgery. For example, the statistic distance between promoter methylation of 11 obese and 16 normal-weight patients was reduced 6 months after a Roux-En Y gastric bypass surgery compared to distance before the intervention. Furthermore, 51 promoters were differentially methylated before and 6 months after the surgery. However, if these results were adjusted for weight loss and fasting plasma glucose only one promoter methylation was still significant different. The authors suggest that the surgery-induced changes in weight and fasting glucose may be responsible for the changes in DNA methylation (84).

Histone modifications have not been studied extensively in humans in association with obesity as DNA methylation because more complex methods and more sample materials are required. The role of histone modifications, especially methylation and acetylation, in adipogenesis and adipocyte differentiation has been mainly investigated in mouse models (85, 86).

In contrast to histone modifications, recent studies have investigated miRNAs in association with obesity. The miRNA pattern in the plasma of obese men and patients with surgery-induced weight loss indicated significant differences in 18 miRNAs between obese and lean individuals. The miRNAs miR-126, miR-140-5p, miR-142-3p and miR-222 were increased in plasma from obese men, whereas miR-15a, miR-21, miR-122, miR-125b, miR-130b, miR-193a-5p, miR-221, miR423-5p, miR-483-5p, miR-520c-3p, miR-532-5p, miR-590-5p, miR-625 and miR-625 were decreased. After surgery-induced weight loss the plasma level of 14 miRNAs had changed significantly after 1 year (87). Further, gender-specific differences in the miRNA profile of obese individuals were shown and miR-125a-3p was more highly expressed in men than in women. The higher levels of miR-125-3p in adipose tissue correlated with the expression of insulin-signaling related genes (88).

Data from mouse and cell-culture studies suggest an important role of diverse miRNAs in the differentiation of adipocytes and thus in adipogenesis and a variety of differences in epigenetic modifications between obese and lean individuals and changes during weight loss have been indicated.

Animal models of epigenetic modifiers in obesity

The main dietary strategies considered in the study of epigenetic changes are high-fat diets and energy-restricted or low-protein diets in animal models and also diets supplemented with specific nutrients including micronutrients and other food components (bioactive compounds) (89). The study of diet-induced obesity requires the development of animal models that reproduce the characteristics of this disease in humans (90). The main advantage of the use of experimental animals is the ability to control potential confounding factors (91). In this context, the consumption of high-calorie diets have an increased percentage of energy from fat often accompanied by a high fructose, *trans* fatty acids and cholesterol, inducing obesity and insulin resistance (92).

Moreover, the effects of chronic caloric restriction are to increase maximal life-span and to prevent some chronic diseases such as type 2 diabetes, cardiovascular diseases, among others (93). Caloric restriction induces epigenetic modifications leading to the development of obesity in adulthood (94). For example, caloric restriction induces epigenetic changes in the *GLUT4* promoter in adipose tissue of mice previously fed a high-fat diet (95). Zhang et al. (2010) determined that caloric restriction during the periconceptional period in both normal-weight and overweight ewes, resulted in hypomethylation of *IGF/H19 DMR* in adrenal gland (96). Furthermore, there is evidenced that caloric restriction mediates its beneficial effects by modulating chromatin function and increasing genomic stability through reversing DNA methylation and increasing global histone deacetylases activity (97).

Accordingly low-protein diets are used most often as a model for maternal malnutrition. In this context, there are some studies that provide evidence for the influence of maternal protein malnutrition on the development of obesity and obesity-related diseases due to epigenetic changes (5). Thus, a maternal low-protein diet has been associated with epigenetic changes in the promoters of *glucocorticoid receptor* (98) and induced methylation changes in the hepatic *PPAR* α promoter of the offspring (99). Likewise Sohi et al. (2011) reported changes in liver histone methylation status in the offspring after a maternal protein-restricted diet (100). Adult mice showed increased hepatic cholesterol/lipid biosynthesis caused by epigenetic changes in PPAR α after a low-protein diet (39).

High-fat diet-induced obesity shows phenotype characteristics such as increased weight and alterations in lipid and carbohydrate profile (101). In obese rats fed

a high-fat diet, a CpG island in the leptin promoter was hypermethylated and was associated with low circulating leptin levels (92). Another study showed evidence of epigenetic changes in key genes regulating energy metabolism in white adipose tissue after chronic high-fat diet intake in male Wistar rats (102). Adult mice showed epigenetic changes in opoid receptor mu subunit gene (*Oprm1*) that participate in the central regulation of food intake and the development of obesity after the transition to a high-fat diet (103). Likewise Vucetic et al. (2011) observed histone modification in Oprm1 after high-fat diet in mice that resulted in altered food behavior (104). In addition, rats fed with high-fat diet showed beta cell dysfunction in islet cells (105). Likewise, chronic high-fat diet altered patterns of DNA methylation of genes associated with appetite and this epigenetic alterations participate on the development of obesity (103).

