EXPERT REVIEWS

Maternal undernutrition and endocrine development

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Maternal undernutrition, whether it occurs before conception, throughout gestation or during lactation, may lead to physiological adaptations in the fetus that will affect the health of the offspring in adult life. The timing, severity, duration and nature of the maternal nutritional insult may affect the offspring differently. Other factors determining outcome following maternal undernutrition are fetal number and gender. Importantly, effects of maternal undernutrition may be carried over into subsequent generations. This review examines the endocrine pathways disrupted by maternal undernutrition that affect the long-term postnatal health of the offspring. Maternal and childhood undernutrition are highly prevalent in low- and middle-income countries, and, in developed countries, unintentional undernutrition may arise from maternal dieting. It is, therefore, important that we better understand the mechanisms driving the long-term effects of maternal undernutrition, as well as identifying treatments to ameliorate the associated mortality and morbidity.

Keywords: developmental origins of health and disease • fetal growth • fetus • glucose–insulin axis • hypothalamic–pituitary–adrenal axis • maternal undernutrition

There is accumulating evidence that events in early life confer risks for the onset of adult diseases: the so-called developmental origins of health and disease [1,2]. Environmental influences may lead an organism along certain pathways during sensitive, and often brief, periods of development, so that it may develop characteristics better adapted to the environment in which it is likely to live [3]. This developmental plasticity during intrauterine development is beneficial if the extrauterine environment is consistent with that signaled via the mother before birth. However, if the extrauterine environment turns out to be very different, plasticity may also result in adaptations *in utero* that are inappropriate for the environment in adulthood and, therefore, detrimental to health.

Any environmental effect on fetal or postnatal development must ultimately act via altered gene expression. Epigenetic regulation of gene transcription is likely to be a key mechanism through which fetal nutrient supply can alter gene expression in the developing fetus in response to environmental cues. Gene expression may be modified by a number of processes, primarily DNA methylation and histone modification [4], both of which may be altered by the availability of amino acids and micronutrients during development *in utero* [5,6]. For example, folic acid deficiency is associated with reduced DNA methylation in humans [7]. Similarly, feeding genetically identical mice a diet high in methyl donors, including folate, results in epigenetic modification of gene expression in the offspring [8]. Importantly, these modified characteristics are transmitted to subsequent generations, demonstrating that epigenetic mechanisms can result in heritable traits that may reflect persistent adaptations to environmental cues over several generations [9]. Thus, DNA methylation-based markers, for example, are seen as promising tools for clinical diagnostics and theraupetics [10], and screening for epigenetic markers in early life may lead to individually customized interventions to reduce the risk of later disease [11].

The intrauterine environment is affected particularly by both overabundance and deficiency of fetal nutrient supply. The proportion of the population in the developed world who are overweight and obese is steadily increasing. In women of childbearing age, this may result in a dietary intake during pregnancy that is high in calories and rich in fat and carbohydrates [12,13]. This can then lead to intrauterine fetal overnutrition, which is associated with numerous deleterious effects for the offspring [14], ranging from higher prevalence of congenital anomalies [15,16] to obesity, metabolic syndrome, and Type 2 diabetes in adult life [12,17–20].

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On the other hand, it has generally been assumed that, except under severe conditions, maternal nutrition is not a limiting factor for fetal development and growth. However, deficiencies in the maternal diet can also have significant effects on the fetus, permanently affecting health in later life [21]. For example, fetal adaptations to intrauterine undernutrition, likely resulting from inadequate materno–placental nutrient supply, increases the risk of cardiovascular diseases in adulthood [22]. Similarly, there are extensive data on the long-term effects of severe food restriction in human pregnancy, resulting from exposure to the Dutch famine of 1944–1945 [23].

Undernutrition may affect the offspring differently, depending on its timing, severity, duration and the nature of the insult. Other factors altering the effects of maternal undernutrition are the number of fetuses in a given pregnancy and fetal gender. Furthermore, although some effects may result directly from alterations in substrate availability, many are thought to be mediated by hormonal effects [22]. In this review, we focus primarily on the endocrine pathways that are disrupted by maternal undernutrition and the consequent long-term effects on postnatal health of the offspring.

Factors modifying the effects of maternal undernutrition on fetal endocrine development

Nature of undernutrition

There is evidence that altered maternal macronutrient balance [24], micronutrient intake [25] and overall caloric intake [23] can each influence postnatal disease risk. Although the majority of studies of the effects of maternal undernutrition on the developing fetus have investigated global undernutrition, many have examined the effects of deficiencies of particular dietary components.

Micronutrient deficiency in human pregnancy has been associated with a number of adverse outcomes, such as preterm birth, intrauterine growth restriction (IUGR) and congenital defects [21]. The importance of an adequate micronutrient supply in pregnancy has been supported by animal experiments, as well as the study of clinical conditions in humans associated with severe deficiency of particular vitamins and minerals [21]. In rats, a maternal vitamin-restricted diet (50% of normal intake of vitamins A, B_1, B_2, B_3, B_5, B_6, B_9, B_{12}, D_3, E, H and K_1) from preconception to weaning led to increased body fat content and plasma triglycerides, as well as lower lean body mass in adult offspring [26]. However, most of these effects appear to be reversed with postinsult vitamin supplementation [26].

Mild iron deficiency during pregnancy in rats led to offspring with significantly increased blood pressure in adult life [27]. Iron deficiency is the most common cause of nutritional anemia in humans, and in pregnancy it leads to elevated IGF-1 and reduced thyroxine concentrations in cord blood [28]. In addition, the more severe the anemia, the greater the effects on fetal growth, which probably result from the numerous effects of anemia on endocrine systems, including the somatotrophic, thyroid and hypothalamic–pituitary–adrenal (HPA) axes [28].

