Choice of Laboratory Rodent Diet May Confound Data Interpretation and Reproducibility

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ABSTRACT

The reproducibility of experimental data is challenged by many factors in both clinical and preclinical research. In preclinical studies, several factors may be responsible, and diet is one variable that is commonly overlooked, especially by those not trained in nutrition. In particular, grain-based diets contain complex ingredients, each of which can provide multiple nutrients, non-nutrients, and contaminants, which may vary from batch to batch. Thus, even when choosing the same grain-based diet used in the past by others, its composition will likely differ. In contrast, purified diets contain refined ingredients that offer the ability to control the composition much more closely and maintain consistency from one batch to the next, while minimizing the presence of non-nutrients and contaminants. In this article, we provide several different examples or scenarios showing how the diet choice can alter data interpretation, potentially affecting reproducibility and knowledge gained within any given field of study. *Curr Dev Nutr* 2020;4:nzaa031.

Introduction

When it comes to designing any experiment in animal models, many factors need to be taken into account (1). In particular, there are a host of environmental factors that require consideration in animal husbandry, including the housing conditions (i.e., group or single housing), temperature and humidity, light and dark cycle timing, bedding, and diet. These factors are reviewed and approved by every Institutional Animal Care and Use Committee (IACUC), but unless directly studied, diet is one factor that is commonly overlooked (even by the IACUC) as a possible variable and frequently is not reported in detail in the methods sections of publications, even in highly ranked journals (2, 3). Terms such as "standard chow," "standard diet," or "normal diet" are commonly used to "describe" the diet, which are inappropriate and tell us nothing about the diet being used. This suggests that many researchers do not consider diet as an important variable in an experiment where diet is not the main focus of the study. So, why should researchers be more considerate of the diet both before conducting an experiment (i.e., design phase) and when reporting it in the methods section of a publication?

Regardless of whether the diet is chosen to promote a certain rodent phenotype or to simply allow for normal growth and health, as researchers we should demand transparency from manufacturers and uphold (when publishing) it by reporting the ingredients and their concentrations (i.e., open to the public). Furthermore, researchers should have assurance that the ingredients will not change and concentrations will remain consistent from batch to batch. If formulas are consistent from batch to batch (i.e., fixed), they should also have a consistent nutrient composition and minimal non-nutrients and contaminants from one batch to the next. The chosen diet may indeed allow the rodents to grow and thrive, but if certain factors are present in the ingredients used (e.g., those naturally occurring in plants, due to climate or location of harvest, or synthetic contaminants), it is not possible to know how one or more of these factors will affect data interpretation. In some cases, they may be acting additively or synergistically, and affecting multiple mechanisms. Furthermore, should the amounts of various non-nutrients and contaminants vary from batch to batch (which they invariably will), data reproducibility would be, at the very least, compromised. Many of these concepts have been described in the most recent National Research Council publication in 1995 (4). As we learn more about how nutrients and other factors within the diet influence data, scientists need to increase their awareness of what is being fed to their animals to avoid costly errors and improve study outcomes, ultimately improving rigor and reproducibility. In this article, we have cited

references that have proper control diets and diet designs to provide evidence that ingredients, non-nutrients, and contaminants present in the diet can alter data interpretation and affect reproducibility in a number of scientific fields.

There are numerous commercially made diets being used in rodent studies at any given moment, and they fall into 2 general categories: grain-based (also called cereal-based) diets and purified ingredient diets (or purified diets).

Diet Types: Grain-Based (GB) Diets and Purified Diets

GB Diets

As the name implies, GB diets contain a combination of cereal grains such as ground wheat, corn, and oats; soybean and alfalfa meals; and wheat middlings (a wheat milling byproduct); and in many cases, animal byproducts including fish meal and porcine meat meal. In most cases, these formulas are "closed" or proprietary, so while a list of ingredients is provided, their concentrations are unknown and can potentially be manipulated by the manufacturer. However, even if the formula is open and fixed, nutrient amounts would be difficult to keep consistent as each ingredient in a GB diet contains multiple nutrients (i.e., soybean meal contains protein, carbohydrate, fat, fiber, vitamins, minerals) and nutrient amounts can vary batch to batch (5). In order to address this potential variation, certain processes are in place to monitor compositional changes in ingredients themselves and the complete diets. Companies preparing GB diets may alter ingredient amounts in order to maintain consistent nutrient amounts across batches of each diet, which is typically proprietary and not disclosed publicly when formulas are not "open" (4, 6, 7). While the maintenance of nutrient amounts may be possible with rigorous quality-control measures in place, other factors need to be considered, such as possible nutrient losses during manufacturing and storage, bioavailability of nutrients in the ingredients, and potential nutrient interactions (4). It is also possible that changes to non-nutrients and contaminants will occur with modifications to ingredient amounts (or even when they are not altered). In fact, it is common to find non-nutrients in most ingredients and they include phytoestrogens (i.e., isoflavones from soybean meal and coumesterol from alfalfa meals) (8–10), heavy metals (i.e., cereal grains and animal byproducts contain arsenic, cadmium, lead) (11, 12), synthetic contaminants (i.e., pesticides, genetically modified organisms, and pollutants including polychlorinated biphenyls and polychlorinated dibenzo-p-dioxins and dibenzofurans), all at highly variable concentrations among GB diets from different manufacturers (12). In addition, other contaminants such as mycotoxins, which are toxic metabolites of fungi known to be present in cereal grains, are present in varying amounts in different batches of the same GB diet, including deoxynivalenol, ochratoxin A, and zearalenone (13). Finally, endotoxins, which are lipopolysaccharides present in the outer cell membrane of gram-negative bacteria, have been observed in variable amounts in different GB diets (14). For some of these non-nutrients and contaminants, it is known that they can affect data at amounts present in GB diets, while for others, there are not enough data in the literature to definitively describe their effects on experimental outcomes. One goal of this article is to make researchers aware of the presence of these

compounds. Individual researchers can then decide if these compounds may have "positive," "negative," or "neutral" effects on their specific endpoints.

