



Conference on ‘Nutrition and Healthy Ageing’ Symposium 3: Nutritional modulations of the ageing trajectory

The impact of early nutrition on the ageing trajectory

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Epidemiological studies, including those in identical twins, and in individuals *in utero* during periods of famine have provided robust evidence of strong correlations between low birth-weight and subsequent risk of disease in later life, including type 2 diabetes (T2D), CVD, and metabolic syndrome. These and studies in animal models have suggested that the early environment, especially early nutrition, plays an important role in mediating these associations. The concept of early life programming is therefore widely accepted; however the molecular mechanisms by which early environmental insults can have long-term effects on a cell and consequently the metabolism of an organism in later life, are relatively unclear. So far, these mechanisms include permanent structural changes to the organ caused by sub-optimal levels of an important factor during a critical developmental period, changes in gene expression caused by epigenetic modifications (including DNA methylation, histone modification and microRNA) and permanent changes in cellular ageing. Many of the conditions associated with early-life nutrition are also those which have an age-associated aetiology. Recently, a common molecular mechanism in animal models of developmental programming and epidemiological studies has been development of oxidative stress and macromolecule damage, specifically DNA damage and telomere shortening. These are phenotypes common to accelerated cellular ageing. Thus, this review will encompass epidemiological and animal models of developmental programming with specific emphasis on cellular ageing and how these could lead to potential therapeutic interventions and strategies which could combat the burden of common age-associated disease, such as T2D and CVD.

Developmental programming: Cellular ageing: Oxidative stress: Mechanisms

The global burden of ageing

Globally, the population is steadily ageing with individuals aged 60 years and older doubling since 1980 and this is forecast to reach 2 billion by 2050. This can be construed as an indicator of improved global health; however, an ageing population also brings increases in the prevalence of age-associated diseases. About 75% of all deaths in the USA and other developed countries now result from age-related conditions such as cancer, diabetes, heart disease, stroke, neurodegeneration and dementia. Environmental factors during any stage of

the life course can influence risk of these conditions. However, this review will focus on the importance of the environment during very early life on modulating risk of age-associated metabolic diseases, such as type 2 diabetes (T2D) and CVD.

Ageing and the thrifty phenotype hypothesis

Over 20 years ago, Hales and Barker proposed the thrifty phenotype hypothesis⁽¹⁾ in which it was postulated that under conditions of suboptimal *in utero*

Abbreviations: CR, caloric restriction; ETC, electron transport chain; IUGR, *in utero* growth restricted; ROS, reactive oxygen species; SOD, superoxide dismutases; SSB, single-strand break; T2D, type 2 diabetes; TOR, target of rapamycin.

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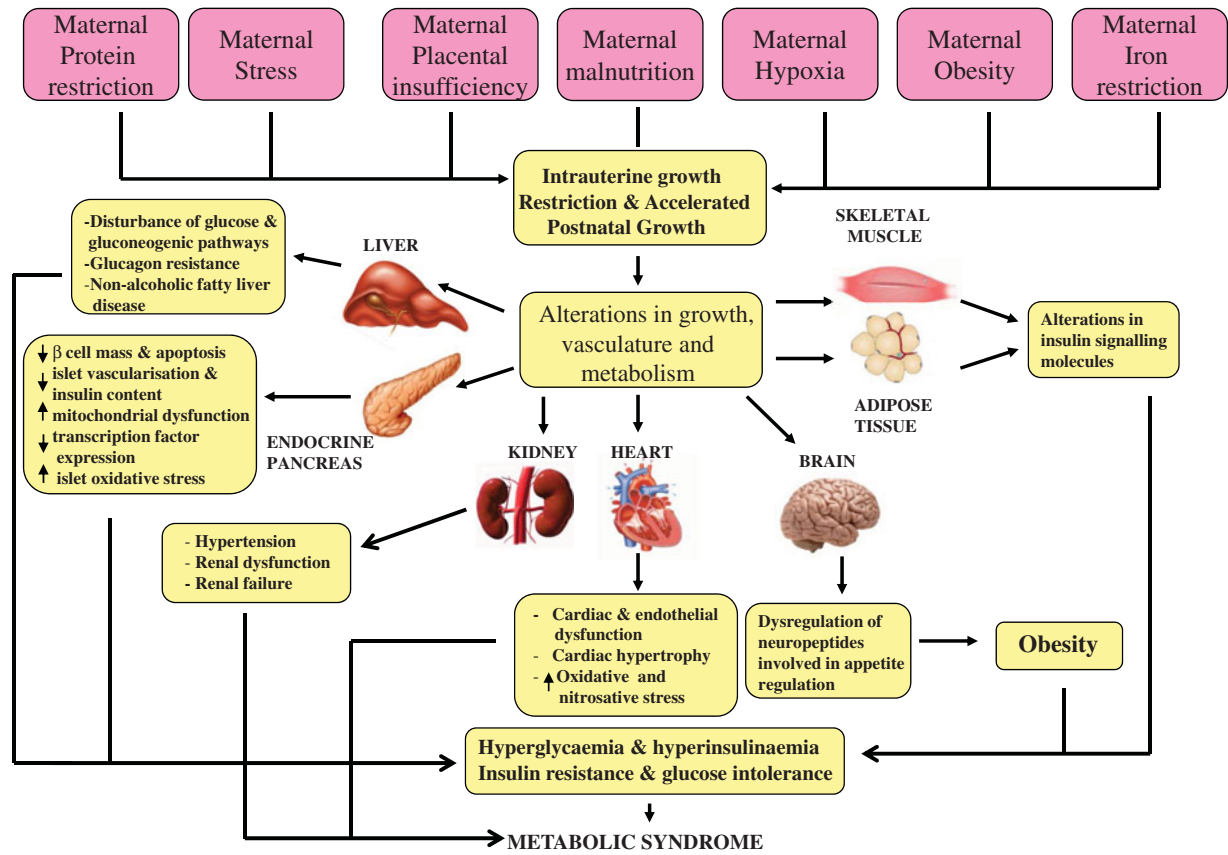


