Modulation of macronutrient metabolism in the offspring by maternal micronutrient deficiency in experimental animals


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Epidemiological evidence indicates that poor early growth is associated with increased susceptibility to visceral obesity, insulin resistance and associated diseases in adulthood. Studies in experimental animals have demonstrated a robust association between nutrient imbalance during foetal life and disease prevalence in their later life specially of those involving macronutrient metabolism. There is very little data on the role of maternal micronutrient deficiencies widely prevalent in India. This review focuses on different animal models of micronutrient restriction, mimicking human situations during pregnancy and lactation that cause aberrations in macronutrient metabolism in the offspring. These aberrations consist of altered body composition, dyslipidaemia and altered insulin sensitivity associated with modulated insulin production. These phenotypic changes were associated with altered lipid profile, fatty acid synthesis / transport, oxidative stress, glucose tolerance / tissue uptake. Further, these were also associated with altered myogenesis and insulin expression and secretion from pancreatic β-islets. While these changes during in utero or early postnatal life serve as essential adaptations to overcome adverse conditions, these become maladaptive subsequently and set the stage for obesity and type 2 diabetes.

Key words Diabetes - insulin resistance - maternal undernutrition - metabolism - micronutrients - minerals - obesity - vitamins

Exposure of the foetus to maternal malnutrition results in intrauterine growth retardation. Intrauterine and early post-natal malnutrition has profound consequences on foetal and post-natal development both in humans and animals. Ample epidemiological evidence exists to show an association between coronary heart disease, hypertension, non-insulin dependent diabetes and impaired glucose tolerance in adults on one hand and low birth weight and altered foetal growth on the other. Diabetes mellitus has become one of the main threats to human health in the 21st century. The risk of diabetic complications commences many years before the onset of clinical diabetes and this includes adiposity and insulin resistance (IR). Neither the mechanistic basis of these common underlying features nor their causes are completely understood. Nevertheless, based on the evidences available it has been hypothesized that intrauterine growth retardation affecting foetal development predisposes the offspring to IR / diabetes in later life.

Of the various factors in utero that influence diseases in adult life, maternal undernutrition is an
important one. By modulating the availability of nutrients for transfer to the foetus, maternal nutrition affects foetal growth and development directly. It may also alter glucose / insulin metabolism permanently. Further, it has varied effects if it occurs at different times in early life. However, studies carried out so far in this area have demonstrated the role of macronutrients such as carbohydrates, proteins and fats (specically the long chain unsaturated fatty acids) in developmental programming for adult onset diseases.

It is well known that micronutrients play an important role in determining the structure and metabolism of the organism. Micronutrient deficiencies, which are common among populations, specially in developing countries, have important public health implications and need to be given immediate attention. Several studies indicate that micronutrients such as vitamins C and E, trace elements like Mg, Cr, Cu, Fe, vanadium, Mn, Zn and Se, and the amino acid arginine (Arg) influence insulin at different levels (biosynthesis, storage, release and ultimate function). For example, Arg derived nitric oxide increases nutrient stimulated insulin secretion. Moreover, deficiencies of these micronutrients have profound and often persistent effects on many foetal tissues and organs, even in the absence of any clinical signs of deficiency in the mother. Further, the consequences of an imbalance of minerals and vitamins during foetal development may not be apparent at the time of the nutritional insult, but may manifest later during development. Multiple micronutrient deficiencies, particularly during pregnancy and / or lactation are common in the developing world and these deficiencies are associated with low birth weight and increased rates of perinatal mortality and morbidity. Further, in developing countries, the prevalence of low birth weight varies from 13 to 30 per cent.

This review highlights the evidence that maternal micronutrient imbalances in utero and during early postnatal life modulate macronutrient metabolism in the offspring which may make a significant contribution to the obesity and IR epidemic.

