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I'm eating for two: parental dietary effects on offspring metabolism

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Abstract

It has long been understood that the pathogenesis of complex diseases such as diabetes includes both genetic and environmental components. More recently, it has become clear that not only does an individual's environment infuence their own metabolism, but in some cases the environment experienced by their parents may also contribute to their risk of metabolic disease. Here, we review the evidence that parental diet influences metabolic phenotype in offspring in mammals, and provide a current survey of our mechanistic understanding of these effects.

Introduction

Metabolic diseases contribute a massive burden to healthcare throughout the world. Although a large number of Mendelian disorders of metabolism have been identified, the vast majority of metabolic disease burden stems from complex diseases such as diabetes, which have both heritable genetic components as well as contributions from a patient's lifestyle and environmental exposures. In recent years, genome-wide association studies (GWAS) have uncovered a large number of sequence variants that significantly contribute to the overall heritability of metabolic diseases, or to various morphological traits such as BMI or adiposity (Travers and McCarthy, 2011). However, an emerging theme from GWAS is that all genetic variants identified typically explain a small fraction of the heritability of a given complex trait such as diabetes. As one of many examples, in human populations of European descent, all significant GWAS "hits" together explain approximately 10% of the heritability of type 2 diabetes, and ~5% of heritability of fasting plasma glucose (Bonnefond et al., 2010; Morris et al., 2012; Scott et al., 2012). In general, a number of factors could explain this so-called "missing heritability," including many rare variants contributing to a given phenotype, and epistasis. In addition to these, it is increasingly appreciated that epigenetics – the inheritance of information beyond DNA sequence – could potentially contribute to the heritability of such diseases. Indeed, a substantial body of evidence links parental nutritional status to metabolic traits in offspring, possibly providing an explanation for a subset of missing heritability in metabolic diseases.

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For example, the development of disease later in life has been linked to exposure to an adverse intrauterine environment, as observed in offspring of pregnancies complicated by intrauterine growth restriction (IUGR), obesity, or diabetes (Hales and Barker, 1992, 2001; Kermack et al., 1934; Ravelli et al., 1998; Ravelli et al., 1976; Valdez et al., 1994). The period from conception to birth is a time of rapid growth, cellular replication and differentiation, and functional maturation of organ systems. These processes are very sensitive to alterations in nutrient availability, and an abnormal intrauterine metabolic milieu can thus have long-lasting effects on the offspring. Perhaps the best example of how nutrient availability during pregnancy affects longterm health and disease in the offspring is exemplified by the Dutch Hunger Winter. This period of famine occurred in the western part of The Netherlands during the winter of 1944-45 – the period of famine was clearly defined, and official food rations were documented. Extensive health care and birth weight registries still exist for this population, and numerous studies on this cohort have clearly shown that prenatal exposure to famine is associated with the later development of diseases such as obesity, diabetes, and cardiovascular disease (Lumey et al., 2007).

David Barker and Nicholas Hales coined the term “fetal origins of adult disease” based on their studies demonstrating a relationship between low birth weight and the later development of cardiovascular disease and impaired glucose tolerance (Hales and Barker, 1992, 2001). This concept has been broadened to include nutritional excess and/or diabetes during pregnancy. Multiple studies in diverse populations throughout the world have demonstrated a significant correlation between low birth weight, maternal obesity, or maternal diabetes, and the later development of chronic diseases such as type 2 diabetes and or obesity (reviewed in (Duque-Guimaraes and Ozanne, 2013; Simmons, 2011)). It must be acknowledged that these epidemiology studies are cross-sectional (rather than longitudinal) and, with the exception of the study by Rich-Edwards and colleagues (Rich-Edwards et al., 1999), typically have small numbers of subjects. Nonetheless, the human data are compelling in aggregate, and are well-supported by animal studies in multiple species including non-human primates.

While most studies have concentrated on the maternal environment, it is also becoming increasingly evident that *paternal* exposures can result in the later development of metabolic disorders in the offspring (Rando, 2012). Further, both human and animal studies demonstrate intergenerational transmission of the maternal or paternal phenotype (see F2 effects in Figure 1), suggesting the possibility that an epigenetic mechanism is mediating these effects (see below). In this review, we will highlight the most relevant animal models and molecular mechanisms underlying maternal and paternal transmission of information about nutritional status to offspring.

The Maternal Environment

Decreased nutrient availability: Fetal growth restriction

A number of animal models have been used to assess the role of gestational environmental effects in inducing chronic diseases in the offspring (reviewed in (Fowden and Forhead, 2004; McMillen and Robinson, 2005)). In such models, pregnant females are subject to a variety of challenges, including 1) caloric or protein restriction, 2) glucocorticoid

administration, and 3) induction of uteroplacental insufficiency via methods such as ligation of the uterine artery. Although the outcomes observed in offspring differ in detail depending on the organism studied and the details of the challenge, a large number of related studies all find that severe maternal undernutrition during gestation can have an impact on offspring glucose metabolism that persists into adulthood. For instance, in the rat, maternal protein restriction (by approximately 40–50% of normal intake) during gestation and lactation can impair insulin sensitivity, induce β-cell defects, and cause hypertension in offspring (Dahri et al., 1991), and as these offspring age they develop hyperglycemia characterized by defects in insulin signaling in muscle, adipocytes, and liver (Fernandez-Twinn et al., 2005; Ozanne et al., 2003; Petry et al., 2001). Beyond the lasting effects of gestational environment on the F1 generation (Figure 1), some reports find that fetal undernutrition can alter phenotypes even in the F2 generation (Jimenez-Chillaron et al., 2009; Radford et al., 2012; Radford et al., 2014), a result that will be discussed further in the paternal effects section.

Mothers can buffer the effects of various dietary regimens on fetal access to nutrients – only in the face of severe maternal malnutrition is fetal growth adversely affected by maternal diet. As a result, IUGR in humans seldom occurs as a result of maternal undernutrition, and instead most often is a consequence of uteroplacental insufficiency. Conditions such as maternal hypertension, pre-eclampsia, anemia, smoking, and poor placentation are common causes of uteroplacental insufficiency. To model uteroplacental insufficiency in the laboratory, bilateral uterine artery ligation at day 18 of gestation in the rat (where term is 22 days) is used to restrict fetal growth. This paradigm recapitulates key aspects of human IUGR, with reduced levels of glucose, insulin, insulin-like growth factor 1 (IGF-I), amino acids, fatty acids, and oxygen being available to the fetus (Simmons et al., 1992; Simmons et al., 2001). In adulthood, IUGR rats develop diabetes with a phenotype similar to that observed in the human with type 2 diabetes: progressive dysfunction in insulin secretion and insulin action (Simmons et al., 2001; Stoffers et al., 2003).

These and many other studies provide a clear link between nutrient availability during gestation and future metabolic phenotype in offspring. A burgeoning field seeks to understand how a fetus' early environment can alter the metabolism of the offspring long after access to adequate nutrition has been restored.

Proximate Cellular Mechanisms: Mitochondrial dysfunction and oxidative stress—How does a resource-poor environment affect developing organ systems? Although many metabolic pathways and signaling systems are of course impacted by levels of hormones and available nutrients, it appears that reprogramming of mitochondrial function represents one of the key adaptations enabling the fetus to survive in a limited nutrient environment (Pejznochova et al., 2010; Sakai et al., 2013). In response to a reduction in energy supply and oxygen secondary to uteroplacental insufficiency, mitochondria are activated in the fetus to satisfy the cellular need for energy (Chang et al., 2013; Lattuada et al., 2008). However, activated mitochondria can lead to increased production of reactive oxygen species (ROS) and to oxidative stress (Figure 2), which can have deleterious effects in cells that have a high energy requirement, such as the pancreatic β-cell (Newgard and McGarry, 1995; Simmons, 2005). Consistent with this, uteroplacental insufficiency has been shown to induce oxidative stress and marked mitochondrial

dysfunction in the β-cells, hepatocytes, myocytes, and placenta of IUGR offspring (Myatt, 2010; Peterside et al., 2003; Selak et al., 2003; Simmons et al., 2005). Islets of IUGR offspring exhibit progressive declines in ATP production and activities of complexes I and III of the electron transport chain, as well as accumulating mutations and decreasing content of mitochondrial DNA with age (Simmons et al., 2005).

Although β-cells are particularly susceptible to oxidative stress, mitochondrial dysfunction is not limited to the β-cell in the IUGR animal. Oxidation rates of pyruvate, glutamate, succinate, and α-ketoglutarate are markedly decreased in isolated hepatic mitochondria from IUGR pups (prior to the onset of diabetes), and this derangement predisposes the IUGR rat to increased hepatic glucose production by suppressing pyruvate oxidation and increasing gluconeogenesis (Park et al., 2003; Peterside et al., 2003). Similar defects are observed in IUGR muscle, where impaired ATP synthesis compromises energy-dependent GLUT4 recruitment to the cell surface, glucose transport, and glycogen synthesis, all of which could potentially contribute to the insulin resistance and hyperglycemia of type 2 diabetes (Selak et al., 2003).

Mitochondrial abnormalities have also been observed in other animal models of IUGR. Mitochondrial DNA content is reduced in liver, pancreas and skeletal muscle of male offspring of rat dams fed a low-protein diet during pregnancy and lactation (Park et al., 2004), and similar findings have been reported in a pig IUGR model (Liu et al., 2012). Finally, more recently, a targeted metabolomics study in a rabbit IUGR model (unilateral uterine artery ligation) revealed a significant increase in metabolites associated with oxidative stress in IUGR rabbit brain (van Vliet et al., 2013). Thus, multiple models of fetal growth restriction in different species show oxidative stress in several tissues, making mitochondrial dysfunction a common feature of fetal growth restriction, and a good candidate to play a mechanistic role in the later development of disease in adulthood (Petersen et al., 2004).

Much remains to be learned about the proximal mechanisms by which an altered intrauterine milieu affects developing animals. Intervention studies will be required to definitively identify whether and how mitochondrial alterations contribute to metabolic changes in offspring. Moreover, while altered mitochondrial function is a common outcome of IUGR, it also is clear that many other signaling pathways are activated, often in a tissue-specific manner, in IUGR offspring as they develop. A key challenge for the field will be to understand how these different pathways interact to result in the long-term outcomes observed in IUGR individuals.

Cellular and molecular mechanisms: Epigenetic regulation—The central question in considering the developmental origins of adult disease is how a transient stimulus occurring early in life can give rise to long-lasting phenotypic consequences that persist many cellular generations after termination of the nutritional challenge. Cellular memory systems such as those involved in cell state maintenance – collectively referred to as epigenetic inheritance mechanisms – play widespread roles in maintaining phenotypes across tens or hundreds of mitotic generations following transient developmental cues, and

thus represent the likeliest candidates underlying persistent effects of the fetal environment later in life.

