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Iron deficiency during pregnancy: the consequences for placental function and fetal outcome

Harry J. McArdle*, Lorraine Gambling and Christine Kennedy
Rowett Institute of Nutrition and Health, University of Aberdeen, Greenburn Road, Aberdeen AB21 9SB, UK

This review examines the importance of the placenta in iron metabolism during development and the effect of iron deficiency on maternal and fetal physiology. Iron is an essential micronutrient, required for a wide variety of biological processes. During pregnancy, the mother has to deplete her iron stores in order to provide the baby with adequate amounts. *Trans*-placental iron transfer involves binding transferrin (Tf)-bound iron to the Tf receptor, uptake into an endosome, acidification, release of iron through divalent metal transporter 1, efflux across the basolateral membrane through ferroportin and oxidation of Fe(II) by zyklopen. An additional haem transport system has been hypothesised, which may explain why certain gene knockouts are not lethal for the developing fetus. Iron deficiency is a common phenomenon during pregnancy, and the placenta adapts by up-regulating its transfer systems, maintaining iron at the expense of the mother. Despite these adaptations, deficiency cannot be completely prevented, and the offspring suffers both short- and long-term consequences. Some of these, at least, may arise from decreased expression of genes involved in the cell cycle and altered expression of transcription factors, such as *c-myc*, which in turn can produce, for example, kidneys with reduced numbers of nephrons. The mechanism whereby these changes are induced is not certain, but may simply be as a result of the reduced availability of iron resulting in decreased enzyme activity. Since these changes are so significant, and because some of the changes are irreversible, we believe that iron prophylaxis should be considered in all pregnancies.

Hepcidin: Placental transport: Zyclopen: DMT1: Fetal programming

Iron deficiency is the most common nutritional deficiency in the world and pregnant women are especially vulnerable. The WHO have suggested that up to 70 % of women may be iron deficient, especially in developing countries. Our own work suggests that, even in a wealthy Western city such as Aberdeen, as many as 30 % of women can have limited iron stores[1]. During pregnancy, the developing fetus is entirely dependent on its mother for its nutritional requirements. All the iron delivered to the baby comes from either maternal iron stores, absorption of iron from the maternal diet or possibly turnover of maternal erythrocytes. Estimates vary, but each pregnancy requires at least 300 mg iron taken from the mother's liver stores[2], and others have proposed that the value is even higher, up to 500 mg. This is a significant consideration when it is suggested that only 20 % of women have this in reserve, and up to 40 % worldwide have no iron stores at all[3].

The consequences of iron deficiency during pregnancy are significant and widespread. Previously, it was thought that they were restricted to increased risk to the mother only, but more recently, work has shown clearly that the fetus and newborn infant will also suffer both short- and long-term consequences, especially in affecting brain function[4–6]. Some of these consequences are outlined in this review. We will also consider how iron is transferred from the mother to her baby, how this

Abbreviations:: DMT1, divalent metal transporter 1; Tf, transferrin; TfR, transferrin receptor.
***Corresponding author**: Professor H. J. McArdle, email h.mcardle@abdn.ac.uk

