
Potential Long-Term Consequences of Fad Diets on Health, Cancer, and Longevity: Lessons Learned from Model Organism Studies

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While much of the third world starves, many in the first world are undergoing an obesity epidemic, and the related epidemics of type II diabetes, heart disease, and other diseases associated with obesity. The amount of economic *wealth* being directly related to a decline in *health* by obesity is ironic because rich countries contribute billions of dollars to improve the health of their citizens. Nevertheless, nutritional experiments in model organisms such as yeast, *C. elegans*, *Drosophila*, and mice confirm that “caloric restriction” (CR), which is defined generally as a 30-40% decrease in caloric intake, a famine-like condition for humans seen only in the poorest of countries, promotes good health and increases longevity in model organisms. Because caloric restriction, and dieting in general, requires a great deal of will power to deal with the feelings of deprivation, many fad diets, such as the Atkins, South Beach, and Protein Power, have been developed which allow people to lose weight purportedly without the severe feelings of deprivation. However, the long-term effects of such fad diets are not known and few experiments have been performed in the laboratory to investigate possible side effects and adverse consequences. In this paper, we review studies with fad-like dietary conditions in humans and model organisms, and we propose a “Dietary Ames Test” to rapidly screen fad diets, dietary supplements, and drugs for potential long-term health consequences in model organisms.

Popular diets have become increasingly prevalent and controversial. More than 1000 diet books are now available, with many popular ones departing substantially from mainstream medical advice. Cover stories for major news magazines, televised debates, and cautionary statements by prominent medical authorities have fueled public interest and concern regarding the effectiveness and safety of such diets (1).

Introduction

Obesity is a major public health problem throughout the developed world, but it is especially troubling in children of the relatively affluent. In the US, the rate of children with obesity has increased from 5% in 1960 to 15% in 2000 (2). Associated with this increase in obesity is an increase in type II diabetes mellitus in children and adolescents, which many researchers believe may be triggered by obesity (3). It is likely that this childhood obesity “epidemic” presages a significant increase in other adult obesity associated maladies, such as blindness, heart disease, renal disorders, and even cancer (4). However, despite the urgent need for more knowledge, the potential long-term effects of childhood obesity on adults are not well understood, nor is it known which of the several dietary regimens are optimal for controlling childhood and adult obesity.

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The quote leading this review is from a recent influential article from Dansinger and colleagues in which they did a comparison of the Atkins, Ornish, Weight Watchers, and Zone diets for effectiveness in terms of weight loss and heart disease reduction in adults (1). Surprisingly, this is one of the few studies where randomized analyses have been conducted, and no studies have yet been completed in children. Dansinger and colleagues did a small trial of 160 participants randomly assigned to either the Atkins (carbohydrate restriction), Zone (macronutrient balance), Weight Watchers (caloric restriction), or Ornish (fat restriction) diet groups, with 40 participants in each group (1). The conclusion of this relatively unique, albeit admittedly underpowered, study is, "Each popular diet modestly reduced body weight and several cardiac risk factors at 1 year. Overall dietary adherence rates were low, although increased adherence was associated with greater weight loss and cardiac risk factor reductions for each diet group" (1).

While this study received a great deal of press coverage, it is unfortunate that the low power of this study, because of the small number of people enrolled, the short time period for data accumulation, and the low participation rate of those enrolled, makes the conclusions tenuous at best. Unfortunately, human clinical studies, such as this, because of the enormous expense and Internal Review Board (IRB) requirements, are difficult for the average laboratory to conduct. Indeed, a more extensive study than that conducted by Dansinger and colleagues would be a major undertaking and take an enormous institutional and financial commitment.

