

# Effect of maternal and paternal nutrition on DNA methylation in the offspring: a systematic review of human and animal studies

## Abstract

Maternal or paternal diet may influence health throughout the life course. This is a systematic review of studies in humans and animals specifically investigating DNA methylation in progeny in relation to diet of the mother or father, or previous generations. There is an overview of the types of diet studied and the metabolic paths affected. Food deprivation in humans and animal models, studies on imprinted genes, hypothalamic pituitary adrenal axis and influence of paternal diet are discussed.

**Keywords:** epigenetics, diet, health, disease, methylation

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**Abbreviations:** CpG sites, cytosine-guanine dinucleotides; Dnmt, dna methyltransferase; SAM, s-adenosylmethionine; ICR, imprinting control region; HPA, hypothalamic-pituitary-adrenal; CRH, corticotrophin releasing hormone

## Introduction

Throughout our lives nutrition contributes strongly to our state of health and plays a role in the aetiology of many common diseases. The diet of our mother and father may also influence our life course, affecting our biochemistry during development at a fundamental level. The Barker hypothesis of the developmental origins of health and disease has highlighted the crucial role of prenatal life in influencing our future.<sup>1</sup> The effect of the pregnant mother's diet on the foetus and life course of the adult has become of particular interest. There is an influence of environmental exposure *in utero* on the establishment of phenotype but relatively little is known of the mechanism by which *in utero* exposure causes a change. In the last decade, interest has grown in epigenetic mechanisms altering the DNA's potential gene expression profile in a heritable way.

This review deals with the most highly characterized epigenetic modification, DNA methylation. The cytosine residue in DNA can be methylated to 5-methyl cytosine, catalysed by DNA methyltransferase enzymes (Dnmt). This occurs mainly at cytosine-guanine dinucleotides (CpG sites). Isolated CpG sites are usually methylated but at the promoter regions there is often a cluster of CpG sites, a CpG island, often unmethylated in promoter regions. Methylation of a CpG island may block access to DNA binding proteins such as transcription factors inhibiting gene expression. Interestingly, on replication of a DNA strand the pattern of methylation may be reproduced.

The donor of the methyl groups for DNA methylation is S-adenosylmethionine (SAM) which is derived from methionine.<sup>2</sup> Loss of the methyl group from SAM forms S-adenosylhomocysteine (SAH) and the plasma SAM: SAH ratio has been used as a marker of DNA methylation capacity. SAH is metabolised to homocysteine,

and this is remethylated to methionine. Dietary components, folate, betaine, choline, and vitamin B12 are important cofactors and methyl group donors for this system.

It is epigenetic mechanisms that give each cell of the early embryo a distinct developmental fate. At two stages in development there is removal of the DNA methylation marks and *de novo* methylation, firstly following formation of the zygote from sperm and oocyte, and secondly days later in the primordial germ cells.<sup>3</sup> Environmental conditions around the time of conception or during gestation may reprogram the developing embryo to follow an altered future path by affecting the epigenetic mark of the genome. Changes in phenotype associated with *in utero* exposure to a change in nutrition may be heritable to the following generation.

Furthermore, there may be an influence of paternal circumstances, such as diet, on the progeny, in which case epigenetic marking of sperm DNA is postulated.<sup>4</sup> Some genes, many of which are important for foetal development, are imprinted.<sup>5</sup> In an imprinted gene only one allele is expressed depending on the parent of origin. The marker for parent of origin is epigenetic, DNA methylation playing a key role. The promoter region for an individual imprinted gene, or an imprinting control region, which may regulate expression for several imprinted genes, may be differentially methylated for each allele. DNA methylation of imprinted genes usually resists the global demethylation of the early zygote, but may be reset in the de-methylation and *de novo* methylation of the primordial germ cells. Periconception is thought to be a critical time in epigenetic programming and in re-establishment of imprinting in the primordial germ cells. Many researchers investigating the effect of parental nutrition on the offspring have looked in particular at imprinted genes.

## Inclusion and exclusion criteria for review

This systematic review is of studies in humans and animals specifically investigating DNA methylation in progeny in relation to diet of the mother or father, or previous generations with the aim of

providing a complete overview of the current research topics in the field.

The National Library of Medicine PubMed database was searched in two stages for published studies, firstly using the MeSH terms “nutrition disorders”, or “diet”, or “food” with the MeSH term “DNA methylation”, or using the keyword “nutrition” with the keyword “DNA methylation”. The results were then sorted by title and abstract to include studies on humans or animals investigating an effect of diet on DNA methylation, and then sorted using the abstract and full text to include specifically only studies investigating DNA methylation in progeny in relation to diet of maternal line or paternal line. Also included are studies referenced to in an included study or a relevant review, which met the inclusion criteria. As the described PubMed search was excluding some studies on folate supplementation in pregnancy, a further search was carried out using the keywords “folate” and “pregnancy” and “DNA methylation”. Secondly, from May 2012 the publications to be included were kept up to date by regular searches of the PubMed database with the keyword “DNA methylation” and inclusion on the basis of title and abstract.

Only studies which assessed or intervened with dietary or nutritional supplement intake are included in the systematic analysis. Studies which use a proxy for nutrition such as low birth weight of progeny or plasma/serum folate were excluded. Alterations in profiles or gene expression of DNA methyltransferase enzymes have not been comprehensively considered in this review. Animal studies published before 2009 were excluded.

## Results

DNA methylation endpoints investigated have been either a general measure of DNA methylation of CpG across the whole genome by analysis of repetitive element DNA methylation, or examination of specific loci in genes of interest. The locus chosen for investigation may be a candidate because the method of analysis for that locus is available, or because there is thought to be involvement of the gene or protein in cardiovascular or metabolic disease.<sup>6</sup>

Although a large number of animal studies satisfied the inclusion criteria, only thirteen published studies in humans, listed in Table 1, looked at the effect of maternal diet on offspring DNA methylation, and all of these involved diet during pregnancy. No human study was found which has yet looked for an effect of paternal diet on offspring DNA methylation. Among the maternal diets observed were very low caloric intake during the Dutch Hunger Winter, an African rainy season diet versus dry season diet, and a Scottish high meat, low carbohydrate diet. Randomised controlled trials compare supplement use or lack of use. The earliest study found which looked at DNA methylation in relation to maternal diet in humans was 2008. The majority of the studies have focused on a possible periconceptual effect of exposure. Offspring have been newborn, young children or mature adults. Most studies have looked at DNA methylation at only one time point but the Gambian supplementation trial has looked at newborn and 9month old infants, allowing an informative comparison.<sup>7,8</sup>

