Nutrition in early life and age-associated diseases

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Abstract

The prevalence of age-associated disease is increasing at a striking rate globally. It is known that a strong association exists between a suboptimal maternal and/or early-life environment and increased propensity of developing age-associated disease, including cardiovascular disease (CVD), type-2 diabetes (T2D) and obesity. The dissection of underlying molecular mechanisms to explain this phenomenon, which is known as 'developmental programming' is still emerging; however three common mechanisms have emerged in many models of developmental programming. These mechanisms are a) changes in tissue structure, b) epigenetic regulation and c) accelerated cellular ageing. This review will examine the epidemiological evidence and the animal models of suboptimal maternal environments, focusing upon these molecular mechanisms and will discuss the progress being made in the development of safe and effective intervention strategies which ultimately could target those 'programmed' individuals who are known to be at-risk of age-associated disease.

Key Words: Developmental programming, mechanism, sub-optimal nutrition, age-associated disease, oxidative stress.

1. Introduction - The global burden of age-related disease

Organismal ageing can be defined as an age-dependent or age-progressive decline in physiological function, leading to an increase in age-specific mortality rate and a decrease in age-specific reproductive rate. With the number of people aged 65 or over estimated to increase from 524 million in 2010 to 1.5 billion in 2050, the prevalence of age-associated diseases (including cardiovascular disease (CVD), glucose intolerance, insulin resistance, type-2 diabetes (T2D), non-alcoholic fatty liver disease (NAFLD), cancer, dementia and obesity) is increasing at an astonishing rate globally. Consequently; this has major implications on worldwide mortality causes, with age-associated diseases making up more than 60% of all deaths worldwide. These sobering statistics can be explained to a certain degree by increases in global longevity, which has partially been caused by shift in causes of mortality; (from infectious and parasitic diseases, to non-communicable diseases), however it is clear that other mechanisms are also important.

2. The concept of developmental programming - Epidemiological evidence

Twenty five years ago, Hales and Barker published seminal papers which described strong associations between suboptimal growth in early life and increased risk of impaired glucose tolerance (Hales et al., 1991), T2D (Hales and Barker, 1992) and metabolic syndrome and CVD (Barker et al., 1993) in later life. Hales and Barker named this phenomenon The Thrifty Phenotype Hypothesis, which suggests that in a poor *in-utero* milieu, 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, liver, kidney and skeletal muscle, a phenomenon known as 'developmental programming' (Figure 1). A common phenotype in these offspring is *in-utero* growth restriction (IUGR).

2.1 Maternal under-nutrition (*in-utero* growth restriction)

These seminal studies have been reproduced in many epidemiological populations throughout the world (reviewed by Hales and Barker, 2001). One of the most compelling pieces of epidemiological evidence for the Thrifty Phenotype Hypothesis came from the Dutch Hunger Winter. Between late November 1944 and early May 1945, people who were previously well-nourished, experienced a very severe famine due to food blockades during World War II. A study by Ravelli and colleagues showed that offspring of mothers who were pregnant during the famine had a low birth-weight and were glucose intolerant in later life (Ravelli et al., 1998). It has also been shown that the time window of exposure to the famine is important: Increased prevalence of coronary heart disease, a raised atherogenic lipid profile and increased adiposity were observed in offspring of mothers exposed to famine in early gestation (Roseboom et al., 2006), whereas those offspring whose mothers were exposed to famine during mid gestation had increased microalbuminurea and deteriorated renal function in adulthood (Painter et al., 2005), whereas those exposed in late gestation had the greatest risk of T2D (Ravelli et al., 1998).

The idea that risk of T2D, CVD and the metabolic syndrome may be altered by the environment *per-se* and not genetic determinants has been supported by studies in monozygotic twins, in which the twin with the lower birth weight developed T2D (Poulsen et al., 1997), glucose intolerance (Grunnet et al., 2007) and impaired insulin secretion and insulin resistance in later life (Poulsen et al., 2002, Poulsen and Vaag, 2006), compared to the genetically identical twin with a normal birth weight. Importantly, the latter studies revealed that these associations occur in an age-dependent manner, which may part; explain the highly age-dependent states of T2D, CVD and the metabolic syndrome.