One non-nutrient found in very high concentrations in GB diets (relative to purified ingredient diets) is fiber, and includes different concentrations of both insoluble and soluble fibers. Sources of fiber in GB diets are derived from plant cell walls of cereal grains (i.e., ground wheat, wheat middlings, ground corn, beet pulp) and include cellulose, hemicellulose (i.e., xylans, mannans, glucans), and lignin, which are mainly insoluble fibers, and pectin, a soluble fiber. The concentrations of these fibers can differ significantly from one GB diet to the next (15). However, other sources that may provide significant amounts of fiber include brewer's dried yeast, which provides β-glucans within the yeast cell walls and is a source of both soluble and insoluble fiber (16). While it is generally considered that dietary fiber has beneficial effects through its influence on gut microbiota (17), it is conceivable that varying fiber amounts/types among and within GB diets could affect microbiome data reproducibility.

Purified Diets

In contrast, purified diets are made with refined ingredients, each providing 1 main nutrient (i.e., corn starch is mainly carbohydrate, soybean oil is mainly fat, casein is mainly protein). Furthermore, these formulas are "open" formulas that remain constant and unchanged, allowing the researcher to "report" the ingredient composition. By using refined ingredients, the nutrient compositions of both macro- and micronutrients in purified diets are well defined, limiting both the variability of nutrients and also contaminants (18). As such, this allows the researcher to selectively manipulate individual nutrients to their advantage.

While a specific purified-ingredient diet may not be appropriate for every research need, they provide researchers with both a defined and "cleaner" option to maintain consistency from one study to the next as well as the ability to adjust nutrients one at a time for studying nutrient amounts from excess to deficiency. The AIN-76A or AIN-93 series diets, established by separate committees in 1976 and 1993 (19, 20), respectively, allowed toxicologists to study the effects of various compounds without the confound of background contaminants that are typically present in GB diets. These diets also provided a means to understand the essentiality of nutrients by removing 1 ingredient, which provides 1 main nutrient, or to understand the development of metabolic disease in mice and rats by adding excess amounts of calories from fat, such as lard in place of calories from a carbohydrate source like corn starch. However, even unadulterated, these AIN diets (or other low-fat diets), which often serve as a control diet for higher-fat diets, may cause some mild metabolic disease (e.g., the early stages of glucose intolerance) or alter other phenotypes compared with GB diets. This may be, in part, due to the refined nature of purified diets, including the presence of sucrose and the typically low amount of total fiber along with the lack of fermentable fiber, which is in contrast to the very high amounts and diverse fiber types found in GB diets. More specifically, fiber in many purified diets consists of only cellulose, an insoluble fiber that is not fermentable by most gut microbes. Hence, researchers should understand the caveats to using certain purified-ingredient diets

that lack soluble fiber and/or contain appreciable amounts of sucrose while at the same time be aware that these formulas can be modified. Modifications include the addition of more total fiber and soluble fiber sources, such as inulin, and replacement of sucrose with sources such as corn starch and dextrose to minimize fructose, an initiator of metabolic disease, including insulin resistance, glucose intolerance, and hypertriglyceridemia (21, 22). It is important for investigators to recognize that not only do purified diets differ in composition from GB diets but how these differences will affect the rodent phenotype. **Table 1** provides an overview of nutrient and contaminant sources in both purified and GB diets.

Non-nutrients and Contaminants in GB Diets That Can Influence Data Interpretation

Phytoestrogens
Endocrine disruptors are chemicals that are either synthetic or naturally found in our environment that may interfere with the endocrine system and affect developmental, neurological, reproductive, and immune functions in humans and animals. Phytoestrogens are one class of naturally derived endocrine disruptors found in soybean meal and alfalfa meal, which are typically included in GB diets. While phytoestrogens are absent in purified diets such as the AIN-76A or AIN-93 series diets, their concentrations in GB diets can range from either low concentrations of <100 mg/kg (if soybean meal and alfalfa meal are omitted) up to 800 mg/kg diet (8, 10, 24). As their name implies, their chemical structures are similar to endogenous estrogens and can target and bind estrogen receptors (ERs), particularly ERβ and to a lesser extent ERα, although more weakly than estradiol. Because of this, phytoestrogens are classified as natural selective ER modulators (SERMs). Studies have demonstrated that the estimated intakes of phytoestrogens by mice and rats fed GB diets can alter many phenotypes, including effects on carcinogenesis development and matu-

ration onset (9, 10, 24, 25). The amounts of phytoestrogens from soy (i.e., isoflavones) in GB diets can vary as much as 3- to 6-fold in different lots of the same diet, with amounts ranging in the hundreds of milligrams per kilogram of diet (8, 10) and differences can be found among lots that are only 1 mo apart (26). The differences in genistein and daidzein (isoflavones in soy) concentrations from lot to lot (from 93, 223, or 431 mg/kg diet) were found to influence maturation onset in 1 rat strain but not another (25). In CD-1 mice, variation in daidzein and genistein from 2 lots of 1 GB diet was >2-fold (159 and 431 mg) and the higher concentration significantly increased the proportion of mice with vaginal opening at day 24. There are several factors that may contribute to these differences including soy variety and variations in location and timing of harvest and climate (27, 28). Variability in phytoestrogen concentrations in different GB diets influence circulating phytoestrogen concentrations (8), but other factors may also contribute, such as transformation of daidzein to S-equol by gut microbiota, which can influence bioavailability and bioactivity (29).