Intriguingly, the metabolic or nutritional state of the organism directly influences epigenetic modifications, as essentially all known epigenetic modifications rely upon substrates derived from intermediary metabolism such as S-adenosyl methionine (SAM), acetyl CoA, α-ketoglutarate, and nicotinamide adenine dinucleotide (NAD^+) (Kaelin and McKnight, 2013). A role for parental nutrition in regulation of DNA methylation in offspring is best exemplified by experiments performed in *agouti viable yellow* (A^{vy}) mice or *axin fused* ($Axin^{Fu}$) mice (reviewed in (Martin et al., 2008)). A^{vy} mice carry an intracisternal A particle (IAP) retrotransposon upstream of the *Agouti* gene, and these animals exhibit a range of coat colors which is linked to repression of *Agouti* transcription in some animals by encroaching cytosine methylation from the IAP element. When pregnant agouti-colored female A^{vy} mice are fed a diet supplemented with methyl donors, a larger percentage of offspring have a wild-type coat color compared to offspring of mothers fed standard chow. These phenotypic changes are associated with changes in DNA methylation at the IAP element (Cooney et al., 2002; Dolinoy et al., 2006; Waterland et al., 2006), suggesting that early-life access to specific metabolites can stably change gene expression via epigenetic modifications, thus affecting the phenotype of the adult.

A number of animal studies have shown that maternal nutritional status and fetal nutrient availability induce epigenetic modifications across the genome – genome-wide DNA hypomethylation, as well as an increase in total histone H3 acetylation, is observed in postnatal IUGR liver (MacLennan et al., 2004). Beyond such global effects of IUGR, focal changes in epigenetic marks have also been described at specific target genes, as for example the promoters of *Ppargc1* and *Cpt1* exhibit local increases in H3 acetylation even relative to the increased background H3 acetylation observed in neonatal IUGR liver (Fu et al., 2004). Among these locus-specific changes, promoters of key developmental transcription factors have been the subject of the greatest interest. Most notably, in the rat model, fetal growth restriction induces epigenetic modifications which alter the expression of the homeodomain-containing transcription factor PDX1 (Park et al., 2008). PDX1 plays a critical role in the early development of both endocrine and exocrine pancreas, and then in the later differentiation and function of the β-cell, making regulation of this target a key focus for understanding the pathological outcomes of IUGR. Levels of *Pdx1* mRNA are reduced by more than 50% in IUGR fetal rats as early as 24 hours after the onset of growth retardation, and altered *Pdx*1 expression persists after birth. Repression of *Pdx1* occurs in two waves, as early repression of this gene involves histone deacetylation by the mSin3A complex, followed later by H3K9 dimethylation and eventual recruitment of DNMT3A and cytosine methylation. Prior to cytosine methylation – at the neonatal stage – this epigenetic process is reversible and may define an important developmental window for therapeutic approaches. Indeed, hormone treatments that result in early reversal of *Pdx1* deacetylation also prevent the onset of diabetes in the IUGR rat (Pinney et al., 2011), although whether epigenetic misregulation of *Pdx1* is responsible for metabolic phenotypes in offspring remains to be directly tested. Intriguingly, *Pdx1* expression is also markedly decreased, with correspondingly increased methylation of a key enhancer element, in islets of humans with

Type 2 diabetes (Yang et al., 2012), further emphasizing this locus as a target of significant therapeutic interest in humans.

Pdx1 represents one of several well-characterized target loci that suffer long-term epigenetic reprogramming in response to maternal dietary conditions. As another example, the pancreatic transcription factor Hnf4α is also epigenetically regulated by maternal diet in rat islets from offspring of protein restricted dams (Sandovici et al., 2011). Here, increased levels of DNA methylation and repressive histone modifications at the P2 promoter of Hnf4α were linked to a significant reduction in expression, while reversal of DNA methylation and histone modifications could re-activate transcription of *Hnf4*α via the P2 promoter. Another relatively well-characterized epigenetic target of caloric restriction during pregnancy and lactation is the *Glut4* promoter in skeletal muscle, where diminished histone acetylation and increased H3K9 methylation occurs in response to maternal caloric restriction, although in this case there is no apparent increase in cytosine methylation (Raychaudhuri et al., 2008). These events effectively create a metabolic knockdown of *glut4*, an important regulator of peripheral glucose transport and insulin resistance. Taken together, these studies show that histone modifications can be stably altered at specific genomic loci in response to a calorie-restricted model of IUGR.

These and ongoing studies identify epigenetic changes associated with pregestational access to nutrients, thus providing compelling hypotheses for the mechanism by which early environment exerts long lasting phenotypic effects (Figure 3). Two outstanding questions are raised by such findings. First, the signaling mechanisms responsible for establishing or altering epigenetic marks at specific target genes largely remain obscure. Does fetal undernutrition affect *Pdx1* histone acetylation by altering global levels of Acetyl-CoA, with genomic variability in activity of specific histone acetyltransferases at individual genes making specific genes more or less responsive to Acetyl-CoA changes in a given concentration window? Or, presumably more likely, are histone-modifying enzymes specifically targeted to individual target genes via signal-induced recruitment by sequence-specific DNA-binding proteins? Second, understanding the epigenetic marks that play the greatest role in contributing to eventual phenotypic outcomes will require directed epigenetic interventions. For example, inducible CRISPR-targeted recruitment of histone acetylases to the *Pdx1* promoter could be used to assess the importance of IUGR-driven deacetylation of this locus in the pathogenesis of β-cell dysfunction in offspring. Both of these areas of research should yield great insight over the coming decade.

Increased nutrient availability: Obesity in Pregnancy

Although it has long been understood that inadequate nutrition during pregnancy can have lasting metabolic effects on children, only more recently has the converse situation of maternal *over*nutrition been appreciated as a contributor to adult disease. Obesity is a growing threat worldwide, and its prevalence has risen dramatically over the last two decades, with many studies indicating that early life exposures are important in promoting adult obesity. There are a number of critical periods during childhood that appear to influence the later development of obesity, including early infancy, 5–7 years of age (known as the adiposity rebound period), and puberty (reviewed in (Dietz, 2004)). It is becoming

increasingly evident that the prenatal stage also represents a window of susceptibility for early life exposures (Bayol et al., 2005; Chang et al., 2008; Guo and Jen, 1995; Jungheim and Moley, 2010; Simmons, 2005; Sullivan et al., 2011), as offspring of obese humans and animals exhibit increased fat mass very early in life. In fact, several studies suggest that obesity can also influence molecular aspects of the oocyte and early embryo (Jungheim and Moley, 2010; Jungheim et al., 2010; Marquard et al., 2011), raising the possibility that exposure to an adverse metabolic milieu even prior to pregnancy (Figure 1) could account for some of the metabolic outcomes observed in offspring. Indeed, by carrying out reciprocal 2-cell embryo transfers between obese mice or lean mice, we have separated the effects of obesity on the oocyte from its effects on the gestational environment. Pre-gestational exposure to maternal obesity impaired fetal and placental growth despite the conceptus being exposed to a normal gestational milieu after transfer, with changes in placental gene expression being observed for offspring generated from high fat oocyte donors (Sasson et al., In press). Alterations are also observed in the brain reward system of offspring generated using this paradigm (Grissom et al., 2014), suggesting that obesity prior to pregnancy may program food preferences and/or intake. These results have profound implications as it is possible that the effects of maternal obesity may be thus transmitted to subsequent generations.

Cellular Mechanisms: Inflammation and oxidative stress—Obesity, in both the non-pregnant and pregnant state, has long been understood to involve increased inflammation and oxidative stress in a multitude of tissues throughout the body. Release of inflammatory molecules occurs not only in metabolic and immune tissues, as the placenta can also contribute to the inflammatory/oxidant state: a number of studies have reported that expression of cytokines, inflammation-related genes, and genes linked to oxidative stress are markedly elevated in placenta of obese women (Hauguel-de Mouzon and Guerre-Millo, 2006; Roberts et al., 2011; Taylor and Poston, 2007; Zaretsky et al., 2004; Zhu et al., 2010). Data from animal studies suggest that oxidative stress is directly linked to the development of obesity in offspring. In rats, exposure to maternal obesity prior to and during pregnancy leads to mild oxidative stress even prior to implantation, and this altered state persists into early life (Sen and Simmons, 2010). Importantly, administration of an antioxidant supplement to the dam completely prevents the development of adiposity and glucose intolerance in the offspring, providing evidence for a causal role for oxidative stress (in the mother or in the embryo) in the later development of obesity.

A key question remains to be addressed – how does inflammation and oxidative stress result in increased accumulation of fat? Multiple studies have shown that inflammatory mediators and oxidants enhance adipogenesis, promote differentiation of adipocyte progenitors, and increase lipid deposition in the adipocyte (Reviewed in (Ortega and Fernandez-Real, 2013)). However, it remains to be determined whether or not these pathways are operative in the early embryo or fetus. It is also well known that a normal redox state plays a fundamental role in embryonic development and it is possible that a more oxidizing enviroment alters lineage specification. Consistent with this notion is the finding that expression of a number of genes regulating adipocyte commitment are altered in blastocysts of obese animals (Sen and Simmons, 2010). As in the case of IUGR, a multitude of signaling cytokines and

metabolites differ in abundance between obese vs. non-obese pregnancies, and much work remains to be done to define the contributions of individual signaling pathways to phenotypic outcomes in the offspring.

Cellular and molecular mechanisms: Epigenetic regulation of adipogenesis— The observation that increased fat mass in offspring of obese animals occurs very early in life suggests that adipocyte development per se may play an important role in the genesis of obesity in the offspring. This is not to say that increased maternal adiposity does not program appetite or energy expenditure later in life — it likely does (Chang et al., 2008; Grissom et al., 2014). However, several lines of evidence also implicate potentiation of adipogenesis in early life as a causal mechanism for the later development of obesity.

Adipocyte precursor cells isolated from fat express high levels of mesenchymal stem cell markers such as Pref-1, Wisp2, and anti-angiogenic factors, which together maintain the adipocyte precursor cell in a committed but undifferentiated state (Rodeheffer et al., 2008; Wagner et al., 2005). Obesity in pregnancy significantly increases expression of similar genes in fat tissue of young offspring, and this altered expression persists in fat tissue of older offspring of obese dams, suggesting the possibility that continuing expansion of the adipocyte precursor pool may help explain the progressive increase in fat mass in the offspring (Sen and Simmons, 2010). Further support for this concept was recently shown in studies by Du and colleagues (Yang et al., 2013), who find that expression of Zfp423, a key transcription factor committing cells to the adipogenic lineage, is significantly increased in e14.5 embryos of obese dams. Expression of many other key adipogenic as well as lipogenic regulators (Zfp423, PPARγ, C/EBPα, C/EBP-β, SREBP-1, FASN, SCD-2, and ELOVL-6) is markedly increased in white adipose tissue of offspring of obese dams (Borengasser et al., 2014). Notably, many of these expression changes in offspring are accompanied by local changes in cytosine methylation, again providing a plausible mechanism by which early fetal experience can induce persistent phenotypic changes in offspring. How the nutritional environment signals to these genes, and whether these epigenetic marks are responsible for long-term phenotypic outcomes, remain to be studied.