The animal models

Different species such as rat, mouse, sheep, pig, rabbit, guinea pig and horse have been used as models for foetal growth restriction, although a majority of experiments are in rats and mice. In view of the shorter life span of laboratory animals and because genetic and environmental influences can be controlled in them, substantial efforts in recent years have centered upon the establishment of animal models for developmental programming which have relevance to the human condition. The experiments have been conducted either by implementing 50 per cent restriction of all minerals / vitamins in the mothers’ diet or total removal of the particular micronutrient (e.g., Mg, Zn, Cr, Mn, Fe, folate and vit B12) from the mineral / vitamin mixture of the diet appropriately. Fig. 1 gives the schematic representation of the feeding protocol used in all experiments. Similarities in the outcomes between these animal models and those encountered in human studies of the metabolic syndrome or type 2 diabetes mellitus are alterations in body weight, body composition and insulin sensitivity. In this review, we shall examine that some of the disorders which together define the metabolic syndrome may be “developmentally programmed” in the offspring subjected to suboptimal maternal micronutrient nutrition.

**Effects of maternal micronutrient restriction on the growth of the offspring**

Earlier literature indicates that micronutrient deficiencies during pregnancy and lactation affect the growth and development of the offspring. We also observed that maternal mineral (only Zn) restriction significantly decreased the birth weight and bodyweight of the offspring at later time points. Although

Fig. 1. Schematic representation of the feeding protocol of different groups of mothers and the offspring. C, control diet throughout; RC, restricted mothers fed control diet from conception and to offspring of such mothers from weaning; RP, restricted mothers fed control diet from parturition and to pups of such mothers from weaning; RW, restricted offspring weaned on to control diet; MNRD, micronutrient restricted diet throughout.
maternal Mg restriction (MgR) did not affect the birth weight of the offspring, when continued postnatally through lactation and weaning, it decreased the body weight of the offspring at weaning and thereafter. Maternal Cr restriction (CrR) had no effect on body weights of the offspring at any time point studied whereas maternal Mn restriction increased offspring's body weight. On the other hand, vitamin restricted offspring weighed lower than controls albeit at weaning but not later. Although maternal folate and/or vitamin B12 deficiencies did not affect the birth weight of the offspring, these increased their body weight by weaning and the increase persisted throughout their life (unpublished observations).

Effects of maternal micronutrient restriction on body composition of the offspring

Adipogenesis, which begins in utero and accelerates in neonatal life, is a prime candidate for developmental programming. Abundant literature indicates that altered body adiposity and lipid metabolism are the earliest changes seen, much before tissue insulin resistance manifests. Indeed, insulin resistance has been hypothesized to originate in impaired adipogenesis and/or lipid metabolism.

We observed that chronic 50 per cent restriction of all vitamins/minerals from the mothers' diet significantly increased body fat per cent and plasma triglycerides in the offspring. Similarly maternal Mg, Mn, Cr, Zn, folic acid and/or vitamin B12 restriction also increased body fat per cent of the offspring significantly (Table I). However, the time point at which the increased body fat per cent was seen in the offspring was different among the micronutrient deficiencies studied. It was interesting that regardless of the micronutrient which was deficient in the diet, the increased body fat per cent in the offspring was associated with significantly higher central adiposity (Table I). Considering the significance of central adiposity in predisposing individuals to insulin resistance and associated diseases in later life, it appears from our results that maternal vitamin/mineral/trace element deficiency may predispose the offspring to IR and associated diseases in their later life. It was of interest that the effects of maternal Cr or Mn restriction were corrected by different rehabilitation regimens while those of Mg and Zn restriction appeared to be irreversible.

Body fat per cent increases normally as a compensation for decrease in the mass of muscle, an insulin sensitive tissue important in postprandial glucose clearance. Maternal restriction of all minerals/trace elements/vitamins persistently decreased lean body mass (LBM) and fat free mass (FFM) (representing muscle and bone mass) in the offspring (Table I). Indeed skeletal muscle fibres are formed prenatally and the total number of fibers and/or relative proportions of different fiber types are largely determined during foetal and early postnatal development. Recently, 50 per cent maternal undernutrition has been shown to decrease total myofibre number and increase fast myosin type Ib isoform in the longissimus dorsi of sheep offspring, whereas a similar challenge decreased the proportion of fast-twitch myofibres in the vastus lateralis of 14 day old sheep. A periconceptional nutrient restriction

