REVIEW

Genetic and Genomic Advances in Developmental Models: Applications for Nutrition Research

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ABSTRACT

There is increasing appreciation that dietary components influence and interact with genes important to metabolism. How such influences impact developmental regulation and programming or risks of chronic diseases remains unclear. Nutrition is recognized to affect development and chronic diseases, but our understanding about how genes essential to nutrient metabolism regulate development and impact risks of these diseases remains unclear. Historically, mammalian models, especially rodents such as rats and mice, have been the primary models used for nutrition and developmental nutrition science, although their complexity and relatively slow rate of development often compromise rapid progress in resolving fundamental, genetic-related questions. Accordingly, the objective of this review is to highlight the opportunities for developmental models in the context of uncovering the function of gene products that are relevant to human nutrition and provide the scientific bases for these opportunities. We present recent studies in zebrafish related to obesity as applications of developmental models in nutritional science. Although the control of external factors and dependent variables, such as nutrition, can be a challenge, suggestions for standardizations related to diet are made to improve consistency in findings between laboratories. The review also highlights the need for standardized diets across different developmental models, which could improve consistency in findings across laboratories. Alternative and developmental animal models have advantages and largely untapped potential for the advancement of nutrigenomics and nutritionally relevant research areas. *Adv Nutr* 2020;11:971–978.

Keywords: comparative genomics, diet, genetics, obesity, mutation, polymorphism, nutrigenomics

Introduction

This review describes recent advances in the genetic manipulations of organisms suitable for developmental studies, with an emphasis on zebrafish and obesity to represent examples related to nutrition. The identification of genes and associated gene variants that contribute toward nutrition-related risks of disease is a critical component for the implementation of nutrigenomics and personalized nutrition.

Although there are many experimental models, each has its benefits and drawbacks that influence the selection of the particular model (**Table 1**). However, with recent studies showing the role of genetics in nutrient metabolism, it is possible that the potential utility of some model organisms could be underappreciated. Sequencing databases are now available for many model organisms (**Table 2**), including *Caenorhabditis elegans* (worms, Wormbase) (1), *Drosophila melanogaster* (fruit flies, Flybase) (2), *Danio rerio* (zebrafish, ZFIN) (3), and *Xenopus laevis* and *X. tropicalis* (frogs, Xenbase) (4). These sequences are particularly valuable for reverse genetic approaches and transcriptome analyses such as RNA sequencing. Whole genome or exome sequencing and other DNA sequencing or RNA sequencing technologies are valuable emerging tools in both developmental biology and genomics (5–7).

Obesity risk is recognized to be polygenic, with >100 different genetic loci linked to adiposity (8, 9), and genetics has a large role in the risk of childhood obesity (10). Because changes in adiposity can be visualized early in development, the manipulation of obesity-associated genes can be analyzed through externally observed phenotypes. Accordingly, nonmammalian models such as zebrafish have frequently been used to study obesity from a variety of perspectives (**Table 3**), particularly how genetics can impact obesity.

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TABLE 1 Comparison of the physiological, anatomical, housing, and dietary considerations relevant to these experimental animal models[1]

	Caenorhabditis elegans	*Drosophila melanogaster*	Zebrafish (*Danio rerio*)	*Xenopus*	Mouse	Human
Organism	Nematode	Fruit fly	Bony fish	Frog	Rodent	Primate
Adipose	No	Fat body	Yes	Yes	Yes	Yes
Liver	No	Fat body	Yes	Yes	Yes	Yes
Pancreas	No	Insulin-producing cells	Yes	Yes	Yes	Yes
Placenta	No	No	No	No	Yes	Yes
Mammary gland	No	No	No	No	Yes	Yes
Housing type	Dishes (group)	Vials (group)	Aquatic tank (group)	Aquatic tank (group)	Monitored dry cages (1–4 mice per cage)	Variable, monitored
Housing costs (11)	Very low	Very low	Moderate	Moderate	Very high	Very high
Generation interval (11)	4 d	1 wk	Months	Months	Months	Decades
Brood size	Hundreds	Hundreds	Hundreds	Hundreds	Dozen or less	One
Diet type for manipulations (defined or semidefined)	Growth media	Moist powder	Pellet/flake (added to water)	Pellet (added to water)	Dry powder or pellet	Variable

[1] Some of these comparisons, particularly housing costs and generation time, have been reviewed previously by Dow (11).

