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Horizons in Nutritional Science

Nutrition in early life, and risk of cancer and metabolic disease: alternative endings in an epigenetic tale?

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There is substantial evidence which shows that constraints in the early life environment are an important determinant of risk of metabolic disease and CVD. There is emerging evidence that higher birth weight, which reflects a more abundant prenatal environment, is associated with increased risk of cancer, in particular breast cancer and childhood leukaemia. Using specific examples from epidemiology and experimental studies, this review discusses the hypothesis that increased susceptibility to CVD, metabolic disease and cancer have a common origin in developmental changes induced in the developing fetus by aspects of the intra-uterine environment including nutrition which involve stable changes to the epigenetic regulation of specific genes. However, the induction of specific disease risk is dependent upon the nature of the environmental challenge and interactions between the susceptibility set by the altered epigenome and the environment throughout the life course.

Cancer: Metabolic disease: CVD: Early life environment: Epigenetics

Cancer is the result of derangement of cellular processes which control cell division and apoptosis[1]. There is a large body of information which shows that environmental exposures, including nutrition, play a significant role in the aetiology of the disease. Such exposures usually precede the appearance of clinical disease by a prolonged period of time, often several decades[1]. While gene mutation has a role in the aetiology of cancer, there is increasing evidence which shows that epigenetic processes such as DNA methylation and covalent modifications to histones are also involved[2]. Such epigenetic changes represent potential for altered gene activity and hence cellular dysregulation, but these may only be manifest when the gene is exposed to an appropriate environmental signal which is enhanced or diminished as a consequence of the epigenetic change compared to normal cells. This suggestion is consistent with a life-course perspective on cancer risk. The early life environment has been shown to be an important factor in determining risk for some types of cancer. Measures of growth in early life show statistical associations with risk of specific cancers. In this context, there appear to be parallels between causal processes in cancer with metabolic disease and CVD in which the early life environment and epigenetic processes lead to a susceptibility which increases the risk to later specific environmental exposures[3]. The purpose of this review is to discuss the hypothesis that the early life environment, in particular nutrition, induces changes in the development of the offspring which lead to increased risk of cancer, or metabolic disease and CVD. The extent to which altered epigenetic processes established during development represent a common mechanism in cancer and metabolic disease, diseases which are generally considered as resulting from widely different pathological processes, will also be discussed.

Early life environment and risk of metabolic disease

Studies in man

Epidemiological studies show that the environment experienced before birth and shortly afterwards has long-term effects on the subsequent risk of metabolic diseases including CVD, type 2 diabetes mellitus and obesity. Barker[4] and Godfrey & Barker[5] showed that adults who were small at birth had a higher risk of developing metabolic disease and CVD, and that increasing birth weight was associated with a graded decrease in risk. Such induction of increased risk as a result of intra-uterine constraint has been termed 'fetal programming'. Importantly, these effects occur over the

Abbreviations: Dnmt, DNA methyltransferase; GR, glucocorticoid receptor; IGF, insulin-like growth factor; HR_{ad}, adjusted hazard ratio; MeCP2, methyl CpG binding protein; PR, protein-restricted.

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usual range of birth weights rather than being the result of pathological states such as intra-uterine growth retardation or, at the opposite end of the spectrum, macrosomy[4]. These findings have been replicated in a substantial number of studies in different populations world wide, although there are important variations in specific outcome measures (see Gluckman & Hanson[6] for examples). Size at birth reflects the growth of the fetus and small size reflects a developmental constraint imposed by the intra-uterine environment. Nutrition, together with hormones and oxygen supply, is an important determinant of fetal growth and has been studied extensively with respect to its role in the induction of risk of metabolic disease. Perhaps the most persuasive example of a specific role for prenatal nutrition in determining subsequence risk of metabolic disease comes from studies of adults who were *in utero* during the Dutch Hunger Winter which occurred during a discrete period of 1944–5[7]. These studies show that compared to adults born before or conceived after the famine, adults who were fetuses during the famine showed increased risk of hypertension, obesity and insulin resistance. Furthermore, the severity of such effects was graded according to the stage of development of the fetus at the time of famine[7]. This suggests that different organ systems are susceptible to nutritional constraint during specific periods of development.

The paradigm that environmental constraint in early life determines subsequent risk on non-communicable diseases has been extended by Gluckman & Hanson[8]. They suggest that this association is an example of a wider spectrum of developmental responses to environmental stimuli which through developmental plasticity induce adaptations to the phenotype of the fetus which predict the postnatal environment. If correct, such predictions confer a survival advantage. This hypothesis has been supported by recent observations between fatness at birth in women, and their subsequent reproductive capacity[9]. It is postulated that a mismatch between the predicted environment and that which the offspring experiences after birth results in a disadvantage that in man leads to increased risk of disease[8]. The extent and nature of the mismatch remains to be determined.