2.2 Maternal over-nutrition (maternal obesity and gestational diabetes)

Given that obesity, particularly in developed countries, has reached epidemic proportions, the issue of maternal obesity is becoming increasingly important. Obese women have increased risk of having large for gestational age and small for gestational age offspring (Djelanik et al., 2011). Maternal obesity (which is associated with gestational diabetes) can cause macrosomic offspring, rather than IUGR as gestational diabetes results in maternal hyperglycaemia. As glucose can cross the placental barrier but maternal insulin cannot, the fetus attempts to regulate its own glucose homeostasis by increasing insulin production from fetal β-cells of pancreatic islets. As insulin is a potent growth factor in fetal life, this can result in macrosomic offspring. The deleterious effects of maternal hyperglycaemia in the offspring are well known. In Pima Indians, a population with high levels of gestational diabetes and T2D (who also have a very high prevalence of obesity), the association of birth weight with T2D has been shown to be U-shaped, with the highest prevalence of T2D and obesity present in both low and high birth weight offspring (McCance et al., 1994). In the general population, it has been shown that children who are large for gestational age at birth and exposed to an intrauterine environment of either diabetes or maternal obesity are at increased risk of developing the metabolic syndrome (Boney et al., 2005) and children from obese mothers are more prone to overweight/obesity, central adiposity and greater fat mass in later life, independent of confounding factors (Daraki et al., 2015, Whittaker, 2004). Maternal obesity during pregnancy has also been linked to increased risk of premature mortality from CVD events (Reynolds et al, 2013) and to coronary heart disease risk (Gaillard, 2015) in the offspring.

2.3 Postnatal catch-up growth

It is evident that the fetal environment is an important determinant in the future prevalence of age-associated disease, including CVD and T2D; however the rate in which an individual grows postnatally is also known to have an impact on age-associated disease risk.

This so-called ‘mismatch’ between a poor maternal environment followed by an adequate or over-sufficient postnatal environment (as evidenced by accelerated postnatal weight gain) has been linked to increased risk of many age-associated diseases including poor glucose tolerance (Crowther et al., 1998), insulin resistance (Ong et al., 2004), endothelial dysfunction (Touwslager et al., 2015), hypertension (Law et al., 2002), CVD (Erikkson et al., 2001) and NAFLD (Faienza et al., 2013) in many human cohorts. Conversely, it has been shown that breast feeding compared to formula feeding can induce slower postnatal growth (Fewtrell et al., 2001) and in large population-based studies, breast fed infants had reduced blood pressure (Martin et al., 2005), reduced risk of childhood obesity (Arenz et al., 2004), reduced cholesterol (Owen et al., 2002) and reduced insulin resistance (Ravelli et al., 2000) compared to those who were formula fed, independent of potential confounding factors.

2.4 Animal Models

Although we have gained some understanding of the concept of developmental programming from epidemiological studies, the underlying mechanisms and potential intervention strategies are impossible to elucidate without the use of animal models. Human studies have many confounding factors and the extensive lifespan make life course studies challenging. The shorter lifespan of most commonly used animal models (rodents) means that studies most relevant for understanding the aetiology of age-associated disease can more easily be attained. Therefore this review will now mainly focus on animal models of sub-optimal nutrition in early life and what they have told us about programming of age-associated disease.

2.4.1 Maternal Protein Restriction

As the Thrifty Phenotype Hypothesis suggests a key role for the supply of proteins and amino acids in fetal growth, maternal protein restriction has become one of the most