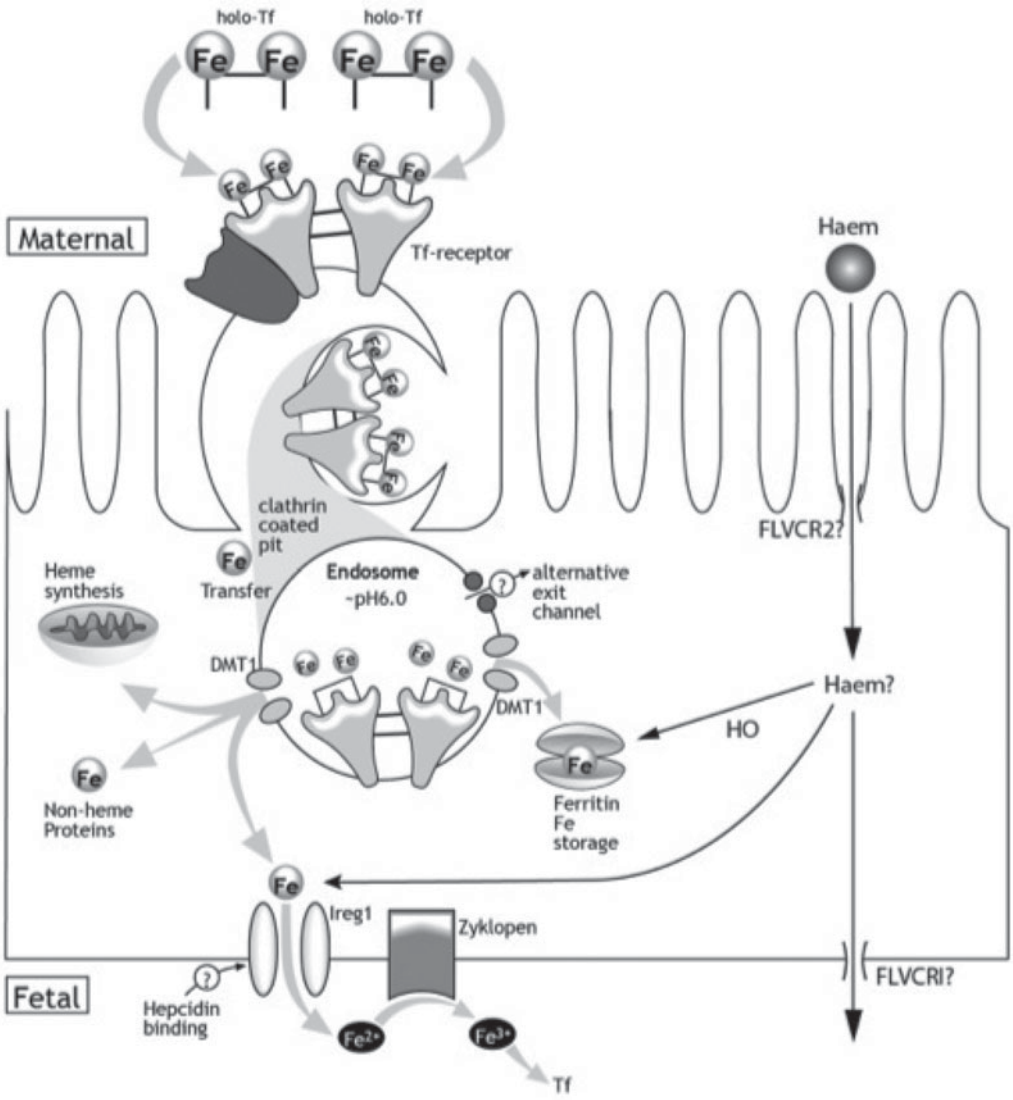

Fig. 1. A model for iron transport across the placenta. Two possible pathways are shown. On the left is the well-characterised model for transferrin-bound iron; on the right is a more speculative transport pathway for haem. The extent to which each may contribute to iron status of the infant is not known. DMT1, divalent metal transporter 1; Tf, transferrin; HO, haem oxidase; FVLCR1, feline leukaemia virus, subgroup C and receptor 1.

process is regulated and what the consequences are of maternal iron deficiency, both in the short and the long term.

Normal mechanisms of transfer of iron

Our present model for iron transfer across the placenta is given in Fig. 1. The overall process is the same for human subjects and rodents, although there may be small differences in detail, so that, unless there are specific points, information from the two species are given interchangeably. The first stage involves iron bound to transferrin (Tf) binding to the transferrin receptor (TfR) on the surface of the placenta, at the syncytiotrophoblast membrane. The Tf–TfR complexes are internalised into vesicles and the endosome is acidified. As the pH drops[7], the affinity of Tf for iron reduces, and the iron is released. It exits the endosome through divalent metal transporter 1 (DMT1)[8]. In most cells, lacking DMT1 means that they cannot use iron and when it is knocked out in the gut, it is a lethal mutation[9]. However, in the placenta, knocking out DMT1 is not lethal for the fetus. Pups are born alive, although somewhat anaemic[9]. Nancy Andrews, whose group demonstrated this, suggested that there could be another channel protein that might operate in the placenta. Znt14[10], or possibly Zip8 have been suggested as candidates[11]. However, as discussed later, it may also be the case that haem can substitute for Tf-bound iron in the DMT1 knockout mouse. Clearly, even in such well-studied phenomenon such as iron transfer from mother to baby, much still remains to be learned.