Iodine is an essential component of the two thyroid hormones triiodothyronine (T_3) and thyroxine (T_4) [29]. In humans, iodine deficiency during pregnancy leads to hypothyroidism, which, when sustained during a critical window of development from conception to 2 years of age, hinders brain development, causing irreversible mental retardation [29].

Dietary zinc restriction in rats during pregnancy and lactation induces functional and morphological adaptations in the fetuses that result in increased blood pressure in adult offspring [30]. Renal effects include reduced glomerular number, glomerular and tubular fibrosis, increased apoptosis and reduced antioxidant activity [30]. Increased reactive oxygen species may also contribute to the apoptosis and renal damage associated with zinc deficiency [30].

A calcium-deficient diet in rats is also associated with hypertension in adult offspring [31]. One randomized controlled trial in humans showed that calcium supplementation during pregnancy is associated with lower systolic blood pressure in the offspring at age 5–9 years, particularly among overweight children [32]. However, an Australian study indicated that the potential long-term benefits of gestational calcium supplementation on offspring blood pressure may be apparent only in the offspring of mothers with high blood pressure during pregnancy [33]. The associated endocrine mechanisms are still unclear.

Protein deficiency during pregnancy has been frequently investigated. Early studies showed an inverse relationship between maternal protein intake and the systolic blood pressure of rat offspring, and that the hypertension in offspring from low-protein-fed dams (9 vs 18% protein) was associated with increased pulmonary angiotensin-converting enzyme activity [34]. Other effects on the cardiovascular system include decreased aortic wall thickness and elastin content [35], as well as structural and functional cardiac changes, predisposing the animals to impairment of diastolic and systolic function in later life [36]. Other studies have consistently demonstrated hypertension in offspring following maternal protein restriction, with some also reporting increased fetal mortality [37]. In humans, an isocaloric low-protein diet in pregnancy has also been associated with increased systolic blood pressure in adolescent boys, independent of birthweight and maternal triceps skinfold thickness during pregnancy [38]. However, one prospective human study showed no association of low protein intake during pregnancy with offspring blood pressure at 6 months [39].

Low-protein diets throughout gestation also have been shown to decrease serum estradiol concentrations in female offspring [37], impair estrous cyclicity [40], alter the offspring's fat distribution and food intake in adulthood [41], and alter glucose tolerance of young rats [42]. Furthermore, the male offspring of rat dams on a protein-restricted diet during pregnancy had elevated cholesterol and triglyceride concentrations, and showed insulin resistance as adults, while the body composition of females had increased relative levels of tissue fat and decreased protein [43]. In mice, a low-protein diet during pregnancy predisposed genetically susceptible mice to the development of atherosclerosis [44].

Note that the deleterious effects on the offspring of a protein-deficient diet in pregnancy are magnified by diet in postnatal life. The offspring of rat dams subjected to protein undernutrition throughout gestation and lactation become significantly more obese, insulin resistant, hyperlipidemic and hypertensive, as well

as more sedentary as adults, when fed a highly palatable diet from weaning [19,45,46]. Furthermore, protein malnutrition throughout gestation shortened the lifespan of mice when dams were allowed to overfeed during lactation [47].

Timing of undernutrition

Retrospective studies on offspring of women exposed to the Dutch famine have shown that early gestational undernutrition primarily affects the cardiovascular system, leading to a greater incidence of coronary heart disease, a more atherogenic lipid profile and disturbed blood coagulation profiles [23]. Furthermore, the offspring of mothers exposed to famine in early gestation had increased stress responsiveness, a greater incidence of obesity, impaired glucose tolerance, and female offspring were more likely to develop breast cancer [23]. The increased incidence of obesity in adults whose to mothers were exposed to the Dutch famine during early pregnancy [23,48], in particular, may be a direct consequence of adaptations in the endocrine sensitivity of fetal adipose tissue [49].

Animal experiments have shown that maternal nutrition before and in very early pregnancy can have important effects on the health of the offspring. In rats, undernutrition of dams for 8 weeks before conception affects growth of vital organs and results in increased blood glucose and cholesterol concentrations in the adult offspring [50]. The induction of hypertension in the offspring was also more marked when the low-protein maternal diet started very early in gestation compared with when it started in mid gestation [37]. Indeed, maternal protein undernutrition for just the first 4.5 days (blastocyst stage) of rat pregnancy (duration: 21 days) results in postnatal hypertension of the offspring [51]. Embryo transfer experiments suggest that the effects are intrinsic to changes in the blastocyst, rather than the intrauterine environment [52].

In sheep, where it is possible to study fetal growth and endocrine function *in vivo*, it is now abundantly clear that maternal undernutrition in the periconceptional period alters many aspects of fetal development in late gestation. Fetal growth trajectory is reduced in late gestation following periconceptional undernutrition [53]. However, fetuses of ewes undernourished only before conception show greater fetal growth and metabolic responses to an acute maternal fast in late gestation than fetuses of ewes undernourished both before and after conception [54], with few changes seen in fetuses of ewes undernourished only after conception. These differences cannot be explained by changes in the fetal glucose–insulin axis or the fetal or maternal HPA axis at the time of the fast [54]. Thus, it appears that maternal nutrition before conception is particularly important in determining fetal responses to late gestational stress, and that effects of maternal undernutrition on fetal development are due neither solely to limitation of substrates for tissue accretion nor to excess fetal glucocorticoid exposure at the time of undernutrition [54].