Because phytoestrogens are SERMs, they can affect typical estrogen actions such as pubertal onset, which is dose dependent in certain rodent models (25, 30). For example, Thigpen et al. (30) found no dose-dependent change in timing of pubertal onset when diets contained a potent SERM (diethylstilbestrol) in the context of a GB diet with 431 mg daidzein and genistein/kg diet but did find a change in a different lot of the same diet with 159 mg daidzein and genistein/kg. The lack of effect of the SERM in their initial study was attributed to the naturally high phytoestrogen amounts in their initial GB diet. When it comes to mitigating the effects of synthetic SERMs known to reduce cancer, the effect by phytoestrogens may not be linear. For example, a lower dose of phytoestrogens abrogated the efficacy of tamoxifen on breast cancer in a mammary tumor mouse model while a higher dose had no effect (31). Furthermore, effects by phytoestrogens may either be pro- or anticarcinogenic, depending on the cancer model being studied. In the ovariectomized, carcinogen-induced mam-

TABLE 1 Typical sources of nutrients and non-nutrients in rodent purified diets and grain-based diets[1]

	Typical sources	
	Purified-ingredient diet	Grain-based diet
Nutrients		
Protein	Casein	Soybean meal, ground corn, wheat, and oats, whey, alfalfa, fish meal, meat meal
Fat	Soybean oil, corn oil	Porcine animal fat, fish meal, meat meal
Carbohydrate	Corn starch, maltodextrin, sucrose	Ground corn, wheat, and oats, wheat middlings
Fiber	Refined cellulose	Ground corn, wheat, and oats, dried beet pulp, alfalfa, wheat middlings
Micronutrients	Mainly vitamin and mineral premixes	Most ingredients, extra micronutrients added
Possible non-nutrients/contaminants		
Phytoestrogens	Absent[2]	Soybean meal (genistein, daidzein), alfalfa meal (coumestrol)
Heavy metals	Trace/not detectable	Grains and animal byproducts (arsenic, lead, cadmium, cobalt)
Pollutants, pesticides, mycotoxins, nitrosamines, endotoxins[3]	Trace/not detectable	Grains (pollutants, mycotoxins) and animal byproducts (pollutants, nitrosamines)

[1]Table adapted from reference 17 with slight modifications.
[2]Unless soy protein isolate is used.
[3]Endotoxin source unknown, but high in grain-based diets (14, 23).

mary tumor model, phytoestrogens can elicit procarcinogenic effects (32) but are antagonistic in certain cancers in mouse models with intact ovaries (33). These examples are by no means comprehensive and other phenotypes known to be affected by phytoestrogens and soy protein include metabolic disease (34, 35) and osteoporosis (36). Therefore, knowing the baseline phytoestrogen amount of any diet being fed may be useful but will not help the researcher predict its impact on the rodent phenotype. This has been discussed at various meetings (37) and still requires attention by investigators across several scientific fields.

Arsenic and heavy metals

Cereal grains and meat meals are typical sources of toxic heavy metals in GB diets (18, 38, 39), including lead, arsenic (As), cadmium, and mercury (12). While present in relatively low concentrations (typically in the hundreds of micrograms per kilogram of diet), As in GB diets may be higher than what is considered safe in drinking water (i.e., 10 ppb inorganic As) (40, 41) and can severely compromise data when trying to evaluate effects of lower doses of exogenous As (i.e., 10–100 ppb inorganic As). Indeed, there were no effects of As (10 or 100 ppb As in the drinking water) in mice fed a GB diet with 390 ppb total and 56 ppb inorganic As, on gene expression of metabolic and detoxifying enzymes as well as immune-signaling pathways in the liver and lung (11). In contrast, this study found clear differences in gene expression due to As in the drinking water when mice were fed the AIN-76A diet (11), a result attributed to the significant differences in dietary As between the purified and GB diets used in this study. When 100 ppb inorganic As was added to the drinking water of mice fed purified diets (AIN-76A), it was possible to identify effects on inflammatory factor activation in the lung (including IL-1 receptor and a number of Toll-like receptors) and compromised immune response to infection by an influenza A virus in C57BL/6 mice (42, 43). In addition, the effect of dosing As in the water at 300 ppb (equivalent to human exposure of 58.5 ppb in water) was found to increase body fat in mice fed the AIN-76A diet (44). These amounts of As are relatively close to what has been measured previously in GB diets (12), and (as may be expected given the variation of grains and animal byproducts in different GB diets), the amounts of As (and other heavy metals) can vary from one GB diet to the next (12). Furthermore, the amounts of these heavy metals in the diet are reflected in a dose-dependent fashion in various tissues of weanling female Sprague-Dawley rats ($n = 9$) fed purified and GB diets for 28 d (dietary As, ppb: LabDiet 5002, 278; NIH-31M, 486; AIN-76A and AIN-93G, not detectable; liver As, mean $\pm$ SEM ppb: LabDiet 5002, 939 $\pm$ 83; NIH-31M, 2806 $\pm$ 176; AIN-76A, 154 $\pm$ 16; AIN-93G, 224 $\pm$ 16; M Ricci, M Pellizzon, J Couse, and E Ulman, unpublished data, 2010). It is beyond the scope of this paper to determine whether certain amounts of heavy metals will or will not affect specific phenotypes but rather to educate the reader as to their presence in GB diets.

Mycotoxins

Mycotoxins are secondary metabolites of fungi that can grow on or within different grain products added to GB diets, including wheat, corn, oats, and soybeans. Over 300 mycotoxins have been identified, and 6 classes of mycotoxins are commonly found in foods, including aflatoxins, fumonisins, trichothecenes (e.g. deoxynivalenol), ergot alka-

loids, zearalenone, and ochratoxin A (mainly of the genera *Aspergillus, Fusarium*, and *Penicillium*). Exposure to certain climate conditions at different stages of plant growth as well as damage caused by insects can allow for growth of fungal species, and growth may persist postharvest and during transportation and storage of grains (45–47). The potential for fungal growth of various species and mycotoxin production in various grains can cause significant differences in mycotoxin amounts from one GB diet to another and from lot to lot within the same diet (13). Several mycotoxins are measurable in GB diets (i.e., deoxynivalenol, ochratoxin, fumonisin, zearalenone, and ergot alkaloids; e.g., ergot alkaloids in Teklad 7012 different lots, ppb: 57, 144, 345; in contrast, all mycotoxins were undetectable in 3 different purified diets: Research Diets, Inc., D11112201, D12450J, and D12492; S Radhakrishnan, M Pellizzon, P Greiss, M Ricci, unpublished data, 2019).