Following release into the cytoplasm, iron is transferred across the cell, by mechanisms that are not

understood, and is released into fetal circulation through a channel called ferroportin. It is oxidised to Fe(III) by zyklopen[12], which is further discussed later, incorporated into fetal Tf and carried to the fetal liver.

Zyklopen is one of a series of copper ferroxidases. The first to be identified was ceruloplasmin, a serum protein. There has been considerable controversy whether ceruloplasmin has a function as a ferroxidase, oxidising Fe(II) stored in the liver to Fe(III) for incorporation into Tf or whether its main function is actually as a copper transporter[13]. In support of the latter hypothesis, possible receptors have been identified in tissues such as placenta[14] and copper from ceruloplasmin can be donated to the fetus in pregnancy[13]. However, it is not essential for this function, because individuals who carry a mutation that results in absence of ceruloplasmin do not show symptoms of copper deficiency. They do, in contrast, show problems associated with iron status, confirming its role in the metabolism of iron.

Three other ferroxidases have been identified. There is a glycosyl phosphatidylinositol-anchored form of ceruloplasmin, found in brain[15], and another called hephaestin, which fulfils the oxidising function in the gut[16]. At the time this was identified, the human genome sequence was completed and from that sequence, a further form of ferroxidase was predicted. We had identified a ferroxidase in placenta which was not ceruloplasmin or its glycosyl phosphatidylinositol-anchored form, or indeed hephaestin[17], and instead was a new form, which we called zyklopen[12].

As mentioned earlier, data recently reviewed also suggest that haem iron may also be a significant source of iron for the baby[18]. Several species of placental mammals can phagocytose and digest maternal erythrocytes and the haem iron is released into fetal circulation (see[19] for a comprehensive review), and O'Brien and her co-worker suggest that the human placenta can also use haem iron. In a recent review[18], they muster the arguments, by suggesting that all the genes and proteins required to take up, and possibly release, haem into fetal circulation, such as the feline leukaemia virus, subgroup C and receptor 1 (FVLCR1), are present at high levels in the placenta. They go further, and suggest that the dogma, that haem iron is digested to inorganic iron in the enterocyte, may not be correct, and that haem in the diet may actually be absorbed as haem iron[18]. While this is yet to be determined, it does open up some very interesting prospects, and certainly there is enough circumstantial evidence to provide support to their contention. If haem iron is, in reality, a significant source of iron for the developing fetus, then the relatively mild effect of the DMT1 knockout mouse may be explained (*FLVCR1* genes are expressed in mouse). More importantly, it may also mean that our calculations of iron requirements during pregnancy may be no longer accurate.

Consequences of deficiency

Several groups have shown that prenatal iron deficiency results in marked and persistent changes in the offspring[20]. Most of these experiments have used rodents, but more recently, studies in human subjects have shown that changes in the cognitive function of babies can be related to maternal iron status[4,21,22].

In rats, iron deficiency during pregnancy results in high blood pressure, changes in lipid metabolism and obesity in the offspring. In female offspring, the prenatal treatment induces a lower systolic pressure before puberty, but afterwards, the results mirror those of the male offspring[23]. The different effects on females have not been followed up, but more recent evidence suggests that there are important differences in the prenatal programming consequences for male and female offspring in response to a variety of nutritional stresses[24].

Trying to understand the mechanisms involved in the increase in blood pressure in offspring born to iron-deficient dams has led to a series of papers. We first tried to identify when, during pregnancy, the developing fetus was most vulnerable to iron deficiency. Studies in the Dutch Hunger Winter, when German occupying troops reduced rations to the civilian population living in the west of the Netherlands, showed that women whose nutritional intake was decreased during the first trimester gave birth to babies that were more likely to develop hypertension, show impaired insulin secretion and a wide variety of serious illnesses later on in life. In contrast, those whose babies were given diminished rations in the third trimester only had an increased risk of developing hypertension and impaired glucose tolerance (reviewed in a commentary by Schulz[25] and de Rooij *et al.*[26]).