Specific nutrients have also been associated with epigenetic modification in obesity. Two major mechanisms by which nutritional factors and diet may affect DNA methylation are (i) changes in the availability of methyl donors and (ii) changes in the activity of the enzymes involved in the process of DNA methylation (methyltransferases). Nutrients involved in this mechanism are methyl donors and micronutrients that act as cofactors for the enzymes involved in the one-carbon metabolism, including folic acid, vitamin B6, vitamin B12, zinc, choline and methionine (89).

Several mouse and rat models deficient in methionine and choline exhibit increased expression of genes related to inflammation such as interleukins and TNFα, whereas there is a decline in plasma levels of triglycerides and cholesterol (106). Moreover, male C57BL/6J mice were fed a lipogenic methyl-deficient diet and showed epigenetic alterations in hepatic steatosis (107). Additionally, Uthus et al. (2006) showed the effects of selenium deficiency on methyl metabolism (108).

However, other nutrients and bioactive compounds may affect the one-carbon metabolism indirectly (21). Likewise, among other modifications, histones undergo acetylation, phosphorylation and methylation. In this context, a number of nutrients and bioactive compounds have also been correlated with changes in the methylation of histones (109). Obesity has been repeatedly associated with epigenetic alterations. Therefore, in order to induce obesity in an animal model to study epigenetic mechanisms, the combination of high-calorie diets supplemented with or lacking nutrients and non-nutrient substances have been developed.

In addition to the studies conducted in rodents, there are other species such as primates and birds that have also been used to study the influence of nutrition on DNA methylation pattern, confirming its role in energy homeostasis (110, 111). Su et al. (2009) evaluated the effects of betaine on fatty liver disease in Landes geese and observed a hypermethylation of the S14α gene (110). Two recent studies showed that *Drosophila* is also a valid model for studies of epigenetic changes and the influence on obesity, underlining the mechanisms involved (112, 113). Matzkin et al. (2013) found evidence that parental diet can influence progeny metabolism in *Drosophila* (113) and Buescher et al. (2013) focused on effects of a high-sugar diet fed to adult females (112).

Conclusion

It is evident that the environment interacts with the genome to influence human health and disease. However, the question remains of whether the altered epigenetic markers in obesity are a cause or a consequence of weight gain. On the one hand, overweight and obesity develops mostly because of high caloric intake and/or low physical activity and it seems plausible that this altered lifestyle impacts epigenetics modifications. On the other hand not all individuals following an 'obese' lifestyle become obese. Thus, epigenetic modifications that already may have been shaped *in utero* or even transgenerational increase the susceptibility to gain weight under distinct conditions and may explain the missing heritability of obesity. Thus, epigenetics have shifted the paradigm in the understanding of complex diseases including metabolic syndrome. Different epigenetic patterns provide not only information about mechanisms but also prospects for interventions in prevention and therapy.

Competing interests: The authors declare that they have no actual or potential competing interests that might be perceived as influencing the results or interpretation of a reported study.

List of abbreviations

Avy	agouti viable yellow
AgRP	agouti related protein homolog
AQP9	aquaporin 9
ARC	arcuate nucleus
ATP10 A	ATPase class V type 10A
BPA	bisphenol A
C/EBPα	CCAAT/Enhancer Binding Protein alpha
Cox2	cytochrome *c* oxidase assembly factor
DAT	dopamine reuptake transporter

DNMT	DNA methyltransferases
DUSP22	dual specificity phosphatase 22
EGCG	epigallocatechin gallate
FAS	Fas cell surface death receptor
GDM	gestational diabetes mellitus
GTL2	Gon-Two Like (TRP subfamily)
HAT	histone acetyl-transferase
HDAC	histone deacetylases
HIF3A	hypoxia-inducible factor 3-alpha
HIPK3	homeodomain interacting protein kinase 3
IAP	intracisternal A-particle
IGF2	insulin-like growth factor 2
IGF2R	insulin-like growth factor 2 receptor
MOR	μ-opioid receptor
MMP9	matrix metallopeptidase 9
MSC	mesenchymal stem cell
PAH	polycyclic aromatic hydrocarbon
PENK	preproenkephalin
PM20D1	peptidase M20 domain containing 1
PPAR γ	peroxisome proliferative activated gamma
PRKG1	cGMP dependent protein kinase type 1
PYY	Peptide YY
RFC5	replication factor C subunit 5
SAM	S-adenosylmethionin
TBT	tributyltin hydride
TNF-α	tumor necrosis factor alpha
TNNT1	troponin T type 1
TNNI3	troponin I type 3.

