Journals of Gerontology: Biological Sciences
cite as: J Gerontol A Biol Sci Med Sci, 2019, Vol. 74, No. 10, 1582–1589
doi:10.1093/gerona/glz154
Advance Access publication June 20, 2019

Editor's choice

Review

Transgenerational Obesity and Healthy Aging in *Drosophila*

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Received: January 2, 2019; Editorial Decision Date: June 12, 2019

Decision Editor: Rozalyn Anderson, PhD

Abstract

Substantial evidence suggests that individuals born to overweight and obese parents suffer detrimental health consequences that dramatically decrease healthy aging. The number of obese individuals worldwide now exceeds the number of under- and malnourished individuals. This obesity epidemic is responsible for approximately 4 million deaths worldwide each year, and predisposes sufferers to a range of age-related diseases such as cardiovascular diseases, and metabolic syndrome. Additionally, obesity is associated with an accelerated onset of age-related ailments, such as cancers and inflammation. The importance of dietary interventions to reduce the incidence of obesity is magnified by emerging evidence that parental physiology can predispose future generations to poor health outcomes. Characterizing and understanding these effects, and how they are mediated, is important if we are to continue to drive improvements to population health. In this article, we synthesize evidence for the intergenerational and transgenerational phenotypic effects of parental obesity. We concentrate on how the fruit fly *Drosophila melanogaster* can be used as a model to study these effects. Fruit flies are highly tractable, and their conserved nutrient signaling and metabolic pathways make them an ideal model for studying nutritional effects on metabolic, reproductive, and aging phenotypes.

Keywords: Fecundity, Life span; Obesity

Over the last century, average life expectancy has been steadily increasing, and shows no signs of slowing (1,2). Dietary interventions to further extend and improve healthy life span into the future are a major focus of biogerontology (3). In general, moderate restriction of food intake, through either restricting calories or restricting overall diet nutrients (CR, DR) or modification of dietary nutrient balance, can augment life span—an effect evident in model organisms from yeast to primates (4). Furthermore, a growing body of evidence points to beneficial metabolic and physiological effects of CR, including reductions in body fat, in both lean and overweight humans when administered during early- to mid-life (5,6). These findings are exciting because they highlight the promise of deploying dietary interventions as a tool to improve healthy aging within the general population. Moreover, the practical utility of such dietary interventions is amplified by recent reports of negative health effects in children born to overweight and obese parents. Negative intergenerational effects of obesity are not well understood, but researching the prevalence and magnitude of these effects will be key to addressing the health and economic costs associated with obesity in contemporary human populations (7,8).

Here, we synthesize evidence from experimental studies that have investigated the effects of parental obesity on reproduction and life span of future generations. In particular, we highlight the utility of the fruit fly *Drosophila melanogaster* as a model for the study of inter- and transgenerational obesity, given its short generation time, small containment footprint, conserved metabolic pathways, well characterized diet, and a readily available suite of advanced genetic tools. We address whether experiments conducted in *Drosophila* can be used to inform likely responses in humans, by critically evaluating whether experimental findings from studies of *Drosophila* are consistent with those that have come from studies of mice. We conclude that the study of *Drosophila* offers a powerful means to better understand the mechanisms involved in the regulation between nutrition and health, across generations.

Why Do We Eat Unhealthy, Imbalanced Diets?

The role of nutrition in health is complex. Diets consist of dozens of components that are required in proportions that vary as a function

of the supply of other nutrients, as well as the consumer's genome, microbiota, life stage, and condition (9). In general, too little or too much of a nutrient is detrimental to an organism's health (10). Thus to maximize fitness, organisms must perform a multinutritional balancing act to ingest and absorb nutrients in suitable proportions. First, this is achieved by nutrient-specific appetites that can alter the relative consumption of different ingredients to achieve and maintain homeostasis (11). When organisms eat, however, the proportion of nutrients in their food may not match requirements. Consumers must therefore make compromises on the balance of nutrients they ingest, and where possible, ameliorate those compromises by mixing foods with complementary nutritional profiles (12,13). The precise nature of these compromises is determined by the relative priority with which each nutrient effects appetite and satiety—a function of the specific evolutionary history of each organism (14).