Fig. 1. (colour online) The thrifty phenotype hypothesis.

nutrition, the fetus permanently alters its organ structure and adapts its metabolism to ensure immediate survival of the organism. This can occur through the ‘sparing’ of certain vital organs, especially the brain, at the expense of other organs, including the heart, pancreas, kidney and skeletal muscle (Fig. 1). This hypothesis was the result of striking epidemiological studies in which Barker and colleagues⁽²⁾ and Hales *et al.*⁽³⁾ determined the prevalence of CVD and T2D in 64-year-old men. It was found that those individuals who were born smaller and weighed significantly less at age 1 year, compared with those with a normal birth-weight, had the highest mortality rates from IHD⁽²⁾, a higher incidence of T2D and abnormal glucose tolerance⁽³⁾. These findings have been robustly reproduced in many populations worldwide^(4,5). Furthermore, studies in monozygotic twins discordant for T2D have strongly implicated the environment playing a pivotal role in mediating the associations between low birth-weight and subsequent development of disease in later life. These studies demonstrated that the diabetic twin had the lower birth-weight^(6–8). Birth-weight has also been shown to be non-genetically linked with insulin sensitivity and glucose intolerance in elderly subjects^(9,10), perhaps suggesting that the ageing process may be exacerbating the effects of low birth-weight. Studies of individuals exposed to famine while *in utero*, have also demonstrated very powerful direct evidence for the importance of maternal

nutrition in early development. Ravelli *et al.* demonstrated that exposure to the Dutch famine of 1944–1945 significantly increased the risk of obesity⁽¹¹⁾ and reduced glucose tolerance in later life⁽¹²⁾.

Accelerated postnatal growth

Although sparing of vital organs such as the brain, at the expense of other organs, is beneficial in continued conditions of poor postnatal nutrition, several epidemiological studies have shown that this is detrimental in postnatal conditions of adequate or overnutrition. A study of a cohort of South African children demonstrated that those who were born small, but who underwent rapid postnatal weight gain had the worst glucose tolerance at age 7 years⁽¹³⁾. Moreover, Indian children with a low birth-weight, who underwent rapid postnatal catch-up growth, were insulin resistant and had increased cardiovascular risk factors at age 8 years⁽¹⁴⁾. In a Finnish cohort, males who were born small, but had higher than average body mass in childhood, had higher mortality from CHD, compared with low birth-weight men with average body masses⁽¹⁵⁾. Moreover, early development of adiposity and insulin resistance was observed in children who were small for gestational age but rapidly gained weight postnatally⁽¹⁶⁾.

Utilisation of animal models in developmental programming

These observations from studies in human subjects have been supported by numerous studies in animal models. These have been carried out in a range of species including non-human primates, sheep and rodents. These have all been important in providing proof of principle that the early environment can influence long-term health and have provided insight into underlying mechanisms. For obvious practical reasons, the majority of studies that have addressed the impact of the early environment on ageing parameters have been carried out in rodents. Conditions that have been associated with early nutrition are also those with an ageing-associated aetiology; therefore, this review will focus upon models of developmental programming and their associations with the ageing process.