Epigenetic epidemiology

The findings described above linking over- or under-nutrition during pregnancy to epigenetic changes in offspring naturally raise the opportunity for epigenomic surveys in humans to potentially identify targets for therapeutic intervention. Indeed, there are numerous studies in humans examining the relationship between fetal nutrient availability and epigenetic modifications in the offspring (Rakyan et al., 2011). At present, many of these are confounded by small sample size, cellular heterogeneity of tissues examined, and lack of validation. For example, most DNA methylation assays are performed in total peripheral blood monocytes, where the unique methylation profiles of the various cellular lineages complicate interpretation of the data. Despite these issues, multiple studies in diverse populations report changes in DNA methylation associated with low birth weight and or altered nutrient availability. Thus, it is likely that an adverse in-utero milieu does indeed induce epigenetic modfications in the offspring, but whether these modifications have biological relevance remains to be determined. The field of “epigenetic epidemiology”

remains an active and growing field of investigation, and we anticipate exciting advances in this area in the coming years.

Multigenerational transmission: Maternal

That the in utero environment influences phenotypes in the offspring is not particularly surprising, given that the offspring directly experience the gestational environment. However, it is also becoming increasingly evident that the effects of an altered in utero milieu may be even transmitted to subsequent generations that did not experience the environment (reviewed in (Aiken and Ozanne, 2014)). For example, in humans, studies done in a Swedish population showed that overnutrition in the grandparents during early childhood is associated with increased risk of cardiovascular disease in grandchildren (Kaati et al., 2002; Pembrey et al., 2006). Further, obesity in the grandchild is linked to obesity of the grandparent independent of parental weight (Davis et al., 2008). The transmission of programmed effects to subsequent generations has been reported in a number of animal models including prenatal glucocorticoid overexposure in rats (this model has clinical relevance as glucocorticoids are commonly given to women threatening preterm delivery) (Drake et al., 2011; Drake et al., 2005), maternal undernutrition in mice and rats (Blondeau et al., 2002; Harrison and Langley-Evans, 2009; Jimenez-Chillaron et al., 2009; Thamotharan et al., 2007), neonatal overnutrition in the mouse induced by a reduction in litter size (Pentinat et al., 2010), and maternal obesity in mice (Dunn and Bale, 2009, 2011; Gniuli et al., 2008). The majority of these studies end at the F2 generation, with relatively few studies having been carried out in the F3 generation and beyond (Dunn and Bale, 2011). Low protein diet fed to pregnant mice decreases β-cell mass in the F1-F3 generations at least until day 21 of life (Frantz et al., 2011), and in a separate study using a similar paradigm F3 animals were shown to develop fasting hyperglycemia in adulthood (Benyshek et al., 2006).

The mechanisms responsible for transgenerational effects in developmental programming are poorly understood in mammals. In the case of transmission via the maternal germline, several possibilities bear mentioning: transmission via alteration of the epigenome (somatic or germline), transmission through factors in the ooplasm (such as mitochondria), or development of the F2 generation in a suboptimal uterine environment provided by the reprogrammed F1 female generation. The last point bears repeating: as both IUGR and maternal obesity have been shown to induce metabolic abnormalities in female F1 offspring, and as this altered metabolic state persists even after the F1 daughter becomes pregnant, this can in turn lead to the development of metabolic abnormalities in the F2 generation, and so forth, thus creating a vicious cycle. In principle, such effects can be experimentally ruled out by transferring the oocytes or fertilized zygotes from F1 animals into control recipient females to separate placental effects from alterations in the F1 oocyte epigenome or mitochondria. However, oocyte and embryo transfer processes themselves have been shown to have adverse affects on the offspring, thus making interpretation of these studies difficult.

Paternal effects

While the impact of maternal environment on children has long been clear, the father's contribution to phenotypes in the offspring is much murkier. Although paternal preconception diet has been the subject of some study, most such studies typically focus on

fertility-related measures such as sperm count and motility, as it is generally believed that sperm contribute nothing but a single haploid genome complement to offspring. However, this view is changing as a burgeoning number of studies have linked paternal environmental conditions – largely stress and dietary paradigms – to offspring phenotypes. Indeed, in humans paternal body mass appears to be a better predictor of childhood metabolic traits than is maternal BMI (Figueroa-Colon et al., 2000). Paternal effect paradigms are of great mechanistic interest, as the basis by which paternal environment influences offspring is unknown at present. Although stress and toxin-related paradigms both have been documented to induce paternal effects on offspring phenotype in mammals (Rando, 2012), here we will limit our focus to dietary paradigms.

Male line effects in human epidemiological studies

As with maternal effects, paternal effects have been described in multiple mammalian species. In humans, the best-known evidence for male line effects of nutrient availability on future generations comes from the Overkalix cohort (Bygren et al., 2001; Kaati et al., 2002; Pembrey et al., 2006). In this remote Swedish town, historical crop records were used to infer nutritional access among the ancestors of the current population. Food supply in grandparents was linked to mortality rates two generations later in a gender-specific manner – paternal grandfathers' food supply was associated with altered metabolism in grandsons, while paternal grandmothers' food supply was linked to outcomes in their granddaughters. Interestingly, in both cases relative protection from, or increased risk of, metabolic disease was dependent on the age of grandparental exposure to inadequate diet. Specifically, inadequate diet in early adolescence (ages 9–13) was correlated with decreased risk of mortality in grandchildren, while the same exposure experienced by young adults (ages 18–22) was associated with increased risk. This finding is of significant interest, as some rodent studies also find contrasting metabolic outcomes depending on the animal's age at the time of administration of an altered nutritional environment (see below).

Although the number of human studies focused on male-line effects is relatively scant, and the numbers of individuals studied tend to be small, the concordance between such male-line epidemiological results and paternal effect studies in rodent models suggest that links between paternal diet and offspring metabolism are potentially evolutionarily conserved.

Rodent and other model systems

A large and increasing number of rodent models also find effects of paternal diet on offspring metabolism. The dietary paradigms used range from relatively poor diets, such as caloric restriction and low protein diet, to diets of nutrient excess such as high fat or "Western" diets. The time of exposure to diet varies considerably between studies – while a subset of paternal effect paradigms focus on the period from weaning to sexual maturity, a larger number of studies focus on in utero undernutrition, in which the males used as the paternal generation were carried by mothers subject to starvation during pregnancy. These of course are a subset of the "F2" effects described above for maternal effect paradigms (Figure 1), in which the sons in such experiments are then considered the F0 generation for a paternal effect study. The timing of exposure is a key factor to take into account when considering paternal effect paradigms, as primordial germ cell (PGC) development occurs

during the last week of gestation in male mice. A number of major epigenomic transitions, such as erasure of previous imprints and establishment of male-specific cytosine methylation patterns, occur during this period (Feng et al., 2010), meaning that dietary paradigms starting after birth (generally after weaning) are presumably less likely to influence the epigenome. That said, much remains to be learned about the plasticity of the epigenome and the ability of spermatogonial stem cells to respond to environmental alterations.

Many different metabolic phenotypes have been reported to change in offspring in response to paternal diet (see below for references). The most common metabolic phenotypes measured are related to glucose homestasis, and include fasting glucose, glucose clearance, insulin release in response to glucose, and glucose clearance in response to insulin. Beyond glucose metabolism phenotypes, cholesterol and lipid metabolism, and other cardiovascular phenotypes such as blood pressure, are reportedly altered in response to paternal dietary conditions.

We will focus first on paternal diets that impact glucose metabolism phenotypes in offspring, as these are easily the most commonly-reported effects of paternal diets. Perhaps the best-studied cases of paternal nutritional effects on glucose homeostasis come from in utero undernutrition paradigms. Here, pregnant females are subject to severe caloric restriction (in some paradigms, up to 50% reduction in caloric intake) during the last week of gestation, and their sons are then maintained on control diet and mated to control females. It is worth noting that this paradigm differs substantially from many of the maternal effect paradigms described above, as the starvation used is unusually severe, and is only used during the last week of pregnancy. Male offspring born following this intervention – effectively, an IUGR “F2” generation transmitted via the paternal germline (Figure 1) – exhibit impaired glucose tolerance, with a 22% increase in the area under the curve following intraperitoneal glucose challenge (Jimenez-Chillaron et al., 2009). These offspring also secrete ~40% less insulin in response to glucose challenge than control offspring at four months of age. Other phenotypes, such as insulin resistance, were observed only in matings between both a male and a female subject to in utero undernutrition, but not in matings with either a control father or mother.

Paternal high fat diets also induce metabolic phenotypes in offspring. Male rats maintained on a high fat diet throughout adulthood are reported to sire daughters with mildly impaired glucose tolerance and abnormal pancreatic morphology (Ng et al., 2010). In mice, fathers consuming a high fat diet (with or without low doses of streptozotocin in two distinct studies) sired both sons and daughters exhibiting impaired glucose tolerance (Fullston et al., 2013; Wei et al., 2014). Finally, paternal consumption of low protein diets (9% vs. 18% protein) has been linked to impaired glucose tolerance in both male and female offspring (Watkins and Sinclair, 2014).

Beyond glucose-related phenotypes, several other metabolic changes have been reported in paternal effect paradigms. Most notably, several studies have shown a link between lipid and cholesterol metabolism and paternal diet. For example, 3 week old offspring of males consuming a low protein diet exhibit significantly decreased hepatic levels of free cholesterol and cholesterol esters relative to control offspring, along with increased hepatic

expression of genes encoding the cholesterol biosynthesis pathway (Carone et al., 2010). Cholesterol and lipid biosynthesis genes also change in expression in embryonic day E16.5 offspring of males subject to in utero undernutrition, although apparently in this system offspring show the opposite phenotype, with increased hepatic cholesterol stores and decreased expression of many components of the cholesterol biosynthesis pathway (Radford et al., 2012; Radford et al., 2014). It will be interesting to determine whether this difference stems from the timing of exposure to poor diet – in utero vs. post-weaning – or reflects the different ages at which offspring were analyzed. Finally, low protein feeding has also been shown to influence systolic blood pressure in offspring (Watkins and Sinclair, 2014). In general, many paternal effect studies will need to be repeated in additional cohorts and in different strains of mice and rats to assure reproducibility and generalization of these findings.