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Table I. Risk factors of metabolic syndrome in the offspring from dietary micronutrient restricted models of developmental programming in the rat

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Parameter measured and outcome</th>
<th>Abnormal result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiposity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50% mineral restriction</td>
<td>Fat%↑, LBM ↓, FFM ↓</td>
<td>Yes Male</td>
</tr>
<tr>
<td>50% vitamin restriction</td>
<td>Fat%↑, LBM ↓, FFM ↓</td>
<td>Yes Male</td>
</tr>
<tr>
<td>Mg restriction</td>
<td>Fat%↑, LBM ↓, FFM ↓, Adiposity index↑</td>
<td>Yes Male</td>
</tr>
<tr>
<td>Cr restriction</td>
<td>Fat%↑, LBM % ↓, FFM % ↓, Adiposity index↑</td>
<td>Yes M + F</td>
</tr>
<tr>
<td>Zn restriction</td>
<td>Fat%↑, LBM ↓, FFM ↓</td>
<td>Yes Male</td>
</tr>
<tr>
<td>Mn restriction</td>
<td>Fat% ↔, LBM % ↔, FFM % ↔, Adiposity index↑</td>
<td>No M + F</td>
</tr>
<tr>
<td>Folate restriction</td>
<td>Fat%↑, LBM % ↓, FFM % ↓, Adiposity index↑</td>
<td>Yes Male</td>
</tr>
<tr>
<td>Adipocytokines</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50% mineral restriction</td>
<td>Leptin ↓, Adiponectin ↓, TNF-α ↔</td>
<td>Yes Male</td>
</tr>
<tr>
<td>50% vitamin restriction</td>
<td>Leptin ↑, Adiponectin ↓, TNF-α ↔</td>
<td>Yes Male</td>
</tr>
<tr>
<td>Mg restriction</td>
<td>Leptin ↑, Adiponectin ↓, TNF-α ↔</td>
<td>Yes Male</td>
</tr>
<tr>
<td>Cr restriction</td>
<td>Leptin ↑, Adiponectin ↑, TNF-α ↑</td>
<td>Yes M + F</td>
</tr>
</tbody>
</table>

LBM, Lean body mass; FFM, fat free mass; TNF-α, tumour necrosis factor-α

↓decreased; ↑ increased; ↔ no change

Source: Ref. 22-27
(~50%) decreased total myofibre number in the foetal semitendinosus muscle\textsuperscript{27}. Our observations in the offspring of Cr restricted dams\textsuperscript{25} of decreased expression of myogenic genes / transcription factors: Pax3, MyoD, Myf5 and Myog were similar to those reported in literature and probably suggest that decreased muscle growth could be a causative factor for reduced LBM and FFM in them. Given that muscle represents the major site of postprandial glucose disposal, it is not surprising that changes in the mass, type, growth patterns, and functional characteristics of the muscle fibers during the perinatal period are important in the programming of insulin sensitivity and diabetes.

It is thus apparent that maternal micronutrient restriction during pregnancy and / or lactation altered the body composition of the offspring in a way suggestive of their predisposal to insulin resistance and associated diseases in their later life. That the phenotypic changes but not the changes in gene expression of the offspring were in general irreversible (except Cr and Mn restriction) by rehabilitation from as early as birth reiterate the importance of maternal micronutrient status in determining the body composition of the offspring and hence their predisposal to adult onset diseases.

