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Improving Laboratory Mice Diets to Increase Relevance to Human Populations

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ABSTRACT:

Mouse models have been an invaluable resource for biomedical research and have been instrumental for advances in our understanding of biology and human disease. However, in using these models there is the inherent issue of the translatability of research findings to human populations. Some differences between humans and rodents can never be reconciled due to key differences in physiology. However, mice models have evolved over time through innovations in genetics and standardized diets, resulting in reduced variability across experiments. Developing mice diets that more closely emulate what humans eat will help increase the translational fidelity of mouse models to human populations. This review will focus on the role of basal laboratory diets for improving mouse models.

Keywords: laboratory mouse diets, total Western Diet, allometric scaling, nutrient density scaling

INTRODUCTION

One of the greatest challenges of biomedical research is increasing the translatability of research generated from model organisms to humans. Advances in biotechnology continue to improve model organisms by replicating the specific genetic components related to the etiology of human diseases. For example, transgenic mice that carry the human version of genes involved in disease are commonplace. Recently, the use of CRISPR technology allows researchers to model disease-specific DNA alterations common for different cancers[1]. Despite these advances, the application of appropriate diets for increasing translational fidelity to humans has not changed appreciably in the same time frame.

Laboratory Mice Diets, a Historical Perspective

The first organized effort to standardize laboratory diets began in the early 1970's at the National Institutes of Health (NIH). Dr. Joseph Knapka developed the NIH-7 diet, the first open source, standardized rodent diet with the aim of decreasing variability across experiments[2]. This diet contained nine different commodity ingredients. Although open source diets eliminate a considerable amount of variation across experiments, standardized diets formulated with complex ingredients can still introduce variation. Complex, plant based ingredients from different geographic regions or harvested in different seasons can have differing amounts of micronutrients[3] or plant secondary compounds[4]. Around this same time, the Ad Hoc Committee on Standards for Nutritional Studies was formed by the Council of the American Institute of Nutrition (AIN) with the goal of developing a standardized diet[5]. The goal of the committee was to design a diet formulated with purified ingredients to decrease experimental variation. Furthermore, this new diet was to be formulated using concentrations of micronutrients at or near recommendations set by the National Research Council. The result of the committee's work was the AIN-76 diet, a purified diet comprised of sucrose, cornstarch, casein, corn oil, cellulose, and a vitamin/mineral supplement [6].

In 1989, an ad-hoc committee convened at the Experimental Biology meeting and discussed whether changes should be made to the AIN-76A diet[7]. These discussions led to series of investigations performed by Dr. Phil Reeves at the USDA Grand Forks Human Nutrition Research Center to improve the AIN-76A diet[8, 9]. Notable changes to the formulation included changing the fat source from corn oil to soybean oil to increase dietary linolenic acid content, inclusion of supplementary L-cystine instead of DL-methionine, and increased amounts of vitamins E, K, B_{12}, in addition to the inclusion of ultra-trace elements. The resulting formulations were the AIN-93G and AIN-93M diets. The AIN-93G diet was designed to provide sufficient energy and protein for growth, while the less energy dense AIN-93M diet was created to provide maintenance energy and protein needs for long-term studies. The AIN-93 formulations are now the standard basal diet for nutrition research.

The creation of these standardized diets from common, purified ingredients was an important advance for research with mouse models. Investigators with limited experience in laboratory animal nutrition could now be confident that their basal diet was not introducing undue variation between their experiments. More importantly, a significant source of experimental variation between different investigators could now be cancelled from the equation if the standardized diets were used.

Are Current, Commonly Used Mouse Diets Relevant to Human Populations?

Unquestioningly, the AIN series diets have improved mouse models by eliminating variation across studies. However, these diets were designed specifically to ensure health, with translatability to human populations generally not being considered. For instance, the most controversial suggestion made at the 1989 AIN-76A ad-hoc panel discussion was to increase the dietary fat concentration to 30% of total energy while also incorporating a diverse set of fat sources, including animal fat [7]. Implementation of these formulation changes would have made the fat portion of the AIN-93 diet much more similar to diets consumed by Western populations[10]. However, this approach was not adopted for the AIN-93 series diets.