No doubt deeper metabolic phenotyping will continue to uncover additional effects of paternal dietary conditions on offspring metabolism. That said, perhaps the most curious aspect of metabolic effects observed following paternal dietary paradigms is that by and large the phenotypes observed in these studies overlap substantially with outcomes of maternal dietary intervention (see above), raising the question of whether paternal dietary interventions may alter fetal provisioning by the mother (discussed below).

Paternal effect models and the sperm epigenome

The male contribution to offspring often amounts to little more than the haploid genome complement in sperm, and as a result a great deal of attention in paternal effect paradigms has focused on so-called epigenetic information carriers in sperm. That said, it is worth noting that fathers can contribute additional information to offspring – such information carriers minimally include 1) paternal transfer of microbiota to mates or to offspring, 2) effects of seminal fluid on maternal behavior or physiology, and 3) cryptic maternal effects in which females judge males and adjust their resource allocation to offspring accordingly (Curley et al., 2010; Pryke and Griffith, 2009). We will first review dietary effects on the sperm epigenome, then will discuss alternative information carriers below.

Five major classes of epigenetic information carriers – transcription factor abundance, chromatin state, small RNAs, DNA modifications such as cytosine methylation, and prions – have been defined in paradigms ranging from microbial environmental memory to metazoan cell state inheritance to transgenerational inheritance systems in plants and worms (Rando, 2012; Rando and Verstrepen, 2007). Of these, the most commonly-studied in paternal dietary paradigms in mammals are cytosine methylation and small RNAs, which will be reviewed here.

Cytosine methylation—Cytosine methylation, which plays well-established roles in epigenetic inheritance paradigms in plant and mammal models, has been the best-studied epigenetic mark in various paternal effect paradigms. Cytosine methylation changes are often reported in offspring of paternal effect paradigms, often at loci plausibly linked to offspring physiology – in our system, offspring of Low Protein fathers exhibit ~10% cytosine methylation changes across an enhancer of the key lipid regulator *Ppar*α (Carone et

al., 2010). However, in this case and many others, methylation changes observed in offspring tissues are often absent in analyses of sperm samples, ruling out the simplistic hypothesis in which diet influences the sperm epigenome, which then escapes erasure during early development. For this reason, we focus here on analyses of cytosine methylation patterns in treated vs. control sperm, and ignore isolated reports of cytosine methylation in offspring tissues. Sperm cytosine methylation patterns have been reported to change at a number of loci in both an in utero undernutrition paradigm (Radford et al., 2014), and in response to paternal prediabetes (Wei et al., 2014). In the former case, 166 differentially-methylated regions (DMRs) were identified using MeDIP-Seq in pooled sperm samples, and bisulfite validation (n of ~12 animals per treatment) of 32 DMRs confirmed 17 regions of hypomethylation in the undernutrition cohort. In general, these regions exhibited ~20% changes in methylation over ~5 adjacent CpGs, with most cases apparently changing from ~40% methylation at each CpG to ~20% methylation. In the case of paternal prediabetes (Wei et al., 2014), thousands of DMRs were reportedly identified by MeDIP-Seq, although validation by bisulfite conversion was only carried out for a small number of loci in pairs of animals. As in the case of in utero undernutrition, methylation differences were generally modest – changes of ~20% between sperm samples. In a handful of these cases, the cytosine methylation changes observed in sperm are also found in F1 tissues of interest, potentially consistent with the hypothesis that these methylation changes are inherited at fertilization and subsequently play causal roles in establishing the reprogrammed state.

However, several considerations dampen enthusiasm for the hypothesis that the reported cytosine methylation changes play causal roles in paternal effects on offspring phenotype. The primary concern is what can be called the “digital sperm problem.” 10–20% changes in cytosine methylation can be meaningful in a multicellular tissue, as for example changing from 90% to 70% methylation at a given cytosine in a liver population could result in tripling the number of cells expressing some systemically-acting growth factor. In contrast, at fertilization, each sperm truly is alone. Thus, a quantitative change in methylation should only alter the penetrance of a given phenotype: a change from 40% to 20% methylation at a specific CpG in sperm (Radford et al., 2014; Wei et al., 2014) means that 1 out of 5, rather than 2 out of 5, sperm carry a methylated cytosine at that position. This means that even if the methylation status of the cytosine in question were *completely* responsible for some phenotype in offspring, at best this 20% methylation change would alter the fraction of a rodent’s litter expressing the phenotype from 2 out of 5 to 1 out of 5. Thus, a 20% change in methylation at a cytosine is unlikely to result in penetrant changes in offspring. That said, given that multiple adjacent CpGs are often reported to change methylation in sperm, if such methylation changes were independent at each position then digital sperm could plausibly carry more continuous, analog, information. Alternatively, it is also plausible that methylation changes occur specifically in a small subset of sperm competent for fertilization (with incompetent or dead sperm nonetheless polluting the ensemble methylation data), or that sperm bearing the methyl marks at this locus exhibit some competitive advantage/disadvantage (superior swimming, oocyte adhesiveness, etc.) in succesful fertilization of an oocyte. Both of these scenarios would make the effective changes in methylation in fertilizing sperm much greater than those measured in the unselected sperm ensemble.

The other key challenge for cytosine methylation as a potential carrier of paternal environmental information comes from the near-global resetting of the paternal epigenome that occurs upon fertilization (Feng et al., 2010). Of course, a small subset of cytosine methylation on the paternal genome escapes erasure, with paternally-contributed methylation at a subset of imprinted loci providing the canonical examples (Bartolomei and Ferguson-Smith, 2011). In addition to imprinted loci, genome-wide analyses of cytosine methylation in gametes and early embryos suggest that additional genomic regions may potentially escape this near-global reprogramming. In mice, sperm-contributed DMRs occur primarily in intergenic CpG-poor regions enriched for LINE elements, and can persist for several cleavages (Smith et al., 2012). Similar enrichment of sperm-specific methylation in intergenic regions is reported in humans, although in contrast to mice much more of this methylation appears to be erased prior to the 2-Cell stage (Guo et al., 2014). These results are consistent with a mechanism in which retention of H3K9-methylated histones over gene deserts in sperm (Carone et al., 2014; Samans et al., 2014) serves to recruit the maternal factor Stella to protect these regions from Tet3-mediated cytosine demethylation (Nakamura et al., 2012). Whatever the mechanism for retention of a subset of the paternal methylome, it appears to occur preferentially at distal intergenic regions rather than at, say, promoters, and in the majority of cases methylation is lost within a few cleavage divisions. Such considerations may help guide more fruitful searches for epigenomic changes responsible for paternal dietary effects.

Beyond correlative evidence for cytosine methylation changes in paternal information transfer, functional studies must be the next step in testing the hypothesis that diet-directed methylation is responsible for offspring reprogramming. Such studies are challenging to consider, although advances in locus-specific recruitment of epigenomic regulators may allow such tests in the near future.

Small RNAs—Since the discovery of microRNAs in 1993 (Lee et al., 1993), an ever-expanding universe of small (<40 nt) RNAs have been described and shown to function in a large variety of biological paradigms. Most interestingly, small RNAs have been implicated in several well-established epigenetic inheritance paradigms, including RNAi in nematodes (Fire et al., 1998), paramutation in maize (Arteaga-Vazquez and Chandler, 2010), and many related epigenetic silencing paradigms in models from Arabidopsis (Chan et al., 2004; Zilberman et al., 2003) to fission yeast (Grewal, 2010). Small RNA families include well-studied species such as microRNAs, siRNAs, and piRNAs, as well as less-characterized entities including tRNA fragments (tRFs) and enhancer-derived RNAs (eRNAs) (Ghildiyal and Zamore, 2009; Sobala and Hutvagner, 2011). In mammals, microinjection of small RNAs into zygotes has been reported to alter coat color phenotypes (Rassoulzadegan et al., 2006), cardiac hypertrophy (Wagner et al., 2008), and other phenotypes.

Several paternal effect studies in mammals have documented changes in small RNA profiles in the sperm of treated vs. control males. The majority of such studies focus on microRNAs, either because they use microRNA-specific profiling methods (such as microarrays, or <24 nt size selection prior to deep sequencing), or because the more extensive literature on microRNAs makes predictions of functional consequences of these changes easier than predictions of the function of a given piRNA or tRF in zygotes. Changes in levels of specific

microRNAs have been reported in sperm in response to paternal conditions both in stress-related and in some nutritional paradigms (Rodgers et al., 2013). Moreover, in the case of early paternal stress, microinjection of a total RNA pool from the sperm of stressed animals was reported to induce a subset of the phenotypes observed in offspring generated by natural mating (Gapp et al., 2014). This result suggests that some component of the RNA payload of mature sperm is capable of altering offspring phenotype when introduced into the early embryo.

That said, the hypothesis that sperm RNAs could be responsible for programming offspring phenotype presents several challenges for current mechanistic models for small RNA function. First, mammalian sperm carry extremely low levels of RNA, and considering the volume of a sperm relative to the oocyte suggests that sperm are unlikely to carry enough RNAs to significantly alter the concentrations of a given RNA species in the oocyte, unless the RNA in question is absent or nearly so from the oocyte. This concern could be alleviated were sperm RNAs to be uniquely modified, or pre-bound by an effector protein, but in any case the simplest model of sperm delivering a pool of soluble small RNAs must contend with the issue of the miniscule sperm cytoplasm. Second, even for uniquely sperm-delivered RNAs, the biochemistry of mammalian Ago proteins strongly supports the idea that only the ~20 or so most abundant microRNAs in a given cell are likely to have regulatory impact on the cell (Wee et al., 2012), an idea that is supported by functional studies in somatic cells (Mullokandov et al., 2012) and in zygotes (Amanai et al., 2006). Finally, in mammals it is unclear how small RNAs introduced at fertilization would have lasting effects on offspring phenotype. In the species where transgenerational inheritance based on small RNAs is best-characterized – worms, plants, fission yeast – RNA-dependent RNA polymerase plays a key role in amplifying small RNA signals. In contrast, mammals do not encode a known RNA-dependent RNA polymerase, suggesting that whatever effects sperm RNAs have on offspring must likely occur within the first few cleavage divisions, and induce longer-lasting effects by their actions during this stage.

Thus, although small RNAs represent a very strong candidate for the molecular basis of dietary information in sperm based on their roles in other model organisms, many mechanistic questions remain to be resolved to consider sperm delivery of small RNAs to be a credible influence over offspring metabolism.