**Table 1** Human studies investigating the effect of maternal diet during pregnancy on DNA methylation in the offspring

Field of Enquiry	Maternal Diet	Time of Exposure	Time of Measurement of Endpoint in Offspring	DNA Methylation Changes	Reference
IGF2/H19 region	Dutch famine exposure	Periconception or Late gestation	60 year old adults	Lower methylation at IGF2 DMR with periconceptual exposure No effect of late gestational exposure	9
Imprinted and non-imprinted loci, candidates for growth and metabolic disease	Dutch famine exposure	Periconception or Late gestation	60 year old adults	Periconceptual exposure: lower methylation of INSIGF and higher methylation of IL10, LEP,ABCA1, GNASAS, MEG3 Sex differences Late gestation exposure: alteration in methylation of GNASAS and in men, LEP	6
Putative metastable epialleles	Rainy season (less food abundance, more folate, harder work) or dry season diet at conception, Gambian population	Periconception with seasonal influence during pregnancy	Children 8-9 years	Methylation at all 5 putative metastable epialleles higher in rainy-season conceived children No effect on global DNA methylation or at IGF2, GNASAS, IL10	10
Genome-wide effect of famine	Dutch famine exposure	Part of the time from conception to birth, analysed as blocks of time: wk 1-10, 11-20, 21-30, 31-birth	60 year old adults	No effect of famine exposure <i>in utero</i> on blood global DNA methylation in white blood cells	11

Table Continued....

Field of Enquiry	Maternal Diet	Time of Exposure	Time of Measurement of Endpoint in Offspring	DNA Methylation Changes	Reference
IGF2/H19 region	Dutch famine exposure	Periconception and first part of pregnancy	60 year old adults	Altered methylation at INSIGF, IGF2 DMR0, IGF2 DMR1, IGF2 DMR2 CTCF locus No effect on H19 DMR Global DNA methylation unchanged	12
Search for affected loci and functional associations	Multinutrient supplementation, Gambian population	Periconception	Newborn and Baby 9 months	Differential methylation of multiple loci at birth and 9 months, associated with eg immune defence, sex differences $\Delta$ methylation at imprinted loci:GNAS, MKRN3, SLC22A18	8
Imprinted genes, including IGF2/H19	Multinutrient supplementation, Gambian population	Periconception	Newborn and Baby 9 months	$\downarrow$ methylation at IGF2R in newborn girls; $\downarrow$ methylation at GTL2-2 in newborn boys $\downarrow$ methylation at PEG1-DMR in infant girls at 9 months; $\downarrow$ methylation at GNASAS-DMR in infant boys at 9 months Methylation at H19 and IG loci not changed	7
Mechanism of action of folic acid	Folic acid supplementation	During pregnancy	Newborn	No effect of folic acid supplementation on newborn cord blood global DNA methylation	9,13
IGF2 DMR and folic acid	Folic acid supplementation	Periconception	Child 17 months	Higher methylation of IGF2 DMR with folic acid supplementation	14
IGF2 DMR and folic acid	Folic acid supplementation	Periconception and during pregnancy or Prenatal	Newborn	Lower methylation at H19 DMR with higher folic acid intake Similar results with periconceptional versus only prenatal folic acid No change in methylation at IGF2 DMR Sex difference	15
Methyl donors	Adequate folate	Periconception and Second Trimester	Newborn	No effect of methyl donor intake on newborn cord blood global DNA methylation Lower global DNA methylation with higher periconceptional choline in males	16
Glucocorticoids / HPA axis	High meat, low carbohydrate diet	Late pregnancy	40 year old adults	Methylation at GR (exon 1F) increased with higher meat, fish, vegetable, lower bread, potato in late pregnancy Methylation at site in HSD2 region2 increased with higher meat, fish in late pregnancy Correlation of methylation at specific CpGs in HSD2 promoter and H19 ICR with neonatal anthropometrics. Higher CpG methylation within all 3 sites with greater adiposity and blood pressure as adult	17
Glucocorticoids / HPA axis / placenta	Choline supplement	Third trimester	Placenta at birth Newborn	In placenta, with higher choline intake, higher methylation at CRH and GR promoters, increased placenta global DNA methylation In newborn cord blood, with higher choline intake, lower methylation at CRH and NR3C1 promoters	18

Functionally, the effect of maternal diet *in utero* in humans on the IGF2/H19 imprinted region has been extensively examined and the hypothalamic-pituitary-adrenal axis has been a focus. Three human studies examined multiple loci with different functionalities and one looked at genome wide repetitive elements, thus moving towards a wider view of how nutrition in pregnancy acts to alter phenotype of the offspring.

The animal studies have spanned a wide range of functional fields of study, involving a number of different dietary regimens. All of the animal studies are presented in Tables 2-8. Many of them directly support or have been a foundation for the human studies and many involve different functional fields of study, diets unlike those tested in humans, and different affected loci. Many of the animal studies that involved methyl donor deficient diets or methyl donor supplementation have investigated tumorigenesis and DNA repair. Ongoing debate over the benefits and mechanism of action of folic acid supplementation programmes is reflected in the large number of animal studies attempting to elucidate the action of folate. In this review, topics of interest covered by the human studies are discussed, with the relevant animal studies being mentioned, while a tabular overview of the areas covered by animal studies alone is provided.

### Famine, protein restriction and restricted feeding

A severe famine of six months suffered by an area of Holland during the war (the Dutch Hunger Winter), affected the health of people exposed prenatally. As recently reviewed,<sup>79</sup> intrauterine famine exposure affected adult glucose tolerance, lipid profile (in women), may be associated with metabolic syndrome, may lead to higher body weight, BMI, waist circumference (in women), and is associated with increased incidence of psychiatric disorders.

There are thought to be windows of sensitivity, or time points during development when the germ cell, gamete or developing embryo is more susceptible to environmental influence on the phenotype. The timing of exposure to famine affected the outcome on the health of the adult offspring. Different organs or systems are more sensitive at different stages of development. There were greater effects of periconceptual exposure on glucose metabolism, schizophrenia incidence, incidence of cardiovascular disease.<sup>79,80</sup> Increased adult mortality of cardiovascular disease, cancer, and breast cancer occurred in women exposed in early gestation.<sup>81</sup> Mid-gestational exposure appeared to have an impact on lung health and

late gestational exposure affected birth weight.<sup>79</sup>

The Dutch Hunger Winter also affected the generation following those exposed prenatally, who had increased neonatal adiposity, were said to have poorer health, and if exposure was over the first and second trimester of intra-uterine life, had lower birthweight than expected.<sup>79</sup>

With this background in mind, four studies of the epigenetic changes in response to the famine have been published, comparing famine exposed offspring with same sex-unexposed siblings (Table 1). Exposure to famine *in utero* did not affect global DNA methylation in white blood cells of adult offspring decades later.<sup>11</sup>

