



## Preconception health 2

# Origins of lifetime health around the time of conception: causes and consequences

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This is the second in a Series of three papers about preconception health

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Parental environmental factors, including diet, body composition, metabolism, and stress, affect the health and chronic disease risk of people throughout their lives, as captured in the Developmental Origins of Health and Disease concept. Research across the epidemiological, clinical, and basic science fields has identified the period around conception as being crucial for the processes mediating parental influences on the health of the next generation. During this time, from the maturation of gametes through to early embryonic development, parental lifestyle can adversely influence long-term risks of offspring cardiovascular, metabolic, immune, and neurological morbidities, often termed developmental programming. We review periconceptional induction of disease risk from four broad exposures: maternal overnutrition and obesity; maternal undernutrition; related paternal factors; and the use of assisted reproductive treatment. Studies in both humans and animal models have demonstrated the underlying biological mechanisms, including epigenetic, cellular, physiological, and metabolic processes. We also present a meta-analysis of mouse paternal and maternal protein undernutrition that suggests distinct parental periconceptional contributions to postnatal outcomes. We propose that the evidence for periconceptional effects on lifetime health is now so compelling that it calls for new guidance on parental preparation for pregnancy, beginning before conception, to protect the health of offspring.

### Introduction

The notion that maternal physiology, body composition, diet, and lifestyle during pregnancy have profound and enduring effects on the long-term health of the offspring, and disease risk into adulthood, has received strong evidential support across the epidemiological, medical, and basic science fields.<sup>1–3</sup> Thus, the Developmental Origins of Health and Disease concept has emerged,<sup>3</sup> suggesting that poor developmental experience can increase the risk of non-communicable diseases in later life, including cardiovascular and metabolic comorbidities (such as hypertension, obesity, and type 2 diabetes), atopic conditions, cancer, and neurological impairment.

Research into the concept has focused on the time during pregnancy when the conceptus is most vulnerable to adverse influences, thereby informing targeted protection and possible intervention. Increasing evidence points to the importance of the time around conception, known as the periconceptional period.

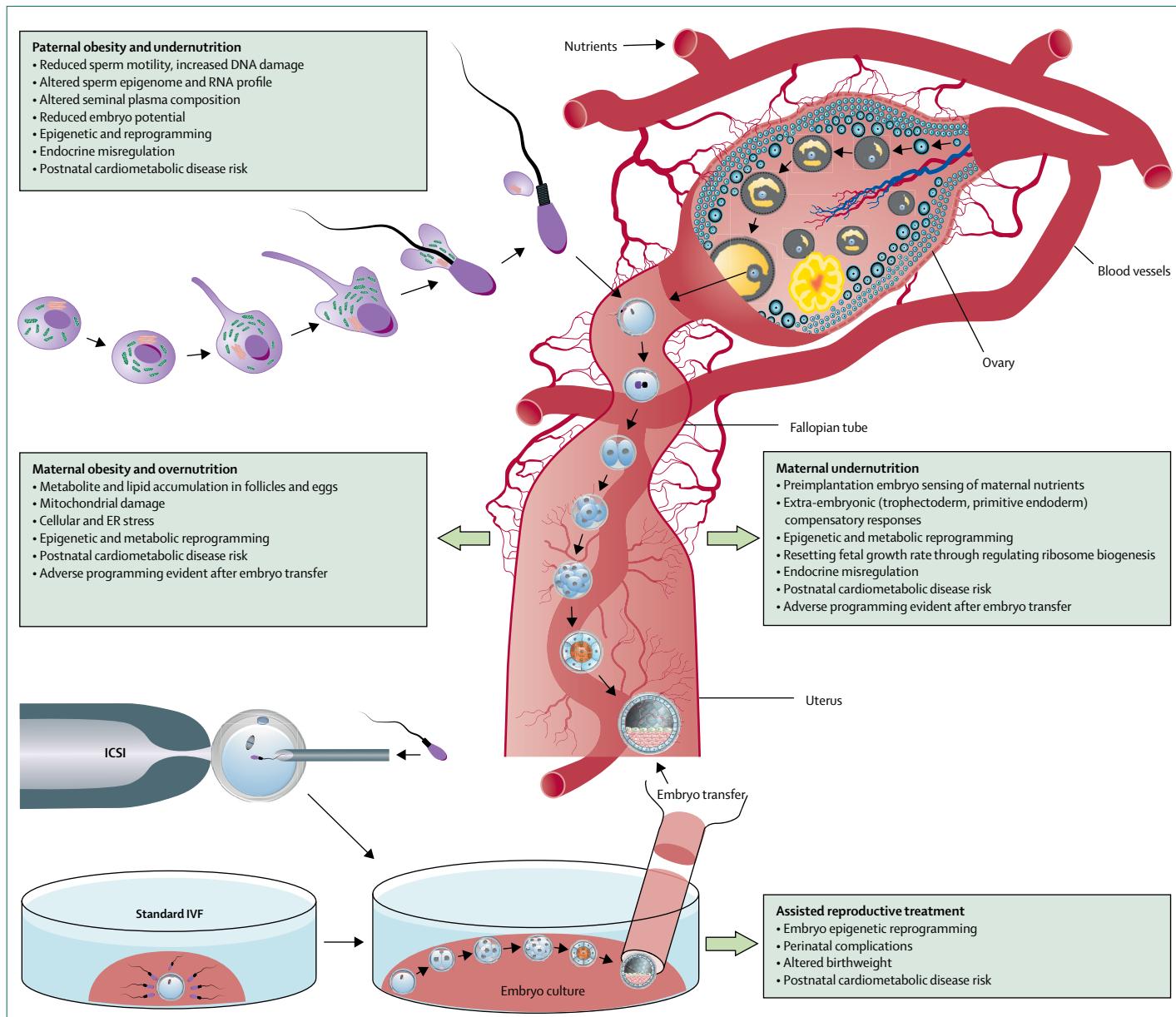
This Series paper focuses on four broad periconceptional environmental exposures shown to adversely affect humans and animal models (figure 1), and discusses mechanistic causes and consequences. We also report a meta-analysis on the relative contributions of maternal and paternal factors on long-term periconceptional influences, in an established low protein diet model of parental undernutrition.