commonly studied models of sub-optimal maternal nutrition. Initial studies in rats utilised an 8% low-protein (LP) diet, compared to a 'normal' 20% protein diet. These studies demonstrated that protein-restricted rat offspring were growth-restricted and underwent an age-dependent loss of glucose-tolerance, so that by 15 months of age, male LP offspring were frankly diabetic which was associated with insulin resistance (Petry et al., 2001). LP rat offspring also demonstrated perturbations in key molecules of the insulin signalling cascade (PKC-ζ, GLUT-4 and p85-α) in skeletal muscle, which occurred before the diabetic phenotype arose (Ozanne et al., 2005). Most importantly, these molecules were also down-regulated in similar proportions in skeletal muscle and adipose tissue of low birth weight young men (Ozanne et al., 2005, Ozanne et al., 2006). The striking similarities between this model and humans highlight the importance of this model in elucidating mechanisms central in intrauterine programming of insulin sensitive tissues. Other models of maternal protein restriction have been associated with hypertension in the offspring (Langley-Evans et al., 1999) and this rise in blood pressure may be due to increased cardiovascular sympathetic tone (Barros et al., 2015), which could lead to age-associated kidney disease in later life. Also, 8% maternal protein restriction accelerated age-related decline in renal and vascular dysfunction in female rat offspring (Black et al., 2015). Maternal protein (8%) restriction has also been shown to induce simple hepatic steatosis in rat offspring (Kwon et al., 2012).

2.4.2 Mismatch of *in-utero* and postnatal environments; impact of postnatal growth

Using the well-established rodent model of maternal protein restriction, cross-fostering techniques have enabled our laboratory to create a model of accelerated postnatal growth. Offspring of LP-fed rodent dams were cross-fostered to control-fed dams. The cross-fostered offspring, termed 'recuperated' had reduced longevity compared to their control littermates in both rats (Jennings et al., 1999) and mice (Ozanne et al., 2004) and a postnatal obesogenic diet in mice exacerbated the shortened lifespan in 'recuperated' offspring

(Ozanne et al., 2004). Propensity to develop age-associated diseases has also been implicated in this model. In mice, an age-dependent development of fatty liver was observed in 'recuperated' offspring, which was associated with increased expression of genes implicated in lipid accumulation (Carr et al., 2014). Additionally, adipose tissue insulin resistance (Tarry-Adkins et al., 2015) and hepatic fibrosis and inflammation were observed in rat offspring of this model (Tarry-Adkins et al., 2016).

Conversely, we have also demonstrated that rodent offspring born to mothers fed a 'normal' (20%) protein diet, and then suckled by mothers fed the low-protein (8%) diet until weaning, underwent slow postnatal growth and demonstrated increased longevity in both rats (Jennings et al., 1999) and mice (Ozanne et al., 2004), compared to animals fed a control (20%) protein diet during both gestation and lactation. The mice were also protected against shortened lifespan when challenged with an obesogenic diet after weaning (Ozanne et al., 2004). Phenotypically, reduced postnatal growth in mice increased thymic growth (an indication of reduced thymic ageing and consequently a marker of immunoprotection) (Chen et al., 2010), reduced splenic ageing (Heppolette et al., 2016), improved insulin sensitivity (Chen et al., 2009) and in rats, increased nephroprotection (Tarry-Adkins et al., 2007). Taken together, this suggests that slow postnatal growth is beneficial to the future health of offspring (a phenotype seen in breast-fed infants) and that a mild stress during early life is beneficial to lifespan maintenance. This supports the 'Hormesis hypothesis' which suggests that exposure of a mild stress (which in greater proportions would be detrimental) can improve the functional ability of organisms. This hypothesis has been successfully tested in many species as diverse as yeast, flies, worms and rodents, where a mild stress such a caloric restriction has been shown to increase longevity (LeBourg, 2009). Other hypotheses also exist to explain the effects of post-natal dietary restriction on health and longevity. The 'Hyperfunction hypothesis' proposed by Blagosklonny (2007) suggests that ageing and age-

associated diseases are driven by processes that contribute to early-life fitness through growth and reproduction and then continue in later life at too high a level. It has been postulated that inhibiting or reducing this overload can help prevent aging/increase longevity (Blagosklonny 2007). It is thought that the nutrient sensing network, including growth hormone, IGF1 and TOR are implicit in promoting these activities (Fontana et al., 2010).