Periconceptional undernutrition also alters several aspects of endocrine regulation in late-gestation fetal sheep [53], and appears to accelerate maturation of the insulin and HPA axes [55,56]. Early gestation nutrient restriction promotes adipose tissue deposition in the offspring [49]. Offspring of ewes undernourished in the periconceptional period have impaired glucose tolerance, which worsens with increasing age [57]. Furthermore, maternal periconceptional undernutrition alters behavioral laterality in offspring in a sexually dimorphic manner [58].

Maternal exposure to the Dutch famine in mid gestation was associated with increased incidence of microalbuminuria and obstructive airways disease, as well as impaired glucose tolerance, in the adult offspring [23]. In sheep, mid-gestation undernutrition results in increased adipose tissue deposition [49] and increased expression of genes that regulate apoptosis in the fetal ovary [59], as well as reduction of large corpora lutea [60].

Exposure to the Dutch famine in late gestation tended to affect intermediary metabolism in the offspring, particularly glucose–insulin homeostasis, leading to an increased risk of Type 2 diabetes [61,62]. Similarly, glucose tolerance was reduced only in the 1-year-old offspring of ewes undernourished in late gestation but not of ewes undernourished in early gestation [63]. Brief, profound, maternal undernutrition in late gestation in sheep also increased central HPA axis responses to corticotrophic stimulation in adult offspring, despite the fact that birthweight was not affected [64]. Interestingly, if the period of undernutrition was prolonged to an extent that did reduce birthweight, the effects on HPA axis function were mitigated, confirming that reduced size at birth following maternal undernutrition is not required for altered postnatal physiology [64].

Fetal number & gender

The effects of maternal undernutrition on the offspring are also affected by fetal number [49,65]. For example, nutrient restriction causes an upregulation of specific mitochondrial proteins within the adipose tissue of twin fetuses but not of singletons [49]. One study found that, in most cases (depending on timing of undernutrition), mRNA expression for *IGF-1* and *-2*, their receptors, the growth hormone (GH) receptor and the glucocorticoid receptor, were all significantly downregulated in the kidneys of twin fetuses but not in those of singletons [66]. Periconceptional undernutrition also results in an earlier prepartum activation of the central HPA axis in twin fetal sheep compared with singleton fetuses [67], but delayed maturation of the adrenal gland was already present in twin fetuses in early gestation, perhaps to prevent preterm onset of parturition [68]. Periconceptional undernutrition also abolished the accelerated maturation of the glucose–insulin axis that is seen in twin fetal sheep compared with singletons [69].

The interactions between maternal nutrition and fetal number persist into postnatal life, with the effects of undernutrition on behavioral laterality being different in singletons and twins [58]. These data suggest that the physiology of twin pregnancies is quite different from that of singletons, and likely to be determined by a combination of factors acting in both early and late gestation [69,70]. The late gestation effects may represent an additional nutritional challenge to twin fetuses compared with singletons, but the effects of fetal number on aspects of development are not the same as early pregnancy undernutrition, suggesting that the early pregnancy effects may be mediated by different mechanisms.

Fetal sex also plays a significant role in determining the outcomes associated with maternal undernutrition. In humans, a low-protein diet during pregnancy was associated with increased systolic blood pressure in adolescent boys but not girls [38]. A low-energy diet in the initial two-thirds of pregnancy in rats produced male offspring that spontaneously increased their intakes of a low-fat diet, gained more weight beginning at weaning and developed diet-induced obesity as adults [71,72]; these effects were not observed in females. Another study in rats observed that gestational undernutrition produced male offspring with reduced bodyweight, while females had increased bodyweight and adiposity as adults [73]. The effects of gestational and postnatal protein restriction on renal angiotensin receptor expression were also sex specific in rats [74] and mice [75], as were the effects of protein restriction during lactation on plasma leptin concentrations in rats [43].

In guinea pigs, acute maternal nutrient restriction for 48 h during the period of maximal fetal brain growth resulted in reduced basal cortisol and adrenocorticotrophic hormone (ACTH) in adult male offspring but led to elevated basal cortisol and normal ACTH in female offspring [76]. Indeed, in young adult lambs, early-gestation undernutrition seemed to have a minor influence on HPA axis function when considered alongside the effect of sex *per se* [77]. These observed differences are dependent upon sex hormones and/or sexually dimorphic development of central pathways regulating energy homeostasis [19]. Similarly, the onset of hypertension in rats following a low-protein diet was delayed in females [37], most likely a result of the effects of estrogen [78]. Epigenetic modification may underlie some of these sex-specific effects, particularly as both the promoters of androgen and estrogen receptor genes, and the expression of their target genes, have been shown to be regulated by epigenetic mechanisms [79].

Effects on endocrine development

Renin–angiotensin system

A well-recognized long-term effect of maternal undernutrition in the offspring is increased propensity to hypertension, which probably results from the interaction of a number of endocrine systems. However, there is accumulating evidence that the effects of maternal undernutrition on nephrogenesis and the renin–angiotensin system (RAS) play a major role.

Maternal food restriction alters gene expression of fetal renal transcription factors and growth factors in rats, impairing nephrogenesis during fetal development [80,81]. Maternal undernutrition in mice [75], rats [81–84] and sheep [85] reduces nephron numbers in the offspring. In humans, a Dutch famine cohort study linked maternal starvation to impaired renal function in the offspring; more specifically, a mid-gestation insult led to higher rates of microalbuminuria, which was likely associated with reduced glomerular endowment [86].