Synthesizing maternal and paternal dietary paradigms—Perhaps one of the most striking aspects of the literature on ancestral dietary conditions is the extensive overlap in phenotypes induced by maternal and paternal effect paradigms. As detailed above, both maternal and paternal effect paradigms influence glucose homeostasis, cholesterol metabolism, and cardiovascular parameters in offspring. In stress paradigms anxiety-related behaviors are often altered in offspring, again both via maternal and paternal transmission. Although these overlapping outcomes of parental exposure history could potentially reflect investigator biases – if you alter the diet of a parent, it makes sense to look for metabolic outcomes in offspring – in many of the studies detailed above genome-wide profiling methods were applied to offspring, with metabolic phenotypes being identified in a relatively unbiased manner. Thus, while these overlaps may not be meaningful, it is

nonetheless worth considering the hypothesis that maternal and paternal dietary paradigms operate via a shared downstream mechanism.

Several mechanisms could unite paternal and maternal effects on offspring. For example, numerous recent studies implicate the gut microbiota in influencing metabolism, so in principle both males and females subject to overnutrition (for instance) could potentially innoculate their offspring with similar microbial communities which efficiently extract nutrients from food. However, in many paternal effect experiments males are removed from the female's cage after only one or two days of mating, and moreover in our system we have found that paternal dietary information can be transmitted to offspring even when using in vitro fertilization (manuscript in preparation), thus removing any interaction between the male and his offspring. This last experiment also argues against a mechanism in which females judge the quality of their mate and alter resource provision to the offspring (Pryke and Griffith, 2009), and makes seminal fluid – which can significantly impact offspring metabolism in mammals (Bromfield et al., 2014) – similarly unlikely.

How then might sperm alter maternal resource provisioning? Intriguingly, numerous studies have found effects of brief embryo culture on metabolic phenotypes in offspring (Rinaudo and Wang, 2012). The earliest cell fate decision in mammalian embryos is cell fate allocation between the inner cell mass (ICM) and the trophectoderm (TE) of the blastocyst, which give rise to the embryo and to the extraembryonic tissues, respectively. It is thus theoretically possible that molecular changes in sperm induced by paternal diet somehow influence cell fate allocation between ICM and TE – by altering cell cycle dynamics for the first few cleavage divisions, for instance – or alternatively, TE cell function, with resulting effects on placental development then resulting in metabolic changes in offspring as detailed above. Indeed, paternal diet and exercise have been linked to preimplantation growth dynamics (McPherson et al., 2013), potentially providing a link between paternal diet and the findings of common maternal effect phenotypes in offspring. Such a link would also help explain how epigenetic marks in sperm such as cytosine methylation or small RNAs, which generally are erased or at least not copied in the embryo, might exert long-term metabolic effects despite operating during a limited time window.

Conclusions

The combined epidemiological, clinical, and animal studies clearly demonstrate that the intrauterine environment influences both growth and development of the fetus and the subsequent development of adult diseases. There are specific critical windows during development, often coincident with periods of rapid cell division, during which a stimulus or insult may have long-lasting consequences on tissue or organ function postnatally. In maternal effect models, mitochondrial dysfunction and oxidative stress are among the earliest molecular events described in offspring subjected to nutrient restriction or uteroplacental insufficiency, and provide a strong candidate mechanism in the pathogenesis of the fetal origins of adult disease. Later in life, a number of changes in epigenetic marks have been identified in multiple tissues in offspring, which provide the likeliest mechanism by which early molecular changes result in persistent phenotypic changes. There are likely

to be many additional mechanisms that will be uncovered as new tools become available to more robustly study these questions.

The near future promises advances on at least three fronts. First, the burgeoning field of epigenetic epidemiology is in its early days, but surveys of epigenetic marks in children who experience adverse placental environments promise to yield a wealth of knowledge regarding the mechanisms responsible for long-term metabolic reprogramming. A number of potential issues with extant studies exist, as for example birthweight is only one marker of an adverse fetal environment, and confining studies to this population only may lead to erroneous conclusions regarding etiology. But this approach has great promise, particularly as it grows in scope and sophistication. Second, advances in epigenomics and in epigenetic "editing" in model organisms should continue to provide mechanistic insights regarding the molecular basis for transgenerational inheritance, and to offer fundamental insights into early development. Finally, prevention of metabolic abnormalities will of course be one of the key goals for future efforts, as mitochondrial function and even epigenetic marks provide promising candidates for therapeutic intervention, and research efforts should be focused in this area.

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REFERENCES

Aiken CE, Ozanne SE. Transgenerational developmental programming. Human reproduction update. 2014; 20:63–75. [PubMed: 24082037]

Amanai M, Brahmajosyula M, Perry AC. A restricted role for sperm-borne microRNAs in mammalian fertilization. Biology of reproduction. 2006; 75:877–884. [PubMed: 16943360]

Arteaga-Vazquez MA, Chandler VL. Paramutation in maize: RNA mediated trans-generational gene silencing. Curr Opin Genet Dev. 2010; 20:156–163. [PubMed: 20153628]

Bartolomei MS, Ferguson-Smith AC. Mammalian genomic imprinting. Cold Spring Harb Perspect Biol. 2011:3.

Bayol SA, Simbi BH, Stickland NC. A maternal cafeteria diet during gestation and lactation promotes adiposity and impairs skeletal muscle development and metabolism in rat offspring at weaning. The Journal of physiology. 2005; 567:951–961. [PubMed: 16020464]

Benyshek DC, Johnston CS, Martin JF. Glucose metabolism is altered in the adequately-nourished grand-offspring (F3 generation) of rats malnourished during gestation and perinatal life. Diabetologia. 2006; 49:1117–1119. [PubMed: 16557373]

Blondeau B, Avril I, Duchene B, Breant B. Endocrine pancreas development is altered in foetuses from rats previously showing intra-uterine growth retardation in response to malnutrition. Diabetologia. 2002; 45:394–401. [PubMed: 11914745]

Bonnefond A, Froguel P, Vaxillaire M. The emerging genetics of type 2 diabetes. Trends in molecular medicine. 2010; 16:407–416. [PubMed: 20728409]

Borengasser SJ, Faske J, Kang P, Blackburn ML, Badger TM, Shankar K. In Utero Exposure to Pre-Pregnancy Maternal Obesity and Post-weaning High Fat Diet Impairs Regulators of Mitochondrial Dynamics in Rat Placenta and Offspring. Physiological genomics. 2014 physiolgenomics 00059 02014.

Bromfield JJ, Schjenken JE, Chin PY, Care AS, Jasper MJ, Robertson SA. Maternal tract factors contribute to paternal seminal fluid impact on metabolic phenotype in offspring. Proceedings of

the National Academy of Sciences of the United States of America. 2014; 111:2200–2205. [PubMed: 24469827]

Bygren LO, Kaati G, Edvinsson S. Longevity determined by paternal ancestors' nutrition during their slow growth period. Acta Biotheor. 2001; 49:53–59. [PubMed: 11368478]

Carone BR, Fauquier L, Habib N, Shea JM, Hart CE, Li R, Bock C, Li C, Gu H, Zamore PD, et al. Paternally induced transgenerational environmental reprogramming of metabolic gene expression in mammals. Cell. 2010; 143:1084–1096. [PubMed: 21183072]

Carone BR, Hung JH, Hainer SJ, Chou MT, Carone DM, Weng Z, Fazzio TG, Rando OJ. High-resolution mapping of chromatin packaging in mouse embryonic stem cells and sperm. Developmental cell. 2014; 30:11–22. [PubMed: 24998598]

Chan, SW.; Zilberman, D.; Xie, Z.; Johansen, LK.; Carrington, JC.; Jacobsen, SE. Science. Vol. 303. New York, NY: 2004. RNA silencing genes control de novo DNA methylation; p. 1336

Chang GQ, Gaysinskaya V, Karatayev O, Leibowitz SF. Maternal high-fat diet and fetal programming: increased proliferation of hypothalamic peptide-producing neurons that increase risk for overeating and obesity. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2008; 28:12107–12119. [PubMed: 19005075]

Chang YL, Wang CN, Wei PC, Peng HH, Chao AS, Chang SD, Cheng PJ, Wang TH. Mitochondrial activation in the growth-restricted fetus of monochorionic twins. Fertility and sterility. 2013; 100:241–246. e241–242. [PubMed: 23557760]

Cooney CA, Dave AA, Wolff GL. Maternal methyl supplements in mice affect epigenetic variation and DNA methylation of offspring. The Journal of nutrition. 2002; 132:2393S–2400S. [PubMed: 12163699]

Curley JP, Mashoodh R, Champagne FA. Epigenetics and the origins of paternal effects. Horm Behav. 2010; 59:306–314. [PubMed: 20620140]

Dahri S, Snoeck A, Reusens-Billen B, Remacle C, Hoet JJ. Islet function in offspring of mothers on low-protein diet during gestation. Diabetes. 1991; 2(40 Suppl):115–120. [PubMed: 1748239]

Davis MM, McGonagle K, Schoeni RF, Stafford F. Grandparental and parental obesity influences on childhood overweight: implications for primary care practice. Journal of the American Board of Family Medicine : JABFM. 2008; 21:549–554. [PubMed: 18988722]

Dietz WH. Overweight in childhood and adolescence. The New England journal of medicine. 2004; 350:855–857. [PubMed: 14985480]

Dolinoy DC, Weidman JR, Waterland RA, Jirtle RL. Maternal genistein alters coat color and protects Avy mouse offspring from obesity by modifying the fetal epigenome. Environ Health Perspect. 2006; 114:567–572. [PubMed: 16581547]

Drake AJ, Liu L, Kerrigan D, Meehan RR, Seckl JR. Multigenerational programming in the glucocorticoid programmed rat is associated with generation-specific and parent of origin effects. Epigenetics : official journal of the DNA Methylation Society. 2011; 6:1334–1343. [PubMed: 22086116]

Drake AJ, Walker BR, Seckl JR. Intergenerational consequences of fetal programming by in utero exposure to glucocorticoids in rats. American journal of physiology Regulatory, integrative and comparative physiology. 2005; 288:R34–R38.

Dunn GA, Bale TL. Maternal high-fat diet promotes body length increases and insulin insensitivity in second-generation mice. Endocrinology. 2009; 150:4999–5009. [PubMed: 19819967]

Dunn GA, Bale TL. Maternal high-fat diet effects on third-generation female body size via the paternal lineage. Endocrinology. 2011; 152:2228–2236. [PubMed: 21447631]

Duque-Guimaraes DE, Ozanne SE. Nutritional programming of insulin resistance: causes and consequences. Trends in endocrinology and metabolism: TEM. 2013; 24:525–535. [PubMed: 23791137]

Feng, S.; Jacobsen, SE.; Reik, W. Science. Vol. 330. New York, NY: 2010. Epigenetic reprogramming in plant and animal development; p. 622-627.