There is emerging data that the effects of prenatal nutrition on health in adulthood can be transmitted to more than one subsequent generation[10]. For example, examination of historical records of the population in Overkalix, Sweden showed that mortality from diabetes increased in men if the paternal grandfather was exposed to abundant nutrition during their pre-pubertal period of slow growth[11]. The children born to women who were in their first trimester during the Dutch Hunger Winter were heavier and those born to women in their third trimester were lighter than the children of women born before the famine[12]. This suggests the effects of the famine during the grandmother's pregnancy were transmitted to their grandchildren.

Studies in animal models

The findings of epidemiological studies have been largely supported by the results of studies of the effects of specific nutritional interventions during pregnancy and/or lactation in laboratory animals, mainly rats and mice (for detailed reviews see Armitage *et al.*[13] and Bertram & Hanson[14]), although studies have also been conducted in other species. The nutritional interventions include feeding a diet with reduced protein content within the physiological range for rodents[15], global reduction in food intake[16] or a high-fat diet[17]. In general terms, feeding these diets to pregnant rodents induced in the offspring aspects of metabolic disease and CVD which in man have been associated with a poor prenatal environment. The offspring of these animal models show, albeit to different degrees, impaired control of blood pressure (hypertension and endothelial dysfunction), insulin resistance, dyslipidaemia, obesity and altered locomotor behaviour. In contrast to man, birthweight in rodents is generally unaffected by nutritional constraint during pregnancy, possibly because of the relative immaturity of the pups at birth and the difference in the timing of fat deposition during development. This emphasises that birthweight is a proxy marker for the effects of the intra-uterine environment on the development of the fetus rather than necessarily being a causal component of the mechanism which links environmental exposure to later risk of disease (also consider Roseboom *et al.*[7]).

Animal models also provide a means of testing the effects of interventions to prevent or reverse the effects of the prenatal environment on the development of the fetus. Two interventions have so far been shown to prevent fetal programming. First, administration of leptin during the neonatal period in rats prevented excessive weight gain in response to a high-fat diet in the offspring of dams which received a 70 % reduction in global nutrition during pregnancy[16]. Secondly, increasing the availability of metabolites involved in one-carbon metabolism, specifically glycine and folic acid, to pregnant rats consuming a protein-restricted (PR) diet prevented induction of hypertension and vascular dysfunction in the offspring[18–20]. These findings suggest that one-carbon metabolism plays a central role in the induction of an altered phenotype.

Prevention of hypertension by increasing the glycine content of the PR diet[18] or impaired lipid and glucose homeostasis by increasing the folic acid content of the PR diet by a similar amount to the daily intake of pregnant women using folic acid supplements[21] decreased the growth of the offspring, which suggests normalisation of vascular function and metabolism may occur at the expense of growth and so represent a developmental trade-off. Although increasing the folic acid content of the maternal PR diet prevented hypertension in the offspring, increasing the folic acid content of the protein-sufficient control diet by the same amount increased blood pressure in the offspring[21,22]. The relative protein and folic acid content of the diet fed to pregnant rats induces in the offspring opposing changes in fasting blood lipid and glucose concentrations[22]. However, the magnitude of such effects also depends upon the amount of fat fed to the offspring after weaning[22]. Transmission between generations of obesity associated with the epigenetically regulated agouti phenotype in mice was also prevented by dietary supplementation with methyl donors[23]. Thus the physiological phenotype of the offspring is dependent upon interactions between prenatal nutrition and diet in later life. One implication of these findings is that altered susceptibility to disease may reflect the early life environment, but risk may be modified by environmental exposures throughout the life course.

Growth before birth and risk of cancer

Information regarding the effect of the prenatal environment on later risk of cancer is rather more limited than that on the early

life origins of CVD and metabolic disease. With the exception of the well-known effects of prenatal exposure to endocrine-disrupting agents such as diethylstilbesterol[24] or ionising radiation[25] on subsequent cancer risk, the precise environmental exposure which leads to cancer is often difficult to define and epidemiological studies are dependent upon testing the strength of associations with proxy measures such as birth weight. Since cancer encompasses a highly heterogeneous group of diseases, the strength of any association between markers of the prenatal environment and subsequent disease risk might be expected to be weak. Nevertheless, associations have been demonstrated between proxy markers of the intra-uterine environment and risk of specific cancers including breast cancer, and leukaemia and some other non-reproductive cancers. To allow comparison between markers of the prenatal environment, and risk of cancer and risk of metabolic disease and CVD, the following discussion will focus on associations with birth weight. However, there are studies which report associations based on other markers of the intra-uterine environment such as length at birth. Many of these are cited in the references listed later.