### Periconceptional developmental conditioning

The periconceptional period has been variously defined, but for the Developmental Origins of Health and Disease concept the key events broadly cover the completion of meiotic maturation of oocytes, differentiation of spermatozoa, fertilisation, and resumption of mitotic cell cycles in the zygote, marking the transition from the parental to the embryonic genome,<sup>4</sup> and the onset of morphogenesis up to implantation.<sup>5</sup> This process represents a period of a few weeks, depending on the mammalian species, and is characterised by extensive change in morphology (emergence of distinct embryonic and placental cell lineages); genomic reorganisation (epigenetic modifications such as DNA methylation to regulate lineage-specific gene expression in the conceptus); and changes in metabolism (setting homoeostatic regulators for growth and energy supply; figure 2). It is, however, recognised that influences at every stage from

### Key messages

- Although evidence for developmental origins of later disease can be found throughout gestation and beyond, there is a growing consensus from both human and animal studies that conception marks a crucial period that merits attention.
- Preconception maternal overnutrition and obesity, maternal undernutrition, related paternal factors, and assisted reproductive treatments can change the phenotype and potential of gametes and early embryos, with enduring consequences across the lifespan.
- Our meta-analysis reveals that suboptimal maternal and paternal nutrition around conception have similar effects on offspring weight, but differing effects on offspring blood pressure.
- These crucial influences on lifetime health occurring so early in development might reflect perturbations or adaptations in epigenetic, cellular, metabolic, and physiological mechanisms. Defining these mechanisms, and the exposures that drive them, is essential for the development of more specific recommendations for preconception health.
- This emerging knowledge has substantial societal and medical implications, providing the basis for a new emphasis on preparation for conception and pregnancy, to safeguard public health and as a means of disease prevention.



**Figure 1: Summary of periconceptional developmental conditioning from the four areas reviewed, with the main mechanisms highlighted in the progression of disease risk**  
ER=endoplasmic reticulum. ICSI=intracytoplasmic sperm injection. IVF=in-vitro fertilisation.

earliest childhood can shape preconception health, and thereby influence eventual pregnancy and birth outcomes.

Adverse developmental processes around the time of conception have been demonstrated in human and animal models in response to diverse environmental situations. In vivo, the quality of the maternal diet, both overnutrition and obesity<sup>8</sup> or undernutrition,<sup>9</sup> and other aspects of her physiological status including hyperglycaemia or lipidaemia,<sup>10</sup> can affect embryo potential with consequences for offspring disease risk over their lifetime. Paternal lifestyle and phenotype can similarly influence long-term offspring health, mediated either through the

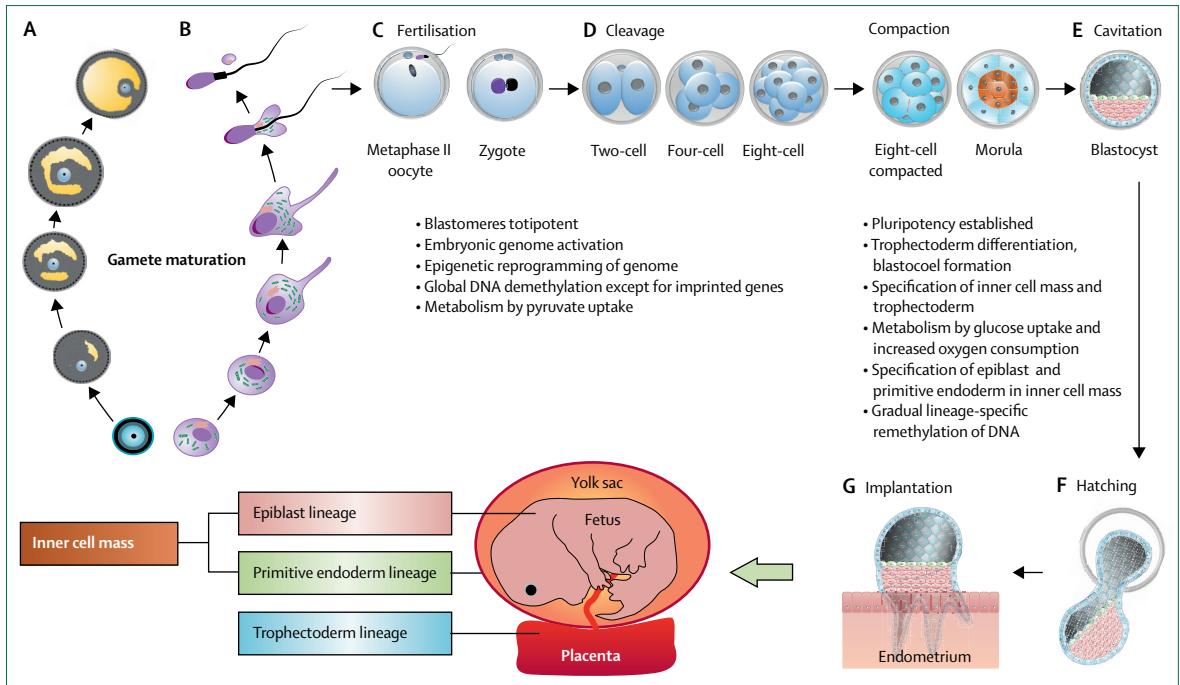
sperm or seminal plasma.<sup>11</sup> Periconceptional parental influences could have particular and differing effects on male and female offspring.<sup>12</sup> In addition, more babies are being born as a result of assisted reproductive treatments, some of which involve embryo culture and exposure to potentially inappropriate environmental factors that could alter offspring phenotype.<sup>12,13</sup> Long-term outcomes are consistent with the Developmental Origins of Health and Disease concept, including cardiometabolic, immunological, and neurological non-communicable disorders.

