Biochemical Society Transactions (2022) **50** 487–497
https://doi.org/10.1042/BST20211069

PORTLAND PRESS

Review Article

Role of macronutrient intake in the epigenetics of obesity

Priyadarshni Patel[1], Jeganathan Ramesh Babu[1,2,3], Xu Wang[4,5,3] and Thangiah Geetha[1,2,3]

[1]Department of Nutrition, Dietetics, and Hospitality Management, Auburn University, Auburn, AL 36849, U.S.A.; [2]Boshell Metabolic Diseases and Diabetes Program, Auburn University, Auburn, AL 36849, U.S.A.; [3]Alabama Agricultural Experiment Station, Auburn University, Auburn, AL 36849, U.S.A.; [4]Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, AL 36849, U.S.A.; [5]HudsonAlpha Institute for Biotechnology, Huntsville, AL 35806, U.S.A.

Correspondence: Thangiah Geetha (thangge@auburn.edu)

Obesity is caused by a combination of hereditary and environmental factors. Despite extensive study, contemporary through diet, exercise, education, surgery, and pharmacological treatments, no effective long-term solution has been found to this epidemic. Over the last decade, there has been a tremendous advancement in understanding the science of epigenetics, as well as a rise in public interest in learning more about the influence of diet and lifestyle choices on the health of an individual. Without affecting the underlying DNA sequence, epigenetic alterations impact gene expression. Previous animal studies have shown a link between the type of diet and expression or suppression of obesity genes, but there are very few human studies that demonstrate the relationship between dietary intake and obesity gene expression. This review highlights the effects of carbohydrates, lipids, and protein intake from the diet on obesity-related genes.

Introduction

Obesity is a global health problem that has been linked to a variety of diseases and disorders, including type 2 diabetes, hypertension, cardiovascular disease, stroke, and cancer [1]. Obesity has complicated pathogenesis, involving various interactions between behavioral, environmental, and genetic factors [1]. The World Health Organization's most recent report states that over 1.9 billion adults are overweight, with over 650 million being obese [2]. In total, this equates to ∼13% of the world's adult population [2]. The rapid rise in childhood obesity is even more concerning, with millions of children affected by it [3]. Obesity is a complex condition characterized by the accumulation of excessive and/or unhealthy body fat, which poses a health risk [1]. From an evolutionary standpoint, the modern development of a sedentary lifestyle, along with an increase in dietary fat and sugar content, may be related to the dramatic rise in obesity rates [4]. However, it has been found to be extremely challenging to precisely understand the determinants of obesity.

Over the last decade, significant advances in epigenetics have coincided with a surge of public interest in the effects of food and lifestyle choices on health [3]. Epigenetic modifications affect the gene expressions without changing the underlying DNA sequences [5]. Epigenetic markers, such as covalent chemical modifications on DNA and histones that determine chromatin structure, are two major components of the human epigenome. As epigenetic marks do not modify the DNA sequence, the entire genomic information or genotype inherited from the parents remains unchanged [6]. Epigenetic marks can alter the local chromatin environment, influencing DNA accessibility and controlling a number of DNA-templated processes, such as gene transcription [5]. If epigenetic signs are misplaced or aberrantly active, it can incorrectly turn on or off the genes affecting its function [6]. Nutrients, contaminants, pollutants, pesticides, and other environmental factors may affect the level of epigenetic marks and turnover. This would result in altered gene expression patterns, which would have an impact on our wellbeing [7–10].

Received: 6 September 2021
Revised: 6 December 2021
Accepted: 8 December 2021

Version of Record published:
7 January 2022

Biochemical Society Transactions (2022) 50 487–497
https://doi.org/10.1042/BST20211069

DNA methylation is the epigenetic mark that has received the most attention, and its role in a variety of diseases has been recognized. Since DNA methylation can be reversed, epigenetics is a promising target for therapeutic intervention [11]. Various studies show a strong correlation between adiposity and methylation at the gene and genome-wide studies in children with obesity [12]. The epigenetic events are influenced by dietary, chemical, and physical influences, resulting in a change in gene expression profile. The variations in DNA methylation patterns may be the cause of inter-individual differences in susceptibility to obesity and other metabolic diseases [13].

A well-balanced diet has long been known to extend life expectancy and help reduce the risk of diseases such as obesity, diabetes, cancer, and mental illnesses [14]. Epigenetic changes can occur as a result of environmental factors, the most significant of which is diet. Obesity is linked to a high intake of energy-dense foods, which has long been recognized as a risk factor. The carbohydrates, fats, and proteins provide us with the building blocks and energy we need to sustain normal physiological processes, but overeating has negative health outcomes [5]. It has been shown that nutrients and bioactive food components can affect epigenetic phenomena either by inhibiting enzymes that catalyze DNA methylation or histone modifications directly or by altering the availability of substrates required for certain enzymatic reactions [15]. As a result, knowing how food consumption affects the epigenome, and how this, in turn, affects gene expression, is crucial for developing improved nutritional therapies in the treatment of various metabolic diseases.

There is solid evidence in the mouse model evaluating the effects of maternal exposure to bisphenol A (BPA) on the epigenome of offspring in ectodermal (brain and tail) cells [16]. Other research demonstrates the link between macronutrient content of the food and epigenetic regulation and obesity development in mice [13]. Another transgenerational mice study showed that male pups developed hyperglycemia and insulin resistance as a result of the maternal high-fat diet (HFD) coupled with the offspring HFD [17]. Male pups exposed to the maternal HFD still had hyperglycemia and glucose intolerance after switching to the control diet. The liver of the pups showed hypermethylation at the gene *Irs2* and hypomethylation at *Map2k4* when exposed to a maternal HFD [17]. Similar to the previous study, the long-term effect of maternal HFD intake on dopamine and opioid gene expression in the hypothalamus of the offspring was studied using a mouse model, the findings showed that maternal HFD can modify the epigenetic markers (DNA hypomethylation) of her offspring, which is linked to long-term gene expression changes [18]. Although long-term transgenerational studies have been performed in animal models, this experiment design is cost-prohibitive in humans. However, a study demonstrated DNAm mediated the association of prenatal famine exposure with adult body mass index (BMI) and triglyceride, but not with glucose. Around 13.4% of the association between famine exposure and BMI was mediated by DNAm at *PIM3*, a gene involved in energy metabolism. DNAm at six CpGs, including *TXNIP*, impacts beta cell function, and *ABCG1*,which regulates lipid metabolism, mediated 80% of the association between famine exposure and serum TG [19].