In our own studies, we reduced iron supply to pregnant rats either during the first half, the second half or the whole of pregnancy. In parallel, we used an *in vitro* culture system to assess if restoring the nutritional status could reverse the effects. We found that iron deficiency in the first half of pregnancy had a more severe and less reversible effect than in the latter[27]. The direct effects of iron deficiency: lower haematocrit, increased TfR expression, etc, were reversible, but reduced birth weight and the expression changes in expression of some genes were not. In the *in vitro* system, we cultured rat embryos from embryonic day 10·5 to embryonic day 12·5[28]. We took rat embryos from deficient dams and cultured them either in control serum or serum taken from deficient rats. Those grown in the deficient medium showed reduced development of their circulation, slowed cardiovascular development and enlarged hearts. These changes could be reversed when embryos taken from deficient dams were grown in control serum. These data suggest quite strongly that the period between embryonic days 10·5 and 12·5, during which circulatory development takes place, is at least one of the critical windows[28].

Regulation of iron status during pregnancy

Iron has the potential to be a toxic element in physiology. It has the capacity to generate free radicals, which can, in turn, cause lipid peroxidation and protein and DNA damage. To reduce the risk of this occurring, a series

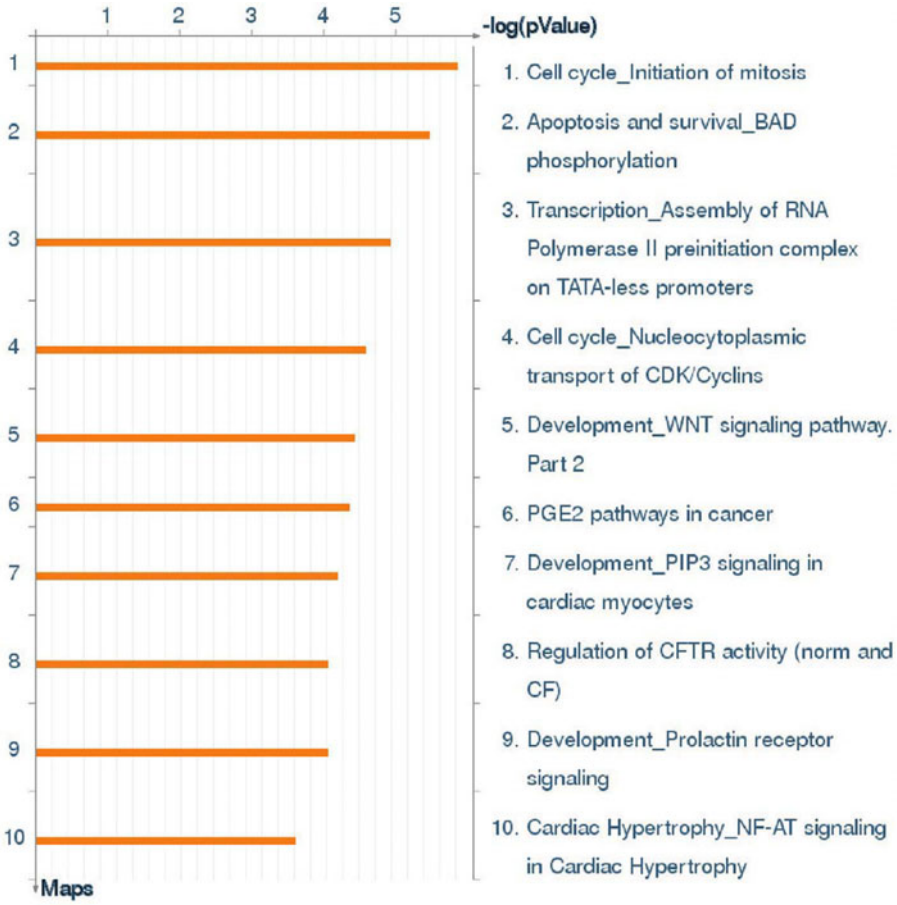

Fig. 2. (Colour online). Gene ontology pathways affected by maternal iron deficiency in the Rowett Hooded Lister rat. These are the pathways that show the greatest degree of change in embryos of iron deficient mothers compared with those of control rats. The processes are ranked according to the extent of the difference (expressed as negative log). Taken from Swali *et al.* [36]). BAD, Bcl2 associated death promotor; TATA, a DNA sequence recognised by transcription factors; CDK, cyclin dependent kinase; PIP3, phosphoinositol tris phosphate; CFTR, cystic fibrosis transmembrane conductance regulator; CF, cystic fibrosis; NF-AT, nuclear factor of activated T cells.