References

1. World Obesity Federation. World obesity 2014 [cited 2014 23 October 2014]; Available from: http://www.worldobesity.org/.
2. WHO. Available from: http://www.euro.who.int/en/health-topics/noncommunicable-diseases/2013. Accessed 18 October, 2013.
3. Remely M, Aumueller E, Jahn D, Hippe B, Brath H, Haslberger AG. Microbiota and epigenetic regulation of inflammatory mediators in type 2 diabetes and obesity. Benef Microb 2014; 5: 33–43.
4. Remely M, Aumueller E, Merold C, Dworzak S, Hippe B, Zanner J, Pointner A, Brath H, Haslberger AG. Effects of short chain fatty acid producing bacteria on epigenetic regulation of FFAR3 in type 2 diabetes and obesity. Gene 2013; 537: 85–92.
5. Jimenez-Chillaron JC, Díaz R, Martínez D, Pentinat T, Ramón-Krauel M, Ribó S, Plösch T. The role of nutrition on epigenetic modifications and their implications on health. Biochimie 2012; 94: 2242–63.
6. Gallou-Kabani C, Vigé A, Gross MS, Junien C. Nutri-epigenomics: lifelong remodelling of our epigenomes by nutritional and metabolic factors and beyond. Clin Chem Lab Med 2007; 45: 321–7.
7. Choi SW, Friso S. Epigenetics: a new bridge between nutrition and health. Adv Nutr 2010; 1: 8–16.
8. Brandl A, Heinzel T, Kramer OH. Histone deacetylases: salesmen and customers in the post-translational modification market. Biol Cell 2009; 101: 193–205.
9. Haberland M, Montgomery RL, Olson EN. The many roles of histone deacetylases in development and physiology: implications for disease and therapy. Nat Rev Genet 2009; 10: 32–42.
10. Sawicka A, Seiser C. Histone H3 phosphorylation – a versatile chromatin modification for different occasions. Biochimie 2012; 94: 2193–201.
11. Brosch G, Loidl P, Graessle S. Histone modifications and chromatin dynamics: a focus on filamentous fungi. FEMS Microbiol Rev 2008; 32: 409–39.
12. Oliver SS, Denu JM. Dynamic interplay between histone H3 modifications and protein interpreters: emerging evidence for a "histone language". Chembiochem 2011; 12: 299–307.
13. Skinner MK, Manikkam M, Guerrero-Bosagna C. Epigenetic transgenerational actions of endocrine disruptors. Reprod Toxicol 2011; 31: 337–43.
14. Karmaus W, Osuch JR, Eneli I, Mudd LM, Zhang J, Mikucki D, Haan P, Davis S. Maternal levels of dichlorodiphenyldichloroethylene (DDE) may increase weight and body mass index in adult female offspring. Occup Environ Med 2009; 66: 143–9.
15. Somm E, Schwitzgebel VM, Toulotte A, Cederroth CR, Combescure C, Nef S, Aubert ML, Hüppi PS. Perinatal exposure to bisphenol a alters early adipogenesis in the rat. Environ Health Perspect 2009; 117: 1549–55.
16. Newbold RR, Newbold RR, Padilla-Banks E, Snyder RJ, Jefferson WN. Developmental exposure to estrogenic compounds and obesity. Birth Defects Res A Clin Mol Teratol 2005; 73: 478–80.
17. Wang G, Walker SO, Hong X, Bartell TR, Wang X. Epigenetics and early life origins of chronic noncommunicable diseases. J Adolesc Health 2013; 52: Suppl 2: S14–21.
18. Grun F, Blumberg B. Environmental obesogens: organotins and endocrine disruption via nuclear receptor signaling. Endocrinology 2006; 147: 6 Suppl: S50–5.
19. He K, Zhao L, Daviglus ML, Dyer AR, Van Horn L, Garside D, Zhu L, Guo D, Wu Y, Zhou B, Stamler J, INTERMAP Cooperative Research Group. Association of monosodium glutamate intake with overweight in Chinese adults: the INTERMAP Study. Obesity (Silver Spring) 2008; 16: 1875–80.
20. Painter RC, Osmond C, Gluckman P, Hanson M, Phillips DI, Roseboom TJ. Transgenerational effects of prenatal exposure to the Dutch famine on neonatal adiposity and health in later life. BJOG 2008; 115: 1243–9.
21. Milagro FI, Mansego ML, De Miguel C, Martínez JA. Dietary factors, epigenetic modifications and obesity outcomes: progresses and perspectives. Mol Aspects Med 2013; 34: 782–812.
22. Clarke-Harris R, Wilkin TJ, Hosking J, Pinkney J, Jeffery AN, Metcalf BS, Keith M. Godfrey, Voss LD, Lillycrop KA, Burdge GC. PGC1α promoter methylation in blood at 5–7 years predicts adiposity from 9 to 14 years (EarlyBird 50). Diabetes 2014; 63: 2528–37.
23. Fraga CG, Galleano M, Verstraeten SV, Oteiza PI. Basic biochemical mechanisms behind the health benefits of polyphenols. Mol Aspects Med 2010; 31: 435–45.
24. Boque N, de la Iglesia R, de la Garza AL, Milagro FI, Olivares M, Bañuelos O, Soria AC, Rodríguez-Sánchez S, Martínez JA, Campión J. Prevention of diet-induced obesity by apple polyphenols in Wistar rats through regulation of adipocyte gene expression and DNA methylation patterns. Mol Nutr Food Res 2013; 57: 1473–8.
25. Milenkovic D, Deval C, Gouranton E, Landrier JF, Scalbert A, Morand C, Mazur A. Modulation of miRNA expression by dietary polyphenols in apoE deficient mice: a new mechanism of the action of polyphenols. PLoS One 2012; 7: e29837.