Breast cancer

The findings of studies which have investigated the association between breast cancer and birth weight are summarised in Table 1. These studies differ markedly in design, the extent to which other variables were included in the analysis, the number of cases studied and whether pre- and post-menopausal women were analysed together or separately. The possible impact of such differences in study design on the reported outcomes have been discussed in detail elsewhere[26,27]. Where associations between birth weight and risk of breast cancer were found they suggest that, in contrast to CVD or metabolic disease, lower birth weight tended to be associated with lower risk of breast cancer, and that cancer risk increased in a graded manner with increasing birth weight (see references in Table 1). While it has been reported that the association between birth weight and cancer risk is strongest in women who develop the disease before their menopause[1], not all studies support this (Table 1). The extent of association between breast cancer risk and maternal age, parity and maternal smoking are less clear as studies have reported conflicting findings[32,34,39,53] and some have reported J-shaped associations[34]. While in some studies the highest birth weights are above 4·5 kg (Table 1), the majority fall below this level. Thus any effect of pathological changes associated with macrosomy cannot account entirely for the positive association between birth weight and risk of breast cancer. A recent meta-analysis supports the overall conclusion that risk of breast cancer is increased in individuals with higher birth weight[54]. Higher birth weight was associated with 12 % increase in relative risk of breast cancer, while higher birth length was associated with 28 % increased risk of disease. Overall, these studies support the suggestion that the intra-uterine environment exerts a persistent effect on the risk of women developing breast cancer.

Two recent studies have investigated the effect of maternal diet on mammary tumorigenesis in their offspring. de Assis *et al.* [55] found that the offspring of rats fed high-fat diet had structural changes to mammary tissue, lower oestrogen receptor-α expression and increased levels of activated mitogen-activated protein kinase. These offspring showed altered mammary gland structure, increased oestrogen receptor-α, insulin receptor and insulin-like growth factor (IGF)-1 receptor expression and increased sensitivity to tumour induction with 7,12-dimethylbenz[*a*]anthracene. Fernandez-Twinn *et al.* [56] reported that the offspring of rats fed a PR diet during pregnancy and lactation had a 2-fold increased sensitivity to mammary tumour induction by nitrosomethylurea. Thus, although these studies used opposing maternal dietary insults, the overall outcome was to increase the sensitivity of mammary tissue to tumorigenic agents. An assessment of maternal folic acid intake in mice on tumour burden in the adult offspring genetically predisposed to intestinal tumours failed to show an effect of prenatal folate exposure or an interaction between prenatal and post-weaning folate exposure[57]. However, since all mice produced tumours and the effects of varying maternal folic acid intake were not assessed in wild-type mice, it is possible that any effects of prenatal folate supply on tumorigenesis were masked by the genetic defect.

Table 1. Associations between breast cancer and birth weight

Reference no.	No. of cases	Direction of trend and menopausal status
28*	153	None; pre-menopausal
29	458	Positive; pre- and post-menopausal
30	582	Positive; pre- and post-menopausal
31	746 and 401	Positive, pre-menopausal; none, post-menopausal
32	1068	None; pre- and post-menopausal
33*	57	None, menopausal status not disclosed
34*	484	Positive; pre-menopausal
35	37	Positive; pre- and post-menopausal
36	90	Positive; pre- and post-menopausal
37	62	None; pre- and post-menopausal
38	177	None; post-menopausal
39	288	None; pre-menopausal
40*	1716	Positive; post-menopausal
41	373	Positive; pre- and post-menopausal
42*	2334	Positive; pre- and post-menopausal
43	359	Positive; pre- and post-menopausal
44	127	Positive; pre- and post-menopausal
45	881	Positive; pre-menopausal
46	59	Positive; pre- and post-menopausal
47	2074	Positive; menopausal status not disclosed
48	196	None; pre- and post-menopausal
49	89	Positive; post-menopausal
50	367	Positive, pre-menopausal; none, post-menopausal
51	312	Positive; pre- and post-menopausal
52	828 and 2312	Positive, pre-menopausal; none, post-menopausal

* Studies in which high birth weight was defined as ≥4500 g.