Differences Between Developmental Models and Human Systems

There are differences between some developmental models and human systems that need to be accounted for when translating results across species. For example, whereas models such as *Xenopus* are excellent models for neural tube closure because of their high conservation with human orthologous genes in this developmental process (12), *Xenopus* (and other nonmammalian) models would obviously be a poor model for placental or mammary gland function. It is possible that gene function differs across species due to evolutionary trends. For example, an ancestral gene with dual functions might be duplicated in other species, leading to subfunctionalization in which gene functions become split between 2 paralogs (13). Different members of the transient receptor potential melastatin type (*TRPM*) channel gene family have varied selectivity for individual ions in humans and other mammals (14), whereas the single *Trpm* gene in *Drosophila* is important for both zinc (15) and magnesium (16) homeostasis during larval development.

Combinations of these genomic and developmental approaches are likely the next phase of gene discovery and characterization. For example, transcriptome analyses combined with loss-of-function approaches have been effective for uncovering novel zinc transporter genes in *Drosophila* (24). Genome-wide screens in developmental models such as *Drosophila* (25), ongoing knockout screens in zebrafish (26), along with knockout mice characterization (27) will aid this effort to identify nutrient metabolism genes required for development and health. The use of these alternative animal models to study the role of nutrition can exploit the advantages of these systems, particularly in relation to how nutrient metabolism genes function and interact with environmental factors such as nutrient intake.

Lack of Standardized Diets for Developmental Models

Nutrient manipulations in many developmental models can be complicated owing to the lack of standardization of the "diets" used for nonmammalian models. This is in contrast to the well-defined diets that are often used for research with the mouse or rat (28). As a result, for many investigators the use of some nonmammalian developmental models for nutrition research is unappealing at first glance. The lack of diet standardization is a limitation that should be addressed to reduce variability in studies in which developmental models are used in nutrition research. Standardized diets, like American Institute of Nutrition rodent diets (AIN-93), specify micronutrient and macronutrient components for diet composition (28). In contrast, there are no current

TABLE 2 Comparison of the estimated number of genes, genome sizes, and the release dates of the genome sequences of selected developmental model organisms, mice, and humans

	Caenorhabditis elegans	*Drosophila melanogaster*	Zebrafish (*Danio rerio*)[1]	*Xenopus tropicalis*	Mouse	Human
Organism	Nematode	Fruit fly	Bony fish	Frog	Rodent	Primate
Number of genes	20,000	15,000	26,000	20,000	22,000	23,000
Genome size,[2] Mbp	97	140	1400	1700	2500	2900
Year of release	1998 (17)	2000 (18)	2013 (19)	2010 (20)	2002 (21)	2001 (22, 23)

[1] Although the release date of the zebrafish genome is later than many other developmental model organisms, it is considered reference quality (like the human and mouse genomes) (19).
[2] Genome size is indicated per million base pairs (Mbp).

TABLE 3 Summary of selected research in zebrafish and obesity[1]