One solution to the enormous expense and difficulties of human clinical studies on dietary interventions to combat childhood and adult obesity would be to increase the number obesity research studies in model organisms. The obesity epidemic justifies the need to study the long-term effects of different dietary regimens on morbidities associated with obesity, such as cancer, cardiovascular disease, and type II diabetes in non-human animal models. While most of the studies on obesity and nutrigenomics, the field of nutrient-gene interactions, have been done with mice and rats (e.g., (5)), in this review we argue that *Drosophila* is also a suitable model for some aspects of obesity and nutrigenomics. *Drosophila* might also be useful to determine the effects of fad diets on the long-term health of an organism (6, 7).

While *Drosophila* apparently do not suffer from obesity, nor do they have a four-chambered heart or even a closed vasculature which one might presume would be a prerequisite for cardiovascular disease research, the advantages of doing nutrigenomics research in flies are nevertheless numerous (6, 7). One advantage of using simple invertebrate models such as flies is that *Drosophila* store triglycerides primarily in their fat bodies (8-10) and, as in humans, a combination

of both diet and genetics affect the circulating and stored triglyceride levels (e.g., (11-14)). Furthermore, over 70% of human disease genes are present in *Drosophila* and many of these diseases have already been modeled in *Drosophila* ((15); www.hemophilia.edu).

Future studies on those diseases that have not yet been modeled in flies are facilitated by the fact that over half of the genes have already been knocked out in *Drosophila*, and deficiencies spanning relatively small chromosomal regions with defined endpoints, many in a well-controlled isogenetic (i.e., genetically uniform) background, uncover over 95% of the genome (15). The *Drosophila* genome is arguably the most-well annotated and accessible such that stocks containing a majority of the mutant alleles are available from public-accessible stock centers (see www.Flybase.org). These advantages, combined with the small sequenced genome, advanced genetics, and short lifespan make *Drosophila* a powerful nutrigenomics model (7). In this review, we present a short summary of dietary studies in *Drosophila* and present exciting future prospects for this organism in the obesity field.

In Drosophila, DR (Dietary Restriction) extends life-span entirely by reducing the short-term risk of death. Two days after the application of DR at any age for the first time, previously fully fed flies are no more likely to die than flies of the same age that have been subjected to long-term DR. DR of mammals may also reduce short-term risk of death, and hence DR instigated at any age could generate a full reversal of mortality (16).

Development of *Drosophila* as a Model for Nutrigenomics

Most of the research in *Drosophila* nutrigenomics is in the area of dietary restriction (DR), which is simply diluting the food with water. In *Drosophila*, as well as in other models such as yeast and *C. elegans*, DR experiments must be conducted rather than the more widely known caloric restriction (CR) experiments, which is reducing the amount of daily food intake, because it is technically very difficult if not impossible to measure the amount of food an individual fly (or yeast cell or worm) eats in one day (17). *Drosophila* DR studies have been extensively reviewed elsewhere (18-21). A common theme among all CR and DR experiments is that these conditions increase both the mean and maximal lifespan and lead to generally improved health and reduced risk of diseases such as cancer and diabetes.

The quote leading this section is from a ground-breaking study from Mair and colleagues which showed that, at least in the *Dahomey* strain of *Drosophila* used by these investigators, that *DR at any stage of life* is able to completely and immediately provide the longevity benefits of continuous DR conditions throughout life (16). In other words, these investigators see

an almost immediate shift in their mortality curves from the elevated mortality rate of control diets to the decreased mortality rate of the DR diet (Fig. 1A). The reverse is also true – they see an almost immediate shift from the decreased mortality rate of the DR diet to the elevated mortality rate of the control diet (Fig. 1B). The implications of these results, if they are applicable to humans, are enormous. The immediate shift in mortality rate by diet suggests that no matter when life-promoting diets are initiated, they will have immediate and long-term effects on the mortality rate and possibly on the other age- and diet-associated diseases discussed above.

We and a small number other laboratories are developing *Drosophila* as a more general model for nutrigenomics (6). One aspect of nutrigenomics is the effect of the type of diet on global gene expression patterns (7). We are also studying the effects of diet on triglyceride levels and longevity (7, 8). However, instead of primarily feeding flies DR diets, we vary the contents of the diets to determine the possible health effects of “extreme diets” (6). While it might be intuitively obvious that “extreme diets,” such as many fad diets, likely have long-term negative health consequences, surprisingly, few studies have investigated the long-term effects of fad diets.