Undernutrition of rats in late gestation did not affect glomerular ultrastructure in the offspring, but rather resulted in reduced nephron number, decreased glomerular filtration rate and increased blood pressure [87]. Other effects of maternal undernutrition include the inhibition of vascular EGF expression in microvascular and aortic endothelial cells early in life, resulting in decreased angiogenesis and increased peripheral vascular resistance, both of which may contribute to offspring hypertension [88]. In experimental animals, the reduction in nephron numbers alone is associated with hypertension in later life [66,84,85], and analogous observations have been made in human studies [89].

However, a reduction in nephron number likely interacts with alterations in the RAS leading to a detrimental outcome. In rats, maternal protein restriction during pregnancy suppressed the newborn intrarenal RAS, with reduced renin mRNA expression in the kidney, as well as suppressed angiotensin II concentrations in renal tissue [90]. Similarly, abnormal kidney RAS ontogeny was observed in the offspring of protein-restricted rat dams, associated with neonatal suppression of angiotensin receptor 1 protein abundance in the newborn kidney but upregulation at 4 weeks of age [91]. However, receptor mRNA levels and sensitivity were upregulated as these animals aged [91–93], potentially resulting in inappropriate reductions in glomerular filtration rate. In sheep, maternal undernutrition also results in reduced nephron endowment and altered renal protein levels of angiotensin II converting enzyme and the type 2 angiotensin receptor in young adult offspring [85]. Intriguingly, treatment of the offspring of undernourished rats with a specific angiotensin II receptor antagonist in early postnatal life prevents the later development of increased blood pressure, providing further evidence that angiotensin II may play a major role in adult hypertension following maternal protein undernutrition during pregnancy [94].

Adipokine axis

The greater incidence of obesity in the adult offspring of mothers exposed to famine during gestation [95] may result from adaptations in the endocrine sensitivity of fetal adipose tissue [49]. Maternal undernutrition during early-to-mid gestation in sheep, for example, led to increased bodyweight and fat deposition in adolescence, as well as dysregulated glucose uptake, despite the absence of any change in birthweight [96].

An undernutrition insult over the period of maximal placental growth in sheep (early-to-mid gestation) resulted in lower maternal plasma cortisol, leptin, T_4 and IGF-1 concentrations, accompanied by increased fetal adipose tissue deposition and an upregulation of mRNA levels of the IGF receptors in this tissue [97]. The offspring of ewes similarly undernourished had increased adiposity at term with upregulation of uncoupling protein 2 (*UCP2*) and *PPAR*-α mRNA levels in adipose tissue [98,99]. It was, therefore, suggested that a combination of raised PPAR-α and UCP2, in conjunction with increased sensitivity to IGFs owing to upregulation of their receptors (but not their ligands) within the adipocyte, could explain the greater fat mass in the fetuses of undernourished mothers [97]. In addition, enhanced UCP2 abundance could enhance apoptosis rate [100] and compromise adipose tissue function during periods of environmental stress [99], increasing adult predisposition to disease [49].

Nonetheless, most fetal adipose tissue is accrued in the final trimester [101]; over this period there is an increased abundance within the fetal circulation of hormones that are important in

regulating fetal adipose tissue growth [49,102], including IGF-1 and leptin [103,104]. Maternal leptin crosses the blood–placenta barrier into the fetal circulation [105], but the female offspring of rats fed a protein-restricted diet solely during lactation also had mean serum leptin concentrations that were nearly half those in controls [43], suggesting that the effects are on the adipokine axis of the offspring rather than the mother. Studies of genetically manipulated mice suggest that the presence of leptin and associated energy regulation are integral to the acceleration of obesity when on a high-fat diet following undernutrition *in utero*, and the premature leptin surge likely plays an essential role as a signal during the early neonatal period in the developmental origins of obesity [106].

It seems that perinatal perturbations of leptin action, either through reduced leptin availability or through hypothalamic leptin resistance, is a mechanism by which perinatal undernutrition may permanently alter hypothalamic circuits [107]. Maternal undernutrition from conception to the end of the period of maximal placental growth in sheep led to an increased leptin response to norepinephrine and angiotensin II infusions [108]. Undernutrition between 30 and 80 days of gestation, a period during which early fetal brain development takes place in sheep, resulted in reduced expression of neuropeptide Y (*NPY*) in the hypothalamus in 1-week-old offspring [109]. If these offspring were placed on an obesogenic diet after weaning, the prenatal effect of maternal undernutrition amplified the effect of postnatal obesity, with obese offspring of undernourished ewes having significantly higher leptin, insulin and nonesterified fatty acid concentrations compared with obese offspring of well-nourished ewes [109]. These changes were accompanied by increased hypothalamic mRNA levels of the melanocortin-4 receptor, a key part of the hypothalamic appetite regulatory pathway. The authors interpreted these changes as reflecting an altered adaptation in energy balance when juvenile obesity follows upon prenatal undernutrition [109].