2.4.3 Uteroplacental Insufficiency

Uteroplacental insufficiency, a condition whereby the fetus is not gaining sufficient nutrients from its mother, is a common cause of IUGR in human pregnancies. Simmons and colleagues have developed an elegant rat model of uteroplacental insufficiency using bilateral uterine artery ligation. They demonstrated that rat dams that underwent this surgery had offspring which were growth restricted, had hepatic insulin resistance in young adult life (Vuguin et al., 2004), developed T2D in later life (Simmons et al., 2001) and had reduced glomerular number which was associated with increased apoptosis (Pham et al., 2003). Female rat offspring of mothers that underwent bilateral uterine artery ligation had selective uterine artery endothelial dysfunction and increased arterial stiffness (Mazzuca et al., 2010) and modest renal insufficiency (Moritz et al., 2009). Conversely, another group, using the same method to induce uteroplacental insufficiency, found that 12 month female offspring were hypertensive; however no evidence of glucose intolerance was observed (Tran et al., 2015).

2.4.4 Maternal caloric/nutrient restriction

Maternal food (nutrient/caloric) restriction is an important issue in developing countries, and consequently several animal models have been utilised to dissect underlying molecular mechanisms of this form of IUGR. Garafano et al. (1999) used a rat model of severe caloric restriction (50% ad-libitum) and found that offspring of food restricted dams

had an accelerated age-dependent loss of glucose tolerance. Others have utilised the same severity of maternal caloric restriction and showed that rat offspring were hypertensive with endothelial dysfunction (Franco et al., 2002) and had differential expression of genes associated with renal hypertension (Tain et al., 2015). In a non-human primate model of maternal nutrient restriction (70% of control food consumption), alterations in the renal transcriptome and kidney morphology was observed in offspring of nutrient restricted mothers (Cox et al., 2006). In an ovine model of 40% caloric restriction, combined with postnatal catch-up growth (which was induced by singleton compared to twin rearing), offspring were growth-restricted and then became obese, had insulin and leptin resistance and raised cortisol, a phenotype which was more severe than following *in-utero* caloric restriction alone (Dallschaft et al., 2014). In addition, hyperinsulinaemia, hyperleptinaemia and compensatory leptin production in pancreatic β-cells was observed in a rat model of 30% caloric restriction. This phenotype was also worsened when these offspring were exposed to hypercaloric nutrition after weaning (Vickers et al., 2001). This exacerbation of phenotype after accelerated postnatal growth is a common mechanism shared in models of maternal protein restriction and caloric restriction. Interestingly, caloric restriction can also ameliorate several hallmarks of cellular ageing including epigenetic alterations, stem cell depletion, cellular senescence, mitochondrial dysfunction, genomic instability (DNA repair mechanisms), proteostasis imbalance and impaired nutrient sensing (Michan et al., 2014).

2.4.5 Maternal Iron Restriction

Iron deficiency (anaemia) is the most common form of nutrient deficiency worldwide, affecting nearly 2 billion people and up to 50% of pregnant women and is a major cause of IUGR. Large placental weights and a high ratio of placental weight to birth weight (known predictors of adult blood pressure) have been observed in offspring of iron-restricted mothers (Godfrey et al., 1991), which may be due to alterations of placental cytokine expression,

which can be regulators of growth and development (Gambling et al., 2002). Three month-old rat offspring of iron restricted mothers have reduced weight at birth which persisted until 3 months of age and had increased blood pressure (Lewis et al., 2001). The hypertensive phenotype has also been shown to be present until 16 months of age (Lisle et al., 2003).