To our knowledge, the first published “extreme diet” nutrigenomic studies in *Drosophila* were done in the late 1970s and early 1980s by Driver and colleagues (22-25). They found that *Drosophila* fed high fat diets, such as a diet rich in the saturated fatty acid palmitic acid (17:0 – 17 carbons and no double bonds) have a dramatically reduced lifespan. These studies are interesting and consistent with contemporary and more recent human epidemiological studies that suggest that high-fat diets contribute to obesity and associated ailments such as cancer, type II diabetes, and shortened lifespan. However, these early studies were limited because the genome was not yet sequenced and whole-genome gene expression and proteomics techniques were not yet available.

In a recently published book chapter, we published the replication of some of Driver and colleagues’ studies with more modern technology, taking full advantage of the genomic studies that have been conducted since the early days of nutrigenomics (24). As in the earlier studies, we found that flies fed diets high in palmitic acid (17:0) have a much shorter lifespan than flies fed a control diet (Fig. 2) (6). We used the same recipe as Driver and colleagues, with the addition of the mold inhibitors propionic acid and tegosept (6). As with the diets used by Driver and colleagues, our control diet contained corn meal, oat meal, agar, and sucrose under isocaloric conditions with the experimental diets (Fig. 2). Also, as with Driver and colleagues, our palmitic acid diet had all of the same ingredients as the control diet, but the sucrose was isocalorically replaced with palmitic acid (Fig.

2) (6). Sucrose is a simple sugar with two hexose rings (one glucose and one fructose), and is therefore easily metabolized to glucose, whereas palmitic acid (17:0) is a long-chain saturated fatty acid found in palm oil and other plants that requires several lipases and other enzymes to convert it to energy sources such as glucose.

In addition to confirming the results published over 25 years ago by Driver and colleagues (24), we found that flies fed the palmitic acid diet had significantly elevated triglyceride levels compared with flies fed the control diet (Fig. 2) (6). Triglyceride levels were not analyzed in the earlier studies by Driver and colleagues (24). Furthermore, using techniques that were not available 25 years ago, we also did whole-genome microarray studies to determine what gene expression changes occur when flies are fed the high palmitic acid diet and compared these changes to those induced by other diets. Most interestingly, we determined the long-term effects of palmitic acid diets fed to flies during the larval stages (6).

In addition to the high palmitic acid diet, we also analyzed diets that approximate “extreme diets” that humans have used to attempt to lose weight. For our Atkins-like diet, we replaced the sucrose in the control diet isocalorically with 95% lean ground beef (Fig. 3) (6). For an Asian-like soy diet, we replaced the sucrose in the control diet isocalorically with soy tofu (Fig. 3) (6). Interestingly, both extreme diets significantly decreased the lifespan of flies compared with the control diet (Fig. 3). We repeated these experiments under more optimal growth conditions (i.e., virgin males and females were measured separately and the flies were kept in less crowded vials rather than cages) and, again, both extreme diets decreased the mean and maximal lifespan by over 50% (D.M.R., unpublished observations).

Why did the three extreme diets reduce the lifespan of flies? The palmitic acid diet reducing longevity is not surprising because diets high in saturated fats increases obesity and cardiovascular diseases in humans. However, the high beef (Atkins-like) and high soy (Asian-like) diets have been shown to combat many age-related diseases including CVD, stroke, and cancer (26-28), and soy isoflavones have been shown to increase longevity in certain strains of mice (29). It is possible that too much carbohydrate (sucrose) was displaced in the extreme diets and this is what shortened lifespan. To address this issue, and to further validate the *Drosophila* model to understand the effects of extreme diets, a range of ratios of beef or soy-to-sucrose should be examined. This will allow investigators to determine whether beef or soy are intrinsically life shortening in *Drosophila*, or whether the absence of sucrose is itself life shortening. Nevertheless, it causes one to be aware that extreme diets in humans might have short term benefits but have long term decreases in longevity or quality of life.