Maternal low-protein restriction

The maternal low-protein rat model is one of the most extensively studied of all programming models and was established by Snoeck *et al.*⁽¹⁷⁾. This involved the administration of a low (8% protein) or a 'normal' (20%) protein diet to pregnant rats. These diets had similar fat content and were made isoenergetic by the addition of carbohydrates to the low-protein diet. The low-protein fed rat offspring were *in utero* growth restricted (IUGR) with significantly reduced birth-weights compared with control-fed offspring; however the placental weights were similar between groups⁽¹⁷⁾. The IUGR rat offspring had severe perturbations in pancreatic islets, including reduced pancreatic islet cell size and proliferation, diminished β -cell mass, and decreased islet vascularisation, and increased islet apoptosis^(18,19). These alterations were programmed *in utero* as they were irreversible after the islets were removed from the disturbed metabolic environment^(18,20). Strong justification for the use of this model to accurately dissect the mechanistic changes in human studies has been demonstrated. Offspring of protein-restricted rat dams had alterations in key insulin signalling molecules in skeletal muscle and adipose tissue, which were strikingly similar in specificity and magnitude to those observed in skeletal muscle and adipose tissue from low birth-weight men^(21,22). Male rat offspring exposed to the low-protein diet *in utero* demonstrate an age-dependent loss of glucose tolerance from insulin-sensitive and glucose-tolerant phenotype in early life⁽²³⁾, through insulin resistance during middle age⁽²⁴⁾ and to the development of frank diabetes in old age⁽²⁵⁾. This age-associated loss of insulin sensitivity and glucose tolerance is also observed in human populations⁽²⁶⁾. Therefore, it is certainly feasible that accelerated cellular ageing may be a potential underlying mechanism of developmental programming. Maternal protein restriction has also been demonstrated to lead hypertension and age-associated deterioration of renal function in offspring⁽²⁷⁾. This was associated with reduced renal mitochondrial respiration rate⁽²⁸⁾

and impairment of recovery of rat hearts subjected to ischaemia/reperfusion injury⁽²⁹⁾.

Cellular senescence and developmental programming

In 1961, Hayflick first demonstrated that all somatic cells have a finite division potential (between 40 and 60 population doublings in normal human fetal cells) before they cease division and enter a cell senescent phase⁽³⁰⁾. A potential explanation for this replicative senescent phenotype of somatic cells may be derived from 'the free radical theory of ageing' hypothesis; established by Harman in 1956⁽³¹⁾, which suggested that free radicals accumulate during the ageing process and cause damage to cellular macromolecules including DNA, proteins and lipids, mediating the development of various pathologies, which could result in cellular senescence and organismal ageing. This has been supported by several studies^(32,33). However, it is known that free radicals such as superoxide (O_2^-) and reactive nitrogen species (ROS) including nitric oxide are formed in physiological as well as pathological processes (reviewed in^(34,35)); therefore it is only when excess O_2^- is produced, that damage to DNA, proteins and lipids through oxidation occurs (reviewed in⁽³⁶⁾). Excess O_2^- can also combine with excess nitric oxide and form peroxynitrite, which can damage proteins through nitrotyrosination and increase DNA single-strand break (SSB) damage⁽³⁷⁾ and damage lipids via peroxidation. Therefore, unsurprisingly, oxidative and nitrosative stress have been implicated in various pathological diseases, all of which have an age-related aetiology, including CVD^(38,39), cancer⁽⁴⁰⁾, neurological disorders^(41,42) and diabetes⁽⁴³⁾. Consequently, organisms have evolved to develop cellular defence mechanisms in order to maintain redox homeostasis; so that levels are sufficient for physiological, beneficial cell functions, but can prevent high pathophysiological levels, which are associated with age-associated pathology.

Antioxidant defence is a major mechanism by which cells can regulate redox homeostasis. Cellular antioxidants can be either enzymatic or non-enzymatic. Enzymatic antioxidants include superoxide dismutases (SOD), which are the first line of cellular defence against excessive O_2^- and are responsible for the catalysis of the surfeit O_2^- into H_2O_2 and O_2 . There are three major isoforms of SOD known to exist in eukaryotic cells and are named according to their cell localisations. Copper-zinc SOD (SOD1) is localised to the cytoplasm, manganese SOD (SOD2) is expressed within mitochondria, and extracellular SOD (SOD3) is found within the extracellular matrix. Although H_2O_2 is less volatile than O_2 , it has the potential to react with transition metals (such as Fe^{2+} or Cu^+) to generate the highly destructive hydroxyl radical (OH^\bullet)^(44,45). Therefore, a further group of antioxidant enzymes, the peroxidases, including peroxiredoxins, catalase, glutathione peroxidases and glutathione reductase convert H_2O_2 into H_2O and O_2 . Non-enzymatic endogenous antioxidants include ascorbate (vitamin C) and ubiquinol (coenzyme Q).

Studies utilising the maternal low-protein restriction of Snoeck *et al.*⁽¹⁷⁾ have demonstrated that pancreatic islets from older rat offspring (age 15 months) had an accelerated cellular ageing phenotype, with evidence of decreased antioxidant defence capacity, increased fibrosis and oxidative stress, compared with age-matched control counterparts⁽⁴⁶⁾. Moreover, a maternal protein restriction model in goats (40% protein restriction) resulted in reductions of SOD in the offspring⁽⁴⁷⁾.