Leukaemia and hepatoblastoma in childhood

A number of studies have reported a positive association between birth weight and risk of both acute myeloid and lymphoblastic leukaemia in childhood (see references in Table 2). As with the reports that show a positive association between birth weight and breast cancer, the majority of studies report associations within the normal range of birth weights. In contrast, risk of hepatoblastoma has been reported to be increased in children born below the normal range of birth weights[75]. This association is strongest in children born below 2500 g,

Table 2. Associations between childhood leukaemia and birth weight

Reference no.	No. of cases	Disease	Direction of trend
58	1323	All leukaemias	Positive
59	802	All leukaemias	Positive
60	72	All leukaemias	Positive
61	681	All leukaemias	Positive, but only less than 2 years of age
62	255	All leukaemias	No association
63	1304	All leukaemias	No association
64	309	ALL and AML	Positive, but only less than 6 years of age
65	337	ALL	Positive
66	71	ALL	Positive, but only above 4 years of age
67*	613	ALL	Positive
68*	98	AML	No association
69	303	ALL and AML	Positive
70	1687	All leukaemias	Positive, only studied children less than 2 years of age
71*	828	ALL and AML	Positive
72*	268	ALL	Positive, only studied children less than 5 years of age
73	65	ALL and AML	Positive
74	2204	ALL and AML	Positive

ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia.
* Studies in which high birth weight was defined as ≥4500 g.

particularly in those born below 1500 g[76–79]. It is difficult to explain the opposing relationship between the intra-uterine environment and hepatoblastoma, compared to other cancers. One possible explanation is that the children at risk of hepatoblastoma were born at the lower extreme of birth weight which suggests intra-uterine constraint outside of the normal range which influences patterns of development in the fetus.

Other cancers

McCormack *et al.* [80] reported detailed statistical analysis of the associations between a number of different cancers and birth weight. When adjusted for birth order and socioeconomic factors at birth and in adulthood, the hazard ratio for each standard deviation increase in birth weight adjusted for gestational age (HR_{ad}) for endometrial cancer was 0·79, while the HR_{ad} for ovarian cancer was not significantly related to birth weight. There was no significant association between birth weight and prostate cancer in men. This study also described the effect of birth weight on risk of non-reproductive cancers. The HR_{ad} for colorectal cancer was 1·16, and lymphatic and haematopoietic tissues 1·19. However, there were no significant associations between birth weight and stomach, liver, pancreatic, respiratory, urinary, neurological, skin and endocrinological cancers. One study has shown an increased OR for osteosarcoma in adults born ≥4000 g compared to those born between 3000 and 3500 g[81]. Also, the OR for all brain tumours in children was 1·05 in those born ≥4000 g compared to those born between 2500 and 3990 g, but was higher (OR 1·40) when astrocytomas were considered separately.

For all cancers, the HR_{ad} was 1·23 in women less than 50 years, but not related to birth weight in older women, while the HR_{ad} for men of all ages was 1·08[80].

Summary

There is a growing body of evidence which supports the suggestion that the intra-uterine environment modifies risk of specific cancers in children and adults. Despite the wide variability of the disease processes including histological origin, time of onset of disease, sex and age of the patients and the means of data collection there is an overall trend towards an increase in risk of specific cancers associated with higher birth weight, particularly at the upper end of the normal range.

Epigenetics and the developmental origins of metabolic disease

Prenatal environment, phenotype induction and gene transcription

The induction of changes to the phenotype of the offspring in response to the prenatal environment that persist throughout the lifespan implies stable changes to gene transcription resulting in altered activities of metabolic pathways and homeostatic control processes, and in differences in the structure of tissues. There are several studies which have investigated the effect of maternal nutrition during pregnancy and/or lactation on gene expression in the offspring in animal models. For example, feeding a PR diet to pregnant rats induced changes to the expression of genes involved in energy balance and glucocorticoid activity in the adult offspring (Table 3). Although the number of genes studied so far is limited, these demonstrate stable effects of nutrient restriction on transcription. Importantly, some of the genes which show altered expression following prenatal under-nutrition are transcription factors which affect multiple pathways in development and nutrient homeostasis; for example PPAR and the glucocorticoid receptor (GR) (Table 3). Thus modifying the regulation of expression of a few key transcription factors may alter

Table 3. The effects of maternal dietary protein restriction during pregnancy, or pregnancy and lactation in the rat on the expression of genes associated with energy balance in the adult offspring

Gene	Tissue	Direction of change compared to control	Reference no.
Glucocorticoid receptor	Liver	Increased	82–85
	Lung	Increased	82
	Kidney	Increased	
	Brain	Increased	
11β-Hydroxysteroid dehydrogenase	Liver	Decreased	82
	Lung	Decreased	
	Kidney	Decreased	
	Brain	Decreased	
Phosphoenolpyruvate carboxykinase	Liver	Increased	83–85
Glucokinase	Liver	Increased	86
Acetyl-CoA carboxylase	Liver	Increased	87
Fatty acid synthase	Liver	Increased	
PPAR-α	Liver	Increased	82–85, 88
PPAR-γ1	Liver	Unchanged	83, 88
PPAR-γ2	Adipose tissue	Decreased	88
Acyl-CoA oxidase	Liver	Increased	83

the activities of a large number of metabolic and developmental pathways.