This review highlights recent studies, which provide insight into the effects of carbohydrates, lipids and protein intake on the epigenetic modifications in various genes (Table 1 and Figure 1). All the human studies that focused on obesity-related genes which play a major role in the metabolic pathways are included in this review and some animal studies are used as additional references. A non-systematic search of electronic bases such as PubMed, Web of Science, and Medline for human studies was done using the following search terms: epigenetics, obesity, childhood obesity, dietary intake, carbohydrates, fats, proteins, DNA methylation, gene expression, epigenetic modifications, HFD, high carbohydrate diet, energy balance, transgenerational epigenetic modifications.

Carbohydrate

Obesity is becoming more prevalent across the world, and the importance of considering diet in the prevention and treatment of obesity is widely known. Carbohydrate (CHO) is one of the macronutrients that provide energy, and as a result, they can lead to excessive energy consumption and weight gain [20]. In the past few years, the association of CHO intake with epigenetic modifications has been analyzed in various studies.

Carnitine palmitoyltransferase-1 (CPT1) converts long-chain acyl-CoAs into long-chain acylcarnitines, allowing them to pass through the mitochondrial membrane and into the mitochondrial matrix, where fatty acid oxidation occurs [21]. Insulin-mediated inhibition of glucose production, insulin secretion, and glycogen synthesis, as well as appetite regulation, are all controlled by *CPT1A* expression. A high level of methylation at *CPT1A* cg00574958 is related to a lower risk of obesity [22]. A research study conducted by Lai et al. looked at the relationship between CHO and fat (FAT) intake as percentages of total diet energy, as well as the CHO/FAT ratio, and the risk of metabolic diseases with *CPT1A*-cg00574958 methylation. A strong link between

Biochemical Society Transactions (2022) **50** 487–497
https://doi.org/10.1042/BST20211069

Table 1 Summary of studies on relation between macronutrient intake and obesity genes Part 1 of 3

Subjects	Parameter	Macronutrient intake	Gene	CpG	Results
Adult Population from: Genetics of Lipid-Lowering Drugs and Diet Network, n = 978; Framingham Heart Study, n = 2331; COR study, n = 645	Carbohydrate intake, fat intake, carbohydrate/fat ratio	CHO (% of total energy): 48.9 ± 6.4; 46.6 ± 8.6. 41.4 ± 7.51 Total fat (% of total energy): 35.4 ± 6.9 33.0 ± 6.6 40.9 ± 6.87	*CPTA1*	cg00574958	CHO intake and the CHO/FAT ratio were positively correlated with cg00574958 methylation, while FAT intake was negatively correlated [23].
Adult population within the Methyl Epigenome Network Association project (n = 473)	Carbohydrate intake, BMI	Carbohydrates (g/day): 239.6 ± 68.1 (with abdominal obesity) 272.1 ± 107.3 (without abdominal obesity)	*ITPR3, PPP2R2D, PPP2R2D, SLC18A1, DRD5, ITPR2, DDC, SLC6A3.*	cg03489495, cg22851378, cg04021127, cg22441882, cg03045635, cg23341970, cg13051970, cg00574958.	A strong correlation was seen between 12 CpG sites and BMI (P < 0.0001). *SLC18A1* and *SLC6A3* gene methylation correlated with total energy (P < 0.001) and carbohydrate (P < 0.001) intakes [26].
Overweight and obese adolescent boys (n = 84)	Carbohydrate, fat, and protein	Carbohydrate intake (g/day): 220.41 ± 86.16 (overweight) 248.49 ± 105.12 (obese) Protein intake(g/day): 166.12 ± 280.18 (overweight) 88.29 ± 48.95 (obese) Fat intake (g/day): 177.33 ± 89.89 (overweight) 170.36 ± 75.43 (obese).	*FTO, IRX3*	Not available	Higher carbohydrates intake significantly up-regulated the FTO gene (P = 0.001) and down-regulated the IRX3 gene (P = 0.01). Protein intake up-regulated the FTO gene (P = 0.00), with no significant effect on IRX3 expression (P = 0.57). There was no significant association between fat intake and IRX3 and FTO expression (P = 0.65, P = 0.26, respectively) [33].
Subjects from Methyl Epigenome Network Association (MENA) project. (n = 474)	Carbohydrate, total energy, protein, and fat.	Not available	*RORA, BHLHE40*	cg09578018, cg01180628	Significant correlations (P < 0.05) between methylation at cg09578018 (RORA) and cg01180628 (BHLHE40) with total energy and carbohydrate intakes. There were no significant associations between these CpGs' methylation patterns with protein or fat consumption [34].
Women followed a nutrition program based on Mediterranean dietary pattern (n = 61)	Carbohydrate intake, total caloric intake.	Not available	*BMAL1*	Not available	Energy (P = 0.047) and carbohydrate (P = 0.017) intake were positively associated with baseline methylation of the CpG 5 to 9 area in the gene BMAL1 [35].
Children aged 10 years. (n = 69).	The ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acids (SFA), the ratio of monounsaturated fatty acids (MUFA) to SFA, and	Total fat intake (% of total energy intake) 39.92 ± 8.96 45.82 ± 9.54. MUFA intake (% of total energy intake)	Not available	Not available	Total fat consumption was strongly associated with the methylation levels of one CpG island shore and four sites. Methylation levels were significantly correlated

Continued

Biochemical Society Transactions (2022) **50** 487–497
https://doi.org/10.1042/BST20211069

Table 1 Summary of studies on relation between macronutrient intake and obesity genes Part 2 of 3

Subjects	Parameter	Macronutrient intake	Gene	CpG	Results
	the ratio of MUFA + PUFA to SFA, cholesterol intake.	19.00 ± 5.97 21.74 ± 5.91 PUFA intake (% of total energy intake) 7.83 ± 7.99 4.77 ± 0.92. SFA intake (% of total energy intake) 13.32 ± 3.62 16.29 ± 3.54. Cholesterol (g/day) 188.90 ± 101.84 304.49 ± 137.54			with PUFA/SFA on 2 islands, 11 island shores, and 16 sites; MUFA/SFA on 9 islands, 26 island beaches, and 158 locations; and (MUFA + PUFA)/SFA on 10 islands, 40 island shores, and 130 sites [37].
Adult normal, overweight, and obese women. ($n = 60$)	Monounsaturated fat, polyunsaturated fat.	Energy intake (kcal/day): 2110 ± 875. Protein (Kcal- % total energy): 357 ± 158. Carbohydrate (Kcal- % total energy): 884 ± 350. Fats (Kcal- % total energy): 883 ± 485.	*CLOCK, BMAL1 and PER2*	Not available	The percentage of methylation of CLOCK CpGs showed associations with the intake of monounsaturated and polyunsaturated fatty acids [5].
Adult participants carrying the rs5082 CC or TT genotypes and consuming low-SFA (<22 g/d) or high-SFA diet (≥22 g/d) in Boston Puerto Rican Health Study ($n = 80$). The findings were validated in the Genetics of Lipid-Lowering Drugs and Diet Network Study ($n = 379$) and the Framingham Heart Study ($n = 243$).	High fat or low-fat diet	Low SFA diet (<22 g/d) High-SFA diet (≥22 g/d)	*APOA2*	cg04436964	Methylation site cg04436964 was found to differ significantly between CC and TT individuals who ate a high-SFA diet and those who ate a low-SFA diet [38].
The POUNDS Lost Trail is a two-year randomized intervention study in which 811 overweight and obese people (BMI 25–40 kg/m).	Fat intake	Fat (%): 36.4 ± 6.1	*NFATC2IP*	cg26663590	A significant associations of fat consumption was associated with genetic (rs11150675) and transcriptional (ILMN 1725441) alterations at the NFATC2IP locus [39].