To some, the concept of periconceptional origins of lifetime health might not be intuitive. Why should this

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**Figure 2: Biological events underpinning periconceptional conditioning**

The periconceptional period (A–G) is one of extensive cellular change comprising the completion of meiotic maturation of oocytes (A), differentiation of spermatozoa (B), fertilisation (C), and resumption of mitotic cell cycles in the zygote (D), marking the transition from parental to embryonic genomes,<sup>4</sup> and the onset of morphogenesis.<sup>5</sup> Periconceptional biology is indeed busy—the morphological and cellular changes occurring during the switch from parental to embryonic generations leading to blastocyst formation (E), are driven by pronounced subcellular and molecular processes. These include global restructuring of the epigenome (mainly DNA methylation and histone modifications that control gene expression), such that expression from the new embryonic genome is distinct from the parental genomes.<sup>6</sup> Epigenetic reorganisation allows the embryo to first exhibit totipotency, a naive cellular state conferring the ability to construct both true embryonic (future fetal) cell lineages and the extra-embryonic (placental) lineages that become evident in the blastocyst. Subsequently, epigenetic modifications underpin embryo pluripotency, the capacity to generate all three germ layers (ectoderm, mesoderm, and endoderm) once gastrulation has taken place. Morphogenesis of the blastocyst is followed by embryo hatching from the zona pellucida coat (F) and implantation (G), mediated through adhesion of the outer trophoblast layer of the blastocyst to the uterine endometrium and subsequent invasion and deciduation. Activation of the new embryonic genome before implantation not only permits de-novo gene expression distinct from parental genomes, but also involves establishment of the embryo's metabolism that matures over time.<sup>7</sup>

short window at the very start of development have such profound consequences for the rest of our lives? Crucially, the essential steps in reproduction over this period occur when the few cells involved are fully exposed to environmental conditions. Therefore, the cells are vulnerable to disturbance of epigenetic mechanisms, leading to an altered profile of embryonic gene expression that persists through subsequent cell cycles, and drives a modified developmental programme. Metabolic and cellular homoeostatic characteristics of the embryo, including mitochondrial activity, can also change in response to nutrient availability. Periconceptional sensitivity to environmental cues also raises the possibility that this window is one of opportunity, providing the embryo with capacity to respond to prevailing conditions and to optimise development to best suit survival and fitness.<sup>9</sup> Thus, periconceptional developmental plasticity (the induction of different phenotypes from a single genotype) could facilitate the setting of suitable growth and metabolic parameters to match the perceived environment, but that, if environmental conditions change, could become maladaptive and lead to later disease.<sup>3</sup>

### Periconceptional developmental conditioning through maternal overnutrition and obesity

The global rise in maternal obesity is associated with reduced female fertility and heightened risk of obesity in the offspring.<sup>2</sup> The adverse effects of high maternal body-mass index (BMI) on the offspring could reflect elevated maternal glucose and insulin concentrations, which drive fetal growth and adiposity (resulting in increased birth and childhood weight), but might also include shared life-style factors within families.<sup>8</sup> Impaired metabolism in offspring might also be associated with increased risk of allergic and atopic conditions, revealing the complexity in phenotype.<sup>2</sup> Animal models have confirmed the link between maternal obesity, and cardiovascular and metabolic disease risk in offspring.<sup>8,14</sup>

Why might the periconceptional period be causal for obesity-related conditioning? Obese women have higher circulating concentrations of inflammatory cytokines, and hormones and metabolites,<sup>15</sup> which accumulate within the ovarian follicular fluid and can adversely affect oocyte maturation and potential. Thus, maternal BMI is positively

associated with increased follicular fluid insulin, lactate, triglycerides, leptin, and other metabolic regulators.<sup>16</sup> In experimental models, this rich follicular fluid influences the developmental competence of exposed animal oocytes, thereby reducing embryo quality.<sup>17</sup> Moreover, oocytes from obese women are smaller and produce blastocysts with increased triglycerides and reduced glucose consumption (markers of poorer potential), than do oocytes from women of healthy weight.<sup>18</sup>

In mice, maternal obesity causes defects in the mitochondrial phenotype of oocytes, including abnormal morphology and cristae structure, altered membrane potential and distribution,<sup>19</sup> and increased mitochondrial DNA content;<sup>19,20</sup> these phenotypes are markers of disturbed mitochondrial function and energy homoeostasis. Oocytes from obese mice also exhibit increased oxidative stress, and spindle abnormalities, suggesting increased risk of aneuploidy.<sup>19,20</sup>

Mitochondrial defects in oocytes could derive from the elevated lipid content and inherent insulin resistance caused by high maternal adiposity. Oocyte hyperlipidaemia, in turn, leads to impaired metabolic regulation and endoplasmic reticulum stress in mice,<sup>17</sup> a condition that causes proteins to misfold during biosynthesis, contributing to metabolic and cardiovascular disease. Bovine and murine in-vitro oocyte maturation models demonstrate that elevated fatty acid concentrations perturb follicular physiology, reduce oocyte developmental competence (including altered transcriptome and epigenome profiles in blastocysts), and lead to early embryos with compromised metabolism and low developmental potential.<sup>13</sup>