Epigenetic regulation of transcription

The methylation of CpG dinucleotides, which are clustered at the 5′ promoter regions of genes, confers stable silencing of transcription[89]. Methylation patterns are largely established during embryogenesis or in early postnatal life. Following fertilisation, maternal and paternal genomes undergo extensive demethylation. This is followed by *de novo* methylation just prior to implantation[89,90]. About 70 % of CpG are methylated, mainly in repressive heterochromatin regions and in repetitive sequences such as retrotransposable elements[91]. Promoter methylation is important for asymmetrical silencing of imprinted genes[92] and retrotransposons[93,94]. DNA methylation also plays a key role in cell differentiation by silencing the expression of specific genes during the development and differentiation of individual tissues, and thus the timing of gene methylation is tissue- and gene-specific[95,96]. For some genes, for example δ-crystallin II and phosphoenolpyruvate carboxykinase, there also appear to be gradations of promoter demethylation associated with developmental changes in the role of the gene product[97,98].

DNA methylation can induce transcriptional silencing by blocking the binding of transcription factors and/or through promoting the binding of the methyl CpG binding protein (MeCP2). The latter binds to methylated cytosines and, in turn, recruits histone modifying complexes composed of deacetylases and histone methyl transferases to the DNA resulting in a closed chromatin structure and transcriptional silencing[99,100]. The precise effect on transcription depends on the nature of the covalent modification and which N-terminal lysine residue is altered[101–105].

Early life environment and epigenetic regulation of transcription in the offspring

Since epigenetic regulation of gene promoters is established during development and is responsible for patterns of transcriptional expression and silencing in adults, perturbations to this process represent a candidate molecular mechanism for induction of persistent alterations in phenotype by the environment experienced in early life. In an elegant study of the effect of maternal behaviour during suckling on the development of stress response in the offspring, Weaver *et al.*[106] showed that pups raised by rat dams which showed poorer nurturing had an increased stress response. The effect was due to hypermethylation of specific CpG dinucleotides within the promoter of the GR gene in the hippocampus of the offspring which were reversed in the adult offspring by intra-cranial administration of Trichostatin A and L-methionine[106–108]. Uterine artery ligation in the rat decreases p53 expression in the kidney of the offspring, which was associated with increased apoptosis and reduced nephron number[109].

Embryo culture and epigenetic regulation of transcription

Nutrition in early life has been shown to alter the epigenetic regulation of transposable elements and of imprinted genes, including IGF-2. These will be summarised here as they are described in detail elsewhere[110]. The composition of the culture medium used to grow mouse embryos alters the expression of IGF-2 and H19 genes by changing the methylation status of their respective promoters[111,112]. In man, *in vitro* fertilisation using the intracytoplasmic sperm injection technique is associated with increased risk of Angelman's syndrome[113,114] and Beckwith–Weidemann syndrome[115] due to loss of methylation of regulatory regions of the UBE3A, and H19 and IGF-2 genes, respectively[113,115]. While such alterations to the epigenetic regulation of imprinted genes produce dramatic alterations to the phenotype of the offspring which are evident in early life, these contrast with the phenotypes induced by variations in maternal nutrition during pregnancy which are more subtle and only become clinically apparent after the neonatal period in childhood or adulthood.

The agouti mouse model

Differences in maternal intake of nutrients involved in one-carbon metabolism, betaine, choline, folic acid and vitamin B_{12}, during pregnancy in the agouti mouse changed the offsprings' coat colour from yellow (agouti) to brown (pseudo-agouti)[116]. This shift is due to increased methylation of seven CpG dinucleotides 600 bp downstream of the A^{vy} intracisternal-A particle insertion site which acts as a cryptic promoter directing the expression of the agouti gene[110]. Differential methylation of the seven CpG dinucleotides was associated with a change in the proportions of mice with the agouti or pseudo-agouti coat colour.

Epigenetic regulation of genes in the offspring of the rat maternal dietary protein restriction mode

Feeding a PR diet to rats during pregnancy induces hypomethylation of the PPARα and GR promoters and increased expression of GR and PPARα in the liver of the recently weaned offspring[83]. This shows that stable changes to the epigenetic regulation of the expression of transcription factors, and hence a phenotype, can be induced in the offspring by modest changes to maternal intake of a macronutrient during pregnancy. However, there is evidence which shows that nutrients involved in one-carbon metabolism play an important role in this process (see later). The expression of the PPARα and GR target genes, acyl-CoA oxidase and phosphoenolpyruvate carboxykinase, was also increased which supports the suggestion that such altered epigenetic regulation of transcription factors modifies the activities of important metabolic pathways[83,84]. Sequence analysis of the PPARα promoter showed that the methylation status of only a few CpG dinucleotides was altered by the PR diet[117]. This suggests that the process of induced epigenetic change is targeted and that the resulting change in transcription may reflect changes in the interaction of the gene with relatively few transcription factors, thus inducing specific changes in the regulation of gene function and hence response to environmental cues. Methylation of the GR and PPARα promoters was also reduced in the heart and the PPARα promoter was hypomethylated in the whole umbilical cord[88]. Hypomethylation of the GR promoter was associated with an increase in histone modifications which facilitate transcription

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while those that suppress gene expression were reduced or unchanged[84]. While this may be primarily the result of reduced binding of the MeCP2 to the GR promoter because of the reduced level of DNA methylation, reduced MeCP2 expression may also have contributed to higher levels of histone acetylation.