cg00574958 methylation and metabolic phenotypes (BMI, triglyceride, glucose) and diseases was observed. The findings revealed that CHO intake and the CHO/FAT ratio were positively correlated with cg00574958 methylation, while the FAT intake was negatively correlated [23]. A similar finding was observed in rodent studies where a high fructose intake or a low-fat diet-induced *CPT1A* methylation in liver tissue [24,25]. The findings of the above-mentioned studies indicate that proportion of CHO and FAT in the diet has an impact on the risk of metabolic diseases via the epigenetic status of *CPT1A*.

Dopamine (DA) is a neurotransmitter that controls the rewarding and motivating mechanisms that control food consumption and eating habits. An association was looked for between obesity, metabolic profiles, and dietary intake with DNA methylation signatures at genes that modulate DA signaling [26]. The results of the study showed 12 CpG sites to be strongly correlated ($P < 0.0001$) with BMI: cg03489495 (*ITPR3*), cg22851378

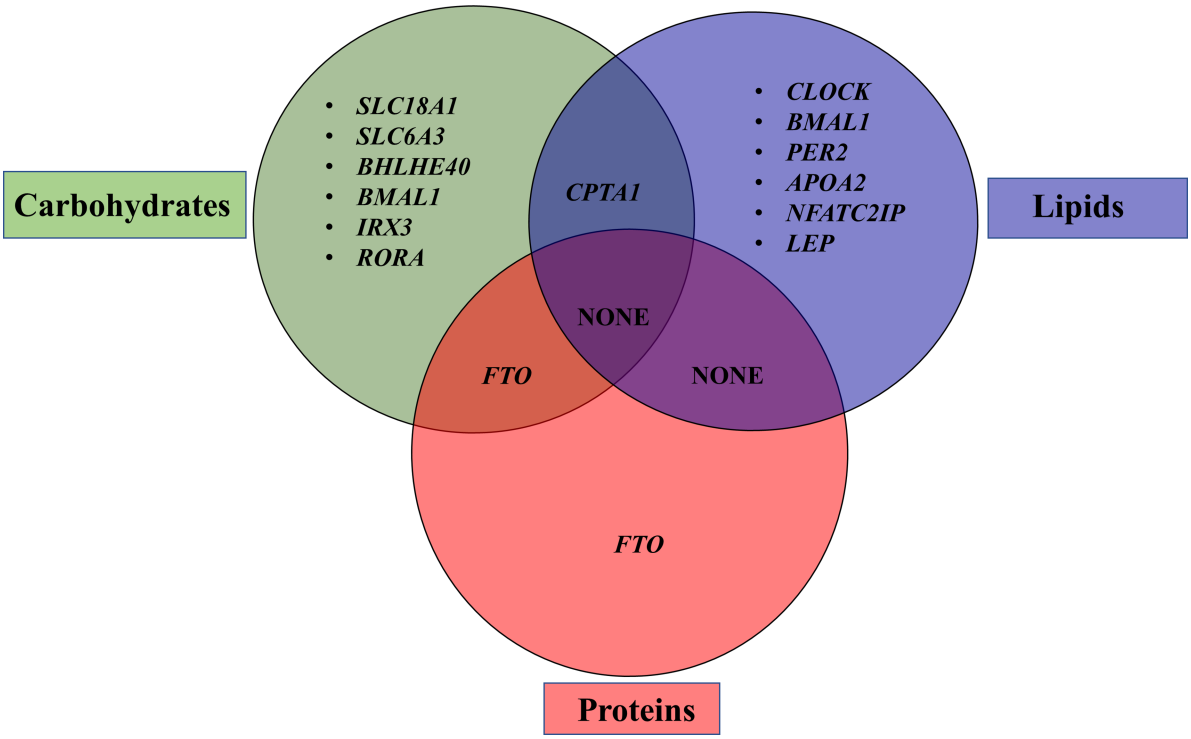

Figure 1. Venn diagram representing various obesity genes and the combined effects of carbohydrates, lipids, and protein intake.

(*PPP2R2D*), cg04021127 (*PPP2R2D*), cg22441882 (*SLC18A1*), cg03045635 (*DRD5*), cg23341970 (*ITPR2*), cg13051970 (*DDC*), cg09691393 (*SLC6A3*). Furthermore, the average methylation levels of these genes varied depending on whether abdominal obesity was present in the subjects. The findings suggest that methylation status on DA signaling genes may play a role in epigenetic mechanisms influencing CHO and calorie intake, as well as fat deposition.

After the age of seven years [27], genes like the fat mass and obesity-associated (*FTO*) gene have been shown to be closely related to obesity and overweight [28]. The *FTO* gene is thought to play a key role in regulating food intake and basal metabolic rate [29]. According to the findings of recent research, the *FTO* gene and dietary macronutrient intake have a mutual interaction. *FTO* gene expression can be influenced by dietary macronutrients such as fats, carbohydrates, and proteins, according to some studies [30,31]. The effects of dietary macronutrients on body weight, hormone secretion, and appetite, on the other hand, are also affected by the *FTO* genotype [31,32]. A field trial study [33] was conducted to determine the effects of *FTO* genotype on the interactions with the macronutrient intake and homeobox transcription factor Iroquois-3 (*IRX3*) gene expression levels. Higher carbohydrate consumption significantly up-regulated the *FTO* gene ($P = 0.001$) and down-regulated the *IRX3* gene ($P = 0.01$), according to the findings. Protein intake increased the expression of the *FTO* gene ($P = 0.001$).

Several physiological and behavioral processes are regulated by the circadian clock [34]. The circadian clock system has been linked to the onset and development of obesity and some other comorbidities. Epigenetic mechanisms, such as DNA methylation, are putatively involved in the regulation of the circadian clock system [34]. Ramoz et al. aimed to determine a link between DNA methylation at circadian rhythm pathway genes and BMI, metabolic profiles, and dietary intakes. The total energy and carbohydrate intakes were associated with methylation status at cg09578018 (*RORA*) and cg01180628 (*BHLHE40*). There were no significant associations between these CpGs' methylation patterns with protein or fat consumption [35]. In another study, energy ($P = 0.047$) and carbohydrate ($P = 0.017$) intake were positively associated with baseline methylation of the CpG 5 to 9 area in the gene *BMAL1* [34].