26. Ayissi VB, Ebrahimi A, Schluesenner H. Epigenetic effects of natural polyphenols: A focus on SIRT1-mediated mechanisms. Mol Nutr Food Res 2014; 58: 22–32.
27. Dolinoy DC, Weidman JR, Waterland RA, Jirtle RL. Maternal genistein alters coat color and protects Avy mouse offspring from obesity by modifying the fetal epigenome. Environ Health Perspect 2006; 114: 567–72.
28. Kilpinen H, Dermitzakis ET. Genetic and epigenetic contribution to complex traits. Hum Mol Genet 2012; 21: R24–8.
29. Tammen SA, Friso S, Choi SW. Epigenetics: the link between nature and nurture. Mol Aspects Med 2013; 34: 753–64.
30. Franks PW, Ling C. Epigenetics and obesity: the devil is in the details. BMC Med 2010; 8: 88.
31. Waterborg JH. Dynamics of histone acetylation in vivo. A function for acetylation turnover? Biochem Cell Biol 2002; 80: 363–78.
32. Hajkova P, Erhardt S, Lane N, Haaf T, El-Maarri O, Reik W, Walter J, Surani MA. Epigenetic reprogramming in mouse primordial germ cells. Mech Dev 2002; 117: 15–23.
33. Hales CN, Barker DJ. The thrifty phenotype hypothesis. Br Med Bull 2001; 60: 5–20.
34. Pembrey ME. Time to take epigenetic inheritance seriously. Eur J Hum Genet 2002; 10: 669–71.
35. Vickers MH. Developmental programming and transgenerational transmission of obesity. Ann Nutr Metab 2014; 64: Suppl 1: 26–34.
36. Kaati G, Bygren LO, Edvinsson S. Cardiovascular and diabetes mortality determined by nutrition during parents' and grandparents' slow growth period. Eur J Hum Genet 2002; 10: 682–8.
37. Pentinat T, Ramon-Krauel M, Cebria J, Diaz R, Jimenez-Chillaron JC. Transgenerational inheritance of glucose intolerance in a mouse model of neonatal overnutrition. Endocrinology 2010; 151: 5617–23.
38. Fullston T, Palmer NO, Owens JA, Mitchell M, Bakos HW, Lane M. Diet-induced paternal obesity in the absence of diabetes diminishes the reproductive health of two subsequent generations of mice. Hum Reprod 2012; 27: 1391–400.
39. Carone BR, Fauquier L, Habib N, Shea JM, Hart CE, Li R, Bock C, Li C, Gu H, Zamore PD, Meissner A, Weng Z, Hofmann HA, Friedman N, Rando OJ. Paternally induced transgenerational environmental reprogramming of metabolic gene expression in mammals. Cell 2010; 143: 1084–96.
40. Armitage JA, Ishibashi A, Balachandran AA, Jensen RI, Poston L, Taylor PD. Programmed aortic dysfunction and reduced Na+, K+-ATPase activity present in first generation offspring of lard-fed rats does not persist to the second generation. Exp Physiol 2007; 92: 583–9.
41. Dunn GA, Bale TL. Maternal high-fat diet effects on third-generation female body size via the paternal lineage. Endocrinology 2011; 152: 2228–36.
42. Blondeau B, Avril I, Duchene B, Bréant B. Endocrine pancreas development is altered in foetuses from rats previously showing intra-uterine growth retardation in response to malnutrition. Diabetologia 2002; 45: 394–401.
43. Thamotharan M, Garg M, Oak S, Rogers LM, Pan G, Sangiorgi F, Lee PW, Devaskar SU. Transgenerational inheritance of the insulin-resistant phenotype in embryo-transferred intrauterine growth-restricted adult female rat offspring. Am J Physiol Endocrinol Metab 2007; 292: E1270–9.
44. Benyshek DC, Johnston CS, Martin JF, Ross WD. Insulin sensitivity is normalized in the third generation (F3) offspring of developmentally programmed insulin resistant (F2) rats fed an energy-restricted diet. Nutr Metab (Lond) 2008; 5: 26.