Of the many nutrients in a diet, carbohydrate, fat, and protein are the three major energy-contributing nutrients. These play a key role in determining evolutionary fitness and are important determinants of diet choice (15). The exact strategy for nutrient mixing varies across species, and not all nutrients have an equal influence on feeding behavior. For some primates (including humans), mice and *Drosophila* larvae, data indicates that protein is the primary determinant of appetite and satiety (14,16–20). Thus, within limits, food is consumed to keep protein intake within a tight range of values, and a much broader range of lipid and carbohydrate intake is tolerated. This can be understood in light of the fact that protein underconsumption leads to a decrease or cessation in reproductive output, while overconsumption can carry metabolic and physiological costs associated with excretion (3,21). By contrast a broader range of sugar and fat consumption can be tolerated as excess energy can be stored as body fat and be advantageous as a buffer against seasonal variations in energy supply (22,23). The capacity for protein levels to shape feeding behavior can be exploited to facilitate loss of body fat by curtailing appetite through consumption of high protein diets. By contrast, meeting a protein intake target when consuming low protein foods can result in overconsumption of sugar and fat, which in the long term can lead to obesity (18). Together, this has led to the hypothesis that the current obesity epidemic in developed countries is fuelled (at least in part) by the unprecedented abundance of low protein, energy dense foods that are highly palatable, yet have little satiety value (24).

Health and Body Fat

The number of obese individuals worldwide now exceeds the number of under- and malnourished individuals. Obesity predisposes sufferers to a plethora of age-related diseases such as type 2 diabetes, cardiovascular diseases, and metabolic syndrome, and is responsible for approximately 4 million deaths worldwide each year (25,26). Additionally, obesity sufferers are more likely to be afflicted by age-related conditions, such as cancers and chronic inflammatory diseases (26).

Body mass index (BMI) estimates body fat, and is calculated by body mass in kilograms divided by the square of the person's height in meters, and is a useful correlate of health outcomes. BMI from 18.5 to 24.9 ("normal" range) is considered "healthy" and without weight-related adverse health consequences (26). The categories of underweight (BMI <18.5), overweight (BMI 25–29.9) to obese (BMI ≥ 30), however, are associated with increased risks of certain diseases, costs to reproductive performance, and reduced life expectancy (27–31). Importantly, for studying the mechanistic bases of these poor outcomes, the obese state can be modeled in mice and flies to varying degrees of accuracy. By providing highly palatable, energy dense diets with low satiety value to model species, the percentage of body fat increases and is accompanied by negative outcomes for reproduction and life span, analogous to the pattern observed in humans (32–34).

Modeling Obesity in Murids and Flies

Murids

Feeding high-energy diets, supplied as excess fat or sugar, to mice or rats leads to a higher percentage of body fat compared to those fed a control diet. These obesogenic diets also lead to a suite of altered metabolic markers similar to changes found in humans, such as: increases in rates of diabetes; poor insulin sensitivity; high circulating blood glucose; and increased incidences of some cancers (35–37). Furthermore, fertility and life span also decrease in overweight mice, similar to what is observed in humans (38–40). These detrimental effects of obesity on female fertility can occur whether the mice are hyperphagic (over-eating), or feeding on diets high in fat and or sugar (41–43). Although obesity has generally negative effects, some recent studies have shown that altering dietary macronutrient balance can modify the propensity of an obese individual to suffer from later-life pathologies such as type II diabetes and heart disease (40,44,45). These effects are fascinating and warrant further investigation.

Flies

Similar to murids, it is possible to manipulate the proportion of adult body fat in flies. In the wild, flies consume a diet of rotting fruit, sourcing carbohydrates from the fruit and the remainder of their nutrients (including additional carbohydrates) from yeast (46). These diets contain very little fat (approximately 1% of mass), and in the lab, growth, reproduction, and life span can be readily supported with diets containing only sugar and yeast. A typical example of one such diet used in our laboratory supplies energy from protein: carbohydrate: fat in proportions ~55:40:5 (corresponding to ~45 g/L protein, 35 g/L carbohydrate, and 2 g/L fat) (47,48). Data from studies that have supplemented this natural nutritional profile with fat show universally detrimental physiological outcomes and shortened life span (49–52). However, it is not yet possible to discern if these unfavorable changes mimic the costs that mammals suffer on high fat obesogenic diets, or if they simply reflect novel pathologies caused by dietary fat levels that are well above what flies have evolved to experience in the wild. We postulate a more ecologically relevant approach to increasing body fat in flies is by increasing the carbohydrate (sugar) component of the diet (53,54), and for this reason we will restrict our discussion to those studies that manipulate parental sugar. Both female and male flies get fatter with increasing dietary sugar concentrations and this does not appear to be acutely toxic since there is no cost to life span for values of dietary carbohydrates of up to ~80% of total energy (3,34,47,55,56). In contrast, female fecundity is maximized at intermediate dietary carbohydrate levels (up to ~50% of total energy), when body fat remains low. As dietary sugar and body fat rise beyond this point, egg laying sharply declines (Figure 1) (3,34,47,55,56).