of regulatory mechanisms are important. However, it is also an essential element, and iron deficiency is much more common than iron overload. During pregnancy, mechanisms have evolved ensuring adequate iron status for the developing fetus, even if this occurs to the detriment of the mother[29].

Iron deficiency causes an increase in percentage of iron transfer from mother to fetus. In early studies, we showed that reduction of iron in the diet before and during pregnancy resulted in a drop of about 75 % in maternal iron levels < 50 % in her fetuses. As a consequence of the deficiency, TfR levels were up-regulated in the placenta[30]. This results in increased iron transfer and a reduction in the severity of deficiency in the offspring. We have attempted to elucidate the regulatory pathway using dietary treatment. In summary, we demonstrated that the primary regulator of fetal iron status is fetal liver hepcidin[30].

Hepcidin is a low-molecular-weight protein, originally identified as an anti-microbial protein[31]. It is now recognised as a major regulator of iron status in non-pregnancy. Increasing hepcidin in serum reduces iron absorption, so it is a negative regulator of absorption. Its mechanism of action has been partially elucidated. Hepcidin binds to ferroportin, the iron efflux channel protein, and the complex is internalised, ubiquitinylated and subsequently degraded[32].

We showed a very tight correlation between fetal hepcidin levels and maternal liver iron levels[30]. Interestingly, this was much more significant than the correlation between maternal iron status and maternal hepcidin, at least until a critical level of iron was reached. When the maternal iron stores began to drop below this level, the mechanisms were activated to try and restore the status[30]. This involves maternal hepcidin levels decreasing and TfR levels increasing in the maternal liver. In turn, this results in an amelioration of the deficiency, protecting the liver against the effects of very severe deficiency on the mother[30].

Table 1. Genes most affected by maternal iron deficiency in 12·5 d embryos of Wistar and Rowett Hooded Lister (RHL) rats. The results are the mean of eight embryos taken from dams fed control or iron deficient diets as described in Gambling *et al.*[30] and Swali *et al.* [36]