45. Pinheiro AR, Salvucci ID, Aguila MB, Mandarim-de-Lacerda CA. Protein restriction during gestation and/or lactation causes adverse transgenerational effects on biometry and glucose metabolism in F1 and F2 progenies of rats. Clin Sci (Lond) 2008; 114: 381–92.
46. Peixoto-Silva N, Frantz ED, Mandarim-de-Lacerda CA, Pinheiro-Mulder A. Maternal protein restriction in mice causes adverse metabolic and hypothalamic effects in the F1 and F2 generations. Br J Nutr 2011; 106: 1364–73.
47. Burdge GC, Slater-Jefferies J, Torrens C, Phillips ES, Hanson MA, Lillycrop KA. Dietary protein restriction of pregnant rats in the F0 generation induces altered methylation of hepatic gene promoters in the adult male offspring in the F1 and F2 generations. Br J Nutr 2007; 97: 435–9.
48. Frantz ED, Aguila MB, Pinheiro-Mulder Ada R, Mandarim-de-Lacerda CA. Transgenerational endocrine pancreatic adaptation in mice from maternal protein restriction in utero. Mech Ageing Dev 2011; 132: 110–6.
49. Torrens C, Poston L, Hanson MA. Transmission of raised blood pressure and endothelial dysfunction to the F2 generation induced by maternal protein restriction in the F0, in the absence of dietary challenge in the F1 generation. Br J Nutr 2008; 100: 760–6.
50. Harrison M, Langley-Evans SC. Intergenerational programming of impaired nephrogenesis and hypertension in rats following maternal protein restriction during pregnancy. Br J Nutr 2009; 101: 1020–30.
51. Benyshek DC, Johnston CS, Martin JF. Glucose metabolism is altered in the adequately-nourished grand-offspring (F3 generation) of rats malnourished during gestation and perinatal life. Diabetologia 2006; 49: 1117–9.
52. Drake AJ, Walker BR, Seckl JR. Intergenerational consequences of fetal programming by in utero exposure to glucocorticoids in rats. Am J Physiol Regul Integr Comp Physiol 2005; 288: R34–8.
53. Burdge GC, Hoile SP, Uller T, Thomas NA, Gluckman PD, Hanson MA, Lillycrop KA. Progressive, transgenerational changes in offspring phenotype and epigenotype following nutritional transition. PLoS One 2011; 6: e28282.
54. Chang HS, Anway MD, Rekow SS, Skinner MK. Transgenerational epigenetic imprinting of the male germline by endocrine disruptor exposure during gonadal sex determination. Endocrinology 2006; 147: 5524–41.
55. Walker DM, Gore AC. Transgenerational neuroendocrine disruption of reproduction. Nat Rev Endocrinol 2011; 7: 197–207.
56. Grun F, Watanabe H, Zamanian Z, Maeda L, Arima K, Cubacha R, Gardiner DM, Kanno J, Iguchi T, Blumberg B. Endocrine-disrupting organotin compounds are potent inducers of adipogenesis in vertebrates. Mol Endocrinol 2006; 20: 2141–55.
57. Vucetic Z, Kimmel J, Totoki K, Hollenbeck E, Reyes TM. Maternal high-fat diet alters methylation and gene expression of dopamine and opioid-related genes. Endocrinology 2010; 151: 4756–64.
58. Archer E. The childhood obesity epidemic as a result of nongenetic evolution: the maternal resources hypothesis. Mayo Clin Proc 2014; 90: 77–92.
59. Ge ZJ, Liang QX, Hou Y, Han ZM, Schatten H, Sun QY, Zhang CL. Maternal obesity and diabetes may cause DNA methylation alteration in the spermatozoa of offspring in mice. Reprod Biol Endocrinol 2014; 12: 29.