In chronic disease research, the relevance of mouse diets to human populations is of critical importance. Diet is widely recognized as an important factor in lifetime chronic disease risk. Americans routinely consume inexpensive, highly processed foods that are energy dense, but lack sufficient levels of many micronutrients. However, there is increasing evidence that persistent low intakes of key micronutrients can cause significant health problems without

triggering symptoms of acute deficiency. For example, as outlined in the current USDA *What We Eat in America* report from the NHANES survey, many Americans routinely consume insufficient amounts of vitamins A, E, and C compared to the Estimated Average Requirement values for these nutrients [10]. Interestingly, low intakes of micronutrients such as vitamins C, E, B_6, and folate can lead to an increased rate of DNA damage, suggesting that these micronutrients may have a role in the etiology of cancer[11]. In support of this view, epidemiological studies have indicated that the calorie-dense, nutrient-poor Western diet is associated with increased risks of cancer and mortality in the U.S. [12, 13].

In order to make rodent diets more applicable to populations consuming a Western diet, several strategies have been used to emulate this dietary pattern. Some of these strategies include the "cafeteria diet" from which rodents can choose highly palatable foods including cookies, candy, cheese, and processed meats. This dietary approach induces obesity and models the Western dietary pattern of consuming energy dense and micronutrient poor foods. However, this dietary model is inherently flawed due to its lack of consistency over time and lack of reproducibility among investigators because of the variability in food selection[14]. Moreover, unless specifically analyzed, it is difficult to assess intakes of energy, macronutrients, and micronutrients using this model. Currently, simple high-fat, diet-induced obesity (DIO) formulations are commonly used and are commercially available to model the Western dietary pattern and to induce obesity. These DIO diets typically contain either 45% or60% of energy from lard and have a higher sucrose and a much higher saturated fat content than the AIN-93 diet. The DIO diets consistently produce an obese phenotype in obesity-prone mouse strains [15]. However, these diets are not representative of the Western dietary pattern in terms of the total amount of dietary fat and the fatty acid composition [10]. Importantly, similar to the AIN diets, the micronutrient content of these high-fat diets is formulated to promote health, which is also inconsistent with the Western dietary pattern[10].Therefore, commonly used diets used as proxies for the Western dietary pattern are not based on actual human intake data.

Formulating Laboratory Mice Diets Using Human Intake Data

A simple way to make mice diets more closely resemble what humans actually eat is to translate data from human intake databases such as NHANES into an animal diet formulation. In order to emulate human dietary patterns, both macro and micronutrients should be considered for diet formulation. Modeling appropriate macronutrient concentrations to rodent diets from human intake data is relatively straightforward. Carbohydrates, fats, and protein can be included into the animal diet at the same ratio as the human diet based on contribution to total energy provided. Moreover, the macronutrient portion of the rodent diet can be further refined by segregating carbohydrate sources into simple and complex carbohydrates or by matching the dietary fatty acid profile of the human intake data.

Conversely, the issue of translating human micronutrient intakes into rodent diets is more complicated as more than one justifiable scaling approach could be used. Arguments can be made for scaling micronutrients by either metabolic body size, body surface area, or nutrient density[16].Metabolic body size scaling is based on Kleiber's law, the observation that an animal's metabolic rate scales to the ¾ power of the animal's mass across a wide range of organisms [17].Body surface area scaling is the preferred method of the Food and Drug Administration and the equation is derived by converting a mass/bodyweight amount to mass/m^2 amount [18]. However, this approach has limitations when scaling for bioactive components, including resveratrol [19]Another way to scale dietary micronutrient concentrations between species is to base the calculation on metabolic rate or more simply, energy intake, instead of organism size [16, 20, 21]. The rationale for this argument is that micronutrients are primarily used as cofactors in energy production and metabolism. Therefore, the concentration of needed

micronutrients is more closely related to organismal energy production rather than body size. In order to compare these methods, Table 1 provides a comparison of micronutrient recommendations for humans[22] scaled to a mouse diet using metabolic body size, nutrient density, and body surface area scaling. These scaled micronutrient amounts are then compared to mouse dietary recommendations[23].

Table 1. Human dietary micronutrient recommendations compared to mouse micronutrient recommendations using metabolic body size scaling, nutrient density scaling, and body surface area. Data are presented as the human recommendation scaled to a mouse diet and then compared to the mouse recommendation as a percentage of that requirement.