In the published studies, we also analyzed global gene expression patterns under control and extreme dietary conditions. Fig. 4 shows a Venn diagram of gene expression changes that are induced by the two diets – soy and beef – compared with the control diet. We found that 480 genes in flies fed a high-soy diet have significantly altered gene

expression patterns compared with control (Fig. 4). Similarly, we found the 400 genes in flies fed a high-beef diet have significantly altered gene expression patterns compared with control (Fig. 4). However, only 46 of these genes are common between these two dietary conditions (Fig. 4). These results, if confirmed in mammalian models, suggest that as many as 2% of the total genes in an organism (~400/~18,000 genes in *Drosophila*) have altered expression levels when the diet of that organism is dramatically altered. Clearly, the potential health consequences of such a dramatic alteration in gene expression patterns deserve further investigation.

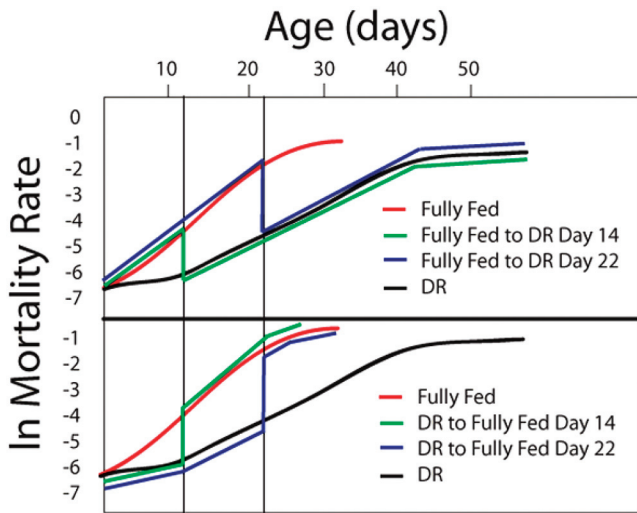


Figure 1: Dietary Restriction (DR) has immediate benefits on mortality rates in *Drosophila*. Top, DR increases the mortality rate (black line) compared with control diets (red line). A shift from control to DR on days 14 (green line) or 22 (blue line) causes an immediate reversion to the DR mortality rate. Bottom, a shift from DR to control diets on days 14 (green line) or 22 (blue line) causes an immediate reversion to the control mortality rate. Adapted from (16).

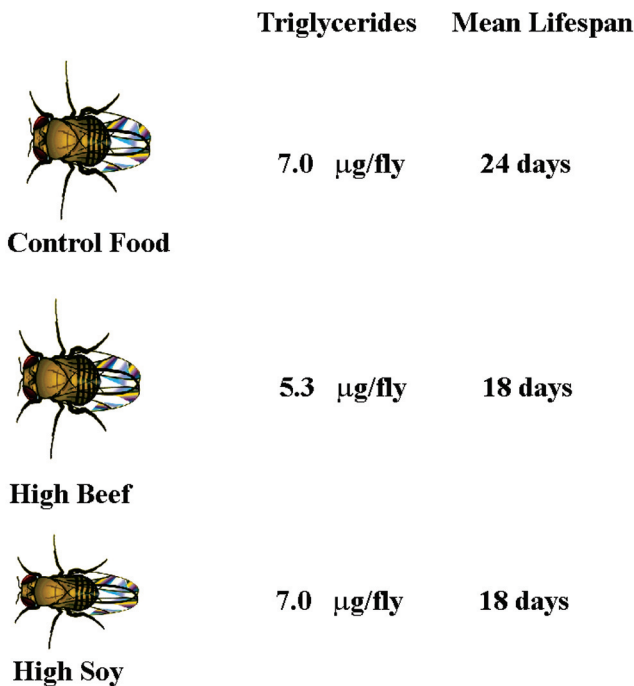


Figure 2: High-soy and high-beef diets dramatically decrease mean lifespan in *Drosophila*. $P < 0.01$ in each case compared with control. Adapted from (6).