Inter- and Transgenerational Consequences of Parental Obesity

It is becoming increasingly apparent that obesity susceptibility is transmitted from generation to generation. These effects can be intergenerational (transmitted from parent (F0) to offspring (F1))

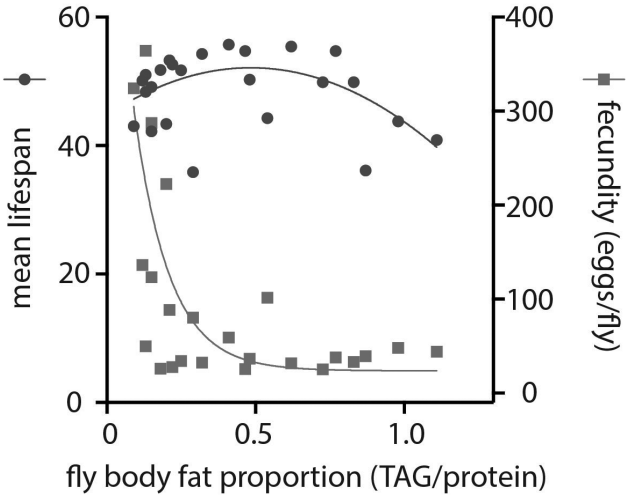

Figure 1. Relationship between body fat, egg laying and life span in an inbred lab strain of female Drosophila melanogaster. Altering the relative proportion of sugar and yeast in a fly diet alters body fat composition. Maximal female egg laying corresponds to nutrient compositions at which body fat content is very low, and as body fat rises, egg laying declines. In contrast, life span remains long, showing only mildly compromised at nutrient compositions that promote higher body fat content (data from Skorupa et al. (34)).

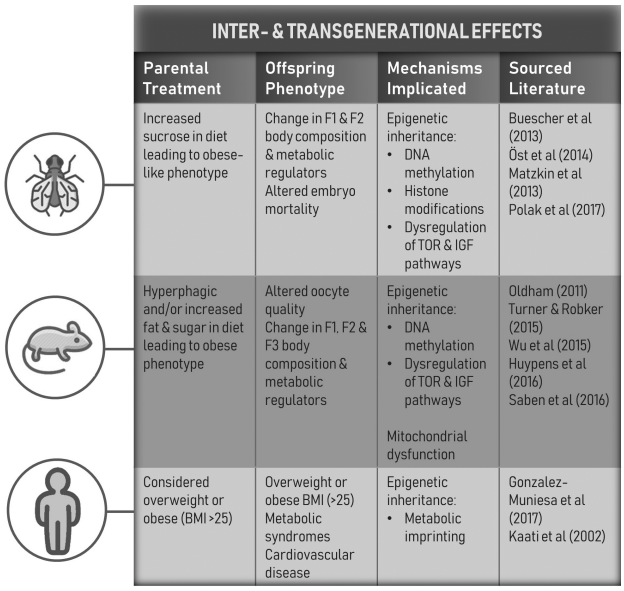

Figure 2. Obesogenic diets in parents can confer detrimental metabolic phenotypes on offspring to the second and third generations. These effects appear to be evolutionarily conserved, meaning we can start to examine their mechanisms in short-lived, easily housed model organisms. The common mechanism to be implicated across taxa involves epigenetic marks that may alter the expression of key nutrient signaling pathways. It will be important in future work to explore additional possible mechanisms (eg, mito-nuclear interactions), using nutrient explicit diets. We also recommend the use of fully-factorial diet designs on mothers, fathers and their offspring in order to parse the effects of maternal and paternal contribution to health of males and females in future generations.

and even transgenerational (transmitted from parent (F0) to the F2 generation and beyond; Figure 2). Distinguishing between these inheritance modes is important for understanding the mechanisms of susceptibility transfer, since in the case of intergenerational effects, the offspring may experience the predisposing parental environment (eg, while in utero), while for transgenerational effects, the affected offspring have no direct experience of the predisposing grand-parental environment. Thus, in oviparous species, like fruit flies, transgenerational effects are those transmitted to the grand offspring (F2) generation, but in mice and humans, they are the effects transmitted to the great-grand offspring generation (F3) because the primordial germ cells of the F2 generation are present in the F1 female fotus while in utero, and are thus subject to the grand-maternal environment.