DNA methylation of several imprinted loci which were candidate genes for metabolic or cardiovascular disease, was different with periconceptual exposure to famine compared to unexposed siblings: DNA methylation of *INSIGF* (part of the proximal promoter of *INS* which encodes the insulin precursor) was lower and DNA methylation of *GNASAS* and *MEG3* was higher.<sup>6</sup> These effects were sex dependent for some of the loci. There was also an effect on some of the non-imprinted loci examined: periconceptual exposure led to higher DNA methylation at *IL10*, *LEP*, and *ABCA1*. Nine of the 15 loci examined showed no alteration in DNA methylation with periconceptual famine exposure. With exposure later in gestation there was altered methylation level at *GNASAS* and in men, *LEP*. Thus there is a sex dependent change at some loci and the change is also dependent on timing of exposure which the group points out does support epidemiologic findings that exposure to famine has a sex and timing specific effect. The group also observes that the differential response to famine at different loci does not conform to a simple view that lower availability of methyl donors directly causes less DNA methylation at the loci. Famine exposure also affected DNA methylation at the IGF2/H19 region which is a topic discussed later in this review.<sup>9,12</sup>

Protein restriction is commonly used as a model of malnutrition or famine. As a result of the search described above, seventeen studies on animals from 2009 onwards were found which have examined protein restriction in the maternal diet during pregnancy and the resulting DNA methylation in the offspring, and are listed in Table 2. Four are on pigs, the rest on rodents. One study continued until the third generation of progeny.<sup>24</sup> A number of functional pathways have been examined, with a rather different focus from that of the human studies.

**Table 2** Animal studies investigating DNA methylation in offspring exposed to maternal protein restriction *in utero*

Field of Study	Animal	Time of Exposure	Time of Measurement of Endpoint in Offspring	DNA Methylation in progeny Exposed <i>in utero</i> to Maternal Protein Restriction	Reference
Key hepatic metabolic genes	Pigs	Gestation	Late gestation, birth, weaning, finisher pig	Influence of DNA methylation in transcription of glucocorticoid receptor gene NR3C1 in late gestation and in cytochrome P450 superfamily CYP2C34 in newborns ↑methylation in some sites in peroxisome proliferator-activated receptor PPARα	19

Table Continued....

Field of Study	Animal	Time of Exposure	Time of Measurement of Endpoint in Offspring	DNA Methylation in progeny Exposed <i>in utero</i> to Maternal Protein Restriction	Reference
Chromosome condensation and segregation	Pigs	Gestation	Late gestation, birth, weaning, finisher pig	↓hepatic global DNA methylation in late gestation, no change postnatally, no change in skeletal muscle global DNA methylation ↓methylation in sites in NCAPG promoter region of foetal liver, different methylation of sites in NCAPG promoter region in foetal muscle, correlations with transcription	20
Mitochondria (dysfunction in malnutrition)	Pigs	Gestation	Newborn	↓mean methylation over 47 CpG sites in somatic cytochrome c gene (CYCS) promoter in newborn piglet liver, negative correlation with transcript amount	21
Glucose homeostasis	Pigs	Before and during gestation	Newborn	Hypomethylation of glucose 6 phosphatase gene promoter in males.	22
Insulin Resistance	Rats	Gestation	Aged adults	↑methylation in the PGC-1 $\alpha$ promoter sequence, a key gene in regulation of insulin resistance	23
Glucose homeostasis	Rats	Gestation and lactation	Three generations at 70d	$\Delta$ methylation in three of the nine CpG sites in Phosphoenolpyruvate carboxykinase promoter in progeny, in F1, F2 and F3 generations, one CpG site showed similar effect in all three generations	24
Lipid metabolism	Rats	Gestation Offspring weaned onto low fat or high fat diets.	Newborn & adult	↓ methylation of PPAR $\alpha$ promoter in neonatal and adult heart, unaffected by post-weaning diet. Methylation of PPAR $\alpha$ promoter in adult heart negatively associated with mRNA level.	25
Tumour suppressor, mammary gland, cyclin dependent kinase inhibitor 1 (p21)	Rats	Gestation	38d pups	No change in DNA methylation at the p 21 promoter in mammary gland of pups	26
Tumour suppressor, mammary gland, p16	Rats	Gestation	38d pups	No change in DNA methylation at p16 promoter.	27
IGF2/H19	Rats	Gestation (from day 2)	Newborn	↑ methylation of imprinting control region at IGF2/H19 locus in liver, attenuated by folate supplementation Global DNA methylation unchanged	28
Hypothalamus	Rats	Gestation and/or lactation	12d pups	↑methylation in hypothalamic neuropeptide gene Pomc promoter with <i>in utero</i> protein restriction, control diet after birth.	4
Appetite	Rats, also folate supplemented group	Gestation	Foetus day 20	Liver global DNA methylation unchanged	29

Table Continued....

Field of Study	Animal	Time of Exposure	Time of Measurement of Endpoint in Offspring	DNA Methylation in progeny Exposed <i>in utero</i> to Maternal Protein Restriction	Reference
Imprinted genes DMRs	Mice	Gestation and/or lactation	3 weeks & adults	Mostly unchanged ↑ methylation in some CpG sites in Grb10 and Nespas/GNasxl DMRs at 3 weeks, not adulthood, in liver; global dna methylation unchanged	30
Cohesin complex and higher order chromatin formation	Mice	Before and during gestation	21d & adult	Δ expression of many genes in liver with low protein ↓ methylation in a few CpG sites in four genes; otherwise generally no change in DNA methylation.	31
Leptin	Mice	Gestation and lactation	Adult	↓ methylation of CpG sites in the CpG island in leptin gene promoter in white adipose tissue Global DNA methylation unchanged in white adipose tissue.	32
Brain renin-angiotensin system	Mice	Late gestation (day 10-day 17.5)	Foetus 17.5day	↓methylation of CpG islands in promoter region of ACE-I gene	33
Microarray approach lipid metabolism	Mice	Before and during gestation	Foetus 19.5day	↑methylation of 106 CpG sites in promoter regions and ↓methylation of 101 CpG sites in promoter regions in foetal liver ↑methylation of some of the CpG sites in the CpG island of the Liver X-receptor- a promoter by up to 39%	34

The diets used in the rodent studies were approximately comparable with regard to protein content. Not only has protein content of the diet decreased in the treatment group, but protein to carbohydrate ratio has altered, there is increased carbohydrate, and in some cases methionine content of the diet is altered. Thus the effect of the diet may not be due solely to a reduction in protein.