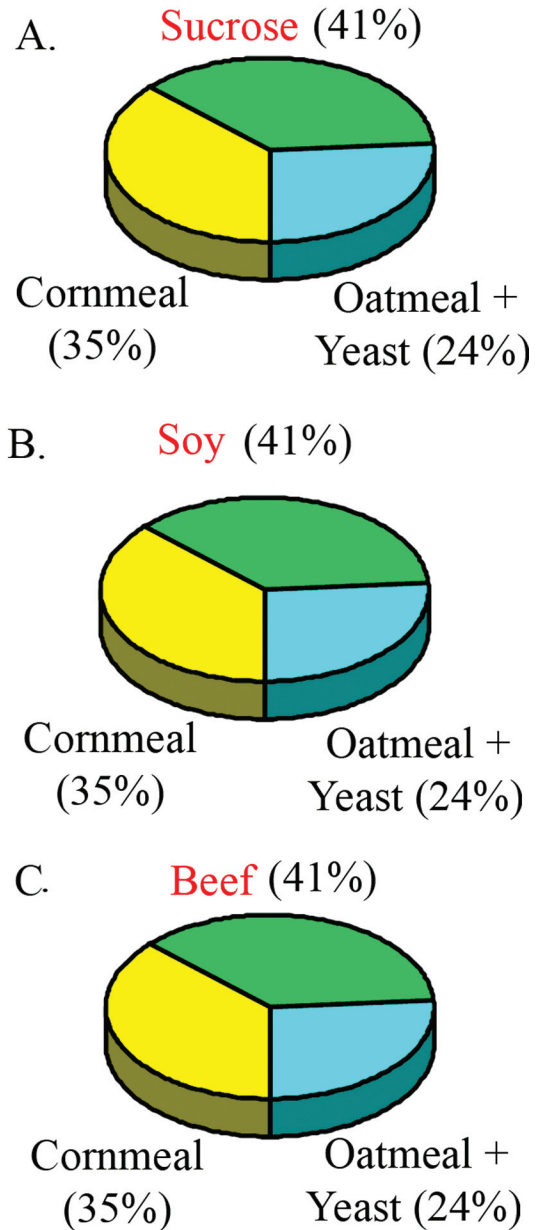


Figure 3: Composition of the “extreme diets” are isocaloric. Composition of the control (Sucrose), high soy (Soy), and high beef (Beef) diets are indicated. In each case, the amount of calories of sucrose, soy, or beef are the same.

Another exciting area of nutrigenomics research that we are pursuing in *Drosophila* is to identify “master modulatory genes” that are affected by a particular diet (Fig. 5). “Master modulatory genes” encode proteins that regulate a large number of other genes in a tissue-specific manner, and were

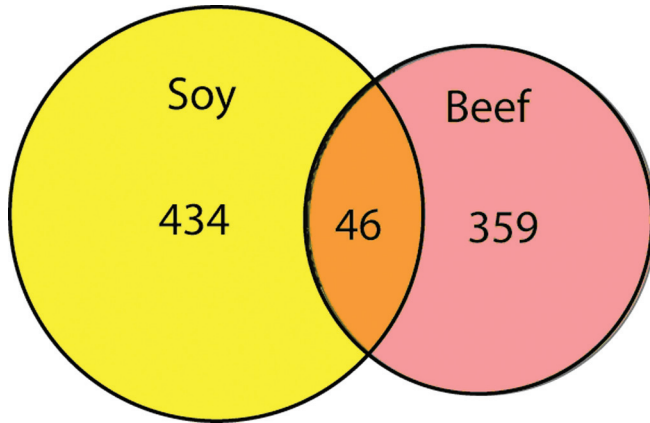


Figure 4: Gene expression analyses of *Drosophila* fed control, high soy, and high beef diets. Microarrays (whole genome spotted arrays) were performed from pooled mRNA isolated from 5-10 day old males and females fed the three diets from egg hatching and throughout larval and adult life. Significant gene expression changes (FDR < 0.05) were observed for 480 genes by the soy diet and 400 genes by the beef diet, but only 46 of the genes had altered expression by both diets. The list of genes and gene lists are published and available online (6).