Effects on the function of adipose and muscle tissues in offspring

Adipose tissue is now recognized not simply a store of excess energy but a major endocrine and secretory organ, releasing a wide range of protein factors and signals, termed adipokines, in addition to fatty acids and other lipid moiities. Adipokines are known to modulate not only adipose tissue function / lipid metabolism but also insulin sensitivity / resistance\textsuperscript{38}. We assessed the alterations if any, in the function of adipose tissue by determining the biochemical changes (plasma and tissue levels of adipokines) associated with increased body adiposity and observed that increased body fat per cent in vitamin restricted (VR) / mineral restricted (MR) offspring was associated with a significant decrease in the plasma levels of adiponectin\textsuperscript{39} (Table I). A simultaneous increase in leptin levels in VR offspring was similar to earlier reports\textsuperscript{40} and corroborated with the increased body fat per cent observed in them (Table I). However, our observation that hypoleptinaemia was associated with high body fat per cent in mineral restricted (MR) and MgR offspring (Table I) was at variance with many earlier studies demonstrating an association between high plasma leptin levels and high percentage of body fat\textsuperscript{40}. Further studies are needed to delineate the role if any, of hypoleptinaemia in maternal MR induced increase in body fat per cent in the offspring. Interestingly, hypoleptinaemia observed by us was in line with leptin deficiency reported in the genetically obese rodent models\textsuperscript{41,42} and in type 1 and 2 diabetic patients\textsuperscript{43}. Further, the changes in adipokine levels in circulation were mitigated only in offspring rehabilitated from parturition but not from weaning, probably confirming the importance of vitamin and mineral nutrition during lactation in modulating these changes. Maternal Cr restriction on the other hand appeared to modulate the tissue (adipose) adipokine levels (e.g., adiponectin, leptin, TNF-\alpha) in only male but not in female offspring (Table I), whereas plasma adipokine levels were affected variably both in male and female offspring. Also the effects of rehabilitation on the different adipokine levels were variable among male and female offspring. Although both maternal vitamin and mineral (trace element) restrictions increased body adiposity in the offspring, it is interesting to note that the probable mechanism(s) leading to adiposity and the associated functional changes may differ at least to some extent.

Regarding the effects of maternal micronutrient restriction on the muscle function, it was observed that although the fold stimulation by insulin was not affected by maternal MgR, basal glucose uptake by muscle (diaphragm) was significantly and irreversibly decreased in MgR offspring\textsuperscript{24} (Table II). It was increased in CrR offspring and the increase was corrected by rehabilitation in only male but not in female offspring\textsuperscript{25} (Table II). Taken together with decreased muscle mass (LBM and FFM) in the offspring, these results suggest an altered basal capacity of the muscle to clear glucose and its differential sensitivity (between male and female offspring) to reversal / mitigation by rehabilitation. Maternal Mn restriction on the other hand had diverse effects on basal glucose uptake by muscle in male and female offspring although it decreased insulin stimulated uptake in both (unpublished observations) (Table II). Not withstanding these observations maternal Mn restriction increased both basal and insulin stimulated glucose uptake by muscle in MnR offspring chronically fed high fat diet. Also the correction of changes by rehabilitation was variable among the two sexes. Overall, it appears that maternal micronutrient restriction affects muscle mass as well as its function (basal glucose uptake) in addition to modulating their susceptibility to other nutritional insults (e.g., high fat...
feeding) and may predispose the offspring to develop hyperglycaemia in later life.

Thus our results appear to indicate that maternal micronutrient restriction not only affects the body composition (proportion of muscle and fat) of the offspring but also the function of these two important insulin sensitive tissues.