Transgenerational Plasticity

Inter- or transgenerational phenotypic plasticity describes the situation when the parental environment or phenotype impacts the phenotype of the offspring, beyond the effects of gene transfer alone (57,58). The most commonly studied effects are those of maternal diet on offspring physiology, which are thought to result from transmission of epigenetic markers, antibodies, hormones, and/or nutrients (59). Since selection typically favors the total lifetime reproductive success of parents (rather than reproductive success during any one bout), both negative and positive maternal effects can be favored if they enhance maternal fitness (57). This will depend on the life expectancy of the mother, resource availability, environmental conditions, and the interaction between the costs of producing an offspring phenotype and the benefits of that phenotype (57). Maternal effects that confer a positive impact on offspring quality are often referred to as "adaptive maternal effects." These are more likely to occur in predictable environments when mothers prime offspring to be suited to the same environmental stresses she experienced (59). Maternal transgenerational plasticity may also be disadvantageous, however, if the postnatal environment differs from the intrauterine one, such as if a fetus is subjected to a malnourished environment and is thus "programmed" for a more energy efficient metabolism, but is raised in a nutrient abundant environment, predisposing the offspring to obesity (60). Although evidence is mounting for adaptive transgenerational anticipatory effects, progress has been hindered by a paucity of experimental studies testing that employ fully-factorial designs in which both parents and offspring are challenged with both a control diet and a novel diet (58).

Inter- and Transgenerational Effects of Parental Obesity in Humans

Several recent studies have shown that many individuals born to overweight and obese parents suffer detrimental later-life health consequences, particularly when born into a nutrient rich environment (61,62) (Figure 2). Developing obesity becomes more likely due to critical developmental periods whereby metabolic imprinting (programming of metabolism) can occur (26). Critical periods identified in the development of obesity are the pre- and neonatal periods (up to 2 years old). In the prenatal period, maternal disproportionate gestational weight gain, especially in the first 20 weeks of pregnancy has been identified as a risk factor in the development of obesity later in the child's life (63–65). Even parental weight gained (or BMI) prior to fertilization is associated with a child's later-life BMI. A mother's prepregnancy and early-pregnancy BMI explains most of the variance in a child's BMI—even when controlling for pregnancy complications such as gestational diabetes, and other lifestyle factors (26). Similarly, paternal BMI could be an important factor for later-life progeny health. Historical records have shown that males

exposed to excess food during the slow growth period of childhood (8–12 years old) exhibit a fourfold greater risk of their grandsons developing later-life type II diabetes and cardiovascular disease (61). We note that parallel poor late-life outcomes have also been reported for individuals that were in utero at the time of extreme energy deprivation (66). In addition, suboptimal fetal nutrition, both from under- or overnourishment leads to an increased risk of cardiovascular diseases and type 2 diabetes (67).

While the evidence for intergenerational effects of obesogenic diets in humans is interesting, it is challenging to separate the effects of this transmission from effects of shared environment. This is because the children are likely to be exposed to the same obesogenic lifestyle as their parents. This is where nonhuman models become critically valuable in helping to us to disentangle trait transmission from shared environmental effects.

Compelling evidence for inter- and transgenerational inheritance of obesity comes from mouse studies where obesity can be restricted to particular windows of offspring development. Specifically, even when oocytes from obese females are used for IVF or embryos transferred to lean mothers for gestation, they exhibit altered fetal development (42,68,69), and become obese and insulin resistant in adulthood (70).