first described in a series of pioneering papers that were published last year (30-33). These papers describe using a combined microarray-quantitative trait loci (QTL) analysis that utilizes the expression levels of all genes in randomized mouse genomes as individual quantitative traits (31). Usually, QTL analyses are conducted with a single trait, such as blood pressure, or a handful of related traits. However, in so called “expression QTL” (eQTL) analyses, every one of the tens of thousands of genes in an organism are used as a quantitative trait. This is a powerful and multi-dimensional approach that is at the cutting edge of genomics research.

The most remarkable, and unexpected, finding in the eQTL analyses that were performed in mice is the discovery of the above-mentioned “master modulatory loci” (Fig. 5). These loci were discovered when the authors plotted their eQTL results on a graph in which the x-axis has the location of the eQTL (+/- 5 Mbp) and the y-axis has the location of every gene in the organism in order (Fig. 5). They found that most of the eQTL were so-called “cis-eQTL” which map in “cis” (+/- 5 Mbp) to the gene that is being regulated (Fig. 5, red circles). In other words, “cis-eQTL” are likely to be polymorphisms in the genes promoter or enhancer regions that alter gene expression (31). More interesting were simple “trans-eQTL” (blue circles) and “modulatory trans-eQTL” (purple circles) which regulate several other genes that are not located near the QTL (Fig. 5). Such “trans-eQTL” were identified in yeast and plant eQTL analyses (34-43) and were not unexpected in the mouse analyses.

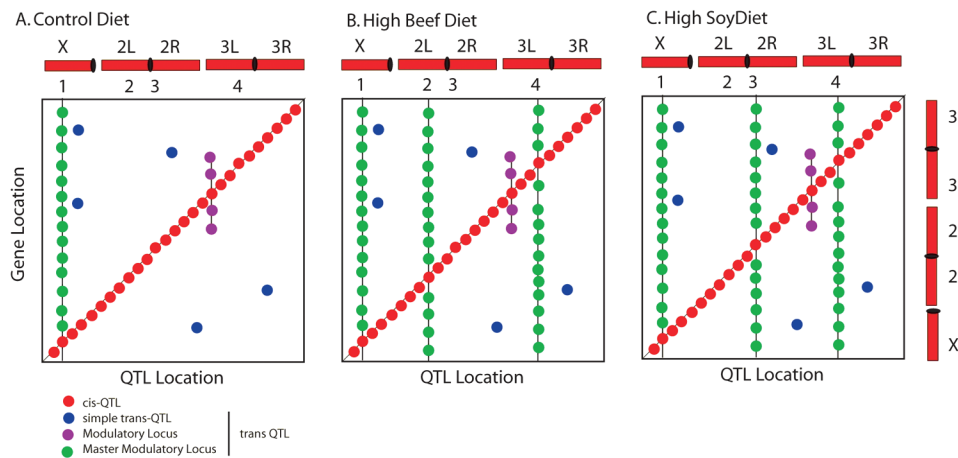


Figure 5: Trans-eQTL analyses in *Drosophila* can identify diet-specific conditional master modulatory genes. A, cis-eQTL (red circles), simple trans-eQTL (blue circles), modulatory loci trans-eQTL (purple circles), and master modulatory trans-eQTL (green circles) are indicated. *Drosophila melanogaster* has three major chromosomes (X, 2nd, and 3rd) and a small 4th “dot” chromosome (not indicated). The QTL location is indicated along the x-axis and the gene location is indicated along the y-axis. 1-4, indicate 4 types of master modulatory trans-eQTL: 1) loci that have effects on the expression of hundreds of target genes in a diet-independent manner; 2) loci that affect target genes in a beef-specific manner; 3) loci that affect target genes in a soy-specific manner; and 4) loci that affect target genes in beef or soy diets, but not in control diets. Such soy- and beef-specific master modulatory trans-eQTL are speculative, but they have been observed for a dietary carcinogen by the authors (D.M.R. and colleagues, unpublished).