Another model for famine or food deprivation is feeding a reduced

diet to animals, and the five published studies using this model to examine an effect on DNA methylation are listed in Table 3. Four of these are studying energy or glucose metabolism. One finds an alteration in global DNA methylation in mouse lung,<sup>35</sup> interesting as famine impacted lung health in exposed human offspring.<sup>79</sup> A key enzyme in gluconeogenesis<sup>36</sup> and organ specificity of global DNA methylation changes have been investigated in baboons.<sup>37</sup> The hypothalamic pituitary axis was examined in sheep.<sup>38,39</sup>

**Table 3** Animal studies investigating DNA methylation in offspring exposed to maternal restricted feeding *in utero*

Field of Study	Animal, Maternal Feeding Protocol	Time of Exposure	Time of Measurement of Endpoint in Offspring	DNA Methylation In Progeny Exposed to Restricted Feeding <i>in Utero</i>	Reference
Pulmonary vascular dysfunction	Mice Restricted diet: 65% of ad lib intake	From day 7 of gestation to delivery	Adult	Δ global DNA methylation in lung pulmonary dysfunction	35
Gluconeogenesis	Baboons Restricted diet: 70% of ad lib intake of controls	Before and during gestation	Foetus late gestation	↓Methylation at 6 sites in promoter of foetal liver phosphoenolpyruvate carboxykinase I gene (PCK1)	36
Organ specific effects	Baboons Restricted diet: 70% of ad lib intake of controls	From early gestation (day 30)	Foetus mid and late gestation	Global DNA methylation per organ in nutrient restriction: ↓methylation in kidney mid gestation, ↑methylation in kidney late gestation, ↑methylation in frontal cortex	37

Table Continued....

Field of Study	Animal, Maternal Feeding Protocol	Time of Exposure	Time of Measurement of Endpoint in Offspring	DNA Methylation In Progeny Exposed to Restricted Feeding in Utero	Reference
Energy balance regulation in hypothalamus	Sheep undernourished	Before gestation and early gestation only	Late gestation	Hypothalamus: ↓methylation proopiomelanocortin (POMC) promoter ↓methylation glucocorticoid receptor (GR) promoter methylation at Oct4 promoter unchanged	38
Energy balance regulation in hypothalamus	Sheep undernourished	Before gestation and early gestation only	Late gestation	Hypothalamus: ↓methylation proopiomelanocortin (POMC) promoter, ↓methylation glucocorticoid receptor (GR) promoter methylation at Oct4 promoter unchanged	39

Further to investigation of an effect on DNA methylation of a target gene, researchers often look for a correlation with gene transcription and study whether there is a regulatory role of methylation. For example, correlations of change in DNA methylation with change in gene transcript suggested a regulatory role for DNA methylation on target genes in pigs exposed to maternal protein restriction *in utero*.<sup>19-21</sup> Liver x-receptor alpha, which regulates cellular lipid homeostasis, was hypermethylated in liver of foetal rats exposed to maternal low protein and there is a relationship between methylation of this gene and transcription, thus this may have been a regulatory factor in the concomitant reduction in mRNA level.<sup>34</sup> PPAR $\alpha$  promoter methylation in adult but not foetal heart was negatively associated with transcription.<sup>25</sup> The hypomethylation of ACE-1 promoter with prenatal low protein exposure was related to gene transcription but not to protein levels.<sup>33</sup> The effect of DNA methylation on transcription may influence effect of *in utero* protein restriction or famine on phenotype.

### DNA methylation at imprinted genes

Several of the human studies reviewed have looked at the insulin-like growth factor 2 (IGF2) gene. IGF2 is important for growth and development *in utero* in humans and thus pertinent to studies of the effect of maternal diet or nutritional challenge on development *in utero*.<sup>82</sup> The gene *IGF2* is in tandem with *H19* on chromosome 11 and is imprinted.<sup>83</sup> A differentially methylated control region for both *IGF2* and *H19* is methylated in the paternal allele and unmethylated in the maternal allele. The mechanism of epigenetic regulation of expression of *Igf2* in mice is reviewed in Chao & D'Amore.<sup>84</sup> The *H19* promoter is methylated and therefore inactive in the paternal allele, so only maternal mRNA is expressed. A region upstream of *H19* promoter, the imprinting control region (ICR) allows binding of a zinc finger protein, CTCF when demethylated, acting as an insulator on the maternal allele. Access to the *Igf2* promoter is consequently blocked in the maternal allele but not in the methylated paternal allele resulting in transcription of *Igf2* mRNA. Other elements are involved in the regulation of IGF2 transcription. As briefly reviewed by Tobi and others,<sup>12</sup> the nearby imprinted insulin promoter *INS* has an influence and *INSIGF* is a fusion transcript of *INS* and *IGF2*. Loss of imprinting at the *IGF2/H19* locus has been associated with disease states. Note that there is to some extent normal variation in the methylation of the DMR as shown in monozygous twins.<sup>85</sup>

The mechanism of regulation of this *IGF2/H19* system and imprinting by DNA methylation is very well characterized therefore, in addition to being associated with growth, this imprinting region has

proven to be a useful tool to investigate the effect of parental diet on DNA methylation.

In the Dutch Hunger Winter cohort, adults whose mothers were exposed to famine around the time of conception, had a lower DNA methylation at CpG sites in the IGF2 DMR compared to unexposed siblings.<sup>9</sup> This difference was not evident in offspring of mothers exposed to famine later in the pregnancy with adequate nutrition at conception suggesting that the timing of exposure to the nutritional factor is important for DNA methylation of this region. The difference between the experimental and control group is small. 5.2% lower methylation is seen with periconceptional famine exposure.

More recently the IGF2/H19 region was examined in more detail in the Dutch Hunger Winter cohort, drawing on the more sophisticated techniques available and the high level of knowledge at this time on the role of the components of the control region.<sup>12</sup> In adults exposed to famine *in utero*, there were small differences in methylation at DMRs compared to unexposed same sex siblings. Methylation was lower at the *INSIGF* locus in the *INS* promoter, methylation was lower in IGF2 DMR0, which is also called IGF2 DMR in other papers. Methylation was higher in IGF2 DMR1, which is a region that binds CTCF. At the IGF2 DMR2 CTCF-binding locus there was lower methylation of three CpG sites with famine exposure but methylation at another locus had no association with exposure. *H19* DMR methylation level was not associated with exposure. The magnitude of the effects of exposure was comparable to those found in other studies reported. Interestingly, it has now become possible to find out whether there is interplay of the gene sequence, in the form of small nucleotide polymorphisms, in the response of the epigenome to nutritional challenge in development. Single nucleotide polymorphisms (SNPs) at the IGF2/H19 region are associated with DNA methylation and the authors suggest that the effect of famine and of SNPs on DNA methylation could be additive at the same locus.