Nutrient	% of Mouse Req. Metabolic Body Size Scaling[1]	% of Mouse Req. Nutrient Density Scaling[2]	% of Mouse Req. Body Surface Area Scaling[3]
Vitamin A	123	228	205
Thiamin	24	44	39
Riboflavin	18	34	31
Niacin	105	195	175
Vitamin B_6	16	28	27
Folic Acid	79	146	132
Choline	27	50	45
Vitamin B_{12}	24	44	39
Vitamin D	59	109	99
Vitamin E	67	125	112
Vitamin K	12	22	20
Biotin	15	27	25
Pantothenate	30	57	51
Calcium	20	37	33
Phosphorus	23	43	38
Magnesium	79	146	132
Iron	23	42	38
Zinc	109	201	181
Copper	15	27	25
Selenium	36	67	60
Potassium	232	429	386
Sodium	296	548	493
Manganese	23	42	38
Iodine	99	183	164
Molybdenum	30	55	49

[1]Calculations are based on human nutrient recommendations for adult males 19-31 [22] and mice [23]. Metabolic body size scaling was computed assuming a human weight of 60 kg, mouse weight of 20 g and scaled to the ¾ power. Human nutrient recommendations are in the format of g/day while mice recommendations are in the format of mg/kg diet, therefore to convert between these formats, a daily mouse intake of 2.5 g was assumed [24]

[2]Nutrient density scaling was calculated by dividing the mass of human recommendation by a daily energy intake of 2079 kcal [10] in order to determine mass of nutrient/kcal. This was then translated to the mouse requirement (mg/kg diet) assuming a mouse food intake 2.5 g/day and an energy intake 9.5 kcal/day [24].

[3]Body surface area scaling computed using the equation: Mouse Equivalent Dose (mg/BW) = human dose (mg/BW) X (human Km,37)/(mouse Km, 3)[18]. Values were computed assuming a human weight of 60 kg, mouse weight of

20 g. Human nutrient recommendations are in the format of g/day while mice recommendations are in the format of mg/kg diet, therefore to convert between these formats, a daily mouse intake of 2.5 g was assumed [24]

In general, when metabolic body size scaling is compared to the body surface area or the nutrient density approach, a lower concentration of micronutrients is calculated. As the data demonstrates in Table 1, one could make the case for using any of these methods. None of the methods systematically scales the human requirement exactly to the mouse requirement. It should also be noted that mouse requirements and human and mouse recommendations are not interchangeable, as these recommendations were implemented to optimize growth and reproduction for mice and in the case for RDA's, to meet the requirements of 97.5% of healthy individuals in each life-stage and sex group. However, because the methodologies and data available to determine appropriate micronutrient recommendations for humans and mice are different, one would not expect a perfect scaling between these recommendations regardless of the method used. An interesting exercise is to compare these scaling methods when the methods used to determine nutritional recommendation is the same in both mice and humans. The nutritional recommendation for selenium is based on the amount of dietary selenium needed to plateau activity of the selenoprotein, glutathione peroxidase. In rodents, that dietary concentration is 0.1 mg Se/kg diet[25, 26]and is 40 μg/day in humans [27]. Using the methods described in Table 1, this human dose scales to 0.04, 0.06, and 0.07 mg Se/kg diet for mice using metabolic body size, body surface area, and nutrient density scaling respectively. This comparison suggests that the nutrient density and body surface area approaches are more accurate than the metabolic body size method for scaling human doses of selenium to mice. However, this observation does not necessarily infer that nutrient density or body surface area are superior scaling methods compared to metabolic body size for all dietary components because micronutrients are distinct with respect to absorption, metabolism, and physiological functions.

Formulation of The Total Western Diet

To address the lack of mice diets that are relevant to human populations, our research group developed the new total Western diet (TWD) for mice with energy and nutrient profiles that emulate a typical Western diet using available NHANES survey data. The TWD was formulated using a nutrient density approach, previously described in detail [28]. Briefly, the amount of each macro and micronutrient in the AIN93G basal diet, a diet routinely used in cancer model studies today, was adjusted to match 50^{th} percentile intakes for Americans as reported in NHANES. These mass amounts were then adjusted for caloric intake. We chose to use the nutrient density scaling approach because previous work from the laboratory of Dr. Harold Newmark demonstrated increased colorectal cancer when mouse diets were scaled to at risk human populations using this method[29-32].