There are well-known effects of individual mycotoxins on the phenotype of various animal species, including hepatotoxicity, immunotoxicity, carcinogenicity, estrogenicity, reproductive toxicity, teratogenicity, gastrointestinal disorders, and reduced growth and food intake. However, it is unknown whether the combinations of different mycotoxins or fungi present in GB diets can alter the rodent phenotype. *In vitro* studies conducted in Caco-2 cells suggest possible additive or synergistic effects for increasing oxidative stress (depending on the combinations of mycotoxins) and mainly additive effects for DNA and protein synthesis (48). *In vivo*, biological effects of mycotoxins depend on several factors including the amounts ingested, number of occurring toxins, duration of exposure to the toxins, and animal sensitivity (47). There is also evidence of lactational transfer of mycotoxins such as fusaric acid from nursing dams to the neonate, as stomach concentrations of mycotoxins in neonates were dependent on the dose given to the dams, which was even observed in those fed the control diet with 300 ppb fusaric acid (49).

Establishing low adverse-effect amounts of mycotoxins in animals and performing these studies in a repeatable manner will require the use of purified diets with minimal contaminants. One study examined the effects of zearalenone on uterine growth in mice and used a GB diet (LabDiet 5002), which contained soybean meal and alfalfa meal (50). The researchers rightly measured zearalenone and found it was <100 ppb, which was lower than the amounts studied (i.e., ≥2000 ppb), although they did not measure dietary amounts of phytoestrogens, which could conceivably confound data interpretation given their similar chemical structure to the mycotoxin being studied. While there are some GB diets that are formulated without soybean meal and alfalfa meal to be "phytoestrogen free," these diets are typically formulated with higher concentrations of corn and wheat products, which commonly contain zearalenone (and some of its metabolites), ultimately leading to more mycotoxins that bind ERs (45, 51). Therefore, to eliminate any possibility that factors from the diet could bind estrogen receptors, and affect data interpretation, a phytoestrogen-free purified diet would be preferred over any GB diet. Furthermore, to establish the dose where no ill health effects are found with a particular mycotoxin (or lowest observed adverse-effect amount), it would be critical to use a purified diet. This conclusion was made in a study using a GB diet to study the effects of ppb concentrations of arsenic in mice (11) and the same rationale can be applied to mycotoxins. Even if the concentration of a particular mycotoxin added to the diet (or gavaged) is in excess of what may be present

in the control diet, it can be argued that it would be prudent to avoid the presence of other mycotoxins, which may be additive or synergistic (or perhaps even counteractive) to the mycotoxin being studied. Only by using a purified diet with minimal contaminants can one truly study the lowest observed adverse-effect amounts of individual mycotoxins, or any other contaminants for that matter.

Endotoxin

Laboratory animal diets can be a source of microbial contamination and this should be considered, particularly when studying the gut microbiota or those using germ-free or specific-pathogen-free mice. GB diets typically contain higher amounts of bacteria than purified diets (LabDiet 5001 vs Research Diets, Inc. D11112201: >10 CFU/g, too numerous to count vs 0 CFU/g; independent laboratory, M Ricci, unpublished observations, 2019) and methods of sterilization, such as γ-irradiation (for both purified and GB diets) or autoclaving (for GB diets), can reduce viable bacterial loads. Besides microbial loads, certain nutrients are highly sensitive to γ-irradiation at ~30 kGy, including thiamin and vitamin A, and lipid peroxide formation is found at doses between 2 and 10 kGy [52, 53]. While it may be difficult to predict just how much the vitamin composition of a given diet is influenced by a given dose of irradiation, it is a common practice by manufacturers to increase dietary amounts of vitamins when higher than typical irradiation doses (e.g., >20 kGy) are required. Irradiation levels used for diets to be fed to germ-free animals require certain doses (depending on the animal facility) and will further reduce microbial loads. However, the bacterial "parts" will still be present, including LPS or endotoxin, which are present in the outer cell membranes of gram-negative bacteria. In line with microbial load, GB diets contain higher endotoxin amounts than purified diets [14], and more variable amounts from one lot to the next (e.g., Teklad 7012, 3 different lots: 17,669, 25,239, 57,701 Endotoxin Units (EU)/g including phytoestrogen-free GB diets (e.g., Teklad 2020X, different lots: 992, 1576, 4692 EU/g); much lower amounts and variability were observed in purified diets (e.g., Research Diets, Inc., D11112201, 4 different lots: 68.2, 77.4, 155.6, and 186.7 EU/g) (analyses performed by Charles River Laboratories, Inc., Charleston, SC, unpublished data, 2019). The presence of endotoxin within irradiated GB diets can impact immune system development and function in germ-free mice. The largest immune organ in the body is the gut-associated lymphoid tissue, and perhaps not surprisingly, this compartment contains fewer immune cells in germ-free mice relative to conventional mice [54]. In germ-free mice, a GB diet can drive expansion of T and B cells in Peyer's patches (found in the mucosal wall of the ileum) and mesenteric lymph nodes [14]. An LPS-containing extract from 2 different GB diets with 10-fold different concentrations of LPS both increased the production of cytokines and maturation of regulatory cells in an *in vitro* test in dendritic cells from wild-type mice, with the greater increases coming from the diet with more LPS [23]. When these same diets were fed to germ-free mice to study their immune response to subcutaneous immunization with birch pollen, those fed the higher-LPS diet had a lower immune response than those fed the lower-LPS diet [23]. While it is unknown if different GB diets or variable amounts of endotoxins affect immune system development in conventional mice, it is conceivable that batch-to-batch variability could differentially affect immune system development of germ-free mice from one study to the next, based on previous data [14]. Thus, these potential

effects by dietary endotoxins deserve to be studied further in both conventional and germ-free mice and the use of purified diets rather than GB diets would eliminate confounding variables to study the effect of endotoxin doses.

Although endotoxin levels are very low in purified diets, the type of casein used in these diets can affect 16S ribosomal RNA (rRNA) sequencing data of fecal samples. For example, typically purified diets use casein precipitated from cow milk with lactic acid cultures. While pasteurization reduces viable bacteria, 16S rRNA sequencing of feces from germ-free or antibiotic-treated mice can provide a strong signal of certain bacterial genera present such as *Lactobacillus* or *Lactococcus* [55, 56]. An alternative to lactic acid casein, mineral acid casein, contains no lactic acid cultures and should mitigate concern of these bacterial components affecting gut physiology.