Fernandez-Twinn DS, Wayman A, Ekizoglou S, Martin MS, Hales CN, Ozanne SE. Maternal protein restriction leads to hyperinsulinemia and reduced insulin-signaling protein expression in 21-mo-old female rat offspring. American journal of physiology Regulatory, integrative and comparative physiology. 2005; 288:R368–R373.

Figueroa-Colon R, Arani RB, Goran MI, Weinsier RL. Paternal body fat is a longitudinal predictor of changes in body fat in premenarcheal girls. The American journal of clinical nutrition. 2000; 71:829–834. [PubMed: 10702180]

Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature. 1998; 391:806–811. [PubMed: 9486653]

Fowden AL, Forhead AJ. Endocrine mechanisms of intrauterine programming. Reproduction. 2004; 127:515–526. [PubMed: 15129007]

Frantz ED, Aguila MB, Pinheiro-Mulder Ada R, Mandarim-de-Lacerda CA. Transgenerational endocrine pancreatic adaptation in mice from maternal protein restriction in utero. Mechanisms of ageing and development. 2011; 132:110–116. [PubMed: 21291904]

Fu Q, McKnight RA, Yu X, Wang L, Callaway CW, Lane RH. Uteroplacental insufficiency induces site-specific changes in histone H3 covalent modifications and affects DNA-histone H3 positioning in day 0 IUGR rat liver. Physiological genomics. 2004; 20:108–116. [PubMed: 15494474]

Fullston T, Ohlsson Teague EM, Palmer NO, DeBlasio MJ, Mitchell M, Corbett M, Print CG, Owens JA, Lane M. Paternal obesity initiates metabolic disturbances in two generations of mice with incomplete penetrance to the F2 generation and alters the transcriptional profile of testis and sperm microRNA content. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2013; 27:4226–4243. [PubMed: 23845863]

Gapp K, Jawaid A, Sarkies P, Bohacek J, Pelczar P, Prados J, Farinelli L, Miska E, Mansuy IM. Implication of sperm RNAs in transgenerational inheritance of the effects of early trauma in mice. Nature neuroscience. 2014; 17:667–669.

Ghildiyal M, Zamore PD. Small silencing RNAs: an expanding universe. Nature reviews Genetics. 2009; 10:94–108.

Gniuli D, Calcagno A, Caristo ME, Mancuso A, Macchi V, Mingrone G, Vettor R. Effects of high-fat diet exposure during fetal life on type 2 diabetes development in the progeny. Journal of lipid research. 2008; 49:1936–1945. [PubMed: 18493032]

Grewal SI. RNAi-dependent formation of heterochromatin and its diverse functions. Curr Opin Genet Dev. 2010; 20:134–141. [PubMed: 20207534]

Grissom NM, Lyde R, Christ L, Sasson IE, Carlin J, Vitins AP, Simmons RA, Reyes TM. Obesity at conception programs the opioid system in the offspring brain. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 2014; 39:801–810. [PubMed: 23924601]

Guo F, Jen KL. High-fat feeding during pregnancy and lactation affects offspring metabolism in rats. Physiology & behavior. 1995; 57:681–686. [PubMed: 7777603]

Guo H, Zhu P, Yan L, Li R, Hu B, Lian Y, Yan J, Ren X, Lin S, Li J, et al. The DNA methylation landscape of human early embryos. Nature. 2014; 511:606–610. [PubMed: 25079557]

Hales CN, Barker DJ. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. Diabetologia. 1992; 35:595–601. [PubMed: 1644236]

Hales CN, Barker DJ. The thrifty phenotype hypothesis. Br Med Bull. 2001; 60:5–20. [PubMed: 11809615]

Harrison M, Langley-Evans SC. Intergenerational programming of impaired nephrogenesis and hypertension in rats following maternal protein restriction during pregnancy. The British journal of nutrition. 2009; 101:1020–1030. [PubMed: 18778527]

Hauguel-de Mouzon S, Guerre-Millo M. The placenta cytokine network and inflammatory signals. Placenta. 2006; 27:794–798. [PubMed: 16242770]

Jimenez-Chillaron JC, Isganaitis E, Charalambous M, Gesta S, Pentinat-Pelegrin T, Faucette RR, Otis JP, Chow A, Diaz R, Ferguson-Smith A, et al. Intergenerational transmission of glucose intolerance and obesity by in utero undernutrition in mice. Diabetes. 2009; 58:460–468. [PubMed: 19017762]

Jungheim ES, Moley KH. Current knowledge of obesity's effects in the pre- and periconceptional periods and avenues for future research. American journal of obstetrics and gynecology. 2010; 203:525–530. [PubMed: 20739012]

Jungheim ES, Schoeller EL, Marquard KL, Louden ED, Schaffer JE, Moley KH. Diet-induced obesity model: abnormal oocytes and persistent growth abnormalities in the offspring. Endocrinology. 2010; 151:4039–4046. [PubMed: 20573727]

Kaati G, Bygren LO, Edvinsson S. Cardiovascular and diabetes mortality determined by nutrition during parents' and grandparents' slow growth period. Eur J Hum Genet. 2002; 10:682–688. [PubMed: 12404098]

Kaelin WG Jr, McKnight SL. Influence of metabolism on epigenetics and disease. Cell. 2013; 153:56–69. [PubMed: 23540690]

Kermack WO, McKendrick AG, McKinlay PL. Death-rates in Great Britain and Sweden: Expression of Specific Mortality Rates as Products of Two Factors, and some Consequences thereof. The Journal of hygiene. 1934; 34:433–457. [PubMed: 20475246]

Lattuada D, Colleoni F, Martinelli A, Garretto A, Magni R, Radaelli T, Cetin I. Higher mitochondrial DNA content in human IUGR placenta. Placenta. 2008; 29:1029–1033. [PubMed: 19007984]

Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell. 1993; 75:843–854. [PubMed: 8252621]

Liu J, Yu B, Mao X, He J, Yu J, Zheng P, Huang Z, Chen D. Effects of intrauterine growth retardation and maternal folic acid supplementation on hepatic mitochondrial function and gene expression in piglets. Archives of animal nutrition. 2012; 66:357–371. [PubMed: 22889112]

Lumey L, Stein AD, Kahn HS, van der Pal-de Bruin KM, Blauw G, Zybert PA, Susser ES. Cohort Profile: the Dutch Hunger Winter Families Study. Int J Epidemiol. 2007

MacLennan NK, James SJ, Melnyk S, Piroozi A, Jernigan S, Hsu JL, Janke SM, Pham TD, Lane RH. Uteroplacental insufficiency alters DNA methylation, one-carbon metabolism, and histone acetylation in IUGR rats. Physiological genomics. 2004; 18:43–50. [PubMed: 15084713]

Marquard KL, Stephens SM, Jungheim ES, Ratts VS, Odem RR, Lanzendorf S, Moley KH. Polycystic ovary syndrome and maternal obesity affect oocyte size in in vitro fertilization/intracytoplasmic sperm injection cycles. Fertility and sterility. 2011; 95:2146–2149. 2149 e2141. [PubMed: 21071018]

Martin DI, Cropley JE, Suter CM. Environmental influence on epigenetic inheritance at the Avy allele. Nutrition reviews. 2008; 1(66 Suppl):S12–S14. [PubMed: 18673479]

McMillen IC, Robinson JS. Developmental origins of the metabolic syndrome: prediction, plasticity, and programming. Physiological reviews. 2005; 85:571–633. [PubMed: 15788706]

McPherson NO, Bakos HW, Owens JA, Setchell BP, Lane M. Improving metabolic health in obese male mice via diet and exercise restores embryo development and fetal growth. PloS one. 2013; 8:e71459. [PubMed: 23977045]

Morris AP, Voight BF, Teslovich TM, Ferreira T, Segre AV, Steinthorsdottir V, Strawbridge RJ, Khan H, Grallert H, Mahajan A, et al. Large-scale association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes. Nature genetics. 2012; 44:981–990. [PubMed: 22885922]

Mullokandov G, Baccarini A, Ruzo A, Jayaprakash AD, Tung N, Israelow B, Evans MJ, Sachidanandam R, Brown BD. High-throughput assessment of microRNA activity and function using microRNA sensor and decoy libraries. Nature methods. 2012; 9:840–846. [PubMed: 22751203]

Myatt L. Review: Reactive oxygen and nitrogen species and functional adaptation of the placenta. Placenta. 2010; (31 Suppl):S66–S69. [PubMed: 20110125]

Nakamura T, Liu YJ, Nakashima H, Umehara H, Inoue K, Matoba S, Tachibana M, Ogura A, Shinkai Y, Nakano T. PGC7 binds histone H3K9me2 to protect against conversion of 5mC to 5hmC in early embryos. Nature. 2012; 486:415–419. [PubMed: 22722204]

Newgard CB, McGarry JD. Metabolic coupling factors in pancreatic beta-cell signal transduction. Annual review of biochemistry. 1995; 64:689–719.

Ng SF, Lin RC, Laybutt DR, Barres R, Owens JA, Morris MJ. Chronic high-fat diet in fathers programs beta-cell dysfunction in female rat offspring. Nature. 2010; 467:963–966. [PubMed: 20962845]

Ortega FJ, Fernandez-Real JM. Inflammation in adipose tissue and fatty acid anabolism: when enough is enough! Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme. 2013; 45:1009–1019.