**Effects on macronutrient metabolism in the offspring: carbohydrate metabolism**

In general, maternal vitamin or mineral restriction per se or their postnatal continuation had no discernable effect on fasting plasma glucose and insulin levels in the offspring till six months of their age. Interestingly fasting plasma insulin levels and homeostasis model assessment of insulin resistance (HOMA-IR) index were significantly higher in 6 months old MgR offspring, whereas their insulin secretion to a glucose challenge [area under curve of insulin (AUC Insulin)] was significantly and irreversibly decreased\(^{23,24}\) (Table II). On the other hand, all these parameters were decreased almost irreversibly in ZnR offspring\(^{22}\) (Table II). Maternal Cr restriction increased fasting plasma glucose, insulin and AUC of glucose and insulin during glucose tolerance test (GTT) albeit the effects were seen late in their life and some of them were of transient nature\(^{25}\) (Table II). The changes were alleviated partly by rehabilitation. In line with consistent fasting hyperglycaemia moderate increases were observed in the expression of phosphoenolpyruvate carboxykinase (PEPCK) in liver suggestive of increased gluconeogenesis (unpublished results). On the other hand, maternal Mn restriction resulted in consistent, fasting hyperglycaemia along with hypoinsulinaemia in the offspring\(^{26,27}\) (Table II). Not withstanding these effects maternal Mn restriction made the offspring susceptible to impaired glucose metabolism when fed high fat diet in their later life, as evident from the increases observed in AUC of glucose and insulin. Thus it is evident that maternal micronutrient restriction and its postnatal continuation modulated the insulin secreting capacity of offspring to a glucose challenge. While the changes in these parameters due to maternal Mn restrictions were alleviated by rehabilitation in general, those due to maternal Mg and Cr restrictions appeared irreversible or partly reversible. These results (except in CrR offspring) are in general agreement with earlier literature, which showed that maternal protein deficiency decreased glucose stimulated insulin secretion in the offspring\(^{44-46}\) and this could either be due to the possible exhaustion of β cells or a reduction in β cell numbers /mass similar to those seen in type 2 diabetes\(^{47}\).

**Effects on lipid metabolism**

Hormones affecting fat/glucose metabolism modulate the activities of several enzymes and proteins involved in lipogenesis /lipid transport\(^{48-50}\) such as fatty acid synthase (FAS) and fatty acid transport proteins (FATPs). The FAS and FATP 1 have been shown to be expressed variably under obese conditions. In our study also expression of fatty acid synthase (FAS), was significantly increased in the liver and adipose tissue of MgR offspring at 6 and 18 months of age and so was the expression of FATP1\(^{24}\). It appears that maternal micronutrient restriction probably increased fatty acid synthesis and transport, which in turn may be responsible for the increase in body fat per cent in the offspring.

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Parameter measured and outcome</th>
<th>Abnormal result</th>
</tr>
</thead>
<tbody>
<tr>
<td>50% mineral restriction</td>
<td>GTT insulin secretion ↓</td>
<td>Yes Male</td>
</tr>
<tr>
<td>50% vitamin restriction</td>
<td>GTT insulin secretion ↔</td>
<td>No Male</td>
</tr>
<tr>
<td>Mg restriction</td>
<td>Plasma insulin↑; GTT insulin secretion↓; Glucose uptake ↓</td>
<td>Yes Male</td>
</tr>
<tr>
<td>Cr restriction</td>
<td>Plasma glucose↑; Plasma insulin↑; Glucose intolerance ↑; insulin secretion ↑; glucose uptake↑</td>
<td>Yes M + F</td>
</tr>
<tr>
<td>Zn restriction</td>
<td>Plasma insulin↑ GTT insulin secretion↓</td>
<td>Yes F</td>
</tr>
<tr>
<td>Mn restriction</td>
<td>Plasma insulin↑; Plasma glucose↑; GTT insulin secretion↓; Glucose intolerance ↑; glucose uptake ↓</td>
<td>Yes Male</td>
</tr>
<tr>
<td>Folate restriction</td>
<td>Plasma glucose↑; Plasma insulin↑; Glucose intolerance ↑; insulin secretion ↑</td>
<td>Yes Male</td>
</tr>
<tr>
<td>B12 restriction</td>
<td>Plasma glucose↑; Plasma insulin↑; Glucose intolerance ↑; insulin secretion ↑</td>
<td>Yes Male</td>
</tr>
</tbody>
</table>

GTT, glucose tolerance test

*Source: Ref. 24-27*
Plasma total cholesterol, high-density lipoprotein cholesterol, free fatty acids, and triglycerides (TG) were comparable among the offspring of MgR and control (MgC) groups at 18 months of age and rehabilitation had no effect as such24 (Table III). On the other hand, in the male offspring of Cr restricted rat dams, plasma lipid profile was in general comparable among groups, whereas CrR female offspring had higher plasma TGs and free fatty acid levels than controls25 (Table III). The changes were alleviated by rehabilitation. While the changes in plasma lipid profile were inconsistent in ZnR offspring22, maternal Mn restriction increased plasma total cholesterol in the offspring of both sexes but had diverse effects on their HDL cholesterol levels27 (Table III). Maternal Mn restriction resulted in higher levels of plasma TGs and free fatty acids in the offspring chronically fed high fat diet (unpublished observations). Thus it is apparent that maternal micronutrient restriction not only affected the adipose tissue content and functions but also lipid metabolism.

