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Invited Commentary

Homocysteine: a role in fetal programming?

Developmental plasticity allows the generation of a number of phenotypes from a single genome (Gluckman & Hanson, 2004*a*). There is a substantial and growing body of evidence from epidemiological studies (Godfrey & Barker, 2001) and from animal models (Bertram & Hanson, 2001) that supports the hypothesis that constraints in the fetal environment, such as undernutrition, induce phenotypes with increased risk of cardiovascular and metabolic disease in later life; so-called fetal programming or phenotypic induction (Godfrey & Barker, 2001; Bateson *et al.* 2004; Gluckman & Hanson, 2004*b*). Gluckman & Hanson (2004*b*) suggest that the phenotype of the fetus reflects adaptations during development that predict the postnatal environment based upon signals from the mother. Phenotypes at increased risk of disease are due to a mis-match between the environment experienced *in utero* and the challenges of postnatal life (Gluckman & Hanson, 2004*b*). However, to some, this remains controversial (Huxley, 2006).

There is an urgent need to identify the mechanism by which information about the maternal environment is transmitted to the fetus and how this is interpreted by the fetus to produce a particular phenotype in response to such developmental cues. Studies using the well-established model of a moderate reduction in dietary protein during pregnancy in the rat suggest that disruption of 1-carbon metabolism may contribute to one or both of these processes. Supply of methyl groups from donors such as glycine via 5-methyltetrahydrofolate (mTHF) is required for a number of critical pathways (for a review, see Muskiet, 2005). Supplementation of the maternal protein-restricted (PR) diet with glycine prevented hypertension in the adult offspring (Jackson *et al.* 2002; Brawley *et al.* 2004). This suggests that phenotypic induction in response to a maternal PR diet is closely associated with altered 1-carbon metabolism.

Cellular differentiation during the development of the embryo involves stable suppression of transcriptionally silent genes by methylation of CpG dinucleotides in gene promoters (Bird, 2002). Such epigenetic regulation of gene expression requires the supply of methyl groups from mTHF via conversion of *S*-adenosylmethionine to *S*-adenosylhomocysteine (Muskiet, 2005). Lillycrop *et al.* (2005) showed that feeding a PR diet during pregnancy results in hypomethylation and increased expression of specific transcription factors, namely the glucocorticoid receptor (GR) and PPARα, which regulate energy homeostasis in the liver of the offspring. This suggests altered methylation of specific genes may be important in the induction in the offspring of a modified metabolic phenotype by maternal dietary constraint. Moreover, hypomethylation of GR and PPARα promoters was prevented by supplementation of the PR diet with folic acid. This suggests a causal link between 1-carbon metabolism, altered epigenetic regulation of gene expression and phenotypic induction.

In this issue, Langley-Evans *et al.* (2006) point out that Bogdarina *et al.* (2004) did not find differences in the methylation of the glucokinase (GK) promoter in the liver of the offspring of dams fed a PR diet during pregnancy, although GK expression was increased, which questions the role of DNA methylation in phenotypic induction. One explanation of this apparent discrepancy may lie in the specificity of the control of gene expression by DNA methylation. Most genes are associated with CpG clusters known as islands, but in the majority of genes these CpG are always unmethylated and the level of expression is determined by the activities of various regulatory proteins such as transcription factors (Bird, 2002). Thus it may not be surprising that the expression of GK differed between offspring without a difference in methylation. Since GK is positively regulated by GR, increased GR activity in the liver of the offspring of rats fed a PR diet (Bertram *et al.* 2001; Lillycrop *et al.* 2005) alone may account for increased GK expression without a difference in the methylation status of the GK gene. If so, there would not be a discrepancy between the findings of Bogdarina *et al.* (2004) and Lillycrop *et al.* (2005). The important point is that in the liver of the offspring of the PR dams, transcription factors with numerous targets that are normally under stable suppression by DNA methylation are activated, presumably in a specific subset of cells as implied by Burns *et al.* (1997), thus changing the overall metabolic activity of the tissue.

If disruption of 1-carbon metabolism is important for phenotypic induction by the PR diet, it might be hypothesised that this diet would be associated with increased homocysteine (Hcyst) concentration since folate is required for the remethylation of Hcyst to methionine (Muskiet, 2005). Exposure of the developing embryo to increased Hcyst concentration may contribute to induction of an altered phenotype by the PR diet as Hcyst directly modifies the activities of several important signal-transduction pathways and transcription factors (Malinow *et al.* 1993; Tsai *et al.* 1994; Dalton *et al.* 1997; Brown *et al.* 1998; Southern *et al.* 1998; Mujumdar *et al.* 2000; Woo *et al.* 2000; Nishimoto *et al.* 2003; Robert *et al.* 2005) and so may change the response of specific tissues to developmental cues. Two studies have reported measurements of maternal Hcyst concentration in pregnant rodents fed a PR diet. Petrie *et al.* (2002) found increased Hcyst concentration in serum from pregnant rats and mice fed a PR diet in early, but not

mid, gestation. Brawley *et al.* (2004) show a trend towards a higher Hcyst concentration at gestational age day 20 in rats fed a PR diet. These findings suggest disruption of maternal 1-carbon metabolism during the early development of the embryo, but not mid and late gestation. Since induction of an altered phenotype in this model is established by embryonic day 4·25 (Kwong *et al.* 2000), exposure of the embryo to increased Hcyst concentration during the early stages of development alone may be sufficient for induction of an altered phenotype. The study reported in this issue by Langley-Evans *et al.* (2006) did not show a difference in maternal Hcyst concentration at gestational day 4, although there is a trend towards higher Hcyst concentration in the PR group, or at later time points. Thus there is a possible disagreement between these findings and those of Petrie *et al.* (2002) in early gestation. It is notable that Hcyst concentration in the study by Langley-Evans *et al.* (2006) is about 30 % higher in the control group than reported by Petrie *et al.* (2002), while the Hcyst concentration in the PR groups is similar. Such discrepancy can only be resolved by further studies. The absence of a difference in Hcyst concentration between the offspring of the control and PR groups at day 20 is consistent with the maternal data. However, if increased Hcyst exposure is involved in phenotypic induction, it would be important to measure Hcyst concentration in the early embryo.

DNA methyltransferases are responsible for establishing and maintaining patterns of DNA methylation (Bird, 2002). Langley-Evans *et al.* (2006) report trends in DNA methyltransferase 1 expression in fetal liver associated with maternal protein intake during pregnancy and the sex of the offspring, although the mechanistic link to Hcyst is not clear. Unfortunately, these experiments appear underpowered and so it is difficult to interpret the conflicting trends in DNA methyltransferase 1 expression.

Altered 1-carbon metabolism is strongly implicated in the mechanism by which different phenotypes are induced in the offspring in response to variations in maternal nutrition. However, until further investigations have been carried out it may be wise to reserve judgement on the precise role of Hcyst in fetal programming.

Graham C. Burdge
Developmental Origins of Health and Disease Research Division
Biomedical Sciences Building
University of Southampton
Bassett Crescent East
Southampton
SO16 7PX
UK
Fax +44 2380 594379
g.c.burdge@soton.ac.uk

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