2.4.6 Maternal Obesity

Animal models of maternal obesity are known to cause a range of age-associated disease pathologies in the offspring. Using a diet rich in simple sugars and saturated fat, male rat offspring born to obese dams have similar body weights between birth and 8 weeks of age, however at 8 weeks of age these offspring had increased biomass in the form of cardiac hypertrophy which was associated with hyperinsulinemia and increased phosphorylation of AKT, ERK, and mTOR activation (Fernandez-Twinn et al., 2012) and cardiovascular dysfunction (Blackmore et al., 2014). It is known that increases in biomass (namely hypertrophy and hyperphagia) underpin many age-associated diseases including CVD and T2D. The observed phenotypes are independent of the offspring's current body weight. This indicates that the observed phenotypes are driven by developmental programming *per-se* and not by differences in offspring weight gain. Female mouse offspring from obese mothers using the same obesogenic diet became hyperphagic between 4 to 6 weeks of age, and developed increased adiposity at 6 months of age which may further exaggerate the programmed metabolic and cardiovascular dysfunction (Samuelsson et al., 2007). Using the same murine model, offspring of obese mothers also develop a fatty liver phenotype which was associated with disrupted lipid metabolism (Alfaradhi et al., 2014), insulin resistance and dysregulation of metabolism (Oben et al., 2010). Hepatic lipotoxicity was also observed in offspring of primates fed an obesogenic diet (McCurdy et al., 2009). A 'junk food' diet

(Bayol et al., 2010) also programs a fatty liver phenotype in rats which was also associated with disrupted lipid metabolism.

2.4.7 Reproductive ageing

The female reproductive system is more susceptible to age-associated functional decline compared to normal somatic ageing, due to menopause (estropause in animals) occurring far earlier than whole body ageing. Epidemiological evidence exists to suggest that female reproductive function can be influenced by the early environment, including timing of menarche (Slobada et al., 2007), fertility (De Bruin et al., 1998) and menopause (Elias et al., 2003). Recently, we have demonstrated an accelerated ageing phenotype, which was associated with decreased ovarian reserve and DNA damage in ovarian and oviduct tissues from maternally protein restricted rats (Aiken et al., 2013) and mice (Aiken et al., 2016) that underwent accelerated postnatal growth. This phenotype has been confirmed by others in rats (Guzman et al., 2014). Zambrano et al. (2005) have demonstrated that maternal protein restriction can also deleteriously affect the male reproductive system, with testicular weight, fertility rate and sperm count decreased in these offspring. Taken together, these findings suggest that reproductive ageing as well as traditional somatic ageing is also susceptible to the effects of 'developmental programming', potentially having implications for future generations.

2.4.8 Trans-generational studies

Recently, several studies have shown that the deleterious effects of suboptimal maternal environments do not merely have a direct effect on the long-term health outcomes of the first generation. It now seems evident that these phenotypes can be transmitted throughout the generations, in the absence of further insult. Offspring of mothers who were exposed to the Dutch Hunger Winter famine *in-utero* had increased adiposity and poor health

(Painter et al., 2008) and children whose grandmothers participated in Ramadan fasting during pregnancy were lighter and had lower placental weights (Alwasel et al., 2013). Epidemiological evidence also suggests that paternal lineage is an important determinant in transgenerational transmission of a programming phenotype. Second generation (F2) offspring whose fathers were exposed to the Dutch Hunger Winter famine had a higher BMI compared to unexposed offspring (Veenendaal et al., 2013). In mice, metabolic changes observed in the offspring of mothers fed a high-fat diet were present in 3 generations after high-fed diet administration (Masuyama et al., 2015) and offspring of mice undernourished *in-utero* had perturbed metabolic profiles which was maintained for 50 generations (Hardikar et al., 2015). In rats, maternal protein restriction followed by accelerated catch-up growth decreased ovarian reserve, increased intra-abdominal fat mass and accelerated ovarian ageing in the second generation (Aiken et al., 2015).

2.5 Common underlying mechanisms of developmental programming

2.5.1 Structural effects

a) The endocrine pancreas:

The structure of the endocrine pancreas seems to be particularly susceptible to changes nutrition in fetal life. Fetal pancreata from rat dams fed a low (8%) protein (LP) diet had increased islet apoptosis (Petrik et al., 1999), reduced β-cell proliferation, decreased pancreatic islet size and diminished islet vascularisation (Snoeck et al., 1990) compared to 20% casein protein-fed (control) offspring. In old age (15 months), these offspring had increased markers of fibrosis compared to controls (Tarry-Adkins et al., 2010) Common aberrations in pancreatic structure is also apparent in models of maternal caloric restriction (50%) whereby age-related loss of β-cell mass are observed in offspring of food restricted mothers (Garafano et al., 1999), and when these offspring were challenged with

streptozotocin, β-cell regeneration was impaired (Garafano et al., 2000). Intrauterine placental ligation also has a deleterious effect on structure of the endocrine pancreas, with an age-dependent loss of relative β-cell mass (Simmons et al., 2001).