Ozanne SE, Olsen GS, Hansen LL, Tingey KJ, Nave BT, Wang CL, Hartil K, Petry CJ, Buckley AJ, Mosthaf-Seedorf L. Early growth restriction leads to down regulation of protein kinase C zeta and insulin resistance in skeletal muscle. The Journal of endocrinology. 2003; 177:235–241. [PubMed: 12740011]

Park HK, Jin CJ, Cho YM, Park DJ, Shin CS, Park KS, Kim SY, Cho BY, Lee HK. Changes of mitochondrial DNA content in the male offspring of protein-malnourished rats. Annals of the New York Academy of Sciences. 2004; 1011:205–216. [PubMed: 15126298]

Park JH, Stoffers DA, Nicholls RD, Simmons RA. Development of type 2 diabetes following intrauterine growth retardation in rats is associated with progressive epigenetic silencing of Pdx1. The Journal of clinical investigation. 2008; 118:2316–2324. [PubMed: 18464933]

Park KS, Kim SK, Kim MS, Cho EY, Lee JH, Lee KU, Pak YK, Lee HK. Fetal and early postnatal protein malnutrition cause long-term changes in rat liver and muscle mitochondria. The Journal of nutrition. 2003; 133:3085–3090. [PubMed: 14519789]

Pejznochova M, Tesarova M, Hansikova H, Magner M, Honzik T, Vinsova K, Hajkova Z, Havlickova V, Zeman J. Mitochondrial DNA content and expression of genes involved in mtDNA transcription, regulation and maintenance during human fetal development. Mitochondrion. 2010; 10:321–329. [PubMed: 20096380]

Pembrey ME, Bygren LO, Kaati G, Edvinsson S, Northstone K, Sjostrom M, Golding J. Sex-specific, male-line transgenerational responses in humans. Eur J Hum Genet. 2006; 14:159–166. [PubMed: 16391557]

Pentinat T, Ramon-Krauel M, Cebria J, Diaz R, Jimenez-Chillaron JC. Transgenerational inheritance of glucose intolerance in a mouse model of neonatal overnutrition. Endocrinology. 2010; 151:5617–5623. [PubMed: 20943806]

Petersen KF, Dufour S, Befroy D, Garcia R, Shulman GI. Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. The New England journal of medicine. 2004; 350:664–671. [PubMed: 14960743]

Peterside IE, Selak MA, Simmons RA. Impaired oxidative phosphorylation in hepatic mitochondria in growth-retarded rats. American journal of physiology Endocrinology and metabolism. 2003; 285:E1258–E1266. [PubMed: 14607783]

Petry CJ, Dorling MW, Pawlak DB, Ozanne SE, Hales CN. Diabetes in old male offspring of rat dams fed a reduced protein diet. International journal of experimental diabetes research. 2001; 2:139–143. [PubMed: 12369717]

Pinney SE, Jaeckle Santos LJ, Han Y, Stoffers DA, Simmons RA. Exendin-4 increases histone acetylase activity and reverses epigenetic modifications that silence Pdx1 in the intrauterine growth retarded rat. Diabetologia. 2011; 54:2606–2614. [PubMed: 21779870]

Pryke, SR.; Griffith, SC. Science. Vol. 323. New York, NY: 2009. Genetic incompatibility drives sex allocation and maternal investment in a polymorphic finch; p. 1605-1607.

Radford EJ, Isganaitis E, Jimenez-Chillaron J, Schroeder J, Molla M, Andrews S, Didier N, Charalambous M, McEwen K, Marazzi G, et al. An unbiased assessment of the role of imprinted genes in an intergenerational model of developmental programming. PLoS genetics. 2012; 8:e1002605. [PubMed: 22511876]

Radford, EJ.; Ito, M.; Shi, H.; Corish, JA.; Yamazawa, K.; Isganaitis, E.; Seisenberger, S.; Hore, TA.; Reik, W.; Erkek, S., et al. Science. New York, NY: 2014. In utero undernourishment perturbs the adult sperm methylome and intergenerational metabolism.

Rakyan VK, Down TA, Balding DJ, Beck S. Epigenome-wide association studies for common human diseases. Nature reviews Genetics. 2011; 12:529–541.

Rando OJ. Daddy issues: paternal effects on phenotype. Cell. 2012; 151:702–708. [PubMed: 23141533]

Rando OJ, Verstrepen KJ. Timescales of genetic and epigenetic inheritance. Cell. 2007; 128:655–668. [PubMed: 17320504]

Rassoulzadegan M, Grandjean V, Gounon P, Vincent S, Gillot I, Cuzin F. RNA-mediated non-mendelian inheritance of an epigenetic change in the mouse. Nature. 2006; 441:469–474. [PubMed: 16724059]

Ravelli AC, van der Meulen JH, Michels RP, Osmond C, Barker DJ, Hales CN, Bleker OP. Glucose tolerance in adults after prenatal exposure to famine. Lancet. 1998; 351:173–177. [PubMed: 9449872]

Ravelli GP, Stein ZA, Susser MW. Obesity in young men after famine exposure in utero and early infancy. The New England journal of medicine. 1976; 295:349–353. [PubMed: 934222]

Raychaudhuri N, Raychaudhuri S, Thamotharan M, Devaskar SU. Histone code modifications repress glucose transporter 4 expression in the intrauterine growth-restricted offspring. The Journal of biological chemistry. 2008; 283:13611–13626. [PubMed: 18326493]

Rich-Edwards JW, Colditz GA, Stampfer MJ, Willett WC, Gillman MW, Hennekens CH, Speizer FE, Manson JE. Birthweight and the risk for type 2 diabetes mellitus in adult women. Annals of internal medicine. 1999; 130:278–284. [PubMed: 10068385]

Rinaudo P, Wang E. Fetal programming and metabolic syndrome. Annu Rev Physiol. 2012; 74:107–130. [PubMed: 21910625]

Roberts KA, Riley SC, Reynolds RM, Barr S, Evans M, Statham A, Hor K, Jabbour HN, Norman JE, Denison FC. Placental structure and inflammation in pregnancies associated with obesity. Placenta. 2011; 32:247–254. [PubMed: 21232790]

Rodeheffer MS, Birsoy K, Friedman JM. Identification of white adipocyte progenitor cells in vivo. Cell. 2008; 135:240–249. [PubMed: 18835024]

Rodgers AB, Morgan CP, Bronson SL, Revello S, Bale TL. Paternal stress exposure alters sperm microRNA content and reprograms offspring HPA stress axis regulation. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2013; 33:9003–9012. [PubMed: 23699511]

Sakai C, Tomitsuka E, Miyagishi M, Harada S, Kita K. Type II Fp of human mitochondrial respiratory complex II and its role in adaptation to hypoxia and nutrition-deprived conditions. Mitochondrion. 2013; 13:602–609. [PubMed: 24008124]

Samans B, Yang Y, Krebs S, Sarode GV, Blum H, Reichenbach M, Wolf E, Steger K, Dansranjavin T, Schagdarsurengin U. Uniformity of nucleosome preservation pattern in Mammalian sperm and its connection to repetitive DNA elements. Developmental cell. 2014; 30:23–35. [PubMed: 24998597]

Sandovici I, Smith NH, Nitert MD, Ackers-Johnson M, Uribe-Lewis S, Ito Y, Jones RH, Marquez VE, Cairns W, Tadayyon M, et al. Maternal diet and aging alter the epigenetic control of a promoter-enhancer interaction at the Hnf4a gene in rat pancreatic islets. Proceedings of the National Academy of Sciences of the United States of America. 2011; 108:5449–5454. [PubMed: 21385945]

Sasson IE, Vitins AP, Mainigi MA, Moley KH, Simmons RA. Pre-gestational versus gestational exposure to maternal obesity differentially programs the offspring. Diabetologia. In press

Scott RA, Lagou V, Welch RP, Wheeler E, Montasser ME, Luan J, Magi R, Strawbridge RJ, Rehnberg E, Gustafsson S, et al. Large-scale association analyses identify new loci influencing glycemic traits and provide insight into the underlying biological pathways. Nature genetics. 2012; 44:991–1005. [PubMed: 22885924]

Selak MA, Storey BT, Peterside I, Simmons RA. Impaired oxidative phosphorylation in skeletal muscle of intrauterine growth-retarded rats. American journal of physiology Endocrinology and metabolism. 2003; 285:E130–E137. [PubMed: 12637257]

Sen S, Simmons RA. Maternal antioxidant supplementation prevents adiposity in the offspring of Western diet-fed rats. Diabetes. 2010; 59:3058–3065. [PubMed: 20823102]

Simmons R. Perinatal programming of obesity. Exp Gerontol. 2005; 40:863–866. [PubMed: 16216461]

Simmons R. Epigenetics and maternal nutrition: nature v. nurture. The Proceedings of the Nutrition Society. 2011; 70:73–81. [PubMed: 21110912]

Simmons RA, Gounis AS, Bangalore SA, Ogata ES. Intrauterine growth retardation: fetal glucose transport is diminished in lung but spared in brain. Pediatric research. 1992; 31:59–63. [PubMed: 1594332]

Simmons RA, Suponitsky-Kroyter I, Selak MA. Progressive accumulation of mitochondrial DNA mutations and decline in mitochondrial function lead to beta-cell failure. The Journal of biological chemistry. 2005; 280:28785–28791. [PubMed: 15946949]

Simmons RA, Templeton LJ, Gertz SJ. Intrauterine growth retardation leads to the development of type 2 diabetes in the rat. Diabetes. 2001; 50:2279–2286. [PubMed: 11574409]

Smith ZD, Chan MM, Mikkelsen TS, Gu H, Gnirke A, Regev A, Meissner A. A unique regulatory phase of DNA methylation in the early mammalian embryo. Nature. 2012; 484:339–344. [PubMed: 22456710]

Sobala A, Hutvagner G. Transfer RNA-derived fragments: origins, processing, and functions. Wiley Interdiscip Rev RNA. 2011; 2:853–862. [PubMed: 21976287]

Stoffers DA, Desai BM, DeLeon DD, Simmons RA. Neonatal exendin-4 prevents the development of diabetes in the intrauterine growth retarded rat. Diabetes. 2003; 52:734–740. [PubMed: 12606515]

Sullivan EL, Smith MS, Grove KL. Perinatal exposure to high-fat diet programs energy balance, metabolism and behavior in adulthood. Neuroendocrinology. 2011; 93:1–8. [PubMed: 21079387]

Taylor PD, Poston L. Developmental programming of obesity in mammals. Experimental physiology. 2007; 92:287–298. [PubMed: 17170060]

Thamotharan M, Garg M, Oak S, Rogers LM, Pan G, Sangiorgi F, Lee PW, Devaskar SU. Transgenerational inheritance of the insulin-resistant phenotype in embryo-transferred intrauterine growth-restricted adult female rat offspring. American journal of physiology Endocrinology and metabolism. 2007; 292:E1270–E1279. [PubMed: 17213472]

Travers ME, McCarthy MI. Type 2 diabetes and obesity: genomics and the clinic. Human genetics. 2011; 130:41–58. [PubMed: 21647602]

Valdez R, Athens MA, Thompson GH, Bradshaw BS, Stern MP. Birthweight and adult health outcomes in a biethnic population in the USA. Diabetologia. 1994; 37:624–631. [PubMed: 7926349]

van Vliet E, Eixarch E, Illa M, Arbat-Plana A, Gonzalez-Tendero A, Hogberg HT, Zhao L, Hartung T, Gratacos E. Metabolomics reveals metabolic alterations by intrauterine growth restriction in the fetal rabbit brain. PloS one. 2013; 8:e64545. [PubMed: 23724060]

Wagner KD, Wagner N, Ghanbarian H, Grandjean V, Gounon P, Cuzin F, Rassoulzadegan M. RNA induction and inheritance of epigenetic cardiac hypertrophy in the mouse. Developmental cell. 2008; 14:962–969. [PubMed: 18539123]