Biochemical Society Transactions (2022) 50 487–497
https://doi.org/10.1042/BST20211069

Lipid

In recent years, the profile of fatty acids consumption has shifted substantially from diets rich in monounsaturated fatty acid (MUFA) and Polyunsaturated fatty acid (PUFA) to a 'Western-style' diet which is rich in saturated fatty acids (SFA) and trans fatty acids. This dietary shift has been related to the increasing obesity epidemic and connected to abnormal epigenetic modifications [5]. Epidemiological evidence indicates that a HFD facilitates the development of obesity and that the amount of dietary fat consumed, and the degree of obesity are directly related [36].

The type and amount of dietary fat have a major impact on the metabolic pathways that lead to obesity, metabolic syndrome, type 2 diabetes, and cardiovascular disease. A study on Greek adolescents was conducted by Voisin *et al.* [37] in which they looked at the effects of cholesterol intake, the proportion of energy intake derived from fat, the ratio of PUFA to SFA, the ratio of MUFA to SFA, and the ratio of MUFA + PUFA to SFA between normal-weight and obese children's genome-wide DNA methylation patterns. The overall fat consumption was significantly associated with the methylation levels of one CpG island shore and four different sites. PUFA/SFA was significantly correlated with the methylation levels of 2 islands, 11 island shores, and 16 sites; MUFA/SFA was significantly correlated with the methylation levels of 9 islands, 26 island shores, and 158 sites; and (MUFA + PUFA)/SFA was significantly correlated with the methylation levels of 10 islands, 40 island shores, and 130 sites. There was a significant gene enrichment in 34 PUFA/SFA pathways, including the leptin pathway, and a significant gene enrichment in 5 (MUFA + PUFA)/SFA pathways. The findings indicate that specific changes in DNA methylation can play a significant role in the physiological responses to various types of dietary fats [37].

The DNA methylation on three genes *CLOCK*, *PER2*, and *BMAL1*, and the association with dietary fat intake was studied by Milagro et al. [5]. The results showed that *CLOCK* CpGs 1 and 8 methylation levels showed a negative correlation with MUFA intake ($P < 0.05$) but, was positively associated with PUFA consumption ($P < 0.05$). These findings imply that dietary fatty acid content may affect the methylation pattern of genes involved in circadian rhythm regulation.

Lai et al. [38] proposed that the genotype-dependent response to a high-SFA diet might entail epigenetic alterations, which would result in altered *APOA2* expression and metabolic alterations in energy metabolism and homeostasis. The study investigated the relationship between epigenome-wide variation and *APOA2* genotype in selected participants of the Boston Puerto Rican Health Study (BPRHS), conditional on dietary SFA intake, and then validated the findings in two additional populations, the Framingham Heart Study (FHS) and the Genetics of Lipid-Lowering Drugs and Diet Network (GOLDN) Study [38]. Epigenome-wide scan was used on 80 people who had rs5082 CC or TT genotype and were consuming either a low-SFA (22 g/d) or a high-SFA (22 g/d) diet, and were matched for age, sex, BMI, and diabetes status. In the BPRHS, the methylation site cg04436964 differed significantly between CC and TT people who ate a high-SFA diet, but not those who ate a low-SFA diet. Furthermore, cg04436964 methylation was shown to be negatively associated with *APOA2* expression in the blood of FHS individuals who consumed a high-SFA diet. Additionally, CC carriers showed lower *APOA2* expression than TT genotype carriers when eating a high-SFA diet. Finally, metabolomic analysis revealed that four pathways, including tryptophan and branched-chain amino acid (BCAA) pathways, were overrepresented by metabolite variations between CC and TT genotypes with high-SFA consumption. In high-SFA consumers, these pathways were related to rs5082-specific cg04436964 methylation variations. This suggests that the epigenetic state of the *APOA2* regulatory region is linked with SFA consumption, inducing an *APOA2* expression difference between *APOA2* genotypes on a high-SFA diet, and altering BCAA and tryptophan metabolic pathways. These findings point to potential processes by which this highly reproducible gene-diet interaction affects obesity risk, and the link between SFA and human health.

The DNA methylation of *NFATC2IP* has been linked to body mass index [39]. In a 2-year weight-loss trial, researchers looked at the implications of genetic variation, methylation, and gene expression at this locus in relation to adiposity variations [39]. DNA methylation was determined from stored blood samples at baseline ($n = 48$) and participants ($n = 692$) were genotyped and randomly allocated to one of four reduced-calorie diets. On a 2-year weight change, a significant genetic association of fat consumption was identified at rs11150675 SNP locus, and a significant transcriptional alteration (ILMN 1725441) was also demonstrated at the *NFATC2IP* locus.

Protein

Another macronutrient that influences body weight and composition is protein. Dietary protein enhances satiety, energy expenditure, and alters body composition in favor of fat-free body mass, making it helpful for

weight management. There is no human research study that determines the association between protein intake and obesity genes except for one [33], which has been discussed in the carbohydrate section. Dietary protein was found to increase the expression of the *FTO* gene. But it was significantly associated negatively with *IRX3* gene expression. Evidence has been seen in animal studies, where dipeptidyl peptidase-4 (*Dpp4*) mRNA expression was found to be regulated by DNA methylation in numerous tissues. In primary hepatocytes, amino acids enhanced Dpp4 expression, whereas glucose and fatty acids had no effect. In mice, dietary protein restriction increased *Dpp4* DNA methylation in the liver, which resulted in decreased *Dpp4* expression and, as a result, lower plasma DPP4 activity. The study concluded that protein restriction in adolescence and adulthood is a sufficient strategy for lowering DPP4, which helps to improve glucose homeostasis [40]. Another finding suggested that during pregnancy, maternal exposure to a low-protein diet and folic acid affects imprinted gene expression of *Igf2* and *H19* gene expression in the liver by modulating DNA methylation of these genes [41]. Yimin et al. determined the *G6PC* activity in maternal malnutrition during pregnancy. Throughout gestation, primiparous, purebred Meishan sows were given either standard protein (SP) or low-protein (LP) diets, and hepatic *G6PC* expression in both male and female newborn piglets was examined. Male LP piglets showed considerably lower blood glucose levels and higher hepatic G6PC mRNA expression and enzyme activity than female piglets [42].