Inter- and Transgenerational Effects of High Sugar Diet-Induced Obesity in *Drosophila*

Most studies that have investigated the effects of high sugar diets in *Drosophila* have focused on the direct effects on trait expression that manifest within an individual's lifetime (3,34,47,55,56,71). A few studies, however, have investigated the effects of altering parental diets on offspring and grand-offspring traits (summarized in Table 1 and Figure 2). Feeding *Drosophila* larvae with isocaloric diets that differed in protein: sugar ratio is sufficient to elicit phenotypic differences in their offspring, even when the offspring were maintained on a common diet (32). The offspring from parents that had received a low protein/high sugar (obesogenic) diet during development, exhibited a lengthened period for metamorphosis, produced less eggs, and had altered body composition (protein, glycogen, TAG), when compared to the offspring from parents that had been raised on a high protein/low sugar diet. Interestingly, many of these effects differed between isofemale genotypes, and in some cases were reversed, indicating intergenerational effects are genotype dependent (32).

In another report, Buescher et al. (53) showed that high sugar diets provided to females produced an obese-like phenotype that persisted in male offspring to the F2 generation. Upon challenge with a high sugar diet, F1 sons of mothers exposed to high sugar exhibited increased levels of trehalose, glycogen, glucose and TAG, as well as exaggerated changes in expression of key genes involved in carbohydrate and fat metabolism. Furthermore, the grandsons (F2) of those same high sugar diet fed females also exhibited a higher proportion of body fat, glycogen and trehalose than the grandsons of females fed a low sugar diet, even though the mothers from the intermediate generation (F1) did not experience high sugar diets. Although no data for the daughters were reported, grand-daughters displayed a higher level of trehalose but not of whole-body TAG. Together, these data suggest that flies exhibit a sex-specific metabolic and gene regulatory response to energy challenge, and that this is sensitive to their grand-mothers' dietary experience.

Although most studies using *Drosophila* focus on how maternal diet affects trajectories of offspring health, the paternal diet can have effects also. Remarkably, even transient changes in the sugar content of the paternal diet, for periods as short as 48 hours prior to mating, can lead to intergenerational obesity and metabolic reprogramming of the offspring (33). When males fed either very high or low sugar diets were crossed to control diet fed females, their sons showed an increase in body fat percentage on high sugar diets when compared to sons from crosses where both mothers and fathers were maintained on diets with intermediate sugar content. This effect was attributed to altered chromatin markers in the sperm of the fathers

Table 1. Inter- and Transgenerational Effects of High Sugar Diets in *Drosophila.*

	F0	F1	F2
Maternal high sucrose Buescher et al. (53).	*Adult mothers*: ↑Trehalose, Glycogen, TAG ↓Body weight	*Larval sons*: ↑Glucose, trehalose, circulating sugars, gene expression: gluconeogenesis, fat body lipolysis ↓Glycogen, cholesterol, gene expression: dFOXO, glycolysis, sugar transport *Adult sons*: ↓Body weight[a], Glucose ↑Trehalose, Glycogen, TAG[a] *Larval and adult daughters:* Not reported	*Larval grandsons*: ↑Glucose and trehalose *Larval granddaughters*: ↑Trehalose ↓TAG
Paternal high sucrose Öst et al. (33).	*Adult fathers*: ↑TAG	*Adult sons*: ↑Body weight TAG, lipid droplet size, glucose ↓Trehalose Altered sperm chromatin state *Adult daughters:* Not studied	*Adult grandsons*: No transgenerational effects found *Adult granddaughters:* Not studied
Both Parents high sucrose (isocaloric) Matzkin et al. (32).	*Adult parents*: ↑Glycogen ↓Protein, fecundity	*Adult offspring*: ↑Glycogen, development time ↓Fecundity, protein, TAG	*Adult grand offspring*: Not studied

Note: TAG = Whole body Triacylglyceride content. [a]Worsened with a high sucrose diet challenge; ↑increased, ↓decreased or reduced.

(33). More recently, it was found that offspring viability during embryogenesis is sensitive to alterations in the ratio of protein: carbohydrate, the caloric density, and the quality of carbohydrates in the paternal diet (72). Exactly how these factors affected the offspring differed when comparing the offspring sired by the father's first mating to the offspring sired by his second mating to a different virgin female. This study is particularly interesting because unlike others that only vary parental dietary sugar, this systematically varied both the dietary protein: carbohydrate ratio as well as total energy density. In doing so, it shows that the better the paternal condition (the sum of energy contained in the father's fat, glycogen, and protein reserves), the higher the proportion of his sired offspring that survived embryogenesis. It would be interesting to examine the mechanistic basis of this transmission to understand if each of the nutrient combinations operate through the same mechanisms to affect the next generation (72).