Mechanisms of ageing and developmental programming

Telomeres are repeating guanine-rich nucleotide DNA sequences, which prevent chromosomal ends from being recognised as double-strand DNA breaks and prevent chromosomal deterioration or fusion with neighbouring chromosomes⁽⁴⁸⁾. They are also particularly susceptible to oxidative damage due to their guanine-cytosine-rich sequences^(49,50). Telomeres shorten in eukaryotic somatic cells after each cellular division (between 20 and 200 base-pairs per division in human cells), due to the ‘end replication problem’⁽⁵¹⁾ and this erosion is an integral part of the ageing process^(52,53). Oxidative stress mediated damage to telomeric DNA is another major mechanism for telomere attrition^(54,55) and may contribute to the development of replicative senescence, as well as mediating oxidative damage induced by cellular senescence. DNA SSB damage has been reported to be a major mechanism of telomere shortening⁽⁵⁶⁾ and oxidative stress can increase frequency of this damage⁽⁵⁷⁾. Interestingly, telomeric DNA is deficient in the ability to repair DNA SSB, in contrast to the majority of genomic DNA⁽⁵⁸⁾. When telomeres reach a critically short length, they become dysfunctional and undergo a conformational change, resembling double-stranded breaks, which causes cells to enter irreversible G₀/G₁ growth arrest (senescence) or apoptosis⁽⁵⁹⁾. This senescence is triggered by activation of the p53/p21/p19 and p16^{INK}/retinoblastoma pathways⁽⁶⁰⁾.

Differences in the telomere length have been implicated in developmental programming. When low-protein-fed offspring were suckled by mothers fed a 20% ‘normal’ protein diet, these ‘recuperated’ animals underwent rapid postnatal growth, had reduced longevity⁽⁶¹⁾ and demonstrated accelerated telomere shortening in aorta⁽⁶²⁾, pancreatic islets⁽⁶³⁾ and renal⁽⁶¹⁾ tissues. This was accompanied by a reduction in SOD2 and increased p21 and p16^{INK} in the pancreatic islets⁽⁶³⁾. Moreover, evidence of accelerated cellular ageing is present in cardiac tissue from recuperated rats from weaning (age 22 d). This included evidence of increased oxidative stress, alterations in antioxidant defence capacity, increased DNA SSB damage, and increased DNA damage repair enzymes⁽⁶⁴⁾. Increased frequency of DNA SSB damage was also observed in renal tissue from age 12-month recuperated animals compared with controls, which was associated with mitochondrial dysfunction⁽⁶⁵⁾.

Ozanne and Hales have also demonstrated that offspring of control (20%) protein-fed dams that were suckled by low (8%) protein-fed dams, until weaning,

had increased longevity in both rats⁽⁶¹⁾ and mice⁽⁶⁶⁾, compared with animals fed a control (20%) protein diet during both gestation and lactation. These mice were also protected against longevity reduction when fed an obesogenic diet after weaning⁽⁶⁶⁾. These data may suggest that the mild stress of reduced protein intake during the suckling postnatal period is eliciting a protective effect of lifespan extension, which is in keeping with a recent ageing theory; ‘the hormesis hypothesis of ageing’, first highlighted by Minus in 2000⁽⁶⁷⁾. This suggests that the exposure of an organism to a mild stress (which in larger doses would be detrimental) can improve the functional ability of organisms. Much support for this theory has been gained from studies conducted in a variety of animal models including yeast, flies, worms and rodents, which have demonstrated that a mild stress, such as caloric restriction (CR) can increase longevity (reviewed in⁽⁶⁸⁾). Indeed, CR is the most potent and reproducible environmental variable capable of extending lifespan.

Uterine placental ligation

Uterine placental ligation^(69,70) is an elegant model of placental insufficiency which results in an IUGR phenotype. This model does not completely restrict blood supply to the fetus, but reduces it adequately to reflect human uteroplacental insufficiency, which can be caused by preeclampsia, maternal smoking and abnormalities in placental development. These neonatal IUGR rats demonstrated reduced glucose, insulin, insulin-like growth factor 1, amino acid and oxygen concentrations^(69–71). These IUGR animals later developed age-associated diabetes⁽⁷²⁾. Other studies demonstrated that bilateral uterine vessel ligation resulted in IUGR rat offspring with increased arterial vascular stiffness and selective endothelial uterine artery dysfunction⁽⁷³⁾ and caused nephron deficits and modest renal insufficiency⁽⁷⁴⁾.

Placental insufficiency in both animal models of uterine placental ligation⁽⁷⁵⁾ and in human studies^(76–79) is strongly associated with the development of oxidative stress, and it is thought that the mitochondria plays an important role in mediating this effect. Mitochondria are a major source of ROS generation in cells and are generated from the mitochondrial electron transport chain (ETC). This couples electron transfer from an electron donor (such as NADH) to an electron acceptor (O₂), with consequential transfer of protons across the membrane and production of an electrochemical proton gradient, which can drive synthesis of ATP. However, during this process, electrons can leak out of the complexes of the ETC and can combine with O₂ to form the free radical ‘O₂[•]. In 1980, Miquel proposed the ‘mitochondrial theory of free radicals in ageing’, in which he suggested that mitochondrially generated ROS can cause accumulation of somatic mutations to the mitochondrial genome and result in cellular senescence and apoptosis in post-mitotic cells⁽⁸⁰⁾. This establishes a ‘vicious circle’ of mitochondrial DNA damage, altered oxidative phosphorylation and overproduction of ROS. Others have supported this theory, whereby

mitochondria isolated from old animals produced more ROS compared with their younger counterparts⁽⁸¹⁾. Furthermore, accumulation of mitochondrial DNA damage^(82,83) and reduction of mitochondrial respiratory chain function in human tissue^(84,85) and that from rats and dogs⁽⁸⁶⁾ have been demonstrated with increased age.