Transgenerational epigenetic changes and macronutrient intake

The risk of developing a chronic disease depends a lot on the early environment provided to the child. Reduced nutritional availability is a key risk factor for the development of metabolic, cardiovascular, neuropsychiatric, immunologic, and gastrointestinal problems later in life [43]. According to recent epidemiological data, maternal hyperglycemia is linked to an increased risk of impaired glucose tolerance, obesity, and elevation in blood pressure in children by the age of seven [44]. Obesity in mothers and poor-quality diets are linked to a higher risk of obesity in children. Placental gene expression and nutrient transport are influenced by maternal nutrition and obesity, but the impact of diet and obesity on epigenetic alterations in the placenta is poorly known [45]. DNA methylation of placental genes may occur due to various environmental exposures, which may affect the development of the fetus [46].

Daniels et al. [46] aimed to examine the effects of maternal nutrition on placenta leptin methylation. Leptin is a pro-inflammatory cytokine generated predominantly by adipose tissue, although it may also be found in other tissues, including the placenta [47]. Leptin is a hormone that regulates appetite, metabolism, and inflammation in the body [48,49]. The placenta produces leptin, and circulating maternal leptin rises throughout the second trimester, resulting in a physiologic hyperleptinemic state with accompanying hypothalamic leptin insensitivity, allowing for increased appetite [47]. It was found that lower levels of leptin methylation were substantially related to higher carbohydrate intake ($P < 0.05$), particularly added sugars ($P < 0.05$), and white or refined carbohydrates ($P < 0.05$). Although total caloric intake was linked to placental leptin methylation ($P < 0.05$), the significance was reduced after adjusting for covariates. There was no significant link between placental leptin methylation and protein or fat consumption of the mother ($P < 0.05$). The findings highlight the importance of carbohydrates intake on the methylation of placental leptin. Because methylation decreases gene transcription, a decreased methylation level might indicate a placental response to a high-calorie, high-carbohydrate diet, resulting in higher levels of leptin throughout fetal development [46].

Thalika et al. [45] hypothesized that maternal characteristics such as maternal obesity and maternal food composition during pregnancy would be linked to altered placental DNA methylation. Multivariable linear regression modeling was used to evaluate the effects of maternal nutrition on placental DNA methylation during pregnancy. Maternal SFA consumption was associated with 302 CpGs of placental DNA methylation, whereas maternal carbohydrate, protein, and lipid intake were substantially associated with methylation of 12, 14, and 28 CpGs, respectively.

The amount of fat consumed by the mother during pregnancy has an impact on fetal development, although the mechanism behind this association remains unknown [50]. Chiu et al. looked at the links between fat consumption during pregnancy and methylation of the insulin-like growth factor 2 (*IGF2)* and *H19* genes in cord blood DNA, which are a pair of imprinted genes with paternal and maternal expression, respectively. During the first trimester, maternal PUFA and total fat intakes were found to be inversely related to *IGF2* DNA methylation. Lower *IGF2* DNA methylation in cord blood was linked to higher first-trimester PUFA consumption. In particular, increased omega-6 fat consumption was linked to decreased *IGF2* DNA methylation [50].

Biochemical Society Transactions (2022) **50** 487–497
https://doi.org/10.1042/BST20211069

Table 2 Summary of transgenerational effects of macronutrient intake

Subject	Parameter	Macronutrient intake	Gene	CpG site	Results
The Rhode Island Child Health Study: mother-infant pair recruited following delivery at Women and Infants Hospital in Providence, Rhode Island, U.S.A. ($n = 135$)	Carbohydrate, fat, and protein.	Carbohydrate mean daily intake(g): 283 Fat mean daily intake(g): 57.8 Protein mean daily intake(g): 75.8	Placental leptin methylation	Not available	Leptin methylation was inversely related to calorie consumption ($r = 0.176$, $P = 0.041$), carbohydrate intake ($r = 0.221$, $P = 0.010$), and added sugar intake ($r = 0.205$, $P = 0.017$). Furthermore, white/ refined carbohydrate consumption ($r = 0.213$, $P = 0.013$) showed significant negative correlations with leptin DNA methylation [46].
Mothers enrolled in the Glowing study ($n = 150$)	Carbohydrate, protein, and fat	Mean % calories from carbohydrate: 49.6 Mean % calories from protein: 15.8 Mean % calories from fat: 35.6	Placental DNA methylation	Not available	Maternal SFA consumption was associated with 302 CpGs of placental DNA methylation whereas maternal carbohydrate, protein, and lipid intake were associated with methylation of 12, 14, and 28 CpGs, respectively [45].
Participants at their initial obstetric care visit at Atrius Harvard Vanguard Medical Associates, a multi-site/ multispecialty practice in Eastern Massachusetts, between 1999 and 2002 for Project Viva, a prospective pre-birth cohort ($n = 96$)	Lipid	SFA intake, % calories: 10.7 (first trimester), 11.2 (second trimester). MUFA intake, % calories: 10.8 (first trimester), 11.3 (second trimester). PUFA intake, % calories: 6.0 (first trimester), 6.3 (second trimester). Omega-3 intake, % calories: 0.5 (first trimester), 0.5 (second trimester). Omega-6 intake, % calories: 5.3 (first trimester), 5.5 (second trimester).	*IGF2, H19*	*IGF2*- site 1 and 2 *H19*- site 1 to 6	During the first trimester, maternal PUFA and total fat intakes were inversely related to *IGF2* DNA methylation [50].
Healthy pregnant women aged 18–39 years from Feeding fetus' low-grade inflammation and insulin-resistance ($n = 1000$)	Lipid	Omega 3 PUFA concentration Low: 2.01% Medium (%): 4.34% High (%): 6.01%	*MSTN, IFNA13, ATP8B3, GABBR2*	Not available	*MSTN, IFNA13,* and *ATP8B3* were differently methylated in low and high n-3 PUFA groups compared with the medium group. The *GABBR2* gene was methylated differently in the medium and high n-3 PUFA groups [51].

The human body does not produce omega-3 PUFA and must obtain from their diet. They are essential for fetal growth and brain development, regulating the inflammatory immune response, to lowering the risk of cardiometabolic illnesses, and reducing subsequent allergy problems in children. The link between the mother's omega-3 PUFA consumption and offspring DNA methylation in cord blood mononuclear cells was studied [51]. Three genes were observed to be differently methylated in both the low and high n-3 PUFA groups when compared with the medium n-3 PUFA group *MSTN, IFNA13,* and *ATP8B3*. The *GABBR2* gene was methylated differently in the medium and high n-3 PUFA groups (Table 2). Figure 2, demonstrates the link between the methionine cycle, methyl donor SAM and epigenetic modification, DNA methylation.

Reversibility of epigenetic modification

The reversal of epigenetic modification in human obesity is not very well understood. Bariatric surgery appears to be capable of partially correcting the obesity-related epigenome [52]. However, no studies addresses whether altering the diet and environment can reverse the epigenetic modifications. This research topic warrants further study.