Probe set ID	Gene symbol	Gene name	Wistar		RHL	
			P-value	Fold-change	*P*-value	Fold-change
1369928_PM_at	*Acta1*	Actin, alpha skeletal muscle	0·02	1·21	0·03	1·16
1372624_PM_at	*Ano6*	Anoctamin 6	0·04	1·11	0·03	1·09
1390005_PM_at	*Asxl2*		0·04	1·06	0·02	1·09
1385414_PM_at	*Cd8a*	Cluster of differentiation 8a	0·03	0·87	0·04	0·92
1370235_PM_at	*Dbi*	Diazepam-binding inhibitor	0·02	0·85	0·04	0·92
1374219_PM_at	*Disp1*	Dispatched homologue 1	0·04	0·84	0·04	0·88
1388449_PM_at	*Eef1b2*	Elongation factor 1-beta 2	0·04	0·96	0·04	0·98
1388297_PM_at	*Eef1g*	Elongation factor 1-gamma	0·01	1·04	0·02	1·05
1368078_PM_at	*Esm1*	Endothelial cell-specific molecule 1	0·04	0·81	0·01	0·86
1374699_PM_at	*Fam84a*	Family with sequence similarity 84, member A	0·04	1·27	0·01	1·17
1373499_PM_at	*Gas5*	Growth arrest-specific 5	0·04	0·85	0·04	0·89
1377577_PM_at	*Gmps*	Guanine monophosphate synthetase	0·04	0·91	0·02	0·93
1377624_PM_at	*Gtpbp10*	GTP-binding protein 10	0·04	0·81	0·00	0·79
1398932_PM_at	*Hint1*	Histidine triad nucleotide-binding protein 1	0·03	0·93	0·01	0·96
1370904_PM_at	*Hla-dma*	Major histocompatibility complex, class II, DM alpha	0·03	1·29	0·02	1·30
1377182_PM_at	*Itgb3bp*	Integrin beta 3 binding protein	0·03	0·78	0·01	0·84
1374912_PM_at	*Kif2c*	Kinesin family member 2C	0·04	0·91	0·05	0·93
1369637_PM_at	*Kif3c*	Kinesin family member 3C	0·01	1·22	0·02	1·19
1391595_PM_at	*Larp6*	La ribonucleoprotein domain family, member 6	0·04	1·12	0·05	1·10
1397471_PM_at	*Mical2*	Microtubule associated monoxygenase, calponin and LIM domain containing 2	0·02	1·18	0·02	1·08
1393085_PM_at	*Mitd1*	Microtubule interacting and transport, domain containing 1	0·03	0·76	0·01	0·79
1372808_PM_at	*Mthfd2*	Methylenetetrahydrofolate dehydrogenase 2	0·05	0·88	0·02	0·93
1372093_PM_at	*Mxi1*	MAX interactor 1	0·04	1·11	0·04	1·09
1374792_PM_at	*Nol7*	Nucleolar protein 7	0·04	0·89	0·03	0·90
1372172_PM_at	*Nphp1*	Nephronophthisis 1	0·04	0·89	0·02	0·88
1388340_PM_at	*Ns5atp9*	NS5A-transactivated protein 9 homologue	0·02	0·88	0·01	0·91
1387225_PM_at	*Opa1*	Optic atrophy 1	0·05	0·92	0·01	0·90
1380071_PM_at	*Parp12*	Poly (ADP-ribose) polymerase 12	0·00	1·18	0·03	1·13
1376025_PM_at	*Prmt2*	Protein arginine methyltransferase 2	0·05	1·13	0·03	1·21
1393081_PM_at	*Rasgef1a*	RasGEF domain family, member 1A	0·04	0·89	0·02	0·90
1389065_PM_at	*Rbm34*	RNA-binding motif protein 34	0·01	0·92	0·05	0·87
1378227_PM_at	*Rbpms2*	RNA-binding protein with multiple splicing 2	0·01	1·10	0·01	1·14
1371638_PM_at	*Rnf7*	Ring finger protein 7	0·05	0·92	0·04	0·94
1371295_PM_at	*Rps20*	Ribosomal protein S20	0·05	0·94	0·03	0·94
1374738_PM_at	*Sdccag10*	Serologically defined colon cancer antigen 10	0·01	0·84	0·03	0·89
1370440_PM_at	*Slc15a4*	Solute carrier family 15, member 4	0·05	1·16	0·02	1·20
1368991_PM_at	*Smpd3*	Sphingomyelin phosphodiesterase 3	0·04	1·16	0·04	1·16
1372167_PM_at	*Snrpg*	Small nuclear ribonucleoprotein polypeptide G	0·02	0·87	0·01	0·89
1370192_PM_at	*Stx12*	Syntaxin 12	0·03	1·12	0·05	1·08
1371679_PM_at	*Synpo2*	Synaptopodin 2	0·02	1·18	0·02	1·14
1374177_PM_at	*Taf13*	TATA-box-binding protein – associated factor	0·04	0·87	0·01	0·92
1390237_PM_at	*Timm8a1*	Translocase of inner mitochondrial membrane 8 homologue a1	0·01	0·82	0·01	0·88
1376719_PM_at	*Tmem38b*	Transmembrane protein 38B	0·04	0·84	0·05	0·92
1398870_PM_at	*Tomm20*	Translocase of outer mitochondrial membrane 20 homologue	0·03	0·90	0·00	0·93
1370238_PM_at	*Usmg5*	Up-regulated during skeletal muscle growth 5 homologue	0·04	0·90	0·02	0·91
1371249_PM_at	*Xbp1*	X-box-binding protein 1	0·01	1·18	0·04	1·27
1386512_PM_at	*Zfp91*	Zinc finger protein 91 homologue	0·03	0·94	0·02	0·90