Simmons and colleagues have demonstrated an impairment of oxidative phosphorylation in both skeletal muscle⁽⁸⁷⁾ and liver mitochondria⁽⁸⁸⁾ of IUGR rats, as well as a progressive accumulation of ROS generation and mitochondrial DNA mutations, and a progressive decline in activities of complex I and III of the ETC in the pancreatic islets of IUGR rats⁽⁸⁹⁾. This may be associated with the low oxygen levels observed in IUGR fetuses, as it has been shown that hypoxia decreases the activity of ETC complexes and increases ROS production^(90,91). Taken together this may suggest that insulin-sensitive tissues of IUGR rats exhibit mitochondrial dysfunction, which may lead to accelerated cellular ageing of these tissues. Several studies have also utilised ovine models of placental insufficiency to induce IUGR in offspring. This model bears striking similarities to the human situation, with decreased fetal and placental weights, reduced fetal oxygen concentrations, decreased placental oxygen transfer rates, decreased fetal glucose and insulin concentrations, decreased glucose-stimulated insulin secretion and diminished β -cell mass (reviewed in⁽⁹²⁾).

Maternal hypoxia

Maternal hypoxia has been studied in a number of human populations. It has been demonstrated that babies born to mothers living at high altitude are also significantly smaller than those born at normal altitude, and that this IUGR was associated with fetal hypoglycaemia and hypoinsulinaemia^(93,94). Placental insufficiency is thought to contribute to this, through impaired placental invasion of maternal blood vessels or through poor placental vascular development^(93,94). Animal models of maternal hypoxia recapitulate these findings, demonstrating that IUGR can result from maternal hypoxia^(95,96). Giussani *et al.* initially, using a chick embryo model⁽⁹⁷⁾ and subsequently a rat model⁽⁹⁸⁾ demonstrated that this IUGR was due to the hypoxia *per se*, and not maternal malnutrition, as severe hypoxia can result in reduced maternal food intake. Maternal hypoxia has also been shown to programme cardiac dysfunction in these rat and chick embryo models^(99,100), including promotion of fetal cardiac overload, leading to ventricular and aortic wall thickening⁽¹⁰¹⁾ and endothelial dysfunction⁽¹⁰²⁾. As observed in the model of protein restriction and placental insufficiency, evidence of oxidative stress has been demonstrated in this model. This has been reported in the placenta (increased levels of 4-hydroxynonenal and heat-shock protein 70)⁽¹⁰³⁾, and in the heart (increased nitrotyrosine staining and heat-shock protein 70)⁽¹⁰²⁾ of rats and in sheep⁽¹⁰⁴⁾. Furthermore, the administration of the antioxidants allopurinol⁽¹⁰⁴⁾ and vitamin C⁽¹⁰⁵⁾ reversed these phenotypes in sheep. Therefore, it is

plausible that hypoxia accelerates ageing in the placenta and cardiovascular systems, potentially leading to eventual cellular senescence, apoptosis and organ dysfunction.

Maternal stress

Severe maternal stress can induce IUGR in offspring, through various pathways including transplacental transport of the stress hormone glucocorticoid, via activation of the hypothalamic–pituitary–adrenal axis. It has also been shown that babies with a low birth-weight have higher plasma cortisol levels throughout life, which also indicates hypothalamic–pituitary–adrenal-axis programming. Indeed, severe maternal stress can deleteriously affect placental physiology, including alterations in blood flow and changes in metabolism, which impact upon oxygen and glucose availability. This has been demonstrated in both human studies^(106–108), and animal models, including rats⁽¹⁰⁹⁾ and sheep⁽¹¹⁰⁾, where these IUGR offspring demonstrated abnormal glucose tolerance^(109,110). Prenatal exposure to rats with glucocorticoids has also been shown to elevate plasma glucose, insulin and hepatic phosphoenolpyruvate carboxykinase in the next generation⁽¹¹¹⁾. Inhibition of 11β -hydroxysteroid (the fetoplacental barrier to maternal glucocorticoids) can also reduce birth-weight, increase the hypothalamic–pituitary–adrenal-axis activity and anxiety-related behaviours, and cause hyperglycaemia, hyperinsulinaemia and hypertension in the offspring (reviewed in⁽¹¹²⁾).