In contrast to yeast and plants, however, when the eQTL analyses were performed in isolated tissues or specific cell types from mice, “master modulatory loci” were identified. “Master-modulatory loci” are trans-eQTL that regulate thousands of genes in a tissue specific manner (34-43). These

investigators also found that different tissues or cell types had different sets of “master modulatory loci.” However, because of the poor resolution of the trans-QTL identified in these mouse studies, it will likely be several years before any “master modulatory genes” are identified.

Recently, we performed eQTL analyses in *Drosophila* to determine whether they also have “master modulatory genes” (D.M.R. and colleagues, unpublished). We found that *Drosophila* seem to have an intermediate condition between mice and plants in which they have “master modulatory genes” that regulate hundreds of genes, as opposed to mice

whose “master modulatory genes” regulate thousands of genes and plants whose “*trans*-eQTL” regulate dozens of genes. Surprisingly, in our *Drosophila* studies, we found that there are diet-specific “master modulatory genes” that appear only when that component is added to the diet, and others that disappear under these conditions. In Fig. 5, we schematize and simplify what the results might look like for “master modulatory genes” for high-soy or beef extreme diets.

The power of *Drosophila* genes greatly simplifies the task of identifying *Drosophila* “master modulatory genes” compared with mammalian systems. Once the “master modulatory genes” are identified in *Drosophila*, one could then determine their evolutionary conservation in mammals by making knock-out (KO) mouse models. The size of the mouse genome, and the relative imprecision of localizing QTLs in this model, makes it prohibitively difficult to identify the master modulatory genes in mice. However, as mentioned earlier, the *Drosophila* genome is ~10-fold smaller than the mouse genome and fine mapping of QTLs can be performed with small deficiency chromosomes.

How might one determine the significance of a master modulatory gene in regulating obesity? Master modulatory genes are “hub genes” that regulate hundreds or thousands of genes in a particular tissue. Therefore, one would predict that a mouse KO for a master modulatory gene that is down-regulated by a particular diet or nutrient would show a similar, or at least partly overlapping, phenotype as if it were on that diet. Similarly, if a master modulatory gene is up-regulated by a particular diet or nutrient, then a mouse over expressing this hub gene would show a similar phenotype as if it were fed the nutrient. Once the hub genes are identified, drugs can be designed that specifically target particular “master modulatory genes” that respond to health- and longevity-promoting dietary conditions. It might take several drugs to target multiple hub genes to show the full effect of the diet, but this should still be feasible. Ultimately, these drugs might allow people to enjoy the benefits of healthful diets without having to endure the misery of subjugating to the diet.

The epidemic of obesity occurs on a genetic background that has not changed significantly in the past 100 years and certainly not since the epidemic began 20 years ago (4).

Discussion

In this short review, we discuss recent nutrigenomics and longevity experiments in *Drosophila* and propose cutting-edge experiments that can be conducted in the near future to identify the healthful and harmful aspects of various diets. Ultimately, *Drosophila* nutrigenomics promises to be a powerful weapon in the arsenal to combat some of the deleterious health effects of the obesity epidemic in modern societies.