Wagner W, Wein F, Seckinger A, Frankhauser M, Wirkner U, Krause U, Blake J, Schwager C, Eckstein V, Ansorge W, et al. Comparative characteristics of mesenchymal stem cells from human bone marrow, adipose tissue, and umbilical cord blood. Experimental hematology. 2005; 33:1402–1416. [PubMed: 16263424]

Waterland RA, Dolinoy DC, Lin JR, Smith CA, Shi X, Tahiliani KG. Maternal methyl supplements increase offspring DNA methylation at Axin Fused. Genesis. 2006; 44:401–406. [PubMed: 16868943]

Watkins AJ, Sinclair KD. Paternal low protein diet affects adult offspring cardiovascular and metabolic function in mice. American journal of physiology Heart and circulatory physiology. 2014; 306:H1444–H1452. [PubMed: 24658019]

Wee LM, Flores-Jasso CF, Salomon WE, Zamore PD. Argonaute divides its RNA guide into domains with distinct functions and RNA-binding properties. Cell. 2012; 151:1055–1067. [PubMed: 23178124]

Wei Y, Yang CR, Wei YP, Zhao ZA, Hou Y, Schatten H, Sun QY. Paternally induced transgenerational inheritance of susceptibility to diabetes in mammals. Proceedings of the National Academy of Sciences of the United States of America. 2014; 111:1873–1878. [PubMed: 24449870]

Yang BT, Dayeh TA, Volkov PA, Kirkpatrick CL, Malmgren S, Jing X, Renstrom E, Wollheim CB, Nitert MD, Ling C. Increased DNA methylation and decreased expression of PDX-1 in

pancreatic islets from patients with type 2 diabetes. Molecular endocrinology. 2012; 26:1203–1212. [PubMed: 22570331]

Yang QY, Liang JF, Rogers CJ, Zhao JX, Zhu MJ, Du M. Maternal obesity induces epigenetic modifications to facilitate Zfp423 expression and enhance adipogenic differentiation in fetal mice. Diabetes. 2013; 62:3727–3735. [PubMed: 23884886]

Zaretsky MV, Alexander JM, Byrd W, Bawdon RE. Transfer of inflammatory cytokines across the placenta. Obstetrics and gynecology. 2004; 103:546–550. [PubMed: 14990420]

Zhu MJ, Du M, Nathanielsz PW, Ford SP. Maternal obesity up-regulates inflammatory signaling pathways and enhances cytokine expression in the mid-gestation sheep placenta. Placenta. 2010; 31:387–391. [PubMed: 20185176]

Zilberman, D.; Cao, X.; Jacobsen, SE. Science. Vol. 299. New York, NY: 2003. ARGONAUTE4 control of locus-specific siRNA accumulation and DNA and histone methylation; p. 716-719.

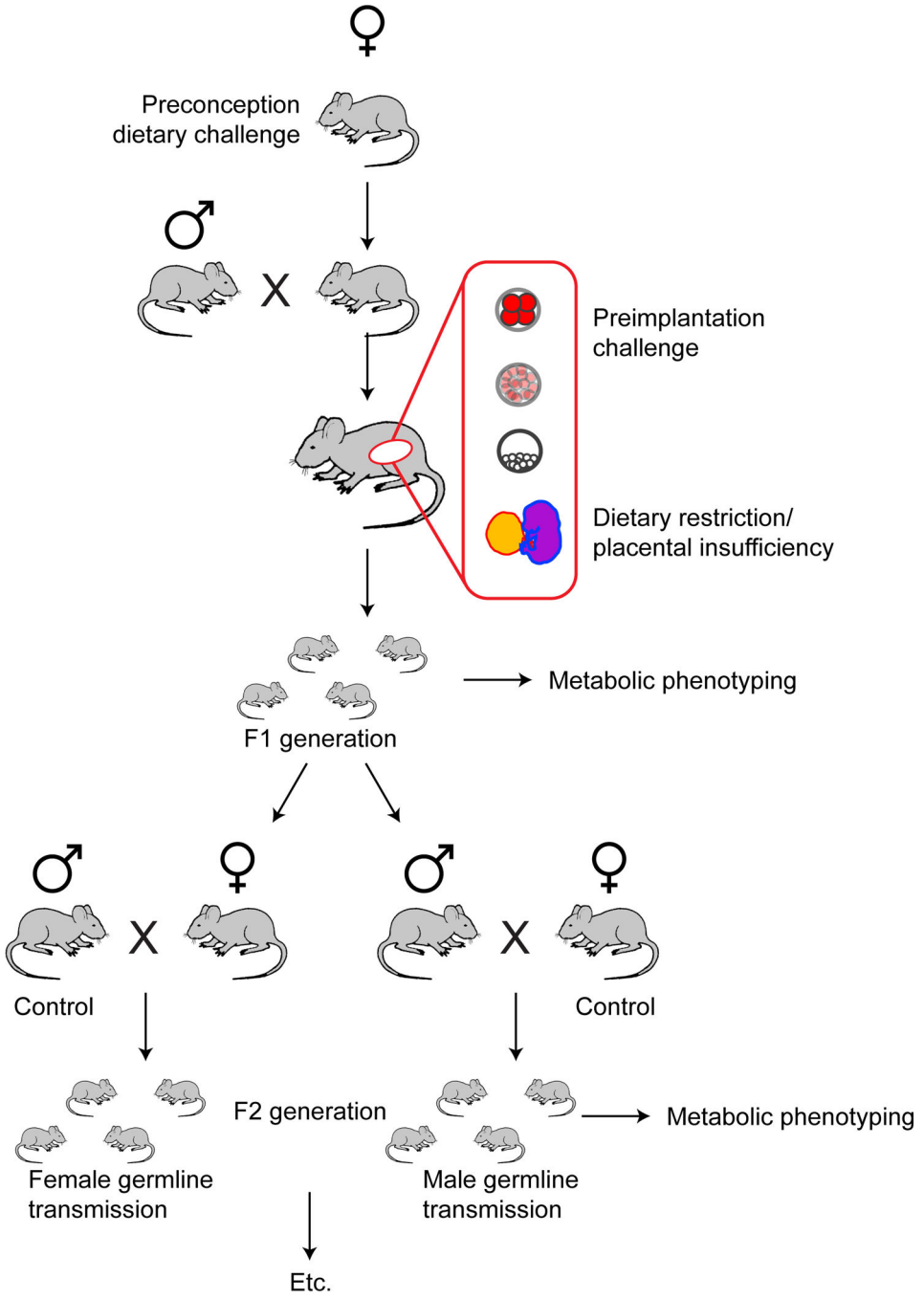
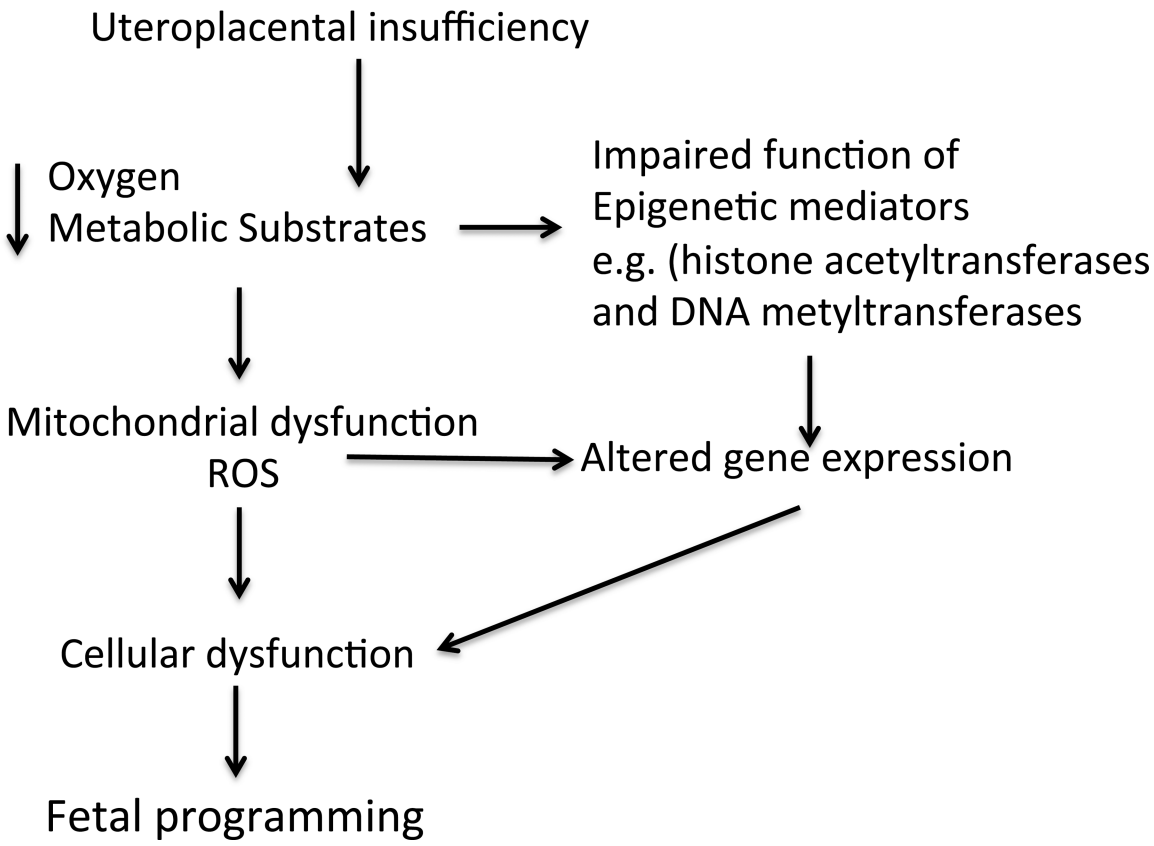


Figure 1. Schematic for maternal effect paradigms

Maternal effect paradigms typically involve altered access to nutrients, with key paradigms including under or overnutrition prior to conception, during preimplantation development, or later in pregnancy. Phenotypes are then typically studied in F1 offspring. In a subset of experimental systems, F1 offspring are bred either with control animals or with one another to identify multigenerational effects of fetal undernutrition.

Mitochondria Function and Fetal Programming



Figures 2/3. Mechanisms linking fetal nutrient supply to later phenotypes

Uteroplacental insufficiency decreases availability of key substrates such as oxygen and glucose to the fetus. Altered substrate availability (e.g. decreased acetyl Co-A, or S-Adenosyl methionine) can directly influence epigenetic mediators resulting in epigenetic modifcations of key genes. Decreased levels of glucose and oxygen can also impair mitochondrial function and increase production of ROS. These processes can have a detrimental affect on numerous cellular pathways culminating in fetal programming.