In rats, the offspring of undernourished dams had premature onset of neonatal leptin surge, impaired leptin transport to the brain, increased density of hypothalamic nerve terminals and elevation of endogenous serum leptin concentrations [110]. The premature leptin surge was associated with adult obesity, perhaps by altering energy regulation by the hypothalamus [110]. Maternal undernutrition in mid-to-late gestation in rats dramatically reduced postnatal plasma leptin concentrations and hypothalamic mRNA levels of the leptin receptor in male pups [111], and affected development of pro-opiomelanocortin (POMC) neurons in the arcuate nucleus [112]. Leptin has also been shown to promote neuronal outgrowth from the arcuate nucleus to the paraventricular nucleus of the developing hypothalamus during the lactation period in rats, thus, potentially 'hardwiring' the hypothalamic appetite regulatory system [18]. Therefore, the evidence from rats shows that maternal undernutrition leads to alterations in the hypothalamus of adult offspring through its effects on leptin pathways, differently programming the long-term appetite regulatory system, especially the responses of POMC neurons to energy status and food-intake rhythm [111]. Interestingly, neonatal leptin treatment in female rats permanently reverses the long-term effects of maternal undernutrition, normalizing caloric intake, locomotor activity, bodyweight, fat mass, as well as fasting plasma glucose, insulin and leptin concentrations in adult life [113]. The authors attributed this effect, at least partly, to the resetting of central and/or peripheral pathways that regulate energy homeostasis [113].

Gonadotrophic axis

There is considerable evidence that reduced maternal nutrient intake during pregnancy can affect reproductive function in the adult offspring [114]. Furthermore, the effects of maternal undernutrition have also been demonstrated on the reproductive tracts of fetuses [114], suggesting one possible explanation for the transmission of effects to the second generation that have been reported in humans [115] and rats [116,117]. Maternal undernutrition in early gestation in sheep led to increased expression of steroidogenic acute regulatory protein (*StAR*) mRNA in the fetal testes, and increased plasma testosterone concentrations [118]. In female fetuses, ovarian development is affected [116,117], with altered proliferation and expression of apoptosis-regulating genes [59] and increased numbers of small follicles in the ovary [60]. There are also effects on the central gonadotrophic axis, with increased pituitary sensitivity to GnRH [60].

Maternal undernutrition affects the gonadotrophic axis of the offspring not only when the insult is restricted to the gestational period alone, but also when it occurs solely during lactation. In rats for example, maternal protein and energy, or solely energy, restriction during lactation lowered serum estradiol and raised testicular testosterone concentrations, and also enhanced androgen receptor expression whilst lowering estrogen receptor and aromatase mRNA expression levels in the testis of offspring at weaning [119]. Protein and energy restriction in lactation also led to significantly elevated concentrations of serum testosterone [119].

The onset of sexual maturity in the offspring is also affected by maternal undernutrition. Little evidence exists for humans, but it has been reported that girls of lower birthweight have an earlier onset of menarche; this effect is enhanced by obesity in childhood [120]. Furthermore, women exposed to the Dutch famine *in utero* delivered their first child at a younger age than those not exposed [121]. In sheep, moderate maternal undernutrition was not detrimental to the onset of puberty (defined as first ovulation) in female lambs, but it delayed the onset and magnitude of sexual activation in male offspring, and lowered peak testosterone concentrations [122]. In rats, late-gestational undernutrition resulted in delayed onset of puberty in both male and female offspring [123]. A more recent study showed that maternal protein restriction solely during lactation also delayed onset of puberty in female rats, associated with decreased luteinizing hormone (LH) and increased testosterone serum concentrations at 70 days, with the latter difference persisting until 1 year of age [124]. Significant reduction in serum LH at 70 days was also observed in rats exposed to undernutrition only during pregnancy [124].

In a recent study in rats, maternal undernutrition during pregnancy led to early onset of puberty, as well as to a reduction in proestrus progesterone levels in later life [125]. The reasons for

this contrasting result are unclear but it appears that, in rats, an undernutrition insult, restricted to or including the lactation period, delays sexual maturation, while undernutrition solely during gestation may have an opposite effect. The severity of the undernutrition insult may also affect the outcome.

Furthermore, maternal undernutrition not only affects the timing of sexual maturation but may also affect reproductive capacity. An earlier study showed that maternal undernutrition during gestation and lactation in rats significantly impaired ejaculatory capacity in male offspring, as well as reducing absolute weight of testicles, and absolute and relative weights of seminal vesicles [126]. However, data from the Dutch famine suggested that human *in utero* exposure to severe food limitation improved rather than impaired reproductive success in women (but not men), with exposed women giving birth to a larger number of children and being less likely to be childless [121]. Interestingly, the rate of twinning was approximately three-times that of unexposed women, suggesting that the increased number of offspring may, in part, reflect alterations in the regulation of ovulation or other aspects of reproductive function [121].

Glucose–insulin axis

In humans, prenatal exposure to famine at any time, but especially during late gestation, is associated with decreased glucose tolerance in adult life [62,127]. Small-for-gestation-age (SGA) babies or those with IUGR are often chronically starved *in utero* [128–130], and, thus, provide some evidence regarding the later effects of intrauterine undernutrition. Short prepubertal IUGR children have been shown to have impaired insulin sensitivity in comparison to their normal-birthweight peers [131]. Young adults born with IUGR also had elevated plasma concentrations of insulin and proinsulin, indicating early development of insulin resistance [132]. Similarly, 25-year-old people born with IUGR displayed decreased insulin-stimulated glucose uptake without major impairment of insulin secretion [133].

In sheep, maternal periconceptional undernutrition appears to accelerate fetal pancreatic maturation [56,69]. However, the postnatal offspring of undernourished ewes demonstrated decreasing glucose tolerance with increasing postnatal age [57], suggesting that the *in utero* adaptation may have resulted in maturation at the expense of differentiation. Late-gestation undernutrition in sheep during the period of maximal fetal growth also results in impaired glucose tolerance and insulin resistance in the adult offspring [63], as does late-gestation maternal undernutrition in rats [46,134].