There is a continuous flow of new fad diet books being written every year. For example, in the first half of 2006 alone, among the top 50 health and fitness books on Amazon.com, one finds the following 8 books: 1) “The Fat Smash Diet : The Last Diet You’ll Ever Need” (44), 2) “The Shangri-La Diet” (45), 3) “Ultrametabolism : The Simple Plan for Automatic Weight Loss” (46), 4) “The South Beach Diet Quick and Easy Cookbook : 200 Delicious Recipes Ready in 30 Minutes or Less” (47), 5) “The Whitaker Wellness Weight Loss Program” (48), 6) “The Reality Diet : Lose the Pounds for Good with a Cardiologist’s Proven Plan” (49), 7) “The Sonoma Diet Cookbook” (50), and 8) “The Rice Diet Solution : The World-Famous Low-Sodium, Good-Carb, Detox Diet for Quick and Lasting Weight Loss” (51). How many of these are potentially healthy or dangerous can only currently be determined by unsupervised human experimentation. Clearly, such uncontrolled human experimentation is not an optimal means of identifying means for improving human health.

What we propose is that fad diets and supplements should be placed under similar regulatory guidelines as drugs. Currently, most fad diets are generally written and promoted by well-meaning but potentially dangerous amateurs with no expertise on the effects of diet on long-term human health. Perhaps what is needed is a validation in model organisms that the fad diet in question is effective in weight loss and has no long-term deleterious effects on health. Only once the effectiveness and safety of an “extreme diet” is determined, first in *Drosophila* and then in mice, should “extreme diets” be performed in humans.

The “Ames test” was developed in the 1940’s by Dr. Bruce Ames to determine the carcinogenicity of compounds (e.g., (52)). When we queried the phrase “Ames test” on MedLine, (May 11, 2006), there were 1848 papers with these key words. Almost all compounds that are in the market in the USA have to go through strict carcinogenicity and toxicity studies before they are approved for human consumption. What we propose is a “Dietary Ames Test” in which fad diets, dietary supplements, and drugs are fed to *Drosophila* and other model organisms to determine their long-term effects on toxicity, longevity, and global gene-expression changes. Perhaps demonstrating long-term safety and efficacy in *Drosophila* and mice should be a necessary prerequisite before one undertakes a dietary or pharmaceutical intervention.

Two important considerations one should make when considering regulating fad diets are whether the diet actually decreases obesity, and if it does, whether the risks of the diet outweigh the risks of obesity. Childhood and adult obesity are huge problems in the developed world and excess adiposity may be a primary culprit in the development of heart disease, diabetes, and cancer. Additionally, there is

growing evidence that excess adiposity per se may shorten lifespan (53-56). In other words, since obesity is itself such a public health problem, would following an extreme diet be such a poor strategy for human health if ultimately caloric intake was reduced and weight loss was achieved? This is a valid question, but when safer alternatives are available, be it a balanced diet or a drug with fewer contraindications, then the less safe alternative should be discouraged by regulations or warnings. For example, become some COX-2 inhibitors, such as Vioxx, have been recently shown to increase risks of heart attacks and other cardiovascular diseases, they have been pulled off the market (57). Why would one want to continue using Vioxx when safer COX-2 inhibitors are available? Similarly, why would one want to continue with an extreme diet when safer diets are available?

The American public needs to be told (and believe) that diets are not followed for 8 days, 8 weeks, or 8 months, but rather form the basis of everyday food choices throughout their life. A diet high in vegetables, fruits, complex CHO's (whole grains and legumes), and low-fat dairy is a moderate-fat, low-calorie diet that prevents weight gain, results in weight loss and weight maintenance. It is associated with fullness and satiety. It reduces risk of chronic disease. It is fast, convenient, and inexpensive. How can we convince people it works, and to try it? (58).

Conclusions

Freedman and colleagues make the point in the above quote that the best way to prolong life is to eat a well-balanced and moderate diet (58). In addition to the review by Freedman and colleagues, the effects of popular diets on health have been reviewed by numerous other investigators (1, 58-68).

Using *Drosophila* nutrigenomics as a "Dietary Ames Test" promises to identify potentially life-shortening or life-enhancing fad diets, supplements, or drugs before they get widespread use. One can then use further multi-dimensional nutrigenomics techniques to identify genes that are affected, either positively or negatively, by the diet or drug. Further confirmation that these genes are conserved in mammals can be done in mouse knockout models. Such sophisticated approaches might be what are needed to successfully combat the obesity epidemic.

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