The mechanistic basis linking maternal stress to offspring metabolism is not clear; however oxidative stress is likely to be a major contributory factor. Entringer *et al.* demonstrated that severe stress in pregnant women was associated with shorter leucocyte telomere length in their children during young adulthood⁽¹¹³⁾. Moreover, a recent study in chick embryos demonstrated that *in utero* exposure to glucocorticoids resulted in higher levels of ROS and increased leucocyte telomere shortening⁽¹¹⁴⁾. In addition, prenatal exposure to high levels of glucocorticoids in rats increased susceptibility of cerebellar granule cells to oxidative stress-induced cell death, increased mitochondrial dysfunction and reduced catalase expression⁽¹¹⁵⁾. These data suggest that severe maternal stress can accelerate cellular ageing. It is common clinical practice for pregnant women at risk of preterm labour to be prescribed with repeat courses of glucocorticoids to aid lung maturation of the premature baby, and indeed studies in sheep treated with glucocorticoids have reported reductions in oxidative stress in the lungs of pre-term lambs^(116,117).

Maternal caloric/nutrient restriction

Several models of maternal CR have been used in a variety of formats, ranging from models of moderate to severe restriction. Rat dams exposed to 50% maternal CR from day 15 of pregnancy until weaning gave birth

to offspring that were growth restricted, with a 50% reduction in body and organ weights at weaning. These offspring also had a 70% reduction in β -cell mass at age 21 d and decreased insulin content in adulthood⁽¹¹⁸⁾. In this model, as in the model of maternal protein restriction, ageing plays a pivotal role in the development of glucose intolerance⁽¹¹⁹⁾. Franco *et al.* also utilised a 50% maternal CR model and demonstrated that both male and female offspring were hypertensive and had endothelial dysfunction⁽¹²⁰⁾. They also demonstrated that enhanced oxidative stress was a potential mechanism for these observations⁽¹²¹⁾. Maternal dietary restriction, using a diet representative of that of Brazil, caused placental oxidative stress in rat dams, which later led to changes in kidney proximal tubule sodium ATPases in the offspring⁽¹²²⁾. In another rat model, 50% maternal CR resulted in offspring that were growth restricted, however when suckled by *ad libitum* fed rat dams, these offspring underwent rapid postnatal growth, becoming heavier than the control offspring⁽¹²³⁾. A non-human primate model of maternal nutrient restriction (70% of control food consumption) resulted in alterations in the renal transcriptome and kidney morphology of the offspring of nutrient restricted primates⁽¹²⁴⁾. Furthermore, the mammalian target of rapamycin (TOR) signalling pathway was found to be central to this phenotype⁽¹²⁵⁾. Mammalian TOR is a serine/threonine kinase that regulates cell growth, cell proliferation and cell survival and it has recently been suggested that ageing can result from overactivation of TOR or mammalian TOR signalling pathways.

Maternal obesity and gestational diabetes

The deleterious effects of low birth-weight on long-term metabolic health are well established; however, it has become apparent that high birth-weight is also a clear indicator of increased risk of disease in later life. This relationship was initially found in epidemiological studies of Pima Indians⁽¹²⁶⁾ in which there is a very high prevalence of both T2D and maternal obesity, demonstrating that risk of developing T2D in later life is increased by both low and high birth-weight. This suggests that a U-shaped curve exists between birth-weight and risk of T2D development. A possible mechanism for the elevated risk of T2D in a high birth-weight population is the increased prevalence of gestational diabetes. Glucose can cross the placental barrier but maternal insulin cannot; therefore the fetus must regulate its own glucose homeostasis by insulin production from fetal β -cells of pancreatic islets. In situations of maternal hyperglycaemia (which occurs in gestational diabetes), higher levels of fetal insulin are produced. Insulin is a potent growth factor in fetal life; therefore this results in gestational diabetic mothers giving birth to macrosomic offspring. Boney *et al.* demonstrated that macrosomic offspring of mothers with gestational diabetes were at increased risk of developing metabolic syndrome in childhood⁽¹²⁷⁾. Human studies have further characterised phenotypes of offspring born to gestationally

diabetic mothers. These individuals were more obese and were hyperglycaemic compared with offspring of women who developed diabetes after pregnancy. They also had an increased propensity to develop diabetes in adulthood⁽¹²⁸⁾. More recently, studies of children exposed to maternal obesity and gestational diabetes *in utero* have shown higher incidence of insulin resistance⁽¹²⁹⁾, heart hypertrophy⁽¹²⁹⁾ and CVD in later life^(130,131).

The detrimental effects of overnutrition during fetal and early postnatal life have also been observed in animal models. Offspring of mice fed a highly palatable obesogenic diet before mating and during pregnancy and lactation were hyperphagic in early postnatal life, had increased adiposity, were hypertensive and insulin resistant, and were heavier in later life^(132,133). In addition, evidence of mitochondrial dysfunction was observed in this model, with reductions in complex II–III linked activity of the ETC in skeletal muscle of male offspring exposed to maternal obesity⁽¹³⁴⁾. Our laboratory has demonstrated that maternal obesity in the mouse programmes cardiac hypertrophy in the offspring which was associated with hyperinsulinaemia, protein kinase B, extracellular signal-related kinase and mammalian TOR activation, which was independent of obesity in the offspring. These animals demonstrated evidence of increased oxidative stress with elevation in 4-hydroxynonenal levels and reduction in SOD2 protein expression⁽¹³⁵⁾. It has also been shown that maternal obesity prior to conception is associated with altered mitochondria in mouse oocytes and zygotes, including increases in mitochondrial potential, mitochondrial DNA content and biogenesis; moreover ROS generation was also increased, again suggestive of increased oxidative stress and an increased ageing phenotype⁽¹³⁶⁾. In addition, rat offspring fed a ‘junk food’ diet during pregnancy and lactation have a greater preference for ‘junk food’ and increased obesity in adulthood⁽¹³⁷⁾. This adiposity was more pronounced in females⁽¹³⁸⁾ and this diet has recently been shown to promote non-alcoholic fatty liver disease in the offspring⁽¹³⁹⁾.