The combination of metabolic, mitochondrial, and chromosomal alterations in oocytes and embryos from obese mothers has important implications for subsequent development. In mice, obese mothers have small fetuses and pups that develop overgrowth, adiposity, and glucose intolerance after birth.<sup>21</sup> Transfer of mouse blastocysts from obese mothers to normal recipients produced similarly growth-restricted fetuses with associated malformations, despite the absence of gestational maternal obesity.<sup>19</sup> Similarly, in sheep, female offspring from embryos of obese natural mothers transferred to non-obese mothers exhibited increased adiposity, with dysregulation in liver and muscle insulin signalling and hepatic fatty acid oxidation.<sup>22</sup> These changes are associated with epigenetic perturbations in the liver, including upregulation of microRNAs regulating insulin signalling.<sup>22</sup> Similarly, mouse embryos transferred from diabetic mothers to control recipients exhibited fetal growth retardation, and congenital anomalies resembling natural diabetic pregnancies;<sup>10</sup> these structural changes are in keeping with clinical practice, in which preconceptional and periconceptional folic acid supplementation, and improved diabetes control, reduce the incidence of anomalies.

The periconceptional effects of maternal obesity are also apparent in pregnancies arising from assisted reproductive treatment. Fertility declines with increasing BMI in women receiving donor oocytes, as in non-donated pregnancies, suggesting reduced uterine receptivity.<sup>23</sup> However, it has also been shown that recipient BMI has no effect on donor oocyte pregnancy success, whereas donor BMI is negatively associated,<sup>24</sup> indicating that preconception oocyte quality is influenced by maternal adiposity.

## Periconceptional developmental conditioning through maternal undernutrition

### Human studies

Poor nutrition in utero and low birthweight remain highly prevalent in low-income and middle-income countries, and are associated with increased risks of chronic diseases in later life across diverse human populations, particularly if followed by accelerated weight gain during infancy.<sup>13</sup> Similar human cardiometabolic and neurological consequences arise from maternal exposure to famine, for example, the Dutch Hunger Winter of 1944–45. In human studies it is difficult to pinpoint gestational windows when heightened sensitivity to maternal undernutrition occurs, but the Dutch famine analyses suggest a poor prognosis for offspring conceived during the famine, rather than offspring experiencing famine later in gestation.<sup>25</sup> Similarly, individuals exposed in utero, particularly during the first trimester, to the Chinese Great Famine (1959–61) had increased risk of hypertension in adulthood.<sup>26</sup> Exposure to the Dutch famine during the periconceptional period caused epigenetic dysregulation, resulting in reduced DNA methylation of the imprinted growth-regulating *IGF2* gene persisting into adulthood, and differential methylation in the regulatory regions of genes affecting growth and metabolism.<sup>25</sup>

In another important human study,<sup>27</sup> dramatic seasonal variation in maternal nutrient consumption in The Gambia affected perinatal outcomes, including birthweight, adult health, and mortality. By studying genomic regions where methylation patterns are highly correlated across tissues derived from all three germ lines, it has been demonstrated that maternal nutrition at conception alters the epigenome prior to gastrulation, with the effects persisting well into childhood and adolescence at a minimum.<sup>28</sup> This periconceptional legacy coincides with seasonal changes in maternal plasma methyl-donor biomarkers, which, along with BMI, are also predictive of childhood methylation patterns.<sup>29</sup> Substantial deviations in the methylation patterns of loci predictive of immune function, tumour suppression,<sup>30</sup> and obesity<sup>31</sup> have been observed.

### Animal models

Animal models have been essential for investigating mechanisms involved in the multistep processes linking periconceptional maternal undernutrition with later-life

disease risk. In rodents fed a low protein diet (LPD)—specifically during the periconceptional period, either exclusively during the final 3 days of oocyte maturation,<sup>32</sup> or the 3–4 day window of preimplantation embryo development (Emb-LPD),<sup>33,34</sup> with normal nutrition at all other times—an altered growth trajectory and cardiovascular, metabolic, and neuro-behavioural dysfunction in adulthood were found. Such targeted dietary models commonly show hypertension in adult offspring coupled with increased adiposity.<sup>32–34</sup> Similar findings have been reported in sheep.<sup>35</sup>

Rodent and sheep models of maternal periconceptional undernutrition suggest that impaired regulation of fetal development could underlie comorbidities. For example, studies in sheep have shown that the late gestation fetal cardiovascular response to hypoglycaemia is modified by prior undernutrition during the peri-implantation period.<sup>36</sup> Moreover, maternal undernutrition during peri-implantation and late gestation affects skeletal muscle development differentially in sheep fetuses,<sup>37</sup> and maternal undernutrition in early gestation alters gestation length, and fetal and postnatal growth.<sup>38</sup>

#### Induction and response mechanisms

The mouse Emb-LPD model has helped to reveal how periconceptional maternal undernutrition might initiate adverse effects during early embryogenesis.<sup>9</sup> Emb-LPD reduces the concentration of circulating maternal insulin and aminoacids, including branched-chain aminoacids within the uterine luminal fluid that bathes early embryos before implantation.<sup>39</sup> Branched-chain aminoacids act as targets for embryo nutrient sensors, enabling nutrient status to be sensed by blastocysts via the mammalian target of rapamycin complex 1 growth-regulating signalling pathway, inducing an altered growth trajectory from before implantation,<sup>39</sup> and shown by embryo transfer to be induced within the blastocyst.<sup>34</sup> Altered induction by Emb-LPD in mice activates compensatory responses that are distinct between extraembryonic (trophectoderm, primitive endoderm) and embryonic (epiblast) lineages of the blastocyst (figure 2). As compared with embryonic development in mice fed a normal protein diet, the Emb-LPD trophectoderm becomes more proliferative, adopts a more invasive migratory phenotype at implantation, and activates increased endocytosis of maternal uterine luminal fluid proteins as an alternative source of nutrients, leading to a placenta that is more efficient in nutrient transfer to the fetus.<sup>39–41</sup> Similarly, the primitive endoderm activates compensatory responses to enhance nutrient delivery via the yolk sac placenta, mediated through epigenetic mechanisms.<sup>41,42</sup>