Pesticides and pollutants—xenoestrogens

Xenoestrogens are foreign estrogens that (like phytoestrogens) are close enough structurally to bind to ER sites. Several examples of xenoestrogen compounds include pesticides (herbicides, insecticides, and fungicides) and dioxins, which is a term used for polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs), an example of which is 2,3,7,8-tetrachlorodibenzo para dioxin (TCDD), a known carcinogen. Other compounds present include dioxin-like compounds such as polychlorinated biphenyls (PCBs). Mesnage et al. [12] assessed 13 different GB diets from 5 different continents and found that all diets were contaminated with pesticides and dioxins (PCDD/Fs and PCBs), as well as genetically modified organisms (GMOs) and heavy metals (lead, cadmium, mercury, and arsenic). This is not necessarily surprising given these diets contain agricultural products and byproducts that are exposed to these environmental contaminants. All GB diets contained pesticides and PCBs at variable amounts from one diet to the next, both with respect to the individual pesticides and PCBs detected as well as the amounts of total pesticides. Measured pesticide residue amounts were as high as 2641 ppb in 1 GB diet and a total of 9 residues were found across different diets, with some containing as much as 6 residues [12]. One diet contained 141 ppb deltamethrin, which can potentially initiate tumor development in mice [57], and the presence of piperonyl butoxide, a synergist that could exacerbate the potential toxicity of deltamethrin (and other pesticide residues) [58], was found in 8 of the 13 diets. Glyphosate, present in the pesticide Roundup (Monsanto Company), is a tumor promoter [59], and Roundup added to water at 0.1 ppb was capable of inducing mammary adenoma growth in rats over a 2-y period [60]. These Roundup residues varied from 0 to >300 ppb in different GB diets and were positively correlated to the percentage of Roundup-tolerant GMOs present in these diets [12]. In addition to pesticides, several of the GB diets contained consistent amounts of PCDD/Fs, while others had relatively higher amounts (in some cases, >0.1 ppb) and should be considered when studying lower-dose exposure amounts of these pollutants on xenobiotic metabolism, immune function, and developmental/reproductive toxicity [61]. Mesnage et al. determined how their combination potentially impacted health in rodents by using chronic non-cancer hazard indexes established by the US Environmental Protection Agency and European Food Safety Authority and found a high potential for these contaminants to impact rodent health. This hazard index was calculated as an additive effect by the mea-

sured substances, which may be an underestimation of true risk given that there are other potential toxic substances present that are either unknown or not measured and some of these substances in combination may have synergistic effects.

Mechanistically, these compounds (among others such as phytoestrogens or certain mycotoxins) in GB diets can influence the phenotype of rodents via ERs and/or aryl hydrocarbon receptors (AhRs) (62). The AhR, in particular, may cause pleotrophic effects as it is a transcription factor controlling multiple genes, including those involved in the immune response and detoxification. Thus, the dietary amount of an AhR ligand should be well controlled, particularly in studies focusing on immunity. Indole-3-carbinol from cruciferous vegetables is a well-known AhR ligand, but did not activate the AhR when added to a GB diet while it did when added to a purified diet, suggesting that factors in the GB diet interfered with its activation (63, 64).

High amounts and diverse types of fiber in GB diets

Fiber is another factor in diets that is not usually considered unless being directly studied. However, given the very high concentration in GB diets, it may be one of the most important factors to consider as cereal grains provide diverse fiber types and amounts, some of which can be fermented by gut microbiota. Insoluble and soluble fibers (mainly plant cell wall material) include a complex array of hemicellulose (partially soluble), cellulose (insoluble), lignin (insoluble), and pectin (soluble), all of which have been shown to be present at variable levels in different GB diets (15). GB diets typically contain amounts ranging from 15–25% total fiber, 18–20% insoluble fiber, and 3–5% soluble fiber (15, 17), likely due to the ingredients (e.g., corn, wheat, and oats), which can vary significantly in total, insoluble, and soluble fiber (65, 66). Therefore, should one need to know the concentration of each type of fiber in a given batch of GB diet it must be directly measured as it is not reported by the manufacturer.

Fibers present in grains such as hemicellulose and pectin can be metabolized by gut microbiota and form metabolites such as SCFAs [known to be a source of energy for colonocytes (67)], which, in turn, can affect the health of the gut. Should there be a change in the GB diet batch during a study, and given the potential variability in fiber in ingredients used in these diets, it is conceivable that this could, in turn, alter the gut microbiota profile. One recent study that fed 3 different GB diets to female Institue of Cancer Research (ICR) mice to study microbiome changes in different regions of the gut including jejunum, ileum, cecum, and fecal samples found differences in α-diversity, but no significant differences in fecal and cecal richness between the 3 diets (68). While it suggests that there may be less concern with feeding different GB diets than expected, it is possible that these diets were compositionally similar given that they had a similar list of ingredients and were prepared by the same company; however, each ingredient concentration is unknown as these are closed formulas. It also should be considered that it may be difficult to differentiate bacterial genera using 16S rRNA sequencing as reports have indicated that ~42% of these genera have 16S rRNA sequences that are >97% similar (69). Given that there are several manufacturers of GB diets and that grains may differ from one supplier to the next in terms of fiber types and amounts, more of these types of studies with different GB diets (and different batches of the same diet) are necessary. Certainly, the gut microbiome is altered

when switching from a GB diet to a purified diet with 5% cellulose (regardless of the amount of fat) in ileal, cecal, and fecal samples (70). Furthermore, there are significant changes in gut morphology, with mice fed purified diets having smaller and shorter ceca and colons compared with those fed a GB diet, even after only 2 d of feeding (71). Therefore, it is vital to know what is being fed when studying gut parameters and microbiota. At present, the study of the effects of different dietary fibers on gut microbiota profiles in laboratory animals is still in its infancy.