The mechanisms underlying the impaired glucose tolerance in offspring of undernourished mothers have not been entirely elucidated, but there may be effects at several levels. These include reduced β-cell proliferation, reduced islet size and vascularization in the fetal pancreas [135], as well as impaired β-cell neogenesis resulting in a reduced pancreatic β-cell mass and insulin content [136,137]. In addition, signaling pathways within the β-cell may be altered [138], and there is evidence that in adult offspring, β-cell telomere length is shortened, indicating greater cell senescence [139]. Peripheral organ responses may also play a role, with altered glucose uptake in adipose tissue, mediated via reduced expression of the glucose transporter GLUT4 [63], and insulin resistance in adipose tissue and muscle [46]. Recently, studies in rats with growth restriction induced by uterine artery ligation have demonstrated that a key pancreatic transcription factor is downregulated by epigenetic mechanisms [140]. Whether the same is true following maternal undernutrition is not yet clear.

Importantly, the effects of maternal undernutrition may be carried over into subsequent generations [19]. For example, when the mothers of an F1 generation were subjected to a low-protein diet, insulin resistance was observed in second-generation rats [116,141]. The female offspring of undernourished rats displayed decreased β-cell mass and were later unable to adapt their endocrine pancreatic mass to pregnancy, while the subsequent F2 generation displayed a reduction in β-cell mass and numerical density of islets [142].

Hypothalamic–pituitary–adrenal axis

Glucocorticoids have been proposed as key factors mediating the long-term effects of an adverse intrauterine environment [143], as they can alter important gene transcription in the developing fetus, trigger cell differentiation in many tissues *in utero*, stimulate the switch from growth to maturation [144–146] and affect the secretion of a number of metabolic hormones [138,147]. An adverse intrauterine environment, such as that resulting from maternal undernutrition or prenatal maternal stress, may result in increased maternal and, thus fetal circulating concentrations of glucocorticoids [143,148,149].

However, although an acute maternal undernutrition insult may elevate circulating glucocorticoid concentrations [148,149], it is less clear that chronic undernutrition has a similar effect. Indeed, in sheep undernourished for several weeks, maternal basal and stimulated glucocorticoid concentrations were actually decreased [97,150]. An additional factor determining the effects of prenatal maternal stressors on fetal exposure to glucocorticoids is placental metabolism of glucocorticoids via the 11β-hydroxysteroid dehydrogenase enzyme (11β-HSD)-1 and -2. Experimental studies demonstrate that blocking activity of the 11β-HSD-2 isozyme, thereby exposing the fetus to increased concentrations of maternal glucocorticoid, has significant effects on the fetal HPA axis that persist into postnatal life [151]. Maternal undernutrition also affects placental 11β-HSD-2 activity [149,152]; it is less clear whether nutritionally induced downregulation of 11β-HSD-2 activity is accompanied by exposure of the fetus to increased glucocorticoid concentrations [153]. As a result, glucocorticoids may mediate some of the effects of maternal stressors, but other factors, such as specific nutrients or other hormonal changes, alter maternal adaptation to pregnancy [154] and, therefore, may also be involved.

Thus, maternal undernutrition may affect the materno–placental HPA axis to mediate effects on the fetus, as well as affect the fetal HPA axis directly. Maternal undernutrition affects mRNA expression levels of corticotrophin-releasing hormone in the hypothalamus, *GR* and *POMC* in the pituitary, as well as *ACTH* receptor in adrenal gland [155]. These effects may alter HPA sensitivity to acute and chronic stressors, as well as HPA axis feedback

regulatory systems [138]. As a result, a suboptimal environment *in utero* may alter both the set point and sensitivity of the fetal HPA axis [138]. Such alterations in HPA axis function are likely to play an important role in the gestational modifiers of disease risk in adult life [147,156].

Maternal undernutrition in rats [149], guinea pigs [76] and sheep [157,158] results in altered basal glucocorticoid concentrations in late fetal or postnatal life. In sheep, moderate undernutrition only in early pregnancy has been shown to result in increased fetal adrenal P450c17 protein expression in late gestation [159] and a precocious fetal cortisol surge leading to preterm birth [55]. Periconceptional and gestational undernutrition in sheep results in elevated fetal plasma ACTH concentrations, and upregulation of mRNA expression of the ACTH receptor (*MC2R*) and *StAR* in the adrenal gland of near-term ovine fetuses [160].

In addition to effects on the fetal adrenal gland, late-gestation fetal ACTH responses to metyrapone (which blocks adrenal cortisol production) demonstrate that central HPA feedback sensitivity is also affected by periconceptional undernutrition [70,158]. These effects are likely to be mediated by alterations in levels of the glucocorticoid and/or mineralocorticoid receptors in the brain, as has been demonstrated in fetal guinea pigs following maternal undernutrition [148] by altered regulation of local glucocorticoid concentrations, by effects on the 11β-HSD isozymes [161,162] or by epigenetic changes in the hypothalamus [163,164].

Once again, fetal perturbations of HPA axis function persist into postnatal life. Maternal undernutrition in late gestation led to increased mineralocorticoid-to-glucocorticoid receptor mRNA expression ratio in the hippocampus, as well as greater *POMC* mRNA expression in the anterior pituitary of 8-month-old rat offspring [165], despite high basal plasma levels of corticosterone. Mineralocorticoid receptor binding capacities in the anterior pituitary gland were decreased, as was the mRNA expression of 11β-HSD isozymes in the pituitary gland [166]. By contrast, *11β-HSD-1* gene expression in the hippocampus was increased [166]. These data indicate both HPA axis hyperactivity and altered glucocorticoid negative feedback [166]. Thus, the effects of maternal undernutrition on HPA feedback sensitivity seen in fetal life can also be demonstrated in postnatal life, potentially leading to the development of adult diseases via persistent glucocorticoid excess [166].