b) The kidney:

Nephron number and size are also commonly affected by models of suboptimal maternal nutrition. Severe maternal protein restriction using a 6% protein diet, leads to permanent reductions in nephron number in rat offspring (Merlet-Benichou et al., 1994), whereas milder maternal protein restriction (9% protein diet, supplemented with methionine) caused reduced glomeruli number and hypertension in rat offspring (Langley-Evans et al., 1999). Both 6% and 9% maternal protein restriction has also been associated with increased apoptosis of renal mesenchymal cells at the start of renal development in the rat offspring (Welham et al., 2002). A rat model of 8% maternal protein restriction also resulted in reduced nephron number, glomerular enlargement, suppression of the renin-angiotensin system and hypertension in the offspring (Woods et al., 2001). A model of rat placental insufficiency has also shown nephron number deficits (Moritz et al., 2009) and a rat model of maternal iron restriction demonstrated reduced nephron number in 3 and 16 month old rat offspring (Lisle et al., 2003). All of these models may lead to age-associated kidney disease in later life.

2.5.2 Epigenetics

The concept that the environment to which a mother or father is exposed can influence health in not only their own offspring, but in future generations has brought about many studies focussing upon epigenetic mechanisms. Epigenetics are gene expression regulating mechanisms which are independent of genomic DNA sequence, and can involve persistent changes in chromatin conformation, such as DNA methylation and histone modifications and microRNAs (miRNAs). Various models of developmental programming

have investigated epigenetic mechanisms in order to explain how a suboptimal maternal environment is capable of inducing effects on future generations.

Adults exposed to famine *in-utero* had reduced methylation of the IGF-2 DMR (Heijmans et al., 2008) and differential methylation of 15 loci implicated in growth and development compared to individuals not exposed to famine (Tobi et al., 2009). In animal models, offspring of rat dams with placental insufficiency had decreased CpG methylation of the cellular senescence marker *p53* and a parallel increase in *p53* expression in the kidney (Pham et al., 2003) as well as genome-wide DNA hypomethylation and increased histone acetylation in liver (MacLennan et al., 2004) and progressively epigenetically silenced *Pdx1* (via histone deacetylation) in pancreatic islets (Park et al., 2008). Administration of Exendin-4 (a glucogon-like peptide-1 analogue) increased *Pdx1* levels in pancreatic islets of these offspring, via up-regulation of histone acetylation activity (Pinney et al., 2011). In a primate model of maternal obesity, hyperacetylation of the histone marks H3K14, H3K9 and H3K was observed in offspring, which was associated with depleted levels of histone deacetylase 1 (HDAC1), however this was not associated with gene repression, (Aagaard-Tillery et al., 2008). In mice, a maternal high-fat diet caused epigenetic alterations in *Adiponectin* and *Leptin* genes, which were detected through 3 generations (Masuyama et al., 2015). Cardiac levels of miR-133 were also elevated in mouse offspring of maternal obesity, which may be associated with the cardiac hypertrophy seen in these offspring (Blackmore et al., 2014). A rat model of maternal protein restriction has shown that Hnf4a (a transcription factor implicated in T2D), is epigenetically regulated by maternal diet and ageing in rat islets and that ageing leads to progressive epigenetic silencing of the entire Hnf4a locus in islets (Sandovichi et al., 2011). Adipose tissue from this model was also associated with increased miR-483-3p which regulates translation of growth differentiation factor-3 (*Gdf3*). This was also observed in adipose tissue of low birth weight young men (Ferland-MCollough et al.,

2012). In the same model, expression of miR-15b was elevated in skeletal muscle of IUGR rat offspring, which was also observed in skeletal muscle of low birth weight diabetic monozygotic twins (Bork-Jensen et al., 2015). DNA methylation of *Pparα* and glucocorticoid receptor (*Gr*) genes was also reduced and gene expression increased in offspring of protein-restricted mothers compared to controls. These epigenetic changes were prevented by folate supplementation (a known methyl donor, important in DNA methylation), (Lillycrop et al., 2005). Taken together, strong evidence exists to support the existence of epigenetic modifications in models of suboptimal maternal environments, which may be an important driver in transgenerational transmission of phenotype.