Topic	Summary of research
Prader–Willi syndrome and associated genes	Zebrafish or other developmental organisms could be attractive systems for studying feeding and obesity related to Prader–Willi syndrome (29). Orthologs for many genes in this region are present in zebrafish (29). In addition, mutations of the *lepr* (30), *mc4r* (31), and *pou3f2* (32) genes have been performed in zebrafish. Although these genes are outside the Prader–Willi locus, they affect pathways implicated in Prader–Willi syndrome (29)
Bardet–Biedl syndrome and associated genes	There are ≥19 separate genes that can lead to Bardet–Biedl syndrome when mutated individually (33), and almost all of these genes are associated with obesity. Zebrafish have been used to confirm the role of candidate genes for Bardet–Biedl syndrome and elucidate the mechanisms by which Bardet–Biedl syndrome proteins affect ciliary function (34–37). However, the impact of Bardet–Biedl syndrome proteins on mechanisms associated with obesity has not been explored in zebrafish or other developmental models
Fat mass and obesity-associated gene (*FTO*) and GWAS fine-mapping	Fine-mapping of causal genes and variants from GWAS studies can benefit from the use of alternative organisms (38). Studies in zebrafish demonstrated that the *FTO* locus contains long-range enhancers that regulate a different neighboring gene, Iroquois-class homeodomain protein 3 (*IRX3*). Noncoding regulatory elements within this haplotype block of the *FTO* gene can activate pancreatic reporter expression in mice and zebrafish. Conserved enhancer elements found within the *FTO* intron physically interact with promoter regions for both *IRX3* and *IRX5* in humans and zebrafish (39, 40)
Melanocortin-4 receptor (*MC4R*)	Zebrafish is a relevant model for *MC4R*-associated obesity. In zebrafish, strain Sa122 lacks a functional *MC4R* gene (31) and has disrupted food intake resembling the hyperphagia found in dominantly inherited obesity in humans with frameshift mutations in *MC4R* (41, 42)
Melanocortin-2 receptor accessory protein (*MRAP2*)	The conserved role of *mrap2* in growth and development in zebrafish provides new insights into the effects of the *MRAP2* gene in human obesity. Heterozygous mutations in *MRAP2* are found in severe, early-onset obesity (43). In zebrafish, paralogs *mrap2a* and *mrap2b* influence growth by affecting the activity of MC4R and its binding ligand α-melanocyte-stimulating hormone (44)
Semaphorins (SEMAs)	Rare variants in semaphorin 3 genes (*SEMA3A–G*) and their receptors (*PLXNA1–4*, *NRP1–2*) have been detected in individuals with severe obesity (45). A mutagenesis screen using CRISPR-mediated genome editing across 21 semaphorin-associated homologs in zebrafish alters adiposity. Of the 21 homologs, 5 mutants have increased adiposity whereas 6 mutants have decreased adiposity. A GWAS in West Africans has linked variants in *SEMA4D* to BMI (46). Studies in zebrafish have studied *sema4d* and its receptors *plxnb2a* and *plxnb2b* but remain unexplored in the context of obesity
Diet-induced obesity in zebrafish	Obesity can be induced in zebrafish by increased frequency of feeding and higher dietary fat content (47). Diets can be administered to zebrafish within 5 d postfertilization (48). Zebrafish contain many of the tissues that are implicated in metabolic syndrome in humans, including adipose, liver, and skeletal muscle (48). Comparative transcriptomic analyses of adipose tissue in diet-induced obesity show similar profiles in zebrafish, rats, mice, and humans (49). Diet-induced obesity in zebrafish in combination with microarray and 2-dimensional gel electrophoresis has uncovered genes that affect hepatic steatosis (49)
Screening of obesity treatment compounds	Pharmacological and dietary treatments can be tested in zebrafish. Chemical activation of NF-κB–inducing kinase disrupts glucose metabolism in zebrafish in a similar fashion to mice (50). The small size of zebrafish larvae allows growth in 96-well plates, which can be used for high-throughput testing of compounds related to energy metabolism (51). Peroxisome proliferator–activated receptor and sirtuin 1 activators, β-adrenergic agonists, and nicotinic acid in zebrafish result in changes in fat and cholesterol concentrations and gene expression similar to those in rodents and humans (51). Dietary compounds can be tested in zebrafish for their ability to prevent diet-induced obesity. Adult zebrafish fed green tea extract added to the high-fat diet had reduced body weight gain and adiposity compared with fish fed only the high-fat diet (52)
High-fat diet and gut microbiome	Gut microbiome, intestinal function, and gnotobiological approaches, such as germ-free housing, can be applied to developmental models like zebrafish in order to study well-defined host–microbial interactions (53). High-fat diets fed to zebrafish can alter gut microbiomes (54–57), including 16S ribosomal RNA microbiome profiles (54, 56), and induce intestinal inflammation (54). High-fat diet resulted in shifts in *Bacteroidetes* populations that correlated with the expression of immunity-related genes for TNF (*tnf*) and IL-1β (*il1b*) and goblet cell count. Furthermore, loss of mucosal architecture, increased mucin production, and greater goblet cell populations were detected in zebrafish fed the high-fat diet, which are consistent with intestinal inflammation (54)
Probiotics and gut microbiome	In zebrafish, the probiotic *Lactobacillus rhamnosus* can offset some effects of diet-induced obesity (57). In zebrafish a high-fat diet results in greater weight gain compared with a low-fat diet. Probiotic treatment results in reduced weight in high- and medium-fat groups compared with corresponding fat groups without *Lactobacillus* supplementation. Both probiotic treatment and dietary fat content result in altered expression of genes related to appetite and lipid metabolism. Although differences in gut microbiota are to be expected between different organisms and even within the same species (58), developmental models can be useful for studying the impact of probiotics on the gut microbiome
Methods for studying obesity and gut function in zebrafish	Feeding intake and patterns can be analyzed in zebrafish (59). Zebrafish-specific benefits, such as tissue transparency, can be combined with rapid, inexpensive, fluorescent lipid staining such as Nile red, lipophilic boron-dipyrromethene dyes, or nitro blue tetrazolium–cholesterol to provide fast in vivo adipose imaging (60, 61). Goblet cells and mucin can be detected by Alcian blue staining (54). Methods for quantifying body composition and bioenergetics, such as quantitative magnetic resonance (62) and Seahorse extracellular flux analyzer (Agilent) (63), are being adapted for zebrafish and are useful for studying normal growth and activity and models of impaired energy metabolism or obesity. BMI, as adjusted to g/cm^2 for zebrafish (64), can be used for body size measurements