In response to Emb-LPD, changes in embryonic lineages could help set the embryonic and fetal growth trajectory to match prevailing nutrient availability. The embryonic lineages use preimplantation nutrient sensing to regulate growth across somatic organs (eg, liver and kidney) through adaptations in the rate of ribosome

biogenesis.<sup>43</sup> In essence, ribosomal RNA expression is suppressed during periods of maternal dietary restriction, but is increased, beyond that of the control rate, when the dietary challenge is removed. This mechanism modulates the level of DNA methylation at the ribosomal DNA promoter, thereby mediating the interaction of RNA polymerase I with the promoter to regulate ribosome biogenesis and growth.<sup>43,44</sup> Ribosomal DNA has also been found to be a genomic target for growth regulation in models of maternal high-fat or obesogenic diets.<sup>44</sup> This exquisite lifetime mechanism, activated in the preimplantation embryo, is likely to be responsive to uterine luminal fluid nutrient concentrations, and appears to utilise a nutrient-sensing ribosome factor, Rrn3, to mediate ribosomal DNA responses.<sup>43</sup> The growth-regulating role of the embryonic lineage is crucial since perinatal weight associates with adult disease risk.<sup>34</sup>

#### Paternal origin of periconceptional developmental programming

Although the connection between a mother's diet and the long-term health of her offspring has been studied in detail, understanding of how a father's diet impacts his offspring remains limited. However, links are emerging between paternal lifestyle, sperm quality, and impaired offspring health.<sup>11</sup> Here, both direct (sperm quality, epigenetic status, DNA integrity) and indirect (seminal fluid composition) paternal mechanisms have been identified; in mice these mechanisms have been shown to affect offspring development across multiple generations.<sup>45</sup>

Mirroring female reproductive fitness, male fertility is closely linked to nutrition and body composition. In humans and rodents, elevated BMI is associated with reduced sperm motility,<sup>46</sup> increased sperm abnormality,<sup>47</sup> increased levels of reactive oxygen species in sperm, reduced serum testosterone, and increased oestradiol concentrations.<sup>48</sup> Consumption of a so-called western-style diet, high in sugar, fat, and processed foods, is associated with reduced sperm motility in men.<sup>49</sup> In addition, consumption of energy-dense diets in men and rodents is associated with poor sperm motility, morphology, and DNA integrity.<sup>50</sup> Reduced sperm DNA integrity, as occurs in obesity and diabetes, correlates with reduced human embryonic development and decreased pregnancy rates.<sup>51</sup> In men undergoing in-vitro fertilisation treatment, obesity is associated with reduced blastocyst development and reduced livebirth rates.<sup>52</sup> In rodents, paternal obesity induced by a high-fat diet increases sperm DNA damage,<sup>53</sup> reduces blastocyst development and implantation rates,<sup>54</sup> and causes subfertility in male and female offspring for up to two generations.<sup>55</sup> These negative effects on offspring development can be prevented through paternal dietary and exercise interventions in mice,<sup>56</sup> indicating that sperm-mediated effects might be transient and even reversible. In rats, a paternal high-fat diet for 10 weeks before mating affected female

(but not male) offspring pancreatic  $\beta$ -cell function, increased bodyweight and glucose intolerance, and impaired insulin secretion.<sup>57</sup> Offspring of male mice overnourished during neonatal life demonstrate glucose intolerance, fasting hyperglycaemia, and insulin resistance, mirroring the metabolic disturbance seen in their fathers.<sup>58</sup>

Similar to the impacts of paternal obesity, paternal LPD in mice induces the expression of genes involved in offspring hepatic lipid and cholesterol biosynthesis.<sup>59</sup> Analysis of offspring hepatic epigenetic status revealed genome-wide changes in DNA methylation, including the key lipid regulator *PPAR $\alpha$* . In adulthood, offspring from male mice fed LPD had high birthweight, a reduced male to female offspring ratio, increased adult adiposity, hypotension, glucose intolerance, and elevated serum tumour necrosis factor  $\alpha$  levels.<sup>60</sup> Furthermore, paternal LPD also affects blastocyst AMPK expression, placental size, fetal growth, and skeletal development.<sup>61</sup>

As for maternal periconceptional nutrition models, epigenetic mechanisms probably mediate the effects of paternal phenotype and exposures on offspring development.<sup>62</sup> Changes in patterns of sperm histone modifications (methylation, acetylation), DNA methylation, and RNA content, are prime candidates for such paternal periconceptional programming. Sperm from infertile men display significant changes in histone populations,<sup>63</sup> with enrichment of active histone markers (ie H3K27me3) at key developmental and pluripotency genes in both mice and humans.<sup>63</sup> Sperm-derived histones are transferred into the oocyte and incorporate into zygotic chromatin following human fertilisation.<sup>64</sup> However, whether any of the 2–15% of histones retained within the mammalian sperm contribute directly to zygotic gene expression regulation is unknown. Human sperm also contain several thousand coding RNA transcripts,<sup>65</sup> and altered expression is linked with infertility.<sup>66</sup> Levels of sperm transfer RNA-derived small RNAs (tsRNAs) are altered by paternal diet in mice;<sup>67</sup> offspring generated by injecting zygotes with sperm tsRNA taken from male mice fed a high-fat diet, showed impaired glucose tolerance and insulin secretion.<sup>67</sup> Although such studies highlight the role of RNA populations in intergenerational programming,<sup>68</sup> the significance of these sperm-derived RNA molecules remains to be elucidated.