2.5.3 Cellular ageing

Telomere length and Cellular Senescence:

Telomeres are non-coding sequences at the ends of chromosomes, consisting of a variable number of tandem repeats of DNA (TTAGGG)n. In normal somatic cells (without telomerase), telomeres shorten with every cell division. When telomeres reach a critical length, they become dysfunctional and undergo a conformational change, resembling double-stranded breaks. This causes the cells to reach irreversible replicative senescence or apoptosis via up-regulation of senescence proteins p53, p21 and $p16^{INK}$ and the apoptosis cascade (Harley et al., 1990). Consequently, many researchers consider that telomeres are associated with cellular ageing and replicative senescence (Harley et al., 1990, Bernodotte et al., 2016) and others have suggested that telomere length may be related to lifespan (Heidinger et al., 2012, Haussmann et al., 2003, Fairlie et al., 2016), however it needs to be noted that some researchers suggest telomere length is not a reliable proxy for biological ageing or lifespan (Bischoff et al, 2006, Mather et al., 2010). Previously, using a well-established Southern blotting based methodology, we have demonstrated accelerated telomere shortening in the aorta (Tarry-Adkins et al., 2008), heart (Tarry-Adkins et al., 2013), female reproductive tract

(Aiken et al., 2013) and pancreatic islets (which was also associated with increased markers of cellular senescence; p21 and $p16I^{NK}$) (Tarry-Adkins et al., 2009) of rat offspring whose mothers were protein-restricted and who then underwent accelerated postnatal growth (recuperated), suggesting that these offspring have an accelerated ageing phenotype which may contribute to their shortened lifespan. Conversely, a model of protein restriction during lactation, which causes slower postnatal growth showed fewer short aortic telomeres, reduced DNA damage and oxidative stress (Tarry-Adkins et al., 2008). Moreover, ovarian telomere length was also shortened in granddaughters of recuperated rat dams, showing that effects can be passed through more than one generation (Aiken et al., 2015).

Oxidative stress:

'The Harman free radical theory of ageing' postulates that oxidative stress is a major driver of accelerated cellular and whole body ageing, however most of the studies that support this hypothesis are associative and do not support a key and independent role of oxidative stress in modulating mammalian ageing. However, it cannot be disputed that high levels of oxidative stress is certainly deleterious to the cell. In particular, the mitochondria is known to be vulnerable to oxidative stress as it is the principle source of intracellular ROS leading to mitochondrial (mt)DNA mutations. These have been widely reported by be involved in the normal ageing process, however it still remains unclear the role mitochondrial dysfunction plays in longevity (Wang and Hekimi., 2015). Under pathophysiological conditions, ROS are over-produced by mitochondrial electron-transport chain (ETC) uncoupling, activation of the xanthine-oxidoreductase system or excessive stimulation of NAD(P)H which can overwhelm endogenous antioxidant defence mechanisms. When this occurs, ROS damages cellular macromolecules including DNA, lipids and proteins. This damage can accelerate telomere shortening in particular as the guanine-rich telomeric sequences are predominantly susceptible to oxidative stress (Kawanishi et al., 2004).