[1] CRISPR, clustered regularly interspaced short palindromic repeats; GWAS, genome-wide association studies; *lepr*, leptin receptor; *NRP*, Neuropilin; *PLXN*, Plexin; *pou3f2*, POU class 3 homeobox 2.

guidelines for standardized diet preparations in many other organisms.

The findings from programs to identify the dietary requirements of key model organisms would address many issues that concern researchers in nutrition and from outside disciplines. Researchers have manipulated micronutrient diet composition to study *C. elegans* (65), *Drosophila* (15), *Xenopus* (66), and zebrafish (67). Chemical chelation of minerals, such as iron, zinc, and copper, has been used in zebrafish, *C. elegans*, and *Xenopus* as alternative models of micronutrient deficiencies (68–70). Defined diets have been created by researchers to study the effects of dietary zinc deficiency or vitamin E deficiency on zebrafish embryonic development (71, 72). Low-boron diets developed initially for rats (66, 73) have been adapted to study *Xenopus* egg development and gastrulation. Relations between growth and size during development and the zebrafish proteome have been reported and are applicable for future nutrition studies (74). Despite the recognition of the potential of zebrafish in nutritional genomics research (75), the variability in commercially available diets highlights a need for standardizing the zebrafish diet (76). Similar concerns have also been raised by Lüersen et al. (77) in the use of dietary-induced obesity and diabetes in *Drosophila*.