Induction of vascular dysfunction in the offspring of rats fed PR diet during pregnancy was prevented by supplementation of the PR diet with glycine or folic acid[18–20]. Hypomethylation of the hepatic GR and PPARα promoters was also prevented by addition of 5-fold more folic acid to the PR diet[83]. Thus one-carbon metabolism plays a central role in the induction of an altered phenotype by maternal dietary restriction as it does in the agouti mouse[110].

DNA methyltransferases and one-carbon metabolism

Methylation of CpG dinucleotides *de novo* is catalysed by DNA methyltransferase (Dnmt) 3a and Dnmt3b, and is maintained through mitosis by gene-specific methylation of hemimethylated DNA by Dnmt1[89] (Fig. 1). Over-expression of Dnmt1 results in hypermethylation of DNA and embryonic lethality[118], while transient depletion of xDnmt1 in *Xenopus* embryos induces DNA hypomethylation producing an altered phenotype and sustained depletion causes apoptosis[119,120]. Thus variations in Dnmt1 expression alter the phenotype of the embryo. Dnmt1 activity is inhibited by homocysteine[121], and Dnmt1 and Dnmt3a expression is modulated by folic acid intake in adult rats[122]. Furthermore, the Dnmt1 promoter contains a GR response element[123] which may induce negative regulation of Dnmt1 expression[124]. This suggests how administration of glucocorticoids during pregnancy may induce stable changes to the expression of gluconeogenic enzymes in the offspring as a result of increasing GR activity[125]. Thus Dnmt activity may be altered by increased glucocorticoid exposure or as a result of changes to one-carbon metabolism, and so represent one candidate mechanism for the induction of altered epigenetic regulation of genes in response to the intra-uterine environment. Feeding a PR diet to rats during pregnancy induced a reduction in Dnmt1 expression and in binding of Dnmt1 at the GR promoter[84], but not the expression of Dnmt3a, Dnmt3b or the putative DNA demethylase methyl binding domain-2[126], and the binding of Dnmt3a at the GR promoter was unaltered. This suggests that hypomethylation of the GR promoter in the liver of the offspring, and probably other genes including PPARα, is induced by the maternal diet as a result of lower capacity to maintain patterns of cytosine methylation during mitosis. Down-regulation of Dnmt1 expression may result from increased exposure to homocysteine[19,127] and/or corticosteroids[128,129] which may act directly or by decreasing folate bioavailability[130]. The central role of one-carbon metabolism is highlighted by the prevention of reduced Dnmt1 expression by increasing the folic acid content of the PR diet[84]. Since Dnmt1 activity appears to be targeted to a subset of gene promoters[131–133], altered Dnmt1 expression provides a mechanism for induction of gene-specific promoter hypomethylation. Since Dnmt1 activity is also required for progression through mitosis[134,135], reduced Dnmt1 activity could also account for the reduction in embryo cell mass[136].