Folic acid supplementation is routinely recommended for pregnant women in many countries and in some countries food is fortified with folic acid as a public health policy. Periconceptional folic acid supplementation increased DNA methylation by 4.6% of the DMR of IGF2 in the 17month-old Dutch children.<sup>14</sup> However in an American trial there was no difference in methylation at IGF2 DMR in newborn cord blood with folic acid supplementation before pregnancy or during pregnancy.<sup>15</sup> Perhaps due to differences in the cohort or study design, the discrepancy may be due to the age of offspring examined. With multinutrient supplementation there was a difference in IGF2 DMR methylation level of effect between newborns and 9month babies.<sup>8</sup>

**Table 4** Animal studies investigating DNA methylation in offspring exposed to maternal methyl group supplementation or restriction *in utero*, published since 2009

Field of Study	Animal, Maternal Diet	Time of Exposure	Time of Measurement of Endpoint in Offspring	Finding	Reference
DNA repair in brain	Mouse, Low folate	Gestation and lactation Offspring high fat from weaning	Adult	Δ in DNA methylation at excision repair genes	40
Behaviour	rats methyl donor deficiency	Before and during gestation	Newborn, young adult, aged adult	↓global DNA methylation in newborn liver No major difference in DNA methylation at glucocorticoid receptor promoter, 11β-hydroxysteroid dehydrogenase type 2, neuronatin, or reelin gene in hippocampus of young adult or aged adult, but ↓methylation at one CpG unit in neuronatin gene in young adult female offspring	41
Intestinal cancer	mice WT and Apc(+/- Min), folate deficient diet	Gestation and lactation	At weaning (32d) and adult	↓DNA methylation of p53 in small intestine. ↑methylation at IGF2 and apc in Apc (+/Min) mice.	42
Intestinal cancer	Mice low, control, high folate	Gestation and lactation	adult	↓ global DNA methylation in low folate exposed adult offspring	43
Intestinal cancer	Mice low, control, high folate	Gestation	Foetus 17.5d	↓methylation of Slc394a and no change in methylation of Esr1 or IGF2 DMR1 in foetal gut	44
Atherosclerosis	Apo E -/- mice Methyl donor supplemented	Before and during gestation and lactation	17 weeks, 28 weeks	↑ global DNA methylation in T-cells	45
Effect of arsenic, microarray	Mice, folate supplement and arsenic <i>in utero</i>	Gestation	Foetus day 18	Δ DNA methylation at 12 genes Increased number of genes Δ DNA methylation in response to arsenic	46
Glucose homeostasis	Rats folic acid supplementation	Gestation	84 - 90d old	Δ methylation of Phosphoenolpyruvate carboxykinase promotor in females, not males	47
Placenta	Rats folic acid supplementation, vitamin B12 deficiency, omega 3 fatty acid supplementation	Gestation	Placenta day 20 gestation	Influence of docosahexaenoic acid on vitamin B12 deficiency-induced Δ global DNA methylation in the placenta	48
Microarray approach, Heritability	mice methyl supplemented diet	Before and during gestation, continued for 6 generations	5 weeks old	↑variation in DNA methylation at multiple loci in liver of supplemented mice, magnified in subsequent generations Unchanged hepatic global DNA methylation	49



Table Continued...

Field of Study	Animal, Maternal Diet	Time of Exposure	Time of Measurement of Endpoint in Offspring	Finding	Reference
Tumour mammary gland	Rats folic acid supplementation	Before and during gestation and lactation	28 weeks	↓global DNA methylation in mammary gland	50
Colitis	Mice methyl donor supplementation	Before and during gestation and lactation	30d and 90d	Δ Colonic mucosa DNA methylation, including at Pptn22-associated Sma I / Xma I interval (associated with diseases eg rheumatoid arthritis, diabetes)	51
Intestinal cancer	Rats folate supplementation	Before and during gestation and lactation	14 weeks	↑colorectal global DNA methylation	52
Multiple effects of fortification	Rats Folic acid supplementation +/- post-weaning supplementation	Gestation and lactation	At weaning and 14 weeks	↓global DNA methylation, ↓methylation at Ppar-γ, Era, p53, Apc in weaning liver ↑methylation at Era and Apc in adult liver Influence of post-weaning supplementation	53
Epigenetics of agouti mouse	Mice, agouti Methyl donor supplementation	Day 8.5 to day 15.5 gestation only	First generation offspring and unsupplemented next generation offspring	No increase in density of CpG methylation in the silent LTR	54
HPA axis	rats methyl donor supplementation	Gestation	Adult	no change in DNA methylation of GR exon 1(7) promoter	55
Placenta, I-carbon metabolism	rats folate and homocystine supplement	Before and during gestation	Placenta day20	↑global methylation in placenta, DNA methylation strongly related to maternal folate levels and hepatic I-carbon intermediates	56
Mammary gland cancer	Rats, choline deficient, control or supplemented	Gestation days 11-17	Adult	On tumour induction, Δ DNA methylation in tumour suppressor gene, stratifin correlated with choline exposure	57
Effect of alcohol on developing hypothalamus	Rats, exposed to alcohol + or - choline	Gestation days 7-21	Adult	↑methylation of proopiomelanocortin gene in hypothalamus with alcohol exposure but not with choline + alcohol exposure.	58

**Table 5** Animal studies investigating DNA methylation in offspring exposed to maternal diet of particular fat content *in utero*

Field of Study	Animal, Maternal Feeding Protocol	Time of Exposure	Time of Measurement of Endpoint in Offspring	Epigenetic Outcome	Reference
Insulin sensitivity	Rats, Fish oil supplementation	Before and during gestation and lactation for 2 further generations	75d old	↓hepatic global DNA methylation in G2, not in G1, no change in muscle global DNA methylation	59
fatty acid status	Rats, low fat, adequate fat, high fat, butter vs fish oil	Before and during gestation and lactation	Adult	↑methylation at hepatic Fads2 promoter with high fat exposure, particularly with fish oil, negative correlation with Fads2 mRNA relationship of methylation at one CpG site in Fads2 promoter with 20:4n-6:22:6n-3 ratio	60
Fatty acid metabolism	Mice, control (containing soybean oil) and deficient (corn oil)	Before and during gestation. Maternal diet from delivery α-linolenic acid supplementation (w-3) as flaxseed oil, or continuation on the control or the deficient diet	At weaning	↑Fads2 promoter methylation in offspring liver only with maternal control diet in gestation and postnatal supplementation. No effect of diet on Fads2 intron 1 methylation in offspring. Δ methylation in Fads2 in maternal liver. methylation status of Fads2 in liver in mothers and offspring associated.	61
Dopamine and opioid circuitry in the brain	Mice, high fat diet through pregnancy	Before and during gestation and lactation	Adults	↓ global DNA methylation in brain ↓ promoter methylation at dopamine reuptake transporter, μ-opioid receptor and preproenkephalin	62
Insulin sensitivity	Mice, high fat diet	Before and during gestation and lactation, for two further generations	Adults	↓methylation at GHSR promoter in second generation offspring brain	63
Bone	Rats, high fat diet to produce obese dams	Before and during gestation	Foetus 18.5d	↑DNA methylation in Homeodomain-containing factor A10 (HoxA10) in foetal rat osteogenic calvarial cells	64
Imprinted genes in placenta	Mice, high fat diet	Gestation	Placenta 15d	Δ methylation IGF2 DMR. Sex difference. HFD led to ↓global DNA methylation in female placenta.	65
Leptin	rats fed various diets,	Gestation and lactation	10 weeks	progeny fed high fat diet or control diet ↑global DNA methylation in adipose tissue with high fat diet after weaning	66
Metabolic disorders	Mice, high fat diet	Gestation and lactation	Pup 2d, 27d	methylation at CpG islands in the promoter and at exon hepatic cell cycle inhibitor at 2d post natal, not 27d.	67
Circadian rhythm	Primates high fat diet	Before and during gestation	Foetus 130d	No difference in DNA methylation at CpG sites in Npas2 promoter Histone difference	68
Vascular tone	Rats, 7% or 21% fat	Before and during gestation and lactation	11 weeks	Hypermethylation at aortic Fads2 promoter in high fat group, correlation with expression	69