**Diverse nutritional insults and identical result in offspring: common associated / underlying mechanisms(s)**

Summarizing the data available on the role of maternal macro- and micronutrient restrictions, one could tentatively conclude that the phenotypic results are relatively similar at least with respect to body composition and insulin secretion. Whilst programming for obesity is undoubtedly a multifactorial process, the diversity of models with a common end-point might suggest some common pathways. Any possible molecular mechanism for developmental programming of an obese / insulin resistance adult phenotype must explain how early environmental stress can set in motion persistent molecular changes that will cause pervasive, damaging effects at a later date. Available literature indicates oxidative stress, taurine metabolism, stress related mechanism involving cortisol and epigenetics as some common pathways considered to be involved in developmental programming for adult diseases in the offspring due to maternal malnutrition during pregnancy and/or lactation. It was therefore considered pertinent to decipher the mechanism(s) associated with the phenotypic changes observed above.

**Oxidative stress / antioxidant status**

 Increased oxidative stress is most often associated with IR. Indeed IR and oxidative stress are hypothesized to be causally related51,52. Similar to reports suggesting a role for oxidative stress in the aetiology of insulin resistance51,52, we observed an increase in oxidative stress and decreased antioxidant status (Table IV) in vitamin restricted (VR) offspring28. That despite a significant increase in the activities of the antioxidant enzymes: (SOD and Gpx in liver), VR offspring had increased oxidative stress probably suggests the importance of non-enzymatic antioxidants (specially the vitamins) as the primary line of defense against oxidative stress53 and/or the limited role the antioxidant enzymes play in maintaining the antioxidant status of the animal. It also perhaps, suggests that the antioxidant enzyme activities may be modulated in the offspring by maternal vitamin restriction, to cope up with the increased oxidative stress.

On the other hand, chronic maternal mineral or Mg restriction did not affect any parameters of oxidative stress and antioxidant defense (Table IV) (enzymatic and non enzymatic)24 whereas maternal Cr restriction increased MDA levels and decreased the activities of SOD and Gpx (Table IV) which were mitigated variably by rehabilitation in male and female offspring. Thus, it appears that maternal mineral restriction induced changes in body adiposity, glucose tolerance and impaired insulin response to a glucose challenge in the

### Table III. Plasma lipid levels in the offspring of dietary micronutrient restricted models of developmental programming in the rat

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Lipid profile measured and outcome</th>
<th>Abnormal result</th>
</tr>
</thead>
<tbody>
<tr>
<td>50% mineral restriction</td>
<td>Plasma triglyceride↑, Total cholesterol ↔, HDL cholesterol ↔</td>
<td>Yes Male</td>
</tr>
<tr>
<td>50% vitamin restriction</td>
<td>Plasma triglyceride↑, Total cholesterol ↔, HDL cholesterol ↔</td>
<td>Yes Male</td>
</tr>
<tr>
<td>Mg restriction</td>
<td>Plasma triglyceride↑, Total cholesterol ↔, HDL cholesterol ↔</td>
<td>No Male</td>
</tr>
<tr>
<td>Cr restriction</td>
<td>Plasma triglyceride↑, free fatty acids↑</td>
<td>Yes Female</td>
</tr>
<tr>
<td>Zn restriction</td>
<td>Plasma triglyceride↓, Total cholesterol ↓, free fatty acids ↓</td>
<td>Yes M + F</td>
</tr>
<tr>
<td>Mn restriction</td>
<td>Plasma triglyceride↓, Total cholesterol ↑, free fatty acids ↓</td>
<td>Yes M + F</td>
</tr>
<tr>
<td>Folate restriction</td>
<td>Plasma triglyceride↑, Total cholesterol ↔, HDL cholesterol ↔</td>
<td>Yes Male</td>
</tr>
<tr>
<td>B12 restriction</td>
<td>Plasma triglyceride↑, Total cholesterol ↔, HDL cholesterol ↔</td>
<td>Yes Male</td>
</tr>
</tbody>
</table>

*Source: Ref. 24, 25, 27*
offspring may be associated with / or due to changes in oxidative stress / antioxidant status in the offspring.