Biochemical Society Transactions (2022) **50** 487–497
https://doi.org/10.1042/BST20211069

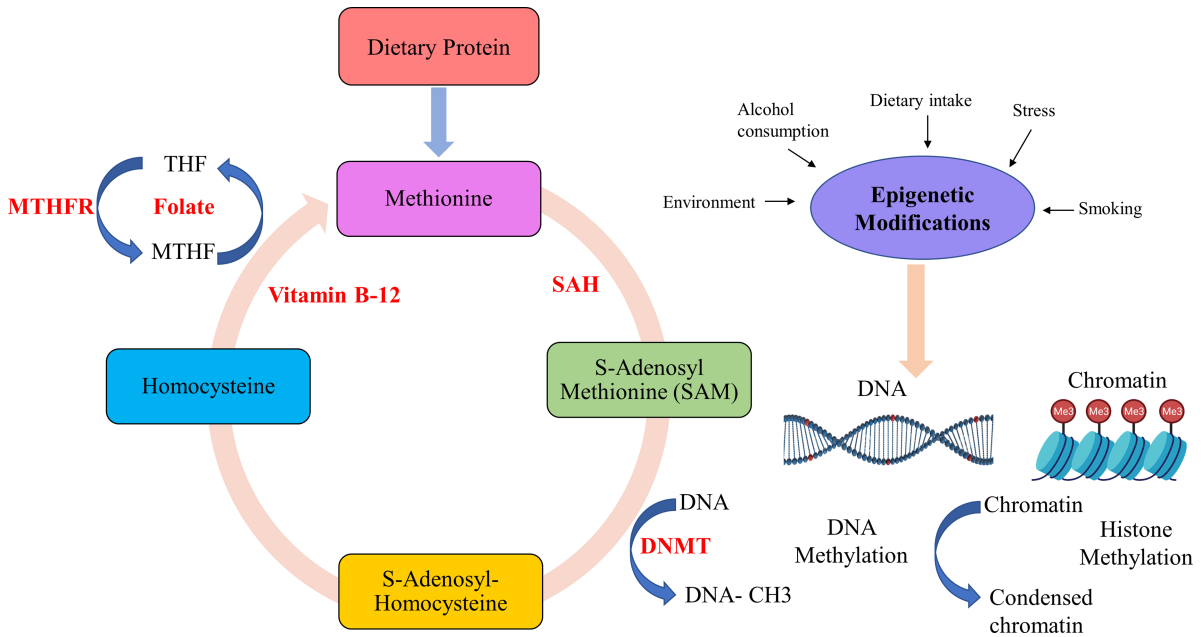

Figure 2. A link between the methionine cycle and epigenetic modification.

Limitations and future directions

There were a few limitations observed in the studies reviewed here; firstly, different methods and tissues were used to analyze the methylation in CpGs. Two of the studies included subjects from various groups, which increases the inconsistency in estimating the methylation associated with population-specific effect size. Also, the dietary habits varied due to the availability of food sources, cultural and regional differences. More intervention studies, including control and various age groups, are needed for a better understanding of DNA methylation and its role in gene regulation leading to an increased risk of obesity.

Conclusion

The current review discusses how the dietary intake of carbohydrates, lipids, and protein affects the methylation of various obesity genes. Overall it can be concluded that dietary lipid and carbohydrate causes alterations at various CpGs affecting the regulation of the genes which in turn affects various metabolic pathways increasing the risk of obesity. However, the results shows mixed results, because epigenetic modifications are tissue-specific and hence more research using human participants in different age groups are needed. This can help to understand the effects of dietary components on epigenetic modifications, allowing us to explore alternative nutrition-based therapeutic approaches and developing tools for a personalized diet to improve the health.

Perspectives

- Genome-wide studies have identified various obesity-related genes associated with epigenetic changes due to dietary intake.
- Epigenetic changes affects the expression of genes involved in the metabolic pathways in children and adults in various ethnicities.
- More human studies are needed to demonstrate the link between dietary intake and epigenetics.

Biochemical Society Transactions (2022) **50** 487–497
https://doi.org/10.1042/BST20211069

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Author Contributions

P.P. researched the literature and wrote the article. All the authors conceptualized, revised and edited the article.

Acknowledgements

This work was supported by the Alabama Agricultural Experiment Station and the Hatch Program of the USDA NIFA to J.R.B., X.W., and T.G. X.W. is also supported by the National Science Foundation EPSCoR RII Track-4 Research Fellowship 1928770. We thank the Miller Writing Center, Auburn University for editing this manuscript.

Abbreviations

BCAA, branched-chain amino acid; BMI, body mass index; BPRHS, Boston Puerto Rican Health Study; CHO, carbohydrate; CPT1, carnitine palmitoyltransferase-1; DA, dopamine; FTO, fat mass and obesity; GOLDN, genetics of lipid-lowering drugs and diet network; HFD, high-fat diet; IGF2, insulin-like growth factor 2; MUFA, monounsaturated Fatty Acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; TG, triglyceride.