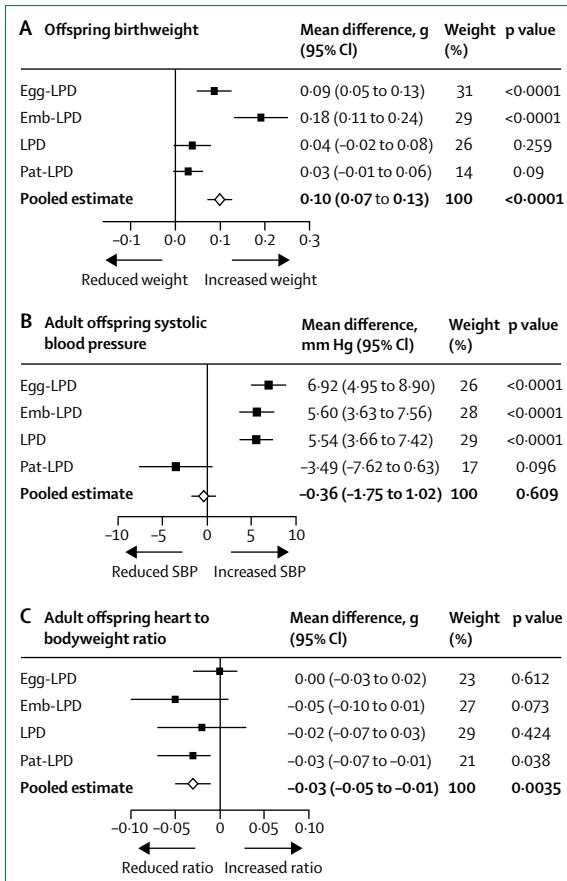
Apart from sperm-specific mechanisms of developmental programming, seminal plasma composition (eg granulocyte-macrophage colony-stimulating factor) can also influence the reproductive process, affecting embryonic, placental, and offspring development in mice,<sup>69</sup> and initiating maternal reproductive tract immunological responses, essential during the establishment and maintenance of human pregnancy.<sup>70</sup> In mice, seminal fluid also impacts on the maternal uterine environment, altering blastocyst development, placental size, and adult offspring glucose tolerance, adiposity, and blood pressure.<sup>71</sup>

#### Panel: Analysis of parental contribution effect

- Data for offspring phenotype were taken from Watkins et al 2008a,<sup>32</sup> 2008b,<sup>34</sup> and 2014.<sup>60</sup> Each study used the same normal protein diet (NPD) and low protein diet (LPD) formulation fed to either female or male mice for distinct periconceptional durations.
- The three studies employed the same rigorous random-effects regression analysis to account for the hierarchical nature of the studies in the statistical analyses.
- Raw data on individual offspring weight at birth, adult tail-cuff systolic blood pressure measurement, and adult heart to bodyweight ratio for all groups were used for the analyses.
- Raw mean differences between offspring in the experimental and study-specific control group (normalised to a value of 0) were calculated for birthweight, systolic blood pressure, and heart to bodyweight ratio.
- Weight (%) refers to the individual contribution (by number of animals) of each study to the total pooled estimate. Heterogeneity (ie, variation in outcomes between studies) was assessed using  $\chi^2$  test on Cochran's Q statistic and by calculating  $I^2$  (ie, percentage of variation across studies attributed to heterogeneity rather than chance). As heterogeneity was significant for all analyses, pooled estimates were calculated by the random effects (Mantel-Haenszel) method.
- The largest effect on offspring birthweight was in response to maternal preimplantation (Emb-LPD) diet (figure 3). Maternal LPD restricted to the terminal stages of oocyte maturation (Egg-LPD) also resulted in increased birthweight. However, maternal LPD throughout gestation had no impact on offspring birthweight, probably reflecting fetal growth regulation during gestation as previously discussed. Paternal LPD also had no effect. Overall, we observed a significant pooled estimate effect of parental LPD on offspring birthweight, representing an increase in LPD offspring weight of 7.8%.
- All maternal LPD groups had elevated systolic blood pressure (figure 3). By contrast, paternal LPD resulted in a non-significant decrease. The differential parental effect on offspring systolic blood pressure meant the pooled estimate showed no overall difference.
- All groups displayed either a negative impact or no effect on adult heart to bodyweight ratio (figure 3). The largest size effect was observed in response to maternal Emb-LPD, but this difference was not statistically significant. Only the paternal LPD offspring heart to bodyweight ratio reached significance. Overall, the pooled effects indicated a reduction in adult heart to bodyweight ratio following both maternal and paternal LPD.

#### Defining the parental contribution to periconceptional developmental effects

Shared maternal and paternal dietary and lifestyle influences could potentially combine for greater impact on periconceptional development. However, most research models to date are uniparental in design, and the combined effects of both parents are unknown. Whether the impact of poor paternal diet on offspring development and wellbeing is of equivalent importance to that of poor maternal diet is also unknown. We did a meta-analysis of our mouse maternal and paternal LPD diet models, using published data for offspring weight at birth, adult systolic blood pressure, and adult heart to bodyweight ratio (a measure of heart capacity), including datasets covering maternal intervention restricted to the periods of oocyte maturation, preimplantation development, or the entirety of gestation (panel and figure 3).<sup>32,34,60</sup> The use of the same robust statistical random-effects regression analysis



**Figure 3: Influence of maternal and paternal factors during periconceptional conditioning in mice following parental low protein diet**