Purified Low-Fat Diets and Metabolic Effects

Purified diets such as the AIN diets (or other low-fat purified diets based on these original formulas) have been modified, often for the development of metabolic disease in rodent models by increasing the fat content in place of carbohydrate. In some cases, even a low-fat purified diet causes mild metabolic impairments relative to GB diets, and certain factors within these diets are to blame, including the very high sucrose content in the AIN-76A diet (50% by weight) and also the very low fiber content. Sucrose at 50–68% of kcals has been found to reduce glucose tolerance in C57BL/6 mice (22) and induce insulin resistance, hypertriglyceridemia, hepatic steatosis, and hypertension in rats (72–74). For example, low-fat diets like the AIN-76A and AIN-93G diets can adversely affect body weight, blood and liver lipids, and blood pressure compared with GB diets (75–78). Although the AIN-93G diet reduced serum cholesterol concentrations in rats regardless of gender compared with those fed the AIN-76A diet, concentrations in males fed both diets were still elevated significantly above those fed GB diets after 1, 6, and 13 wk. Similar effects were found for plasma triglycerides, but in this case, the AIN diets (particularly the AIN-76A diet) elevated plasma triglycerides (at all time points in females) compared with those fed a GB diet (77). The main factor present in sucrose that influences these metabolic changes is fructose (21), and replacing sucrose with glucose-derived carbohydrate such as dextrose or corn starch can reduce these metabolic effects of purified diets (74).

Other factors that may explain the differences observed between those fed the AIN diets and GB diets include the lack/presence of soluble fiber and phytoestrogens. Fiber is commonly added to purified diets as cellulose at ~5% by weight (called for by the AIN diets and others), which, as stated previously, is far less than what is commonly found in GB diets. For example, the addition of soluble fibers such as inulin, in the context of a diet higher in fat, can reduce body weight, adiposity, and blood and liver lipids, and improve glucose tolerance of rodents, and these changes may be due in part to increases in SCFAs produced by fermentation of inulin by gut microbiota (79, 80). Prebiotic fibers like inulin have direct effects on gut microbiota by increasing IL-22 and, in turn, increase epithelial cell formation and mucosal layer thickness for improved barrier function (81, 82). In addition, these beneficial effects with soluble fiber may lead to improvements in metabolic health of mice fed these diets (17, 82). Therefore, further improvements to purified diets may be as simple as the addition of soluble fiber source(s). However, to more closely mimic gut microbiota in mice fed GB diets, the addition of multiple, diverse fiber sources will likely be required, which we have discussed previously (17). **Table 2** provides a short list of questions to

TABLE 2 Questions to ask when considering the diet choice for rodent studies

	Purified-ingredient diets	Grain-based diets
Open formulas?	Yes	No[1]
Defined/consistent ingredients?	Yes	No
Can modify 1 nutrient at a time?	Yes	No
Non-nutrient chemical entities?	No	Yes
High/diverse fiber?	No[2]	Yes

[1]Only a select few are open.
[2]Can modify by adding more total fiber and different fiber sources.

ask when choosing to use either purified or GB diets in in vivo studies with rodent models.

Improper Use of Control Diets

After learning of the differences between GB and purified diets, it should be clear that these diets should never be compared to one another when determining a given dietary effect on the rodent phenotype. When designing any experiment, scientists try to limit the differences between the groups to those being purposely studied. In the case of diet, it is impossible to interpret the data if the diets are completely mismatched—it would be similar to comparing data from mice to those from rats. Yet, comparing mismatched (often, purified to GB) diets is an all too common phenomenon and unfortunately appears to be generally accepted by preclincal research authors and reviewers. It is particularly disturbing that this occurs in journals that are highly cited, thus leading to more researchers to perpetuate what is essentially subpar science (2, 3). It is our hope that information like that contained in this article will help to reduce the use of mismatched diets and therefore lead to a better use of valuable experimental resources, stronger conclusions, and accelerated scientific progress.

Conclusions

Diets—specifically the nutrients and non-nutrients/contaminants that they contain—affect the rodent phenotype. Given the diversity of diets in use today and that many studies use GB diets (which contain contaminants at biologically relevant levels), it is easily conceivable that this diversity is playing a role in the data-reproducibility crisis. Should there be an interest in replicating a particular study, then all aspects of the study would need to be replicated, including the diets used. That said, even if one uses the same GB diet from one study to the next, it is difficult to say this diet is the "same" given the well-known batch-to-batch variations in contaminants and, potentially, even some nutrients. This was the reason why the first AIN committee went to great lengths to establish the "open formula" purified-ingredient AIN-76A diet—to allow researchers across the globe to use the same diet. However, there are still factors to consider when using a purified diet (e.g., fiber) and therefore it is critical for the investigator to understand these issues prior to beginning their study. Therefore, the details of the diet(s) used should be known during the stage of study design and reported in detail (i.e., diet number, company, complete formulation, if available) both in grant ap-

plications as well as in the methods section of manuscripts so that the scientific community can critically evaluate the validity of the findings. In order to have a fighting chance of reproducing an in vivo study, all known variables that can be controlled, including the diets used, should be controlled.