Recent dietary and metabolic studies relevant to nutrition using zebrafish attempt to fill such voids. For example, specific dietary protein sources for zebrafish can affect body size and composition during growth. Fish fed wheat gluten had shorter length, lower body weight, and higher body fat compared with other diets (78). In contrast, casein-fed fish had shorter length and lower body weight, like gluten-fed fish, but had lower body fat. Fish protein hydrolysate in the diet results in higher body fat and lower lean mass in zebrafish. This information, such as protein source origins, is important when formulating or comparing the effects of semidefined diets in fish. Another study investigating the effects of a range of dietary crude protein and lipid content on the growth and behavior of zebrafish found that a diet consisting of 32% crude protein and 8% crude lipid, fed at 5% of the fish body mass, was sufficient to support growth and normal fish behavior (79). Researchers have also conducted initial studies to determine the optimal diet composition necessary to support maximal growth of zebrafish larvae and provide accompanying complete body composition data resulting from the feed, including measurements of essential amino acids, various fatty acids, minerals, and trace elements (80). Other studies have analyzed the proteomic changes that occur during posthatching zebrafish development and determined that the period between 20 and 40 d postfertilization is the optimal timeframe for zebrafish fed *Artemia nauplii* because the ingested energy is used primarily for nonreproductive growth during this period, based upon changes in protein profiles (74). Similar studies might also be helpful for other types of diet or feed. Development of diets that promote growth and development in laboratory settings, followed by the standardization of diets, could reduce variable results and simplify data interpretations in alternative organisms such as zebrafish.

Zebrafish researchers have noted that variable nutrition practices can lead to variability or impaired reproducibility of studies that can hinder the adoption of alternative model organisms by nutrition scientists (81). Watts et al. (81) have noted that strict guidelines and references adopted for diet formulations in rodent models have improved consistency in research studies by different scientists. Watts et al. (81) have proposed a number of factors, such as temperature, photoperiod, oxygenation, water flow, and life stage (larvae or adult), that should be considered by zebrafish researchers, particularly if nutrition is a critical component. In particular, the daily nutritional requirements of zebrafish need to be established so that standardized reference diets can be set. Many of these factors also apply to other model organisms, and as zebrafish diets are set, perhaps these studies can provide a path for researchers using other developmental and alternative model organisms to follow.

As an example of how varied commercial diets can affect growth, body fat deposition, and reproduction, Fowler et al. (82) fed zebrafish 1 of 5 commercial diets commonly used by researchers or a formulated reference diet for 16 wk starting from 21 d postfertilization. Differences in weight and length were observed across all diets. Mean lipid content, calculated as total lipid weight as a percentage of dry body weight, also differed in fish fed different diets. The reference diet closely matched the *Artemia* diet in spawning success, which the authors attributed to the high dietary fatty acid composition of *Artemia* brine shrimp. Because the exact composition of dietary components of these commercial aquatic diets is unknown, it is difficult to confidently compare results between studies when diet is a major and unaccounted environmental factor. Crude protein was relatively consistent across all diets, ranging from 48% to 61%. However, crude fiber in diets varied widely from 0.4% to 5%, and total carbohydrate differed from $<$2% to $>$23%. The authors also noted that commercial aquaculture diets can prioritize fast growth in the early zebrafish lifespan (83), and it is unclear whether growth driven by overnutrition occurs at the expense of long-term health (83). Two commercial diets resulted in greater body lipid content compared with the control reference diet, which is notable because commercial diets have been used by researchers as the basal control diet upon which high-fat or overnutrition treatments are based. The formulated diet fed in this study, with defined macronutrient, vitamin, and mineral compositions, appeared to meet zebrafish growth requirements and would provide significant dietary consistency and transparency.