The postnatal effects of exposure to maternal undernutrition on the HPA axis also change with postnatal age. Undernutrition of ewes in the first month of gestation led to a significant increase in resting plasma cortisol of female offspring at 12 months [77]. Similarly, early gestational undernutrition led to greater ACTH and cortisol response to corticotropin-releasing hormone challenge in the offspring at 2 months and higher basal cortisol levels at 5.5 months [167]. However, periconceptional undernutrition results in suppressed HPA axis responses to corticotrophic stimulation and to isolation stress in male offspring, which worsens with advancing age [168]. A similar suppression of HPA axis function with increasing age is seen in sheep exposed to exogenous glucocorticoids *in utero* [169,170]. This pattern of advanced maturation in the fetus, followed by suppressed or impaired function in adult postnatal life, is consistent with that seen in pancreatic function.

Therefore, the HPA axis may play a critical role in the postnatal endocrine effects of maternal undernutrition. The effects on fetal endocrine development may be mediated, at least in part, by exposure of the developing fetal organs to altered circulating or tissue concentrations of glucocorticoids. Furthermore, altered postnatal HPA function may mediate many of the adverse health effects seen in the offspring of mothers undernourished during pregnancy.

Somatotrophic axis

The fetal somatotrophic axis is functional *in utero* [171] and, during prenatal development, GH acts on many tissues at all developmental stages, although it appears to have a relatively minor influence on fetal growth [172,173]. IGF-1 and IGF-2 are the hormones primarily regulating fetal growth, acting in both paracrine and endocrine fashions [174–176]. IGF-2 is thought to be most important in embryonic and early gestational growth [177], while IGF-1 is considered the main endocrine growth regulator in late gestation [178,179].

The fetal somatotrophic axis is substantially regulated by the nutritional status of the mother [171]. Maternal undernutrition reduces fetal plasma concentrations of IGF-1 and IGF-2, and reduces their mRNA expression in a number of tissues [180]. In sheep, this reduction in fetal plasma IGF-1 concentrations appears to occur throughout gestation, and has been demonstrated after maternal undernutrition from early-to-mid gestation [157], early-to-late gestation [181] and mid-to-late gestation [182]. More detailed studies in chronically catheterized sheep have shown that fetal plasma IGF-1 concentrations are regulated by fetal glucose and insulin but not amino acid concentrations [183,184], and that this is the probable mechanism by which maternal nutritional status regulates fetal growth [185].

Fetal plasma concentrations of IGF binding proteins (IGFBPs) also have been shown to be nutritionally regulated [186]. In rats, maternal undernutrition increased fetal plasma IGFBP-1 and IGFBP-2 concentrations, thus restricting the availability of circulating IGF-1. Similarly, IGFBP-2 concentrations were higher in the fetuses of undernourished ewes [181], as were *IGF-1R* and *IGF-2R* mRNA expression in fetal adipose tissue at term [49].

There is also some evidence that these nutritionally induced changes in the IGF axis are prolonged beyond the period of maternal undernutrition. Fetuses of ewes undernourished only in the periconceptional period showed enhanced responsiveness of IGF-1, IGFBP-1 and IGFBP-3 to an undernutrition insult in late gestation [187]. In rats, plasma IGF-1 was reduced and IGFBP-1 and -2 increased in early postnatal life in the offspring of undernourished dams [188].

Growth hormone is also nutritionally regulated both before and after birth. Fetuses of ewes severely undernourished in late gestation had higher GH concentrations and GH mass secreted per burst, as well as increased GH peak and nadir levels [171]. In addition, in sheep, the number of GH secretory episodes increased,

while the response to an exogenous GnRH challenge was significantly lower in male nutrient-restricted fetuses [189]. After birth, sheep that had experienced fetal undernutrition as a result of placental restriction had increased plasma GH concentrations in pubertal and adult males, but lower levels in adult females [190].

Human data on the postnatal effects of prenatal undernutrition are conflicting. SGA children have been shown to have lower serum IGFBP-1 levels and higher overnight GH secretion, with an apparent shift in the set point of their GH–IGF-1 axis [191], consistent with other reports that IUGR children are partially IGF-1 resistant [192]. However, prepubertal children with short stature after IUGR have also been reported to have decreased GH secretion due to impaired GH pulse amplitudes, as well as having reduced plasma IGF-1 and IGF-2 concentrations, probably indicating GH insufficiency [193]. Boys (but not girls) also had reduced overnight urinary GH excretion [193]. Similarly, both low birthweight and low placental weight were associated with reduced GH excretion in men and women at 20 years of age [194].

Such changes may be the result of permanent effects in the hypothalamus, as adult growth-restricted-born rats showed altered mRNA expression in the hypothalamus, with increased somatostatin in the periventricular nucleus and decreased NPY in the arcuate nucleus [195,196]. However, these alterations were sex specific, with male but not female rats showing decreased galanin mRNA expression in GHRH neurons that control GH secretion [197].