Taken together, these studies provide evidence that varying the composition of diets consumed by *Drosophila* parents not only affect their own phenotype but also that of their offspring and grand offspring, and that these effects can be altered by genetic background. While interesting, these data are the result of relatively few studies and each differs in experimental design. Furthermore, because the traits being investigated are sensitive to environmental conditions, transgenerational effects can be difficult to reproduce, even within the same laboratory (73). Thus, a limitation is that it is currently difficult to measure the magnitude of these inter- and transgenerational effects, and to identify specific nutritional triggers. This highlights the need for further research, and for future studies to make explicit the nutritional composition of the diets they employ, and ideally adopt a range of diets over which to generate the effects. This will enable phenotypic responses to be mapped to diet within a structured framework, such as the geometric framework of nutrition (9) as reported in Polak et al. (72). In this way, transgenerational phenotypes whose manifestation might appear to be weak or variable between two studies can be unified into a continuum of responses across nutrient space. This will give us the power to understand what nutritional treatments are required to elicit transgenerational plasticity and therefore target work to understand the mechanisms.

Mechanisms Underpinning Intergenerational Effects of Diet-Induced Obesity

Determining the molecular mechanisms underlying inter- and transgenerational metabolic imprinting using human clinical samples is challenging, due (at least in part) to the variable nature of conditions and environments from which samples originate. We therefore look to model organisms for answers. For murids and flies, both genetic and dietary models of obesity are available. Here, we concentrate on diet-induced obesity and evolutionarily conserved mechanisms. Although our understanding is far from complete, inheritance of epigenetic markers that alter offspring metabolic physiology via transcriptional changes are consistently identified across taxa as being critical to altering transgenerational plasticity. These markers include DNA methylation and histone modifications (Figure 2). These alter the expression of nutrient-sensing pathways such as insulin-like-growth factor (IGF), and TOR (Target of Rapamycin) (74). The dysregulation of these nutrient-sensing pathways under obesogenic conditions has been linked to age-related metabolic changes, and altered mitochondrial function (75–77). Both the TOR and the IGF pathways are evolutionarily conserved, TOR is present in yeast and animals and IGF is present in animals. There is also evidence that small RNAs and other molecular modifiers could be responsible for transmission (33) but many in vivo studies investigating the role of transgenerational metabolic imprinting are unable to parse these various possible causes.

One study demonstrating the importance of DNA methylation for transmitting phenotypic plasticity in mice used agouti viable yellow (A^{vy}) mutant mice, which are genetically predisposed toward hyperphagic obesity. In this work, maternal obesity influenced fat accumulation in the offspring to the F3 generation, suggesting a cumulative effect of obesity across generations (78). Interestingly, these effects were ameliorated by providing pregnant mothers with a pro-methylation diet enriched for the methyl donors folic acid, betaine, vitamin B12, and choline. This diet exacerbated the mottled yellow coat of the A^{vy} mice, which is indicative of its efficacy to increase DNA methylation, but the observed effect to suppress transgenerational body weight gain was independent of coat color variations indicating DNA methylation elsewhere in the genome is important (78). An epigenetic basis of transmission is also supported by mouse studies that have used in vitro fertilization (IVF) with gametes from obese parents and implanted the embryos into normal weight surrogates, thus removing any potentially confounding effects of the environments at conception, in utero, during lactation, or in transmission of the maternal microbiome at birth. These data show that both oocytes and sperm from obese parents can contribute to intergenerational obesity and development of type II diabetes of offspring challenged with a high fat diet (70).

Obese male flies fed a high sugar diet have also been shown to predispose their offspring to obesity, transmitted via epigenetic marks. Unlike mammals, flies possess negligible levels of DNA methylation (79), but they do have a heritable system for modifying DNA accessibility, and thus gene expression, via post-translational modifications of histones (80). Sperm from male flies fed a high sugar diet showed evidence of repressive histone methylation marks and importantly, modifiers of these marks were correlated with sustained repression of lipid biosynthetic genes in the embryo. Moreover, these modifiers are required for intergenerational transmission of a predisposition to obesity when offspring were maintained on a high sugar diet (33). This study on flies provides direct evidence that histone modifications that alter the expression of metabolic genes are required to transmit a phenotype from parent to offspring in a manner similar to what has been shown for mice.