**Table 6** Animal studies investigating DNA methylation in offspring exposed to maternal overfeeding *in utero*

Field of Study	Animal, maternal feeding protocol	Time of exposure	Time of measurement of endpoint in offspring	Epigenetic outcome	Reference
Methyl donor metabolism, gluconeogenesis	overfed mice	Gestation and lactation, sustained for 4 generations	adult	Δ methylation at PEPCK, relationship with mRNA ↑hepatic Dnmt methylation of Dnmt promoter	70
Chromosome condensation and segregation	Pigs, protein excess	Gestation	Late gestation, at birth, at weaning, finisher pig	Δhepatic global DNA methylation; Δmethylation of NCAPG	20
Triglyceride biosynthesis in liver	Mice, high fat/high sucrose diet (high calorie lipogenic diet)	Before and during gestation and lactation	Pups 5d	methylation of hepatic glycerol-3-phosphate acyltransferase 1 promoter, inversely correlated with mRNA	71

**Table 7** Animal studies investigating DNA methylation in offspring exposed to other maternal dietary regimens *in utero*

Field of Study	Animal, Maternal Feeding Protocol	Time of Exposure	Time of Measurement of Endpoint in Offspring	Epigenetic Outcome	Reference
Iron metabolism	Mice, iron-chelating flavonoid quercetin throughout gestation	Before and during gestation	Foetus 14.5d, adult	↑global DNA methylation	72
Glucocorticoid metabolism	Rats, magnesium deficient diet	Before and during gestation and lactation	Pup 21d	↑ methylation at the 11 beta-hydroxysteroid dehydrogenase-2 promoter	73
Renin angiotensin system	Rats, high salt diet during pregnancy	Gestation	Foetus 21d	mean methylation at five CpG sites linked to AT1b promoter in heart	74
Zinc metabolism	Mouse, low zinc diet	From day 7 gestation	5 weeks	↑ methylation in two CpG sites of hepatic metallothionein2 promoter region	75
IGF2/H19	Sheep, Isoenergetic diets of either alfalfa haylage (high in fibre), corn (high in starch) or dried corn distillers grains (protein, fat, fibre) mid to late gestation	Mid-late gestation	Foetus late gestation (130d)	With fibre and dried corn distillers (protein fat fibre) based diets, increased methylation of CpG islands of IGF2 and H19 compared to corn (starch based, low amino acid, ie low methyl group) in muscle tissue	76
Congenital heart defects	Rats, Vitamin A deficient diet	Before gestation ± during gestation	Foetus 13.5d	↑methylation at CpG loci of GATA-4 gene in embryo heart	77
Obesity	Rats, high multivitamin diet then further diet groups after weaning	Gestation	29 weeks	No change in global DNA methylation in adult offspring	78

**Table 8** Animal studies investigating DNA methylation in offspring following paternal diet exposure

Field of Study	Animal, Paternal Feeding Protocol	Offspring	Epigenetic Outcome	Reference
Genome wide approach	Mice, protein restriction	3 week pups	Δmethylation at many CpG sites  ↑ methylation at putative enhancer locus for PPARα	4
Wide approach	Pigs, high methyl groups	Two generations later (F2)	Δmethylation at IYD gene	79
Obesity	Rats, high fat diet	Female offspring	methylation of the I113ra2 gene	80

Methylation at H19 DMR in newborns exposed to folic acid supplementation before and during pregnancy was lower compared to unexposed newborns.<sup>15</sup> The size of this effect is similar to other studies, and the decrease was greater for male babies than female babies, adding to the evidence of a sex difference in the epigenetic response to intrauterine nutritional factors. In rats prenatally exposed to low protein the ICR at the IGF2/H19 locus was hypermethylated in newborn liver, expression of IGF2 was increased and this increase was significantly associated with the methylation of the ICR.<sup>28</sup> There was no change in methylation at DMR2.

In a sheep model Lan and co-workers, 2013, investigated a variety of energy sources in the maternal diet and imprinted gene DNA methylation in offspring exposed late in gestation.<sup>76</sup> Different diets did affect gene expression in a number of imprinted genes and DNA methyltransferase genes. There was a higher DNA methylation at CpG islands of IGF2R and H19 with the fibre-based diet and a diet of fibre, protein and fat, than the starch-based, low amino acid diet. DNA methylation at the CpG island in intron 2 of IGF2R positively correlated with gene expression.

More recent studies have a more epigenomic approach and also can take advantage of a more detailed knowledge of the molecular systems involved. A randomised controlled trial was carried out in the Gambia.<sup>7</sup> Women who were not yet pregnant took a supplement containing vitamins A, D, E, C, B1, B2, B6, B12, Niacin, Folic acid, Iron, Zinc, Copper, Selenium, Iodine, or they took a placebo, until confirmation of pregnancy (average 9.5 weeks gestation), then all subjects were switched to iron and folate supplement only. The group looked at patterns of DNA methylation at 13 imprinted loci: 2 paternally methylated germline DMRs, 3 paternally methylated somatic DMRs, and 7 maternally methylated germline DMRs. Results were adjusted for season of conception as season has been found to have an impact on nutrition in the Gambia. Changes in DNA methylation were found to be gender specific. In maternally supplemented groups there was lower methylation at IGF2R in newborn girls and lower methylation at GTL2-2 in newborn boys. These changes were not found at the second time of analysis, 9months, and the authors suggest this may be due to the small sample size. Methylation at the paternally methylated germline DMRs at H19 and IG loci were not changed. At 9months other changes are found: a lower methylation at PEG1-DMR in girls and a lower methylation at GNASAS-DMR in boys at 9months.