60. Mathers JC. Session 2: personalised nutrition. Epigenomics: a basis for understanding individual differences? Proc Nutr Soc 2008; 67: 390–4.
61. Waterland RA. Assessing the effects of high methionine intake on DNA methylation. J Nutr 2006; 136: 6 Suppl: 1706S–10S.
62. Waterland RA, Travisano M, Tahiliani KG, Rached MT, Mirza S. Methyl donor supplementation prevents transgenerational amplification of obesity. Int J Obes (Lond) 2008; 32: 1373–9.
63. Scarlett JM, Zhu X, Enriori PJ, Bowe DD, Batra AK, Levasseur PR, Grant WF, Meguid MM, Cowley MA, Marks DL. Regulation of agouti-related protein messenger ribonucleic acid transcription and peptide secretion by acute and chronic inflammation. Endocrinology 2008; 149: 4837–45.
64. El Hajj N, Schneider E, Lehnen H, Haaf T. Epigenetics and life-long consequences of an adverse nutritional and diabetic intrauterine environment. Reproduction 2014; 148: R111–20.
65. Guerrero-Bosagna CM, Sabat P, Valdovinos FS, Valladares LE, Clark SJ. Epigenetic and phenotypic changes result from a continuous pre and post natal dietary exposure to phytoestrogens in an experimental population of mice. BMC Physiol 2008; 8: 17.
66. Dolinoy DC, Huang D, Jirtle RL. Maternal nutrient supplementation counteracts bisphenol A-induced DNA hypomethylation in early development. Proc Natl Acad Sci USA 2007; 104: 13056–61.
67. Sinclair KD, Allegrucci C, Singh R, Gardner DS, Sebastian S, Bispham J, Thurston A, Huntley JF, Rees WD, Maloney CA, Lea RG, Craigon J, McEvoy TG, Young LE. DNA methylation, insulin resistance and blood pressure in offspring determined by maternal periconceptional B vitamin and methionine status. Proc Natl Acad Sci USA 2007; 104: 19351–6.
68. Fernandez JR, Klimentidis YC, Dulin-Keita A, Casazza K. Genetic influences in childhood obesity: recent progress and recommendations for experimental designs. Int J Obes (Lond) 2012; 36: 479–84.
69. Soubry A, Murphy SK, Wang F, Huang Z, Vidal AC, Fuemmeler BF, Kurtzberg J, Murtha A, Jirtle RL, Schildkraut JM, Hoyo C. Newborns of obese parents have altered DNA methylation patterns at imprinted genes. Int J Obes (Lond) 2015; 39: 650–7.
70. Zheng J, Xiao X, Zhang Q, Yu M. DNA methylation: the pivotal interaction between early-life nutrition and glucose metabolism in later life. Br J Nutr 2014; 112: 1850–7.
71. Kyle UG, Pichard C. The Dutch Famine of 1944–1945: a pathophysiological model of long-term consequences of wasting disease. Curr Opin Clin Nutr Metab Care 2006; 9: 388–94.
72. Khulan B, Cooper WN, Skinner BM, Bauer J, Owens S, Prentice AM, Belteki G, Constancia M, Dunger D, Affara NA. Periconceptional maternal micronutrient supplementation is associated with widespread gender related changes in the epigenome: a study of a unique resource in the Gambia. Hum Mol Genet 2012; 21: 2086–101.
73. Cooper WN, Khulan B, Owens S, Elks CE, Seidel V, Prentice AM, Belteki G, Ong KK, Affara NA, Constância M, Dunger DB. DNA methylation profiling at imprinted loci after periconceptional micronutrient supplementation in humans: results of a pilot randomized controlled trial. FASEB J 2012; 26: 1782–90.
74. Li J, Chen L, Yu P, Liu B, Zhu J, Yang Y. Telmisartan exerts antitumor effects by activating peroxisome proliferator-activated receptor-gamma in human lung adenocarcinoma A549 cells. Molecules 2014; 19: 2862–76.