In evidence of other mechanisms, mitochondrial and nuclear genotypic interactions have also been shown to alter whole-body metabolism and gene expression under diet-induced obesogenic conditions (81,82). In murids, obesogenic prenatal diets that lead to metabolic syndrome are associated with reduced mitochondrial DNA (mtDNA) content (reduced copy number of mtDNA molecules per cell) and altered expression of the mtDNA genome in offspring (43,83). It is not clear from these studies if the reduced mtDNA frequency is due to an overall loss of—or dysfunction of—the mitochondria. Studies attempting to elucidate what causes the loss have implicated mitochondrial dysfunction in oocytes of obese mothers as causal in mitochondrial loss in offspring, due to endoplasmic reticulum stress. Endoplasmic reticulum stress reduces protein secretion and disrupts mitochondrial activity in oocytes, thus impairing its function. This indicates that a maternal obesogenic diet can influence offspring metabolism by altering the mitochondrial content and quality in the oocyte at periconception (43,84). It is interesting to note that another study has also shown altered mitochondrial morphology and cellular metabolism in the muscle of F2 and F3 offspring of female mice fed high fat or high sugar diets (69). How

mitochondria may interface with the epigenetic alterations observed in offspring of obese mothers is yet to be determined (68). Emerging evidence using fruit flies has demonstrated that the mtDNA interact with the nuclear genome of the organism to affect phenotypic outcomes that can have long-term consequences on components of health and fitness, such as life span (85,86). Building upon this work will be revealing to understand how mito-nuclear interactions contribute to inter- and transgenerational effects of obesogenic diets.

Perspectives and Future Directions

Transgenerational effects of obesity may cap healthy aging improvements and life expectancy outcomes into the future, but to what extent and by what mechanisms is currently unknown. By studying model organisms, we have learned that both the quantity and quality of nutrients consumed by either parent can contribute to varying offspring phenotypes via epigenetic mechanisms that modify offspring nutrient-sensing pathways (Figure 2). We note also a connection between the metabolic pathways affected and mitochondrial metabolism as well as a possible connection between affected pathways and interactions between the mitochondrial and nuclear genomes, the importance of which is well worth further exploration.

We also note that future studies can benefit from employing a fully-factorial manipulation of both the parental prereproduction diets and those used to challenge the offspring, so that the specific and potentially synergistic effects of obesogenic diets can be identified (59). To date, only one study has attempted this in *Drosophila* (53) but it manipulated maternal diet only. By mating the parental generation in a fully-factorial design and providing offspring with diet challenges that are either matched or mismatched to the diet their parents received, we can parse several effects. First, we can determine the relative contributions of maternal and paternal diet on offspring health. Second, we can evaluate whether offspring health and fitness improves when the diet is matched to their mother's or their father's diet (when compared to mismatched), which elucidates any potential parental anticipatory effects. Conversely, if offspring from parents fed obesogenic diets display poorer health and fitness when challenged with an obesogenic diet, this may indicate obesogenic diets have a compounding effect across generations. It is also imperative for future studies to define both the energy content as well as the macronutrient balance administered in inter- and transgenerational obesity studies, since parental diet quality has the power to impact transgenerational phenotypes, even when diets are isocaloric (32). Using a structured framework, such as the geometric framework of nutrition (9) may reveal obesogenic phenotypes that afford differing inter- and transgenerational risks to health and longevity.

Finally, examining the interactive effects of genotype and diet on transgenerational obesity by using genetic models, such as those used in (53), may be helpful to elucidate mechanisms. Palu et al. (2017) (73) studied metabolic phenotypes in the wild-type grandoffspring of a fly with obesity that is caused by loss of the glucagon receptor orthologue adipokinetic hormone receptor (AKHR). These flies have a defect in fat catabolism. The data show that wild-type grand-offspring had an altered fat phenotype only when they descended from AKHR null grandfathers and heterozygote mothers. Thus, different metabolic causes of obesity may have different modes of transmission. By combining carefully designed diet studies with genetic models of obesity, it will be possible to generate greater resolution for mechanistic studies so we may better understand these complex phenotypes in humans.

Funding

This work was supported by the Australian Research Council (FT160100022 and DP170100165) to D.K.D. and (FT150100237) to M.D.W.P. and by the National Health and Medical Research Council (APP1117976) to R.R.

Conflicts of Interest

None reported.

Acknowledgments

All authors contributed to writing and editing the manuscript.

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