With the same cohort of Gambian women described above,<sup>8</sup> a genome-wide investigation using a microarray chip containing individual CpGs in promoters associated with 14000 genes was able

to make powerful comparisons of changes in methylation status of CpG loci in blood from infants at birth and 9months, finding effects of periconceptual supplementation, age, and gender at multiple loci.

14 genes exhibited changes with periconceptual supplementation in boys and 21 genes in girls. A greater number of genes are differentially methylated with supplementation at 9months. 50% of the changes seen in newborns are present at 9months. There is little overlap in differentially methylated loci between the sexes, and the group suggests that there are sex-specific developmental paths. The greatest number of changes was between birth and 9months independent of treatment. An interesting point to note that most of the sites differentially methylated were lone CpG sites outside CpG islands.

By means of databases a functional analysis of the differentially methylated loci showed that a number of them were associated with defence against infection and the immune response. In male newborns, interestingly, affected genes were involved in nervous system development and skeletal development. For female newborns, particular affected genes were involved in immune and non-immune defence against infection, and in cardiovascular function. In 9month infants, both sexes had changes in immune response genes and genes involved in defence against infection, cancer and development, neurological function and in males, cardiovascular function. In three imprinted genes there is an effect of supplementation: GNAS, involved in intrauterine growth, MKRN3, involved in obesity and SLC22A18, associated with various tumours.

Metastable epialleles are regions where epigenotype, such as DNA methylation, is stochastically set in the early embryo to be retained in all differentiated cell lineages. There is thus high variation between individuals in levels of DNA methylation at these regions. Based on what is known of murine metastable epialleles, Waterland and co-workers, 2010, found putative human metastable alleles.<sup>10</sup>

They postulate that level of DNA methylation at these loci may affect susceptibility to disease and they describe a study in a rural Gambian population where seasonality dramatically affects the diet. 9year old children, who had been conceived in the dry season, when food is abundant, were compared with children conceived in the rainy season when there is a short supply of food as well as a greater amount of physical work to be done. Blood folate levels in this population increase during the rainy season. Data was retrospective from years with a strong effect of seasonality on birth weight. Interestingly, DNA methylation of all 5 putative metastable epialleles was significantly higher in children conceived in the time of hardship. There was no

change in DNA methylation at IGF2, GNASAS, and IL10 in relation to season of conception. The effect of season of conception in the Gambia on metastable epialleles was larger than the previously discussed effect of conception during famine on DNA methylation in adults.<sup>6</sup> The epigenotype at the metastable epialleles is in tissues from all three germ layers which means it is established before gastrulation. This is consistent with a sensitivity to conditions around the time of conception.

### Hypothalamic-pituitary-adrenal axis

Hypothalamic-pituitary-adrenal (HPA) axis is a complex system regulating glucocorticoid production. Alterations in the foetus have been implicated in development of later life disease.<sup>86</sup> Animal models have shown increased maternal glucocorticoids during gestation leads to a metabolic syndrome-type profile in adult offspring. In adult humans high glucocorticoid level is associated with cardiovascular disease and neurobehavioral problems and high maternal cortisol is associated with low birth weight. Glucocorticoids are involved in glucose, protein and fat metabolism and are important in foetal development. The HPA also plays a key role in the response to stress and chronic stress has long since been linked to metabolic disease states.

Retrospective summaries of dietary intake of mothers advised to eat a high meat, low carbohydrate diet during pregnancy were used to investigate the relationship with DNA methylation at candidate genes in blood of the 40year old offspring.<sup>86</sup> Methylation at the glucocorticoid receptor gene (exon 1F) was increased in offspring whose mothers had higher meat, fish and vegetable intake and lower bread and potato intake in late pregnancy. This exon has a role in control of glucocorticoid receptor expression, which in turn mediates glucocorticoid action. The authors suggest that the increased methylation may impact on transcription factor binding. Methylation increased at a specific site in 11 $\beta$ -hydroxysteroid dehydrogenase enzyme 2 gene (HSD2) region 2 with increased meat and fish intake in late pregnancy. HSD2 modulates access of glucocorticoid to the glucocorticoid receptor. The group has previously found that methylation at this locus increases its gene expression and is associated with hypertension. Thus maternal diet *in utero* may influence the pathway of glucocorticoid action decades later via DNA methylation changes.

A randomised controlled trial tested the effect of intake of high or moderate choline during the third trimester of pregnancy.<sup>18</sup> With higher choline intake there was a 33% lower plasma cortisol in cord blood. There was higher methylation in the proximal promoter region of corticotrophin releasing hormone (CRH) promoter and in glucocorticoid receptor promoter region including exon1-F in the placenta at birth following higher level of consumption of choline. Transcription of CRH was lower with more choline consumption maybe due to decreased binding of transcription factors because of DNA methylation of the promoter region. In contrast, in cord blood leukocytes DNA methylation of the promoter regions of the same two genes was lower. CRH regulates HPA axis reactivity and cortisol production, thus this study suggests that the quantity of choline in the maternal diet may impact a system which could affect the response to stress over the life course and affect the susceptibility to metabolic disease.

This study also examined the effects of the experiment on DNA methylation at GNAS-AS1, IGF2, IL10 and LEP. Only at one site was a difference found: one CpG unit in GNAS-AS1 in placental tissue was less methylated with high choline intake. The authors suggest that choline supplementation could be given to pregnant mothers where

stress might adversely affect the HPA axis reactivity of the foetus.

Sheep have been chosen as an apt animal model because the hypothalamic pituitary axis and placental function are similar to humans.<sup>38,39</sup> Sheep were nutritionally deprived before conception and for early pregnancy, then were fed ad lib. Proopiomelanocortin (POMC) is a neuropeptide involved in energy balance at the hypothalamus, acting to decrease food taken in and increase energy extended. In late gestation foetus where pregnant mothers were food deprived around conception, there was a decrease in DNA methylation of the POMC promoter and the glucocorticoid receptor promoter in the hypothalamus. They make a comparison to twinning also. Authors postulate that a similar alteration in regulation at the POMC promoter and the GR promoter a human could lead to dysregulation of food intake in adulthood and consequently obesity or dysregulation of glucose homeostasis and consequently diabetes. In rats a methyl supplemented diet in pregnant dams altered the stress response of the offspring but no change in DNA methylation of glucocorticoid receptor gene exon 1 promoter was observed.<sup>55</sup>

### Global DNA methylation

Various methods are used to determine genome-wide DNA methylation level such as the Luminometric DNA Assay or methylation of a repetitive element sequence, LINE-1 or Sat2. Lower global DNA methylation in white blood cells has been associated with a raised risk of various cancers.<sup>87</sup> Researchers are looking at associations with other diseases, such as Alzheimer's, or with risk factors during the life course that may predispose to later life diseases, such as smoking, or dietary factors, in particular folate. Some studies have found a relationship with folate intake in adults and LINE-1,<sup>88</sup> however this does not seem to be a straightforward. Level of global DNA methylation in white blood cells from 58year olds who had been exposed to the Dutch famine *in utero* and their unexposed siblings bore no relationship to the exposure.<sup>11</sup>

Fryer's group found no correlation between maternal folic acid supplementation and cord blood DNA methylation level.<sup>13</sup> In their trial they found that cord blood homocysteine is inversely correlated with cord blood DNA methylation and suggest that homocysteine level may be a better indicator of available methyl groups than folate level. McKay and co-workers,<sup>89</sup> measured red blood cell folate and serum vitamin B12 in pregnant mothers and in cord blood at birth and found an inverse correlation of global DNA methylation with vitamin B12 in cord blood and, in agreement with the Dutch Hunger Winter cohort and the folate supplementation trials, found no correlation of folate status and global DNA methylation.