References

1. Smith AJ, Clutton RE, Lilley E, Hansen KEA, Brattelid T. PREPARE: guidelines for planning animal research and testing. Lab Anim 2018;52(2):135–41.
2. Pellizzon MA, Ricci MR. The common use of improper control diets in diet-induced metabolic disease research confounds data interpretation: the fiber factor. Nutr Metab (Lond) 2018;15:3.
3. Warden CH, Fisler JS. Comparisons of diets used in animal models of high-fat feeding. Cell Metab 2008;7(4):277.
4. Subcommittee on Laboratory Animal Nutrition. Committee on Animal Nutrition, Board on Agriculture, National Research Council. Nutrient requirements of laboratory animals, Washington (DC): National Academies Press; 1995.
5. Rao GN, Knapka JJ. Contaminant and nutrient concentrations of natural ingredient rat and mouse diet used in chemical toxicology studies. Fundam Appl Toxicol 1987;9(2):329–38.
6. Barnard DE, Lewis SM, Teter BB, Thigpen JE. Open- and closed-formula laboratory animal diets and their importance to research. J Am Assoc Lab Anim Sci 2009;48(6):709–13.
7. Weiskirchen S, Weiper K, Tolba RH, Weiskirchen R. All you can feed: some comments on production of mouse diets used in biomedical research with special emphasis on non-alcoholic fatty liver disease research. Nutrients 2020;12(1):163.
8. Thigpen JE, Setchell KDR, Saunders H, Haseman J, Grant M, Forsythe D. Selecting the appropriate rodent diet for endocrine disruptor research and testing studies. ILAR J 2004;45(4):401–16.
9. Thigpen JE, Setchell KD, Kissling GE, Locklear J, Caviness GF, Whiteside T, Belcher SM, Brown NM, Collins BJ, Lih FB, et al. The estrogenic content of rodent diets, bedding, cages, and water bottles and its effect on bisphenol A studies. J Am Assoc Lab Anim Sci 2013;52(2):130–41.
10. Jensen MN, Ritskes-Hoitinga M. How isoflavone levels in common rodent diets can interfere with the value of animal models and with experimental results. Lab Anim 2007;41(1):1–18.
11. Kozul CD, Nomikos Athena P, Hampton TH, Warnke LA, Gosse JA, Davey JC, Thorpe JE, Jackson BP, Ihnat MA, Hamilton JW. Laboratory diet profoundly alters gene expression and confounds genomic analysis in mouse liver and lung. Chem Biol Interact 2008;173(2):129–40.
12. Mesnage R, Defarge N, Rocque LM, De Vendômois JS, Séralini GE. Laboratory rodent diets contain toxic levels of environmental contaminants: implications for regulatory tests. PLoS One 2015;10(7):e0128429.
13. Waldemarson AH, Hedenqvist P, Salomonsson AC, Häggblom P. Mycotoxins in laboratory rodent feed. Lab Anim 2005;39(2):230–5.
14. Hrncir T, Stepankova R, Kozakova H, Hudcovic T, Tlaskalova-Hogenova H. Gut microbiota and lipopolysaccharide content of the diet influence development of regulatory T cells: studies in germ-free mice. BMC Immunol 2008;9:65.
15. Wise A, Gilburt DJ. The variability of dietary fiber in laboratory-animal diets and its relevance to the control of experimental conditions. Food Cosmet Toxicol 1980;18:643–8.

16. Kwiatkowski S, Thielen U, Glenney P, Moran C. A study of Saccharomyces cerevisiae cell wall glucans. J Inst Brew 2009;115(2):151–8.

17. Pellizzon MA, Ricci MR. Effects of rodent diet choice and fiber type on data interpretation of gut microbiome and metabolic disease research. Curr Protocols Toxicol 2018;77(1):e55.

18. Wise A. Interaction of diet and toxicity—the future role of purified diet in toxicological research. Arch Toxicol 1982;50(3–4):287–99.

19. Bieri J, Stoewsand G, Briggs G, Phillips R, Woodard J, Knapka JJ. Report of the American Institute of Nutrition ad hoc committee on standards for nutritional studies. J Nutr 1977;107(7):1340–8.

20. Reeves PG, Nielson FH, Fahey GC. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition Ad Hoc Writing Committee on the reformulation of the AIN-76A rodent diet. J Nutr 1993;123:939–51.

21. Thresher JS, Podolin DA, Wei Y, Mazzeo RS, Pagliassotti MJ. Comparison of the effects of sucrose and fructose on insulin action and glucose tolerance. Am J Physiol Regul Integr Comp Physiol 2000;279(4):R1334–40.

22. Sumiyoshi M, Sakanaka M, Kimura Y. Chronic intake of high-fat and high-sucrose diets differentially affects glucose intolerance in mice. J Nutr 2006;136(3):582–7.

23. Schwarzer M, Srutkova D, Hermanova P, Leulier F, Kozakova H, Schabussova I. Diet matters: endotoxin in the diet impacts the level of allergic sensitization in germ-free mice. PLoS One 2017;12(1):1–15.

24. Brown NM, Setchell KD. Animal models impacted by phytoestrogens in commercial chow: implications for pathways influenced by hormones. Lab Invest 2001;81(5):735–47.

25. Thigpen JE, Setchell KDR, Padilla-Banks E, Haseman JK, Saunders HE, Caviness GF, Kissling GE, Grant MG, Forsythe DB. Variations in phytoestrogen content between different mill dates of the same diet produces significant differences in the time of vaginal opening in CD-1 mice and F344 rats but not in CD Sprague-Dawley rats. Environ Health Perspect 2007;115(12):1717–26.

26. Muhlhauser A, Susiarjo M, Rubio C, Griswold J, Gorence G, Hassold T, Hunt PA. Bisphenol A effects on the growing mouse oocyte are influenced by diet. Biol Reprod 2009;80:1066–71.

27. Eldridge AC, Kwolek WF. Soybean isoflavones: effect of environment and variety on composition. J Agric Food Chem 1983;31(2):394–6 .

28. Mead MN. The feed factor: estrogenic variability in lab animal diets. Environ Health Perspect 2006;114(11):A640–2.

29. Landete JM, Arqués J, Medina M, Gaya P, de Las Rivas BD, Muñoz R. Bioactivation of phytoestrogens: intestinal bacteria and health. Crit Rev Food Sci Nutr 2016;56(11):1826–43.

30. Thigpen JE, Haseman JK, Saunders HE, Setchell KD, Grant MG, Forsythe DB. Dietary phytoestrogens accelerate the time of vaginal opening in immature CD-1 mice. 2003;53(6):607–15.

31. Liu B, Edgerton S, Yang X, Kim A, Ordonez-Ercan D, Mason T, Alvarez K, McKimmey C, Liu N, Thor A. Low-dose dietary phytoestrogen abrogates tamoxifen-associated mammary tumor prevention. Cancer Res 2005;65(3):879–86.

32. Allred CD, Allred KF, Ju YH, Virant SM, Helferich WG. Soy diets containing varying amounts of genistein stimulate growth of estrogen-dependent (MCF-7) tumors in a dose-dependent manner. Cancer Res 2001;61(13):5045–50.