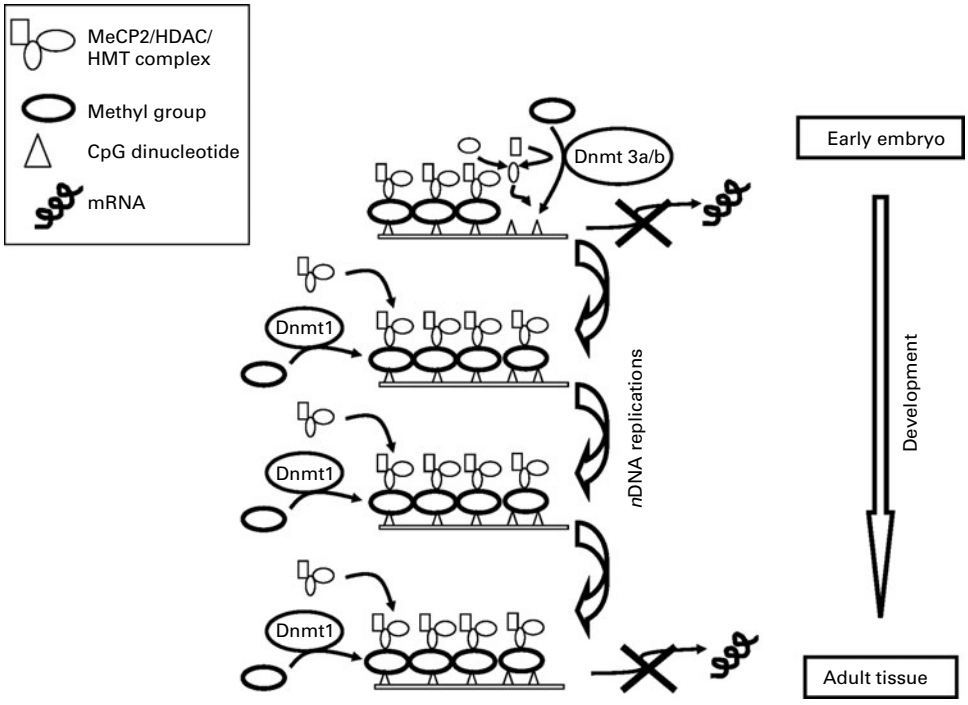

Fig. 1. Gene silencing by DNA methylation. Gene expression is silenced in the early embryo by the activities of DNA methyltransferase (Dnmt) 3a and Dnmt3b which catalyse methylation of CpG dinucleotides *de novo*. This recruits methyl CpG binding protein-2 (MeCP2) which in turn recruits the histone deacetylase (HDAC)–histone methyltransferase (HMT) complex which induce condensation of chromatin at the promoter. Methylation of CpG dinucleotides, and hence gene silencing, is maintained through mitotic cycles by Dnmt1 activity.

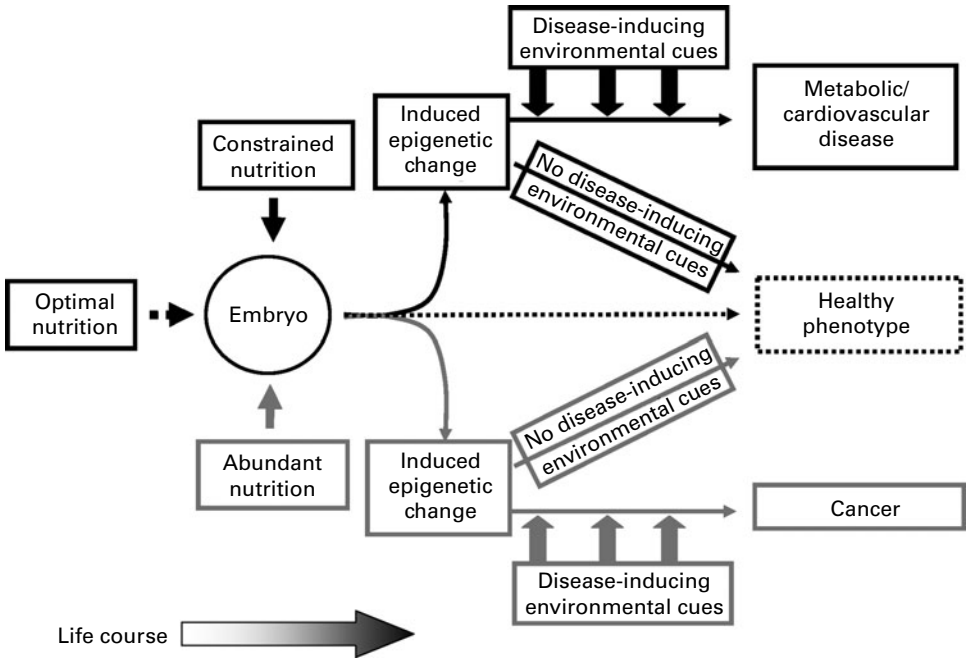

Fig. 2. A model for induction of increased risk of CVD/metabolic disease or cancer by different nutritional exposures acting on the same genome during development. Optimal nutrition during development facilitates establishment of an epigenotype which is expressed as a healthy phenotype. Nutritional constraint induces altered epigenetic regulation in genes associated with increased risk of CVD/metabolic disease. Conversely, nutrient abundance during development induces epigenetic changes in genes associated with increased risk of cancer. However, for both altered epigenotypes, the disease phenotype is only manifest when the organism is exposed to appropriate environmental signals, such as poor diet, during the life course. If these later environmental cues are avoided, possibly by lifestyle choice, then a healthy phenotype is maintained.

A model for the induction of an altered metabolic phenotype in the offspring by prenatal under-nutrition

Based upon current data, we have suggested a mechanism for the induction of an altered phenotype in the offspring by nutrient constraint during pregnancy in which promoter methylation is lost in a gene-specific manner during mitosis due to decreased Dnmt1 expression and activity[84,85]. This is accompanied by reduced binding of the MeCP2–histone deacetylase–histone methyltransferase complex leading to persistence of histone modifications that permit transcription.