Cortisol pathway / 11β-hydroxysteroid dehydrogenase (HSD)-1 expression

Persistent changes in the expression of proteins which influence adipocyte development and lipolysis, e.g. peroxisome proliferator-activated receptor-γ (PPAR-γ), could permanently influence adipocyte proliferation and hypertrophy. Altered expression of 11β-HSD-1 has been reported in response to maternal undernutrition, e.g., adipocytes of prenatally nutrient-restricted lambs show increased expression of 11β-HSD-1, which could lead to increased cortisol exposure and proliferation of adipocytes. Though not significant, our unpublished observation that 11β-HSD-1 expression in liver was altered in Cr R offspring appear to imply a role for this pathway in maternal micronutrient restriction induced increase in the adiposity of rat offspring.

Epigenetic changes

Nutrients play essential roles in the following epigenetic events. Folate has a unique role in generating S-adenosylmethionine. Methylenetetrahydrofolate reductase deficiency results in hypomethylation of DNA, consistent with a role for folate in DNA methylation. Nutrients such as choline and methionine are the major dietary sources of methyl groups, whereas vitamins B6, B12, riboflavin and zinc are coenzymes and cofactors, respectively in one-carbon metabolism. Methylation of CpG islands leads to transcriptional repression and controls gene expression. The best-characterized epigenetic modification of DNA is methylation of cytosine within CpG dinucleotides. DNA methylation plays a role in allele-specific gene expression (genomic imprinting), heritable transcriptional silencing of parasitic sequence elements and X-chromosome inactivation. Growing body of evidence from in vitro embryo culture indicates that the methylation status of genomically imprinted genes (including IGF2, H19, IGF2R, etc.), can be altered with consequences for subsequent organ growth and function. Impaired DNA methylation is associated with perinatal death, decreased fertility, abnormal foetal development and tumourigenesis. Persistent changes in the methylation status of nuclear DNA (nDNA) have been proposed, and studies from several animal models support this hypothesis. Although we are yet to determine whether epigenetic changes such as DNA methylation are associated with altered gene expression observed in the offspring, our recent findings (unpublished observations) that maternal folate and / or vitamin B12 deficiency resulted in changes in the offspring appear to suggest that epigenetic mechanism(s) such as DNA methylation may underlie maternal micronutrient deficiency induced phenotypic and physiological changes in the offspring.

Covalent attachment of biotin to histones (DNA-binding proteins) silences gene expression and plays a role in cellular response to DNA damage. Post translational modifications of histones can alter chromatin conformation thereby regulating gene expression. Modifications such as acetylation, phosphorylation, methylation, de-amination, ubiquitylation, sumoylation, ADP-ribosylation and proline isomerization generate diversity in histone structure. Any given modification can either activate or repress gene transcription based on the location of the modified amino acid residue. Some modifications, such as acetylation/deacetylation activate/repress gene transcription are mediated by histone acetyl transferases/histone deacetylases (HDACs) respectively, which are dynamic, reversible and associated with inducible genes.

Further, tryptophan and niacin are converted to nicotinamide adenine dinucleotide, which is a substrate for poly (ADP-ribosylation) of histones and other DNA-binding proteins. Poly (ADP-ribosylation) of these proteins participates in DNA repair and apoptosis. Recent studies show that microRNAs (miRNAs) also contribute to epigenetic processes.