406 Oxidative stress is a common factor in many models of developmental programming (Table
407 1). In a model of maternal protein restriction, combined with rapid postnatal growth,
408 increased indices of ROS, (including increased lipid peroxidation, protein nitrotyrosination
409 and altered antioxidant defence capacity) was observed in the heart (Tarry-Adkins et al.,
410 2013) and aorta (Tarry-Adkins et al., 2008) of male rats and in ovarian tissue of
411 'programmed' offspring (Aiken et al., 2013) In each case, these were associated with
412 telomere shortening. Enhanced levels of oxidative stress (as evidenced by a decreased
413 superoxide dismutase activity and increased superoxide anion concentrations) have also been
414 implicated in a rat model of caloric restriction (50%) in both male (Franco et al, 2002) and
415 female offspring (Franco et al., 2007). Mitochondrial dysfunction in particular has been
416 common in several models of 'developmental programming': Rat offspring from placental
417 insufficiency pregnancies have impaired oxidative phosphorlyation in both hepatic (Peterside
418 et al., 2003) and skeletal muscle mitochondria (Selak et al., 2003), as well as progressive
419 accumulation of ROS, increased mtDNA mutations and a decline in complex I and III ETC
420 activities in pancreatic islets (Simmons et al., 2005). Mouse offspring of obese pregnancies
421 also demonstrate hepatic oxidative stress and mitochondrial dysfunction (Alfaradhi et al.,
422 2014) and rat offspring of a maternal 'junk food' diet also demonstrate increased oxidative
423 stress and mitochondrial dysfunction (Bayol et al., 2010). Defects in components of the
424 mitochondrial ETC have also been observed in the skeletal muscle of both rat (Shelley et al.,
425 2009) and mouse (Latouche et al., 2014) models of maternal obesity.

426 **2.6 Intervention strategies**

427 It is apparent that over-production of ROS is a common underlying consequence of
428 sub-optimal maternal nutrition; therefore some animal studies have focussed on the use of
429 antioxidant therapies to attempt to reverse the deleterious phenotypes observed in models of
430 developmental programming. These included the use of high concentrations of vitamins A, C,

E and selenium to reduce adiposity and improve glucose tolerance in rat offspring, which resulted from a maternal exposure to a high-fat diet (Sen et al., 2010). Prevention of vascular dysfunction and microvascular rarefaction in rat offspring in a model of maternal protein restriction was also induced by antenatal treatment with the antioxidant Lazaroid (Cambonie et al., 2007). In an ovine model of nutrient restriction, pregnant ewes that were supplemented 5mg of melatonin had increased umbilical artery blood flow (Shukla et al., 2014). These studies all show proof of principle that antioxidant therapy can reverse deleterious phenotypes of developmental programming, however the doses used are far higher than used clinically and these studies focus on maternal supplementation, where in practice, phenotypes of developmental programming are more likely to be apparent at the time of or just after delivery, therefore postnatal antioxidant supplementation needs to be addressed. Our laboratory has demonstrated that a postnatal supplementation of a clinically relevant dose of Coenzyme Q_{10} (CoQ_{10}) (an endogenous antioxidant) prevented cardiac (Tarry-Adkins et al., 2013), and aortic (Tarry-Adkins et al., 2014) accelerated ageing phenotypes, prevented adipose tissue insulin signaling dysregulation and inflammation (Tarry-Adkins et al., 2015) and ameliorated hepatic fibrosis and oxidative stress (Tarry-Adkins et al., 2016) in a rat model of maternal protein restriction followed by accelerated postnatal growth. It needs to be noted however although antioxidant therapy seems to have had some success in ameliorating some factors associated with age-related disease, no studies to date have successfully increased lifespan in laboratory rodents or reduced cancer morbidity/mortality in human random controlled trials and this warrants further investigation.

3. Conclusions

The increased risk of development of age-associated disease, including CVD, T2D, NAFDL and obesity is strongly associated with growth in conditions of a suboptimal maternal milieu, both as a direct transmission to the 1st generation and via transgenerational

transmission. It is evident that several common mechanisms exist between models of suboptimal nutrition, irrespective as to whether these models are based on maternal under-nutrition or over-nutrition. Structural perturbations in tissue, epigenetic modifications and accelerated ageing, usually involving generation of ROS are all common to these models, in both animal models and epidemiological populations. Although studies have begun to address methods of intervention to reverse or prevent deleterious programming phenotypes, especially antioxidant intervention, more research is needed to tease out further mechanisms underpinning the phenomenon of developmental programming to tailor interventions suitable for use in human populations.

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