There are different feeding models of obesity for zebrafish, and a recent study has shown that different dietary models of obesity can lead to distinct phenotypes (64). Some of these methods result in phenotypes that better resemble obesity and adipose dysfunction in humans. Landgraf et al. (64) compared a control diet with 5 mg *Artemia* (22% fat, 44% protein,

16% carbohydrate) per fish per day with 2 experimental diets, 1 group fed 60 mg *Artemia* per fish per day (normal-fat, high-caloric group) and another group fed 5 mg *Artemia* and 30 mg egg yolk powder (59% fat, 32% protein, 2% carbohydrate), resulting in higher fat, isocaloric content compared with the normal-fat group. Both experimental diets produced zebrafish with heavier, longer fish with a higher BMI (g/cm^2) compared with control diet–fed fish. Both overfed groups showed signs of adipocyte hypertrophy in both visceral and subcutaneous adipocytes compared with the control group. The normal-fat, high-caloric group had larger adipocytes in the subcutaneous adipose than the high-fat group, whereas the high-fat dietary group had larger adipocytes in the visceral adipose compared with the normal-fat group. Blood triglycerides and cholesterol were high in the high-fat group compared with both normal-fat and control groups. Fish in the high-fat–diet group also showed ectopic accumulation of lipids in both liver and muscle compared with the normal-fat and control groups, as shown by Oil Red O staining, MRI, and hematoxylin-eosin staining. Livers of high-fat–diet fish also had increased *col1a1a* (collagen 1a) mRNA abundance, which is consistent with increased liver fibrosis (84). In summary, attention to dietary approaches in experimental animals, such as the high-fat diet used by this study, can result in metabolic phenotypes that are better models for human obesity. Greater consistency in the composition and use of obesity-inducing diets could improve the interpretation of studies conducted across different laboratories.

There are also essential considerations for environmental reporting given that whereas mammals are endothermic, many alternative animal models are ectothermic and their whole-body heat regulation is dependent on both metabolism and the external environment. As a consequence, the use of ectothermic animal models allows treating temperature as a variable in experiments designed to elucidate physiological mechanisms that are linked to temperature regulation and contribute toward conditions such as obesity. Despite the immense variation in body size and physiology in ectotherms, when adjustments are made for environmental temperature (85, 86), their metabolic rates, relative heat production, and oxygen consumption follow the same quarter-power scaling laws as those for endothermic animals. Similar arguments focusing on planarian energy requirements and storage have been recently published (87).

The ability to respond to changes in the environment, however, does dictate the need for detailed reporting of methods. The rationale for stricter reporting guidelines in nutritional studies with mammals is even more important for studies that employ ectothermic animal models (88). For example, diet components outside macronutrient and micronutrient composition can impact physiological responses. Phytochemicals present in commercial rodent diets can induce hepatic phase I and II biotransformation enzymes many-fold compared with more purified diets (89). Commercial and fixed-formula diets can also differentially affect the gut and oral microbiota and obesity development (90). Until there is standardization in diet composition and environmental housing (including temperature) for non-mammalian models, data interpretation and comparisons between studies will rely on detailed diet and methodological reporting.

Conclusions

Most disorders of nutritional importance, including obesity, are multifactorial, with interactions between polygenic and environmental factors. The ability to manipulate diet in combination with multiple genes in alternative developmental models complements current capabilities in traditional organisms such as mice and rats. The identification of genes that are required for health and function in nutrient metabolism provides possible courses of action or improves study designs for disease risk. For example, genetic scoring of disease risk or treatment outcomes, such as in developmental disorders like autism (91) or metabolic conditions such as obesity (92), relies on the identification of specific, causal gene polymorphisms. In the case of mendelian randomization, these studies can leverage well-validated genetic risk scores related to nutrient metabolism to improve understanding of the impact of nutrient status on disease pathogenesis (93). The use of developmental models for continued identification of genes affecting adiposity could eventually yield a more comprehensive index of obesity risk. In addition, the study of gene function in combination with dietary treatments or environmental factors could enable targeted interventions designed to prevent childhood obesity (94). It is acknowledged by the Academy of Nutrition and Dietetics that precision nutrition holds significant promise for dietitians and the future of clinical practice (95). However, significant knowledge gaps need to be addressed before precision nutrition meets the threshold for evidence-based practice and can be implemented in a clinical setting (96). Consequently, developmental models could be invaluable tools for improving knowledge about gene–health relations to further nutrigenomics and personalized nutrition.

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