The TWD has fewer calories from protein and carbohydrate sources and twice that amount from fat as compared to the AIN-93G diet. The new diet contains more saturated and monounsaturated fats, less polyunsaturated fat, more complex carbohydrates and twice the level of simple sugars. The TWD includes less calcium, copper, folate, thiamine, and vitamins B_6, B_{12}, D, and E, but much more sodium. Overall, the TWD is not necessarily extreme in the level of

any given nutrient, but rather reflects the overall dietary pattern of the U.S. This same approach can be used to design diets for other laboratory animals including rats, hamsters, and ferrets with the caveat that nutritional requirements unique to these animals are met. Thus, we believe this newly devised diet that better represents the typical U.S. nutrition is highly useful for studies employing mouse models of human chronic disease.

Effects of the Total Western Diet on Metabolism

In our first study with the TWD[33], we sought to compare the impact of the TWD to that of a typical 45% fat DIO formulation and the optimal AIN-93G diet on various health parameters, including weight gain, insulin resistance, and systemic inflammation in mice. Mice fed diets with a Western micronutrient profile did not develop a metabolic syndrome or obese phenotype, compared to mice consuming the optimal AIN-93G diet formulation. This was a surprising finding considering the TWD is high in fat relative to AIN-93G (35 vs 17.5% energy from fat for the TWD and AIN93G respectively). Interestingly and importantly, when mice were fed a version of the TWD with the same micronutrient content as the AIN-93G diet, they developed an a more obese phenotype, and were comparable to mice fed the standard DIO diet. These data suggest that, in the context of the TWD, suboptimal vitamin and mineral intakes in mice specifically restrict the hyperphagia and resulting increased weight gain associated with the higher fat content of the TWD. Additionally, it is important to note that the micronutrient profile of the TWD did not limit lean mass accretion, suggesting that the animals were not stunted. These findings are important in that they reveal a role of dietary micronutrients in moderating the hyperphagic behavior shown by C57BL/6J mice consuming a moderately high fat diet.

Effects of the Total Western Diet on Colon Cancer

In another study with the TWD [34], we tested the hypothesis that the TWD basal diet would influence effects of green tea extract (GTE) on colorectal cancer, and short chain fatty acid metabolism compared to mice fed the standard AIN-93G diet. Green tea was chosen as a model bioactive because it has been well studied in relation to these endpoints. Indeed, in our study we discovered interactions between the basal diet, GTE, and our selected endpoints. For instance, GTE reduced bodyweight but only in mice fed the TWD. Fasting glucose was reduced by GTE treatment in mice fed the TWD but not the AIN-93G diet. Cecal short chain fatty acids were reduced by GTE, but only in mice fed the TWD. Conversely, GTE decreased liver triglycerides but only in mice consuming the AIN-93G diet. Perhaps the most compelling observation was the marked increase in the multiplicity of aberrant crypt foci (ACF, colonic pre-neoplastic lesions) in mice consuming the TWD compared to AIN-93G fed mice, suggesting that the American dietary pattern promotes colorectal cancer. Interestingly, GTE markedly reduced ACF only in animals fed the TWD, but not the AIN-93G diet. In a similar investigation from another laboratory, Nakanishi et al. [35]found that the inclusion of walnuts suppressed tumor development in mice fed the TWD, but not the AIN-76A diet.

All of these observations point to a complex, yet critical interaction between basal diet and the activity of a well-established bioactive dietary component, GTE. Our results suggest that standardized basal diets, including the AIN-93G, may underestimate or fail to demonstrate the efficacy of bioactives such as GTE. Importantly, in this study, if the AIN-93G diet had been used

as the only basal diet to investigate the effects of GTE on colorectal cancer, we would have reported that GTE had no effect.

SUMMARY

The consideration of the role of basal diet in pre-clinical studies to model human disease has matured from what once was largely an afterthought to what is now a more careful, systematic consideration of basal nutrition and its impact on physiology. Continued emphasis is also maintained on the reproducibility of diet effects across laboratories. Making mouse diets more relevant to at risk human populations should be the next big evolution in laboratory diet design, as continued use of diets that are wholly unrepresentative of human nutrition patterns in pre-clinical studies makes little sense. As we demonstrated with the GTE investigation, an appropriate basal diet may be especially important for functional food research. The TWD approach is a step forward in this regard and will help increase the translational fidelity of pre-clinical mouse models.

List of Abbreviations: aberrant crypt foci, ACF; American Institute of Nutrition, AIN; diet-induced obesity, DIO; green tea extract, GTE; National Health and Nutrition Examination Survey, NHANES; National Institutes of Health, NIH; Total Western Diet, TWD.

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Authors' Contributions: All authors contributed to this review.

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