References

1 Wang, S., Song, J., Yang, Y., Zhang, Y., Wang, H. and Ma, J. (2015) HIF3A DNA methylation is associated with childhood obesity and ALT. *PLoS ONE* **10**, e0145944 https://doi.org/10.1371/journal.pone.0145944
2 West, S., Caterini, J., Banks, L. and Wells, G. (2018) The epidemic of obesity and poor physical activity participation: will we ever see a change? *J. Funct. Morphol. Kinesiol.* **3**, 34 https://doi.org/10.3390/jfmk3020034
3 Obri, A., Serra, D., Herrero, L. and Mera, P. (2020) The role of epigenetics in the development of obesity. *Biochem. Pharmacol.* **177**, 113973 https://doi.org/10.1016/j.bcp.2020.113973
4 Hochberg, Z. (2018) An evolutionary perspective on the obesity epidemic. *Trends Endocrinol. Metab.* **29**, 819–826 https://doi.org/10.1016/j.tem.2018.09.002
5 Milagro, F.I., Gomez-Abellan, P., Campion, J., Martinez, J.A., Ordovas, J.M. and Garaulet, M. (2012) CLOCK, PER2 and BMAL1 DNA methylation: association with obesity and metabolic syndrome characteristics and monounsaturated fat intake. *Chronobiol. Int.* **29**, 1180–1194 https://doi.org/10.3109/07420528.2012.719967
6 Huang, H., Lin, S., Garcia, B.A. and Zhao, Y. (2015) Quantitative proteomic analysis of histone modifications. *Chem. Rev.* **115**, 2376–2418 https://doi.org/10.1021/cr500491u
7 Miska, E.A. and Ferguson-Smith, A.C. (2016) Transgenerational inheritance: models and mechanisms of non-DNA sequence-based inheritance. *Science* **354**, 59–63 https://doi.org/10.1126/science.aaf4945
8 Dawson, M.A. and Kouzarides, T. (2012) Cancer epigenetics: from mechanism to therapy. *Cell* **150**, 12–27 https://doi.org/10.1016/j.cell.2012.06.013
9 Shen, H. and Laird, P.W. (2013) Interplay between the cancer genome and epigenome. *Cell* **153**, 38–55 https://doi.org/10.1016/j.cell.2013.03.008
10 Zenk, F., Loeser, E., Schiavo, R., Kilpert, F., Bogdanovic, O. and Iovino, N. (2017) Germ line-inherited H3K27me3 restricts enhancer function during maternal-to-zygotic transition. *Science* **357**, 212–216 https://doi.org/10.1126/science.aam5339
11 Zardo, G., Cimino, G. and Nervi, C. (2008) Epigenetic plasticity of chromatin in embryonic and hematopoietic stem/progenitor cells: therapeutic potential of cell reprogramming. *Leukemia* **22**, 1503–1518 https://doi.org/10.1038/leu.2008.141
12 Lima, R.S., de Assis Silva Gomes, J. and Moreira, P.R. (2020) An overview about DNA methylation in childhood obesity: characteristics of the studies and main findings. *J. Cell. Biochem.* **121**, 3042–3057 https://doi.org/10.1002/jcb.29544
13 Lomba, A., Milagro, F.I., Garcia-Diaz, D.F., Marti, A., Campion, J. and Martinez, J.A. (2010) Obesity induced by a pair-fed high fat sucrose diet: methylation and expression pattern of genes related to energy homeostasis. *Lipids Health Dis.* **9**, 60 https://doi.org/10.1186/1476-511X-9-60
14 Zhang, Y. and Kutateladze, T.G. (2018) Diet and the epigenome. *Nat. Commun.* **9**, 3375 https://doi.org/10.1038/s41467-018-05778-1
15 Choi, S.W. and Friso, S. (2010) Epigenetics: a new bridge between nutrition and health. *Adv. Nutr.* **1**, 8–16 https://doi.org/10.3945/an.110.1004
16 Dolinoy, D.C., Huang, D. and Jirtle, R.L. (2007) Maternal nutrient supplementation counteracts bisphenol A-induced DNA hypomethylation in early development. *Proc. Natl Acad. Sci. U.S.A.* **104**, 13056–13061 https://doi.org/10.1073/pnas.0703739104
17 Zhang, Q., Xiao, X., Zheng, J., Li, M., Yu, M., Ping, F. et al. (2019) A maternal high-fat diet induces DNA methylation changes that contribute to glucose intolerance in offspring. *Front. Endocrinol (Lausanne)* **10**, 871 https://doi.org/10.3389/fendo.2019.00871
18 Vucetic, Z., Kimmel, J., Totoki, K., Hollenbeck, E. and Reyes, T.M. (2010) Maternal high-fat diet alters methylation and gene expression of dopamine and opioid-related genes. *Endocrinology* **151**, 4756–4764 https://doi.org/10.1210/en.2010-0505
19 Tobi, E.W., Slieker, R.C., Luijk, R., Dekkers, K.F., Stein, A.D., Xu, K.M. et al. (2018) DNA methylation as a mediator of the association between prenatal adversity and risk factors for metabolic disease in adulthood. *Sci. Adv.* **4**, eaao4364 https://doi.org/10.1126/sciadv.aao4364
20 van Dam, R.M. and Seidell, J.C. (2007) Carbohydrate intake and obesity. *Eur. J. Clin. Nutr.* **61**, S75–S99 https://doi.org/10.1038/sj.ejcn.1602939
21 Zammit, V.A. (2008) Carnitine palmitoyltransferase 1: central to cell function. *IUBMB Life* **60**, 347–354 https://doi.org/10.1002/iub.78
22 Aslibekyan, S., Demerath, E.W., Mendelson, M., Zhi, D., Guan, W., Liang, L. et al. (2015) Epigenome-wide study identifies novel methylation loci associated with body mass index and waist circumference. *Obesity (Silver Spring)* **23**, 1493–1501 https://doi.org/10.1002/oby.21111
23 Lai, C.Q., Parnell, L.D., Smith, C.E., Guo, T., Sayols-Baixeras, S., Aslibekyan, S. et al. (2020) Carbohydrate and fat intake associated with risk of metabolic diseases through epigenetics of CPT1A. *Am. J. Clin. Nutr.* **112**, 1200–1211 https://doi.org/10.1093/ajcn/nqaa233
24 Moody, L., Xu, G.B., Chen, H. and Pan, Y.X. (2019) Epigenetic regulation of carnitine palmitoyltransferase 1 (Cpt1a) by high fat diet. *Biochim. Biophys. Acta Gene Regul. Mech.* **1862**, 141–152 https://doi.org/10.1016/j.bbagrm.2018.12.009