The effect of parental low protein diet (LPD; 9% casein) on offspring weight at birth (A), adult offspring systolic blood pressure (B), and adult offspring heart to bodyweight ratio (C), compared with offspring from parents fed a normal protein diet (18% casein). Analysis of three studies involving female MF1 mice being fed LPD exclusively during the terminal stages of oocyte maturation (3.5 days prior to mating; Egg-LPD), exclusively during preimplantation embryo development (Emb-LPD) or throughout gestation (LPD). Forest plots also include offspring data in response to a paternal low protein diet (Pat-LPD) fed to C57BL6 males prior to mating. For Egg-NPD, n=44–95 males and 69–94 females from 19 litters; Egg-LPD, n=30–89 males and 37–112 females from 19 litters; Emb-LPD, n=39–70 males and 39–64 females from 19 litters; NPD, n=36–60 males and 40–71 females from 19 litters; LPD, n=38–55 males and 47–61 females from 19 litters; Pat-NPD, n=34–44 males and 36–41 females from 16 litters; and Pat-LPD, n=24–29 males and 41–44 females from 16 litters (litters were adjusted after being weighed at birth to a mean of three males and three females, therefore, number ranges reflect both total number of offspring at birth [A] as well as number of offspring post-adjustment [B, C]). (A) Plots present differences between means (95% CI) of birthweight (g) to study-specific NPD group. Between-study heterogeneity  $I^2=33\%$ . (B) Plots present differences between means (95% CI) of adult systolic blood pressure (mm Hg) to study specific NPD group. (C) Plots present differences between means (95% CI) of heart to bodyweight ratio to study specific NPD group.  $I^2=61\%$ . Data combining all LPD and all NPD treatment groups were used to determine the pooled estimate.

across each of these studies, strengthens our comparison of parental effects in the current analysis. However, such rigorous statistical approaches are rarely adopted, especially in animal model studies, and so we have

restricted our analysis to data from these three studies alone. Offspring birthweight was increased in response to maternal LPD during the terminal stages of oocyte development (Egg-LPD), and Emb-LPD (figure 3). Overall, the pooled estimate demonstrated that parental LPD increased offspring birthweight. Our second analysis explored the impact of parental LPD on adult offspring systolic blood pressure (figure 3). All maternal challenges resulted in offspring hypertension, but paternal LPD resulted in a non-significant decrease in blood pressure in the adult offspring. Our final analysis examined the impact of parental diet on adult heart to bodyweight ratio (figure 3). Only paternal LPD had a significant effect, reducing offspring heart to bodyweight ratio. These data demonstrate differential effects from paternal and maternal periconceptional developmental exposures on offspring phenotype. It is essential that further studies define the precise impacts and underlying mechanisms by which parental diet regimes affect offspring development and wellbeing. Studies examining concurrent paternal and maternal interventions on shared offspring outcomes are also warranted.

### Periconceptional developmental programming and assisted reproductive treatment

Direct evidence for human periconceptional effects comes from assisted reproductive treatments, during which mature gametes, and the preimplantation embryo, are exposed to precisely timed in-vitro manipulations. Several million, apparently healthy, children have now been born worldwide using assisted reproductive treatments, but relatively little is known about the possible impact of the technology-associated exposures during conception and very early development on their health status during childhood and later life. The spectrum of human demographic confounders (including parental infertility), changes and improvements in assisted reproductive treatment techniques, and the relative sample sizes used, make analyses complex and the reported outcomes need to be interpreted with caution. Nevertheless, it is well established that singleton assisted reproductive treatment pregnancies have increased risk of low birthweight, congenital abnormalities, and increased mortality rate, although disentangling confounding by parental infertility is difficult.<sup>72</sup> Human embryo culture media have changed over time, with the predominant current practice to use commercially sourced media of proprietary (unspecified) composition.<sup>13</sup> Comparison of perinatal outcome following use of different commercial media, including a multicentre randomised controlled trial,<sup>73</sup> has indicated that birthweight is significantly affected, with effects on growth still manifest at age 2 years.<sup>74</sup>

Compared with naturally conceived offspring, the cardiovascular phenotype of children and adolescents born as a result of in-vitro fertilisation reveals increased risk of high blood pressure,<sup>75,76</sup> vascular dysfunction with abnormal blood flow and vessel thickness,<sup>77</sup> and evidence

of cardiovascular remodelling during development in utero, affecting heart shape and chamber size.<sup>76</sup> Metabolic consequences include increased fasting glucose and peripheral insulin resistance,<sup>75,78</sup> raised plasma lipids, and obesity.<sup>78</sup> A systematic review<sup>79</sup> found no difference in cognitive outcomes among children conceived with conventional in-vitro fertilisation and those conceived naturally, but did identify conflicting findings that require clarification among studies of children conceived with intracytoplasmic sperm injection.

Collectively, the evidence suggests that assisted reproductive treatment, like the in-vivo nutritional models discussed previously, could alter the development and growth trajectory of human embryos, and increase the risk of postnatal chronic cardiometabolic dysfunction. This legacy is unlikely to be due to parental infertility in isolation, since controls in some studies<sup>75,77</sup> comprise naturally conceived offspring from subfertile parents. Moreover, animal models exposed to assisted reproductive treatment demonstrate similar long-term consequences to human studies, despite normal parental fertility; in-vitro fertilisation embryo culture and transfer in mice resulted in offspring with altered growth trajectory, relative hypertension, cardiovascular abnormalities, and glucose and insulin dysfunction.<sup>80</sup>