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Oxidative stress related parameters measured and outcome</th>
<th>Abnormal result</th>
<th>Source: Ref. 24, 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>50% mineral restriction</td>
<td>GSH ↓</td>
<td>Yes Male</td>
<td></td>
</tr>
<tr>
<td>50% vitamin restriction</td>
<td>MDA ↑, GSH ↓, SOD ↑, GPx ↑</td>
<td>Yes Male</td>
<td></td>
</tr>
<tr>
<td>Mg restriction</td>
<td>MDA ↔, GSH ↔SOD ↔, GPx ↔, catalase ↔</td>
<td>No Male</td>
<td></td>
</tr>
<tr>
<td>Cr restriction</td>
<td>MDA ↑, SOD ↓, GPx ↓, catalase ↓</td>
<td>Yes M + F</td>
<td></td>
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</table>

GSH, reduced glutathione; MDA, malondialdehyde; SOD, superoxide dismutase; GPx, glutathione peroxidase
MicroRNAs are double-stranded, noncoding RNA molecules (with an average size of 22 bp) that serve as post-transcriptional regulators of gene expression in higher eukaryotes. miRNAs have been implicated in many processes in invertebrates, including cell proliferation and apoptosis, fat metabolism, and neuronal patterning.

**Summary**

In summary, our initial studies showed that maternal restriction of vitamins and minerals increased body fat percent, plasma triglycerides and modulated adipokine expression which are important in the development of diseases related to carbohydrate and lipid metabolism. These also decreased lean body mass, fat free mass and impaired the offspring’s capacity to secrete insulin to a glucose challenge. Although maternal Mn restriction per se altered only the body fat percent and plasma lipid profile in offspring, interestingly it made them susceptible to impaired glucose metabolism when fed high fat diet in their later life. In general, the increase in body fat percent was associated with increased central adiposity, altered plasma lipid profile and modulated expression of adipokines, fatty acid synthase and fatty acid transport protein1, suggesting alterations in lipid metabolism. On the other hand, decreased lean and fat free mass were associated with altered expression of myogenic genes and glucose uptake by diaphragm indicating modulations in muscle development and function. Further, alterations seen in fasting plasma insulin levels and insulin secretion to a glucose challenge probably suggest alterations in carbohydrate metabolism. Taken together with our finding that the phenotypic changes in the offspring were mostly irreversible by rehabilitation from as early as birth, our observations demonstrate that maternal micronutrient deficiency not only alters the body composition of the offspring irreversibly but also modulate carbohydrate and lipid metabolism and may predispose them to adult onset diseases. The probable biochemical mechanism(s) by which maternal micronutrient restriction modulates adiposity and / or insulin resistance and macronutrient metabolism in the offspring are represented schematically in the Fig. 2.

**Future directions**

This review has focussed on a limited array of programmed outcomes: those most relevant to the metabolic syndrome and the triad of cardiovascular disease, type 2 diabetes, and obesity. There are other important and informative studies highlighting the impact of early life experiences in determining a range of other health outcomes including malignancies, mental health, and musculoskeletal health.

It is clear that the nutritional environment during early life is a determinant of postnatal health. Whereas the dominant focus of experimental studies to date has been on defining the phenotypic consequences of perturbations of maternal nutrition, the emphasis has now shifted to determining those initiating mechanisms through

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**Fig. 2.** Probable biochemical mechanism(s) by which maternal micronutrient restriction modulate adiposity and insulin resistance in the offspring.
which early nutrition and associated growth patterns result in cardiovascular and metabolic dysfunction. The size and scope of this field has grown to include the interests of geneticists, physiologists, and evolutionary biologists and is supported by clinical, epidemiological, and experimental studies in equal measure. There are emerging areas of critical interest including the extension of studies on the nutritional environment of the early embryo to include a better understanding of the impact of the perturbations of the environment of the gametes and embryo during a range of assisted reproductive technologies on later adult health. The application of epigenomic approaches and the determination of those imprinted or nonimprinted genes and transposon insertion sites that are targets for early nutritional effects on epigenetic gene regulation, are important new areas of investigation. An understanding of the generation and maintenance of epigenetic changes in gene regulation in the developing germline will provide insights into the mechanisms underlying developmental programming. It is likely that these studies will indicate the importance of nutritional programming which is greater than that predicted from earlier epidemiological studies and identify novel therapies and preventive strategies that will be important in a public health context.

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