25 Ohashi, K., Munetsuna, E., Yamada, H., Ando, Y., Yamazaki, M., Taromaru, N. et al. (2015) High fructose consumption induces DNA methylation at PPARalpha and CPT1A promoter regions in the rat liver. *Biochem. Biophys. Res. Commun.* **468**, 185–189 https://doi.org/10.1016/j.bbrc.2015.10.134
26 Ramos-Lopez, O., Riezu-Boj, J.I., Milagro, F.I., Martinez, J.A. and Project, M. (2018) Dopamine gene methylation patterns are associated with obesity markers and carbohydrate intake. *Brain Behav.* **8**, e01017 https://doi.org/10.1002/brb3.1017
27 Herrera, B.M., Keildson, S. and Lindgren, C.M. (2011) Genetics and epigenetics of obesity. *Maturitas* **69**, 41–49 https://doi.org/10.1016/j.maturitas.2011.02.018
28 Spadafora, R. (2018) The key role of epigenetics in human disease. *N. Engl. J. Med.* **379**, 400 https://doi.org/10.1056/NEJMc1805989
29 Hakanen, M., Raitakari, O.T., Lehtimaki, T., Peltonen, N., Pahkala, K., Sillanmaki, L. et al. (2009) FTO genotype is associated with body mass index after the age of seven years but not with energy intake or leisure-time physical activity. *J. Clin. Endocrinol. Metab.* **94**, 1281–1287 https://doi.org/10.1210/jc.2008-1199
30 Kalantari, N., Keshavarz Mohammadi, N., Izadi, P., Doaei, S., Gholamalizadeh, M., Eini-Zinab, H. et al. (2018) A haplotype of three SNPs in FTO had a strong association with body composition and BMI in Iranian male adolescents. *PLoS ONE* **13**, e0195589 https://doi.org/10.1371/journal.pone.0195589
31 Fischer, J., Koch, L., Emmerling, C., Vierkotten, J., Peters, T., Bruning, J.C. et al. (2009) Inactivation of the Fto gene protects from obesity. *Nature* **458**, 894–898 https://doi.org/10.1038/nature07848
32 Esfahani, F.H., Asghari, G., Mirmiran, P. and Azizi, F. (2010) Reproducibility and relative validity of food group intake in a food frequency questionnaire developed for the Tehran lipid and glucose study. *J. Epidemiol.* **20**, 150–158 https://doi.org/10.2188/jea.je20090083
33 Doaei, S., Kalantari, N., Izadi, P., Salonurmi, T., Jarrahi, A.M., Rafieifar, S. et al. (2019) Interactions between macro-nutrients' intake, FTO and IRX3 gene expression, and FTO genotype in obese and overweight male adolescents. *Adipocyte* **8**, 386–391 https://doi.org/10.1080/21623945.2019.1693745
34 Samblas, M., Milagro, F.I., Gomez-Abellan, P., Martinez, J.A. and Garaulet, M. (2016) Methylation on the circadian gene BMAL1 is associated with the effects of a weight loss intervention on serum lipid levels. *J. Biol. Rhythms* **31**, 308–317 https://doi.org/10.1177/0748730416629247
35 Ramos-Lopez, O., Samblas, M., Milagro, F.I., Riezu-Boj, J.I., Crujeiras, A.B., Martinez, J.A. et al. (2018) Circadian gene methylation profiles are associated with obesity, metabolic disturbances and carbohydrate intake. *Chronobiol. Int.* **35**, 969–981 https://doi.org/10.1080/07420528.2018.1446021
36 Golay, A. and Bobbioni, E. (1997) The role of dietary fat in obesity. *Int. J. Obes. Relat. Metab. Disord.* **21**, S2–S11 PMID:9225171
37 Voisin, S., Almen, M.S., Moschonis, G., Chrousos, G.P., Manios, Y. and Schioth, H.B. (2015) Dietary fat quality impacts genome-wide DNA methylation patterns in a cross-sectional study of Greek preadolescents. *Eur. J. Hum. Genet.* **23**, 654–662 https://doi.org/10.1038/ejhg.2014.139
38 Lai, C.Q., Smith, C.E., Parnell, L.D., Lee, Y.C., Corella, D., Hopkins, P. et al. (2018) Epigenomics and metabolomics reveal the mechanism of the APOA2-saturated fat intake interaction affecting obesity. *Am. J. Clin. Nutr.* **108**, 188–200 https://doi.org/10.1093/ajcn/nqy081
39 Sun, D., Heianza, Y., Li, X., Shang, X., Smith, S.R., Bray, G.A. et al. (2018) Genetic, epigenetic and transcriptional variations at NFATC2IP locus with weight loss in response to diet interventions: the POUNDS lost trial. *Diabetes Obes. Metab.* **20**, 2298–2303 https://doi.org/10.1111/dom.13333
40 Saussenthaler, S., Ouni, M., Baumeier, C., Schwerbel, K., Gottmann, P., Christmann, S. et al. (2019) Epigenetic regulation of hepatic Dpp4 expression in response to dietary protein. *J. Nutr. Biochem.* **63**, 109–116 https://doi.org/10.1016/j.jnutbio.2018.09.025
41 Gong, L., Pan, Y.X. and Chen, H. (2010) Gestational low protein diet in the rat mediates Igf2 gene expression in male offspring via altered hepatic DNA methylation. *Epigenetics* **5**, 619–626 https://doi.org/10.4161/epi.5.7.12882
42 Jia, Y., Cong, R., Li, R., Yang, X., Sun, Q., Parvizi, N. et al. (2012) Maternal low-protein diet induces gender-dependent changes in epigenetic regulation of the glucose-6-phosphatase gene in newborn piglet liver. *J. Nutr.* **142**, 1659–1665 https://doi.org/10.3945/jn.112.160341
43 Ravelli, A.C., van Der Meulen, J.H., Osmond, C., Barker, D.J. and Bleker, O.P. (1999) Obesity at the age of 50 y in men and women exposed to famine prenatally. *Am. J. Clin. Nutr.* **70**, 811–816 https://doi.org/10.1093/ajcn/70.5.811
44 Tam, W.H., Ma, R.C.W., Ozaki, R., Li, A.M., Chan, M.H.M., Yuen, L.Y. et al. (2017) In utero exposure to maternal hyperglycemia increases childhood cardiometabolic risk in offspring. *Diabetes Care* **40**, 679–686 https://doi.org/10.2337/dc16-2397
45 Thakali, K.M., Zhong, Y., Cleves, M., Andres, A. and Shankar, K. (2020) Associations between maternal body mass index and diet composition with placental DNA methylation at term. *Placenta* **93**, 74–82 https://doi.org/10.1016/j.placenta.2020.02.018
46 Daniels, T.E., Sadovnikoff, A.I., Ridout, K.K., Lesseur, C., Marsit, C.J. and Tyrka, A.R. (2020) Associations of maternal diet and placenta leptin methylation. *Mol. Cell. Endocrinol.* **505**, 110739 https://doi.org/10.1016/j.mce.2020.110739
47 Ashworth, C.J., Hoggard, N., Thomas, L., Mercer, J.G., Wallace, J.M. and Lea, R.G. (2000) Placental leptin. *Rev. Reprod.* **5**, 18–24 https://doi.org/10.1530/ror.0.0050018
48 Friedman, J.M. (2011) Leptin and the regulation of body weigh. *Keio J. Med.* **60**, 1–9 https://doi.org/10.2302/kjm.60.1
49 Harris, R.B. (2014) Direct and indirect effects of leptin on adipocyte metabolism. *Biochim. Biophys. Acta* **1842**, 414–423 https://doi.org/10.1016/j.bbadis.2013.05.009
50 Chiu, Y.H., Fadadu, R.P., Gaskins, A.J., Rifas-Shiman, S.L., Laue, H.E., Moley, K.H. et al. (2021) Dietary fat intake during early pregnancy is associated with cord blood DNA methylation at IGF2 and H19 genes in newborns. *Environ. Mol. Mutagen.* **62**, 388–398 https://doi.org/10.1002/em.22452
51 Bianchi, M., Alisi, A., Fabrizi, M., Vallone, C., Rava, L., Giannico, R. et al. (2019) Maternal intake of n-3 polyunsaturated fatty acids during pregnancy is associated with differential methylation profiles in cord blood white cells. *Front. Genet.* **10**, 1050 https://doi.org/10.3389/fgene.2019.01050
52 Izquierdo, A.G. and Crujeiras, A.B. (2019) Obesity-related epigenetic changes after bariatric surgery. *Front. Endocrinol. (Lausanne)* **10**, 232 https://doi.org/10.3389/fendo.2019.00232