The effect of iron deficiency on gene expression

As discussed earlier, iron deficiency during pregnancy results in symptoms of metabolic syndrome in rats, with increased blood pressure, obesity and changed lipid metabolism in the offspring[23]. These results mimic those seen in rodents fed a low-protein diet[33], and the similarities led us to the hypothesis that there may be common pathways or gene changes affected by disparate nutritional stresses, which we called the

gatekeeper hypothesis[34]. To test this hypothesis, we carried out a series of high throughput experiments, comparing the genes and pathways changed in two different strains of rats given two dietary treatments; low protein or low iron. We considered that this approach would identify common genes or pathways that could be implicated in the development of post-natal hypertension. It also gave us the opportunity to identify genes that were altered in iron deficiency and to see if the changes were strain specific or shared between species of rat.

The first part of the experiment demonstrated that nephron number was decreased in fetuses of both strains of rat where the mother was given either deficient diet[35]. Extensive electron microscopy analysis showed no changes in the kidney structure, implying that only nephrogenesis was compromised.

In order to test this hypothesis further, we examined gene expression at the early stages of nephrogenesis. We isolated male embryos at 13·5 d of gestation. We conducted microarray and proteomics analyses to test whether there were genes or pathways affected that are common to both nutritional stresses. It is beyond the scope of this review to consider further the gatekeeper hypothesis and more details can be found in the paper published in 2011[35]. Instead, we will concentrate on the changes induced in iron deficiency, which were also examined more closely in a companion paper[36].

In the iron-deficient animals, 979 genes were significantly up-regulated and 1545 were down-regulated. The magnitude of change was not large, but the variation between embryos was small, so that the statistics were rigorous. The data were then analysed in terms of the gene pathways that may be expected to be affected by iron deficiency. We also conducted proteomics analyses, to identify non-transcriptionally mediated alterations. The results are presented in the supplementary material for the 2011 paper published by our groups[35,36].

In general, pathways identified as altered by iron deficiency were associated with down-regulation of gene expression (Fig. 2). Top of the list were genes associated with the cell cycle, such as initiation of mitosis[36]. A list of the genes most altered in their expression is given in Table 1. Consistent with the pathway analysis, the genes had a range of functions, including regulation of cell cycle, cell growth and proliferation and cancer development. We also demonstrated changes in transcription factors such as *c-myc*, which decreased in expression and *p53*, which increased in expression. These data are consistent with others, who showed that iron loading increased *c-myc* and decreased *p53*.

The data also show marked increases in expression of *SOX4* and *Map1b*. These genes are involved in regulation of embryo development and the up-regulation may result in increased apoptosis. *Map1b* is important in microtubule assembly, which is critical for correct operation of the cell cycle. These results support our idea that the decreased number of nephrons found in the kidney of offspring born to iron deficient dams are due to a decrease in the cell cycle and hence a reduced number of cells. Why this occurs is not clear. Possibilities include changes in methylation patterns and epigenetics, but may also be as simple as reduced enzyme activity because of a decrease in iron supply.

Summary and conclusions

In this short review, we have examined the effects of iron deficiency on pregnancy outcome and consequences. We have only selected parts of a very large literature, but trust that we have managed to demonstrate the central role that iron plays in growth and development. We have also, in our more recent work, identified that there are still questions to be answered, especially whether the changes in pathways that we have shown in our high-throughput experiments are of real concern, or whether the developing organism has the capacity to compensate, either at the time of stress or later, in an adaptive process.

One important aspect that has not been covered relates to tissue specificity. We have not yet shown whether the changes in gene expression occur in all tissues or only in those undergoing growth and differentiation at the time of the nutritional stress. This is of particular importance when considering the Scientific advisory committee on nutrition (SACN) recommendations for iron prophylaxis. At the moment, SACN considers that prophylactic treatment with iron during pregnancy is not required. Whether this decision needs to be re-examined or not is still open to debate. The recent work demonstrating that deficiency alters cognitive function certainly suggests that consideration should be given to the option. Demonstrating that the changes occurring as a result of iron deficiency are irreversible will certainly encourage this re-examination.

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Conflicts of Interest

None.

Authorship

H. J. McA. wrote the initial draft. L. G. and C. K. provided corrections and comments and H. J. McA. wrote the final draft and revised the paper.

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