Hypothalamic–pituitary–thyroid axis

Thyroid hormones play important roles in embryogenesis and fetal maturation [198], having a crucial role in the development of the fetal CNS in all mammals, including humans [199,200]. Maternal undernutrition in late gestation in rats led to decreased fetal plasma T_4 concentrations [148], as did maternal undernutrition throughout gestation in sheep [189]. However, others have reported little effect of maternal undernutrition on fetal plasma thyroid concentrations in sheep [182] and guinea pigs [201]. After birth, lambs born with IUGR following placental restriction had persistently reduced total T_4 and elevated total T_3 plasma concentrations [202]. These changes may reflect altered tissue hormone conversion, since elevated mRNA expression of the prohormone deiodinase type 2 was found in the hippocampus and cerebellum of fetuses of undernourished guinea pigs [203]. Again, the effects appear to be sex-specific, with increased *TR-α1* and *-β1* mRNA expression in male brains, but decreased expression levels in female brains [203].

Plasma thyroid hormone concentrations are also reduced in IUGR human fetuses, while thyroid-stimulating hormone (TSH) levels are increased [204], as are *TR-α1* and *TR-β1* mRNA expression in the placenta [205]. However, there is reduced expression of all thyroid receptor isoform proteins in the cerebral cortex and cerebellum of severe IUGR human fetuses [206]. By contrast, another study showed that, at birth, cord blood T_4 concentrations were higher, while recombinant T_3 concentrations were lower in neonates born to malnourished and/or anemic mothers [28,207]. Nonetheless, postnatal serum T_3 and T_4 concentrations were reduced in SGA babies in the first few weeks after birth [208,209].

Maternal undernutrition in rats during late gestation and lactation led to considerable reductions in plasma concentrations of free T_3, free T_4 and TSH in 14-day-old offspring, as well as histological changes and weight reduction in the thyroid gland [210]. Maternal refeeding early in postnatal life (4 days) restored thyroid weight and plasma thyroid hormone concentrations to control levels at 14 days, but plasma TSH failed to fully recover [210]. Similarly, protein restriction during lactation in rats led to hypothyroidism, and although refeeding restored serum T_3, T_4 and TSH to normal levels by 60 days, there was continuing high radioiodine uptake, suggesting permanent changes in thyroid function [211]. Indeed, a subsequent study showed that the same undernutrition regime led to hyperthyroidism in the adult offspring [212]. Thus, in rats, lactation seems to be a critical period for long-term effects of maternal undernutrition on thyroid function [213].

However, there is a paucity of data on the long-term effects of maternal undernutrition on the hypothalamic–pituitary–thyroid axis, and most of the available data refer to the effects of deficiency of iodine, which is an essential component of T_3 and T_4. The fetal supply of T_4 during the first half of human pregnancy is primarily of maternal origin, so that maternal iodine deficiency may have severe and irreversible effects on fetal neurological development [198,214] and permanent neurological impairment in the neonate offspring [198,214,215].

Conclusion

Maternal undernutrition, whether it occurs before conception, during gestation or during lactation, may alter endocrine development in the offspring, ultimately affecting health in later life. However, the effects of the maternal undernutrition insult vary according to its timing, severity, duration and nature. Fetal number and gender also influence fetal endocrine development and the long-term effects of maternal undernutrition. Importantly, effects of maternal undernutrition may evolve over the lifetime of the individual and, therefore, are carried over into subsequent generations.

Clearly, the effects of maternal undernutrition on the endocrine development of the offspring are complex, and involve multiple inter-related mechanisms. Epigenetic regulation of gene transcription is likely to be one important mechanism, but many aspects are still obscure. Since poor maternal and child nutrition is common in both developing and developed countries [216,217], it is paramount that we not only better understand the mechanisms underlying its long-term effects, but also identify treatments to ameliorate the associated mortality and morbidity.

Expert commentary

In view of the numerous effects on the offspring endocrine development associated with maternal undernutrition, it is desirable to develop appropriate preventive strategies to minimize both incidence and severity of associated long-term health effects. Improving the nutritional status of women prior to and during

pregnancy would be an obvious intervention. However, such measures would have to be appropriately targeted to specific groups, based on cultural, social and economic status, which constitutes a considerable public health challenge.

Other possible clinical strategies include nutrient supplementation, and at least one study has shown that folic acid supplementation during pregnancy prevented epigenetic modification of hepatic gene expression in the offspring of protein-restricted rats [218]. Furthermore, additional research is needed into postnatal interventions that can reverse the alterations in the endocrine system induced by maternal undernutrition. Leptin treatment in early postnatal life, for example, normalized the undernutrition-induced phenotype in adult rats, indicating that some prenatal adaptations resulting from fetal undernutrition can be reversed postnatally [113].

Five-year view

In the next 5 years, we will understand the epigenetic mechanisms, use them as diagnostic markers and be looking at early postnatal interventions based on reversing the nutritional and endocrine consequences of prenatal events. Public health focus will turn to optimal nutrition of women before pregnancy and in the offspring in early postnatal life.

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Key issues

- Maternal undernutrition affects the endocrine development of the offspring, not only when it occurs during gestation but also during the preconceptional period and during lactation.
- Both micro- and macro-nutrient restriction may have long-term effects.
- The effects vary according to its timing, severity, duration and nature of the undernutrition.
- The effects also vary according to the sex of the offspring.
- In some cases, accelerated fetal maturation of the endocrine axis is associated with early activation but later suppression of that axis in postnatal life.
- The hypothalamic–pituitary–adrenal axis is one of several endocrine axes involved but the effects cannot be explained by simple exposure to excess glucocorticoids.
- Epigenetic mechanisms may underlie some of the long-term effects of maternal undernutrition.

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