75. Menale C, Piccolo MT, Cirillo G, Calogero RA, Papparella A, Mita L, Miraglia Del Giuduce E, Diano N, Crispi S, Mita DG. Bisphenol A effects on gene expression in children adipocytes: association to metabolic disorders. J Mol Endocrinol 2015 Apr 15. pii: JME-14-0282.
76. Dick KJ, Nelson CP, Tsaprouni L, Sandling JK, Aïssi D, Wahl S, Meduri E, Morange PE, Gagnon F, Grallert H, Waldenberger M, Peters A, Erdmann J, Hengstenberg C, Cambien F, Goodall AH, Ouwehand WH, Schunkert H, Thompson JR, Spector TD, Gieger C, Trégouët DA, Delouka P. DNA methylation and body-mass index: a genome-wide analysis. Lancet 2014; 383: 1990–8.
77. Xu X, Su S, Barnes VA, De Miguel C, Pollock J, Ownby D, Shi H, Zhu H, Snieder H, Wang X. A genome-wide methylation study on obesity: differential variability and differential methylation. Epigenetics 2013; 8: 522–33.
78. Davis KE, Neinast MD, Sun K, Skiles WM, Bills JD, Zehr JA, Zeve D, Hahner LD, Cox DW, Gent LM, Xu Y, Wang ZV, Khan SA, Clegg DJ. The sexually dimorphic role of adipose and adipocyte estrogen receptors in modulating adipose tissue expansion, inflammation and fibrosis. Mol Metab 2013; 2: 227–42.
79. Feinberg AP, Irizarry RA, Fradin D, Aryee MJ, Murakami P, Aspelund T, Eiriksdottir G, Harris TB, Launer L, Gudnason V, Fallin MD. Personalized epigenomic signatures that are stable over time and covary with body mass index. Sci Transl Med 2010; 2: 49ra67.
80. Na YK, Hong HS, Lee DH, Lee WK, Kim DS. Effect of body mass index on global DNA methylation in healthy Korean women. Mol Cells 2014; 37: 467–72.
81. Campion J, Milagro FI, Goyenechea E, Martínez JA. TNF-alpha promoter methylation as a predictive biomarker for weight-loss response. Obesity (Silver Spring) 2009; 17: 1293–7.
82. Moleres A, Campión J, Milagro FI, Marcos A, Campoy C, Garagorri JM, Gómez-Martínez S, Martínez JA, Azcona-Sanjulián MC, Martí A, EVASYON Study Group. Differential DNA methylation patterns between high and low responders to a weight loss intervention in overweight or obese adolescents: the EVASYON study. FASEB J 2013; 27: 2504–12.
83. Milagro FI, Campión J, Cordero P, Goyenechea E, Gómez-Uriz AM, Abete I, Zulet MA, Martínez JA. A dual epigenomic approach for the search of obesity biomarkers: DNA methylation in relation to diet-induced weight loss. FASEB J 2011; 25: 1378–89.
84. Nilsson EK, Ernst B, Voisin S, Almén MS, Benedict C, Mwinyi J, Fredriksson R, Schultes B, Schiöth HB. Roux-en Y gastric bypass surgery induces genome-wide promoter-specific changes in DNA methylation in whole blood of obese patients. PLoS One 2015; 10: e0115186.
85. Okamura M, Inagaki T, Tanaka T, Sakai J. Role of histone methylation and demethylation in adipogenesis and obesity. Organogenesis 2010; 6: 24–32.
86. Farmer SR. Transcriptional control of adipocyte formation. Cell Metab 2006; 4: 263–73.
87. Ortega FJ, Mercader JM, Catalán V, Moreno-Navarrete JM, Pueyo N, Sabater M, Gómez-Ambrosi J, Anglada R, Fernández-Formoso JA, Ricart W, Frühbeck G, Fernández-Real JM. Targeting the circulating microRNA signature of obesity. Clin Chem 2013; 59: 781–92.
88. Yeh CL, Cheng IC, Hou YC, Wang W, Yeh SL. MicroRNA-125a-3p expression in abdominal adipose tissues is associated with insulin signalling gene expressions in morbid obesity: observations in Taiwanese. Asia Pac J Clin Nutr 2014; 23: 331–7.
89. McKay JA, Mathers JC. Diet induced epigenetic changes and their implications for health. Acta Physiol (Oxf) 2011; 202: 103–18.