There was no effect of maternal periconceptional or second trimester intake of methyl donor nutrients on cord blood white blood cell global DNA methylation measured at LINE-1 in a prospective trial population known to be consuming enough folate.<sup>16</sup> However, in male babies, higher maternal periconceptional intake specifically of choline was associated with lower global DNA methylation.

The trial by Jiang and co-workers of choline supplementation in the third trimester found global DNA methylation in placental tissue at birth was higher with a higher maternal choline intake in the third trimester, however leukocyte global DNA methylation in cord blood was not altered with choline intake.<sup>18</sup> Maternal leukocyte global DNA was not altered with choline intake. Interestingly, supplemented choline was deuterium labelled at the methyl groups and it was seen that the supplemented choline molecules were used for placental DNA methylation.

In pigs, global DNA methylation in liver *in utero* was affected by the maternal low protein diet but after birth there was not a difference between low protein and control groups.<sup>20</sup> No change in global DNA methylation of skeletal muscle was observed but global DNA methylation was found to decrease with age in all treatment groups. In adult mice progeny exposed to low protein *in utero*, global DNA methylation of white adipose tissue was not different from the control group.<sup>32</sup> Thus there are tissue differences in the response to low protein exposure, and it may be that an early effect is lost by adulthood.

In spite of a high level of folate supplementation, rats exposed to low protein *in utero* had no change in global DNA methylation, and with their other results this group conclude that any effect of folate on appetite regulation is likely to be gene specific.<sup>29</sup> The relationship of dietary intake of methyl donors to global DNA methylation is not straight forward and may be affected by targeted gene specific methylation changes.

### Timing of exposure

The majority of the human studies involve exposure to the dietary factor around the time of conception, as conditions at this time may affect the *de novo* methylation of the DNA of the embryo or the imprinting of certain genes. In some cases an effect of periconceptional exposure has been found but without an effect of late gestational exposure.<sup>89</sup> In some cases a different pattern of genes are affected by exposure to the dietary factor periconceptionally or at a later time in gestation.<sup>6</sup> Choline supplementation only late in gestation in a human trial affected promoter methylation,<sup>18</sup> and for a small period in gestation in an animal study and affected methylation of a tumour suppressor gene.<sup>57</sup> Methyl donor supplementation of the agouti mouse is given for a mid-gestation window only.<sup>54</sup> Feeding sheep different diets late in gestation affected IGF2/H19 methylation.<sup>76</sup> Thus exposure to a maternal dietary factor at periconception or at a later stage of *in utero* development can affect DNA methylation.

### Paternal epigenetics

A fascinating aspect of epigenetics is the ability to transfer a circumstance of the father, adverse or otherwise, to first generation or even subsequent generation offspring. To date there are no published investigations in humans as far as we are aware where the father's diet has been observed or trialled and epigenetic changes in the offspring analysed. Soubry and co-workers (2013) examined obesity in fathers prior to conception and found an association with hypomethylation at IGF2 DMR.<sup>90</sup> The authors point out that the difference is of a similar magnitude to the effect of the Dutch famine. There was no significant association of obesity with DNA methylation level at H19 DMR. Animal studies have examined paternal diet effect on the DNA methylation in progeny.

Whilst the effect of grandmaternal famine exposure during the Dutch Hunger Winter on the F2 generation was not observed through the paternal F1 generation,<sup>91</sup> transgenerational influence of paternal and grandpaternal nutritional exposure has been observed elsewhere, as reviewed by Curley and others.<sup>92</sup> In an animal study, male mice were fed a protein restricted diet then mated with control-fed dams and the effects of the dietary intervention on the subsequent generation were examined.<sup>4</sup> Hundreds of genes were differentially expressed in the three week old F1 generation. In particular there was upregulation of pathways of DNA replication, and lipid and cholesterol biosynthesis. About 1% of the mouse genome was mapped for differential methylation of cytosines. There was an effect of the paternal low protein diet on methylation of CpG islands, albeit a small one, and the

promoters where methylation changed were not correlated with those of altered gene expression. There was a 30% increase in methylation at a likely enhancer locus for Ppara, a key lipid regulator, which did correlate with Ppara gene expression down regulation. Interestingly, preliminary investigations were carried out on a possible epigenetic mechanism of action in sperm. A change in global DNA methylation was not detected.

Braunschweig and co-workers investigated paternal inheritance with respect to methyl donor exposure in pigs.<sup>93</sup> The F0 generation of boars was fed a diet high in methyl donors, their offspring F1 boars and their offspring F2 pigs were fed normally. As well as other effects of grandpaternal methyl donor exposure, there was a change in DNA methylation at IYD gene promoter in liver. This interesting finding suggests that there may be an epigenetic effect of increased methyl donors for generations.

As a rat model of paternal obesity, male rats were fed a high fat diet. Female offspring had impaired glucose homeostasis, and a large number of pancreatic islet genes were differentially expressed, I13ra2 to the greatest degree. Lower DNA methylation at the I13ra2 gene suggests that the high fat diet of the father may have affected the progeny by epigenetic means.<sup>94</sup>

### Conclusion

The study of nutrition and epigenetics in development is a fast moving new field. Up to now human studies are few but the animal model is burgeoning. The areas of biochemistry studied are very diverse and likely to remain so. In pace with progress in techniques more genome wide microarray studies are emerging enabling a picture to be built up of the effect of *in utero* conditions. Imprinted genes of offspring are affected by exposure to maternal diet *in utero*, and the IGF2/H19 region has been particularly extensively examined. The hypothalamic pituitary adrenal axis is another area of active study.

Future work is needed to understand any role of paternal diet on DNA methylation, and as obesity and undernutrition remain significant health issues an understanding of how a parent's diet can affect future generations is of great interest.

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### Conflict of interest

The author declares no conflict of interest.

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