Epigenetics and cancer

A change in the epigenetic regulation of genes has been implicated as a causal mechanism in specific cancers including lung, prostate and breast cancer[137], colon cancer[138] and haemopoietic cancers[139]. Specifically, increased cancer risk is associated with global hypomethylation of the genome with concurrent hypermethylation or hypomethylation of the promoters of specific genes. The mechanism by which global hypomethylation is induced is unclear, but may reflect the global decline in DNA methylation associated with increasing age[137]. The age-related decline in global methylation is related to a reduction in Dnmt1 activity[140] which, in turn, may induce expression of oncogenes such as c-*Myc* and c-N-*ras* [140]. Thus it appears that modulation of Dnmt1 activity is a key regulatory step in both fetal programming and in the induction of the tumorigenesis. This may be accompanied by methylation *de novo* of tumour suppressor genes[141] by increased Dnmt3a activity leading to aberrant activation of genes involved in cell proliferation and cell differentiation[142]. Together these changes represent a shift in the regulation of gene control which, in turn, may predispose the genome to further changes in methylation which result ultimately in neoplasia[143]. The mechanism leading to gene-specific hypermethylation in cancer is unclear, although it is possible that, as in fetal programming, the balance of nutrients involved in one-carbon metabolism, including folate, vitamin B_6 and B_{12}, may modulate the activities of Dnmt[143]. Hypermethylation of specific genes in cancer appears to be similar to the effect of increasing the folic acid content of the PR diet fed to pregnant rats on the methylation status of CpG in the liver PPARα promoter in the offspring. While the methylation of CpG which were hypomethylated in the offspring of dams fed a PR diet was normalised by increasing the folic acid content of the maternal PR diet, hypermethylation was also induced in other specific CpG dinucleotides[117]. The observation that nutrition in early life can induce both hypomethylation and hypermethylation of specific CpG dinucleotides suggests a mechanism for induction of different disease endpoints (for example, metabolic disease or cancer) by variation in the same environmental exposure, which is marked by differences in the direction of association between birth weight and disease risk. Prenatal under-nutrition results in hypomethylation of specific CpG dinucleotides in individual gene promoters which would tend to increase binding of regulatory proteins. It is possible therefore that higher nutrient availability, such as increased folic acid intake, may induce hypermethylation of other CpG which would result in different DNA–protein interactions and so induce a shift in regulation. One key example of the role of epigenetics in modulating gene activity by shifting the balance between agonist and suppressor proteins is the induction of

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tumorigenesis by activation of telomerase in differentiated cells. Telomerase activity is down-regulated in most cells during terminal differentiation in embryogenesis as a result of methylation of the GC-rich promoter region, but is often active in cancer cells. It has been proposed that activation of telomerase in preneoplastic cells is due to a shift in regulation between the activator c-*Myc* and the suppressor WT1 by changes in the methylation status of specific CpG within the binding domains of these transcription factors in the promoter of the catalytic subunit with confers RT activity (hTERT)[144]. One consequence of hTERT activation is to increase Dnmt1 activity[145] leading to copying of aberrant patterns of cytosine methylation. This suggests a synergistic role for hTERT and Dnmt1 in controlling cell proliferation and the methylation status of the genome.

Summary

The findings of epidemiological studies of the relationship between prenatal growth and risk-specific cancers, metabolic disease and CVD suggest that the early life environment is a causal component of the aetiology of these conditions. This is further implied by the common role for altered epigenetic regulation of specific genes and of altered Dnmt activity. Thus, risk of what may generally be considered to be very different disease entities may reflect a continuum of developmental changes which operate via the same enzymes and pathways which induce alterations to the epigenetic regulation of specific genes. Risk of specific diseases may reflect the nature and/or the magnitude of the environmental exposure during early life. It is not known how these environmental cues may be targeted in a manner which induces altered epigenetic regulation of specific genes or of individual CpG dinucleotides and so lead to increased risk of different disease processes. However, such specificity is implied by emerging evidence that the magnitude of the maternal nutritional challenge and the relative amount of specific nutrients in the maternal diet induce directionally opposite changes in the physiology and epigenotype of the offspring[21,83,146].

Overall, these findings support the concept that a range of prenatal nutritional environments from constraint to abundance may induce risk of ultimately different pathological processes (Fig. 2). The induced epigenetic changes are likely to be permissive for altered gene expression and hence determine the interaction between an organism and its environment over the life course and, in turn, determine whether increased risk due to the early life environment becomes disease in later life. However, this is an emerging field of research and a substantial number of studies will be required to demonstrate directly a causal association between variations in early life nutrition, induced epigenetic change and differential disease risk, to characterise and understand the underlying mechanisms and to develop prognostic markers in order translate the research findings into clinical tools.

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