Maternal iron restriction

Iron deficiency is very common with 2 billion people affected globally and causes many abnormalities including long-term cognitive impairment (reviewed in⁽¹⁴⁰⁾). Epidemiological studies have shown that maternal iron deficiency is associated with increased incidence of IUGR^(141,142). Large placental weights and a high ratio of placental weight to birth-weight are observed⁽¹⁴³⁾. A potential mechanism for these altered whole body and organ growth trajectories may be the alterations of placental cytokine expression, which was observed in rat offspring born to iron-restricted mothers⁽¹⁴⁴⁾. Other laboratories have shown that maternal iron restriction causes long-term problems for the offspring, including hypertension^(145,146), changes in renal morphology⁽¹⁴⁷⁾ and changes in placental vascularisation⁽¹⁴⁸⁾. So far,

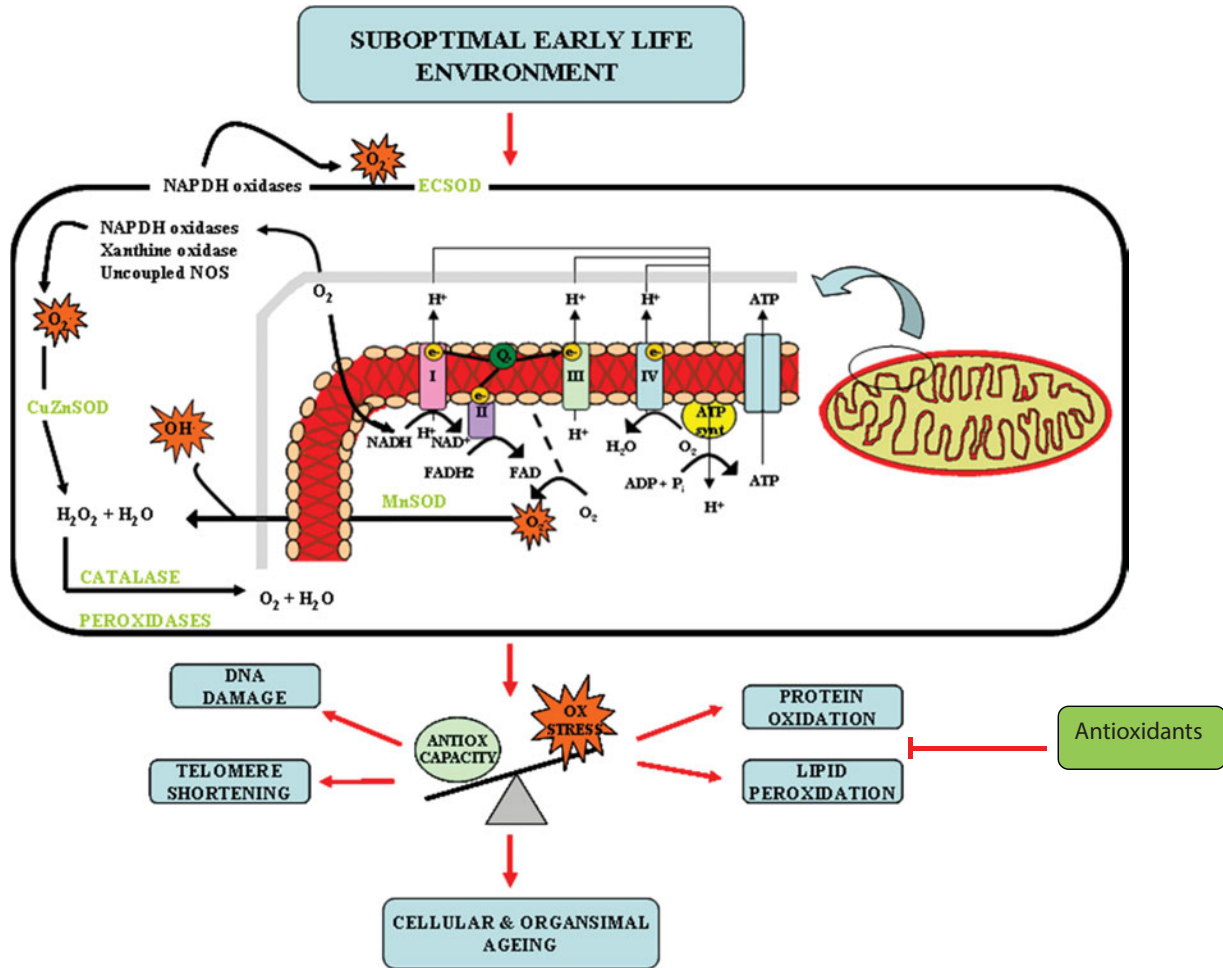


Fig. 2. (colour online) Oxidative stress (Ox stress) as an underlying mechanism of developmental programming and ageing.

models of maternal iron restriction have not addressed the potential role of oxidative stress.