90. Panchal SK, Brown L. Rodent models for metabolic syndrome research. J Biomed Biotechnol 2011; 2011: 351982.
91. Speakman J, Hambly C, Mitchell S, Król E. The contribution of animal models to the study of obesity. Lab Anim 2008; 42: 413–32.
92. Milagro FI, Campión J, García-Díaz DF, Goyenechea E, Paternain L, Martínez JA. High fat diet-induced obesity modifies the methylation pattern of leptin promoter in rats. J Physiol Biochem 2009; 65: 1–9.
93. Fontana L, Klein S, Holloszy JO. Effects of long-term calorie restriction and endurance exercise on glucose tolerance, insulin action and adipokine production. Age (Dordr) 2010; 32: 97–108.
94. Seki Y, Williams L, Vuguin PM, Charron MJ. Minireview: Epigenetic programming of diabetes and obesity: animal models. Endocrinology 2012; 153: 1031–8.
95. Wheatley KE, Nogueira LM, Perkins SN, Hursting SD. Differential effects of calorie restriction and exercise on the adipose transcriptome in diet-induced obese mice. J Obes 2011; 2011: 265417.
96. Zhang S, Rattanatray L, MacLaughlin SM, Cropley JE, Suter CM, Molloy L, Kleemann D, Walker SK, Muhlhausler BS, Morrison JL, McMillen IC. Periconceptional undernutrition in normal and overweight ewes leads to increased adrenal growth and epigenetic changes in adrenal IGF2/H19 gene in offspring. FASEB J 2010; 24: 2772–82.
97. Vaquero A, Reinberg D. Calorie restriction and the exercise of chromatin. Genes Dev 2009; 23: 1849–69.
98. Lillycrop KA, Phillips ES, Jackson AA, Hanson MA, Burdge GC. Dietary protein restriction of pregnant rats induces and folic acid supplementation prevents epigenetic modification of hepatic gene expression in the offspring. J Nutr 2005; 135: 1382–6.
99. Lillycrop KA, Phillips ES, Torrens C, Hanson MA, Jackson AA, Burdge GC. Feeding pregnant rats a protein-restricted diet persistently alters the methylation of specific cytosines in the hepatic PPAR alpha promoter of the offspring. Br J Nutr 2008; 100: 278–82.
100. Sohi G, Marchand K, Revesz A, Arany E, Hardy DB. Maternal protein restriction elevates cholesterol in adult rat offspring due to repressive changes in histone modifications at the cholesterol 7alpha-hydroxylase promoter. Mol Endocrinol 2011; 25: 785–98.
101. Boque N, Campión J, Paternain L, García-Díaz DF, Galarraga M, Portillo MP, Milagro FI, Ortiz de Solórzano C, Martínez JA. Influence of dietary macronutrient composition on adiposity and cellularity of different fat depots in Wistar rats. J Physiol Biochem 2009; 65: 387–95.
102. Lomba A, Martínez JA, García-Díaz DF, Paternain L, Marti A, Campión J, Milagro FI. Weight gain induced by an isocaloric pair-fed high fat diet: a nutriepigenetic study on FASN and NDUFB6 gene promoters. Mol Genet Metab 2010; 101: 273–8.
103. Vucetic Z, Carlin JL, Totoki K, Reyes TM. Epigenetic dysregulation of the dopamine system in diet-induced obesity. J Neurochem 2012; 120: 891–8.
104. Vucetic Z, Kimmel J, Reyes TM. Chronic high-fat diet drives postnatal epigenetic regulation of mu-opioid receptor in the brain. Neuropsychopharmacology 2011; 36: 1199–206.
105. Ng SF, Lin RC, Laybutt DR, Barres R, Owens JA, Morris MJ. Chronic high-fat diet in fathers programs beta-cell dysfunction in female rat offspring. Nature 2010; 467: 963–6.
106. Rinella ME, Elias MS, Smolak RR, Fu T, Borensztajn J, Green RM. Mechanisms of hepatic steatosis in mice fed a lipogenic methionine choline-deficient diet. J Lipid Res 2008; 49: 1068–76.
107. Pogribny IP, Tryndyak VP, Bagnyukova TV, Melnyk S, Montgomery B, Ross SA, Latendresse JR, Rusyn I, Beland FA. Hepatic epigenetic phenotype predetermines individual susceptibility to hepatic steatosis in mice fed a lipogenic methyl-deficient diet. J Hepatol 2009; 51: 176–86.
108. Uthus EO, Ross SA, Davis CD. Differential effects of dietary selenium (se) and folate on methyl metabolism in liver and colon of rats. Biol Trace Elem Res 2006; 109: 201–14.
109. Campion J, Milagro FI, Martinez JA. Individuality and epigenetics in obesity. Obes Rev 2009; 10: 383–92.
110. Su SY, Dodson MV, Li XB, Li QF, Wang HW, Xie Z. The effects of dietary betaine supplementation on fatty liver performance, serum parameters, histological changes, methylation status and the mRNA expression level of Spot14alpha in Landes goose fatty liver. Comp Biochem Physiol A Mol Integr Physiol 2009; 154: 308–14.
111. Unterberger A, Szyf M, Nathanielsz PW, Cox LA. Organ and gestational age effects of maternal nutrient restriction on global methylation in fetal baboons. J Med Primatol 2009; 38: 219–27.
112. Buescher JL, Musselman LP, Wilson CA, Lang T, Keleher M, Baranski TJ, Duncan JG. Evidence for transgenerational metabolic programming in Drosophila. Dis Model Mech 2013; 6: 1123–32.
113. Matzkin LM, Johnson S, Paight C, Markow TA. Preadult parental diet affects offspring development and metabolism in Drosophila melanogaster. PLoS One 2013; 8: e59530.