Adverse effects on long-term health associated with assisted reproductive treatment appear to have an epigenetic origin induced during the periconceptional period. Children born as a result of assisted reproductive treatment have an increased risk of rare imprinting disorders associated with DNA methylation errors on imprinted genes;<sup>81</sup> aberrant methylation of imprinted *H19* gene has been reported in human cultured embryos.<sup>82</sup> In mouse models, embryo culture could cause imprinted genes to lose their allele-specific expression (particularly at the growth regulating *H19/IGF2* locus), together with aberrant methylation patterning in embryos, placental, and fetal tissues.<sup>83</sup> Assisted reproductive treatment-induced aberrant epigenetic profiles might also be propagated during human pregnancy in fetal and placental tissues, and persist into childhood affecting genes regulating growth, such as the *IGF2/H19* locus.<sup>84</sup> Media composition—particularly albumin or serum components, or ammonium ion accumulation from amino-acid catabolism—could contribute to altered mouse epigenetic status.<sup>85</sup> Importantly, even a very limited culture period is sufficient to induce epigenetic changes.<sup>83</sup> Embryo culture exposure also modifies expression and methylation of non-imprinted genes in mice, and alters expression of DNA methyltransferases.<sup>86</sup> For example, in mouse models assisted reproductive treatment affects the endothelial nitric oxide synthase (*eNOS*) gene, implicated in vascular dysfunction, however, modification of culture media composition might prevent this effect.<sup>87</sup> Although provocative, more studies in both animal models and humans are required to replicate findings to date.

## Diversity and commonality in periconceptional effects

The evidence reviewed previously suggests that periconceptional experience can induce lifelong changes in phenotype, affecting disease risk. Beyond these nutritional and assisted reproductive treatment conditions, studies in rodents show broad examples of periconceptional effects, such as from maternal stress.<sup>88</sup> Moreover, maternal alcohol consumption exclusively around conception induced metabolic dysfunction in rat adult offspring with evidence of epigenetic disturbance.<sup>89</sup> Maternal systemic inflammation at conception in mouse models, although not affecting cardiometabolic health, suppressed innate immunity after challenge in adult offspring, possibly reflecting a self-protection mechanism in a predicted pathogenic postnatal environment.<sup>90</sup> In addition, mouse embryo transfer experiments suggest that advanced maternal age might adversely affect offspring cardiometabolic health,<sup>91</sup> but the mechanisms underlying this age-associated effect are unknown.

The diversity of periconceptional induction conditions identified across mammalian species, coupled with clear evidence of both maternal and paternal pathways, implicates an early window when environmental exposures, combined with an inherent capacity for developmental plasticity, might confer advantage when offspring are exposed to a similar environment postnatally. During the periconceptional period there is rapid and radical molecular, cellular, and morphogenetic restructuring; the signalling pathways that control these processes are sensitive to multiple molecules and other factors within the cellular environment, and could provide a mechanistic underpinning for this concept.<sup>92</sup> However, the periconceptional setting of metabolic homoeostasis could become maladaptive if conditions change, or if nutrient levels induce perturbations in metabolism, generating the circumstances underlying adverse health risk. A consistent mechanism identified across conditions and species has been epigenetic variation, a plausible pathway for biological embedding of early life exposures, and transmission of phenotypic effects throughout life. This mechanism has been demonstrated directly through manipulation of maternal one-carbon metabolism during early embryogenesis, potentially reducing the availability of methyl donor groups necessary for DNA and histone methylation;<sup>93</sup> however, such epigenetic changes are not necessarily directly linked with changes in gene expression.<sup>94</sup> In a sheep model, a periconceptional maternal diet deficient in one-carbon metabolite substrates and cofactors (vitamin B12, folate, methionine) modified offspring DNA methylation, and led to adverse cardiometabolic and immune dysfunction.<sup>95</sup> Similarly, addition of folate to rodent maternal LPD rescued normal expression and DNA methylation of metabolic regulators in offspring, possibly protecting against cardiovascular dysfunction.<sup>96</sup> In mice, a paternal

low folate diet altered the profile of sperm DNA methylation, changed the placental transcriptome, and resulted in offspring with craniofacial and musculoskeletal malformations.<sup>97</sup> Moreover, the negative impact of mouse paternal undernutrition on sperm quality, testicular oxidative stress, fertility, and offspring fat accumulation and dyslipidaemia, are reversed through vitamin and antioxidant supplementation.<sup>98</sup> As with assisted reproductive treatment, additional studies are warranted to define the critical windows and pathways linking perinatal one-carbon metabolism, epigenetic variation, and programming of later offspring health.

## Conclusion

We propose that there is sufficient evidence from human and animal research showing that the periconceptional period is a key window during which poor maternal and paternal physiology, body composition, metabolism, and diet can induce increased risk of chronic disease in offspring—a lifetime legacy and major driver of health burden in the 21st century. The evidence that similar consequences can result from assisted reproductive treatment practices sharpens the focus on this window. Environmental factors might perturb gametes or early embryos, affecting homoeostatic mechanisms, or might induce adaptations to developmental environmental signals with consequences persisting into adulthood.

This evidence calls for a major re-examination of public health policy to protect against future disease risk through societal advice on, and greater provision of, preconception care,<sup>99</sup> as also promoted in the two accompanying reviews in this Series. Although a focus on parental risk factors during the preconception period, such as smoking and excess alcohol intake, is wise and well established, new drives to prepare nutritionally for pregnancy are crucial, including healthy body composition, physical activity, and diet for both parents.<sup>100</sup> Further definition of the underlying epigenetic, cellular, metabolic, and physiological mechanisms, and the exposures that drive them, is an important research agenda that is pivotal to the characterisation of more specific recommendations for preconception health.

### Contributors

TPF, AJW, MAV, and KMG drafted the manuscript. All authors provided input into the manuscript and approved the final version of the manuscript.

### Declaration of interests

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