Developmental programming, epigenetics and oxidative stress

Epigenetics can be defined as any change in phenotype or gene expression caused by modifications (including DNA methylation or histone methylation, acetylation, phosphorylation and ubiquitination), which is independent of changes in genotype. It is known that environmental cues can be ‘remembered’ during the lifespan and changes to the epigenetic landscape are associated with the ageing process. It is now emerging that epigenetic modification of transcription factors is a common underlying mechanism in many models of developmental programming. This includes epigenetic silencing of the pancreatic development gene *Pdx1* in offspring of mothers with placental insufficiency⁽¹⁴⁹⁾. Moreover, rat offspring of a maternal low-protein diet have demonstrated epigenetic alterations in the *PPAR-α*⁽¹⁵⁰⁾. In addition, we have also demonstrated that *in utero* exposure to a low-protein diet can alter the dynamics

of age-associated epigenetic changes at the hepatocyte nuclear factor 4- α locus⁽¹⁵¹⁾. Oxidative stress can induce epigenetic modifications, including DNA methylation and histone modification. DNA breaks caused by oxidant damage can provide access sites to DNA methyltransferases, which promote DNA methylation; moreover ROS can directly interact with histones resulting in disruption of normal gene expression. During oxidative stress, guanine residues are replaced with the oxygen radical adduct 8-hydroxyguanine and this can profoundly alter methylation status of adjacent cytosines and cause alteration of gene expression⁽¹⁵²⁾. Therefore, it is feasible that a molecular mechanism for the observed changes in epigenetic modification in several models of developmental programming may well be the development of oxidative stress.

The potential of antioxidant therapy in models of developmental programming

A common mechanism for the observed phenotypic outcomes in most of these animal models of



developmental programming is oxidative stress and this finding fully recapitulates epidemiological studies in which IUGR children demonstrate increased lipid peroxidation^(153–155), increased DNA damage⁽¹⁵⁵⁾ and reduced antioxidant enzyme capacity⁽¹⁵⁵⁾ compared with children of a normal birth-weight. Therefore, several animal models of developmental programming have focused upon using antioxidants as a therapeutic intervention in order to reverse the observed phenotypic changes. These included the reduction of adiposity and improvement of glucose tolerance resulting from the exposure to a high-fat diet, by maternal supplementation with high concentrations of vitamins A, C, E and selenium⁽¹⁵⁶⁾. Moreover, cardiac dysfunction in both rat⁽¹⁰¹⁾ and sheep⁽¹⁰⁴⁾ models of acute hypoxia has been demonstrated to be improved by maternal supplementation with vitamin C. In addition, prevention of hypoxia-induced placental oxidative stress with vitamin C has also been observed in rats⁽¹⁰³⁾. In a model of maternal protein restriction, hypertension, vascular dysfunction and microvascular rarefaction were prevented by antenatal treatment with the antioxidant Lazaroid⁽¹⁵⁷⁾ however, the physiological relevance of this antioxidant dose for translation into human studies is not known. Prenatal exposure to hypoxia in sheep also increased oxidative stress in the offspring, and maternal administration of an antioxidant; allopurinol reversed this phenotype^(105,158). These studies show proof of principle that maternal antioxidants can prevent detrimental programming effects; however, the doses used to achieve these effects may not be able to be used safely in pregnant women. In addition, they focus on interventions to the mother.

Future perspectives

Many epidemiological studies and animal studies have demonstrated that growth and nutrition during early life development can influence the long-term physiology and health and as a result, the lifespan of the individual. However, the molecular mechanisms that underpin this phenomenon are only starting to be dissected. Studies in human populations, animal models and cell systems all seem to be pointing to the accumulation of oxidative stress and consequently accelerated cellular ageing, as an important underlying molecular mechanism (Fig. 2). Several developmental programming studies have demonstrated proof of principle that maternal antioxidant therapy may reverse some of deleterious effects of a sub-optimal early life exposure. However, both animal^(25,27) and human studies^(159,160) demonstrate that evaluation of suboptimal *in utero* exposure may only be possible until later postnatal life and therefore further studies are required to address the potential beneficial effects of targeted postnatal antioxidant supplementation. This targeted intervention has the potential to combat the burden of common age-related diseases such as T2D, CVD and the metabolic syndrome that represent the major health-care issues of the 21st century.

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Conflicts of interest

None.

Authorship

Both authors contributed equally to the writing of this manuscript.

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