33. Fritz WA, Coward L, Wang J, Lamartiniere CA. Dietary genistein: perinatal mammary cancer prevention, bioavailability and toxicity testing in the rat. Carcinogenesis 1998;19(12):2151–8.

34. Ascencio C, Torres N, Isoard-Acosta F, Gómez-Pérez FJ, Hernández-Pando R, Tovar AR. Soy protein affects serum insulin and hepatic SREBP-1 mRNA and reduces fatty liver in rats. J Nutr 2004;134(3):522–9.

35. Torre-Villalvazo I, Tovar AR, Ramos-Barragán VE, Cerbón-Cervantes MA, Torres N. Soy protein ameliorates metabolic abnormalities in liver and adipose tissue of rats fed a high fat diet. J Nutr 2008;138(3):462–8.

36. Picherit C, Chanteranne B, Bennetau-Pelissero C, Davicco M-J, Lebecque P, Barlet J-P, Coxam V. Dose-dependent bone-sparing effects of dietary isoflavones in the ovariectomised rat. Br J Nutr 2001;85(03):307.

37. Heindel JJ, vom Saal FS. Meeting report: batch-to-batch variability in estrogenic activity in commercial animal diets—importance and approaches for laboratory animal research. Environ Health Perspect 2008;116(3):389–93.

38. Newberne PM. Influence on pharmacological experiments of chemicals and other factors in diets of laboratory animals. Food Sources Incidental Drug Expo 1975;34:209–18.

39. Greenman DL, Oller WL, Littlefield NA, Nelson CJ. Commercial laboratory animal diets: toxicant and nutrient variability. J Toxicol Environ Health 1980;6:235–46.

40. National Research Council. Arsenic in drinking water. Washington (DC): National Academies Press; 1999.

41. National Research Council. Arsenic in drinking water: 2001 update. Washington (DC): National Academies Press; 2001.

42. Kozul CD, Hampton TH, Davey JC, Gosse JA, Nomikos AP, Eisenhauer PL, Weiss DJ, Thorpe JE, Ihnat MA, Hamilton JW. Chronic exposure to arsenic in the drinking water alters the expression of immune response genes in mouse lung. Environ Health Perspect 2009;117(7):1108–15 .

43. Kozul CD, Ely KH, Enelow RI, Hamilton JW. Low-dose arsenic compromises the immune response to influenza A infection in vivo. Environ Health Perspect 2009;117(9):1441–7.

44. Castriota F, Zushin P-JH, Sanchez SS, Phillips RV, Hubbard A, Stahl A, Smith MT, Wang JC, La Merrill MA. Chronic arsenic exposure impairs adaptive thermogenesis in male C57BL/6 J mice. Am J Physiol Metab 2020; doi: 10.1152/ajpendo.00282.

45. Alshannaq A, Yu JH. Occurrence, toxicity, and analysis of major mycotoxins in food. Int J Environ Res Public Health 2017;14(6):632.

46. Whitlow L, Hagler W, Diaz D. Mycotoxins in feeds. Feedstuffs 2010;74–84.

47. Yiannikouris A, Jouany J-P. Mycotoxins in feeds and their fate in animals: a review. Anim Res 2002;51:81–99.

48. Kouadio JH, Dano SD, Moukha S, Mobio TA, Creppy EE. Effects of combinations of Fusarium mycotoxins on the inhibition of macromolecular synthesis, malondialdehyde levels, DNA methylation and fragmentation, and viability in Caco-2 cells. Toxicon 2007;49(3):306–17.

49. Porter JK, Wray EM, Rimando AM, Stance PC, Bacon CW, Voss KA. Lactational passage offusaricacid from the feed of nursing dams to the neonate rat and effects on pineal neurochemistry in the F1 And F2 generations at weaning. J Toxicol Environ Health 1996;49(2):161–75.

50. Underhill KL, Rotter BA, Thompson BK, Prelusky DB, Trenholm HL. Effectiveness of cholestyramine in the detoxification of zearalenone as determined in mice. Bull Environ Contam Toxicol 1995;54(1):128–34.

51. Yakar S, Nunez NP, Pennisi P, Brodt P, Sun H, Fallavollita L, Zhao H, Scavo L, Novosyadlyy R, Kurshan N, et al. Increased tumor growth in mice with diet-induced obesity: impact of ovarian hormones. Endocrinology 2006;147(12):5826–34.

52. Kilcast D. Effect of irradiation on vitamins. Food Chem 1994;49:157–64.

53. Caulfield CD, Cassidy JP, Kelly JP. Effects of gamma irradiation and pasteurization on the nutritive composition of commercially available animal diets. J Am Assoc Lab Anim Sci 2008;47(6):61–6.

54. Cebra JJ. Influences of microbiota on intestinal immune system. Am J Clin Nutr 1999;69(Suppl):1046S.

55. Dollive S, Chen Y, Grunberg S, Bittinger K, Hoffmann C, Vandivier L, Cuff C, Lewis JD, Wu GD, Bushman FD, et al. Fungi of the murine gut: episodic variation and proliferation during antibiotic treatment. PLoS One 2013;8(8):e71806.

56. Irvin A, Cockburn A, Primerano DA, Denvir J, Boskovic G, Infante AM, Wu GD, Cuff CF. Diet-Induced alteration of the murine intestinal microbiome following antibiotic ablation. Biomed Life Sci 2017;7(7):545–64.

57. Shukla Y, Arora A, Singh A. Tumourigenic studies on deltamethrin in Swiss albino mice. Toxicology 2001;163(1):1–9.

58. Rodriguez-Vivas RI, Li AY, Ojeda-Chi MM, Trinidad-Martinez I, Rosado-Aguilar JA, Miller RJ, Pérez de León AA. ln vitro and in vivo evaluation of cypermethrin, amitraz, and piperonyl butoxide mixtures for the control of resistant Rhipicephalus (Boophilus) microplus (Acari: Ixodidae) in the Mexican tropics. Vet Parasitol 2013;197(1–2):288–96.

59. George J, Prasad S, Mahmood Z, Shukla Y. Studies on glyphosate-induced carcinogenicity in mouse skin: a proteomic approach. J Proteomics 2010;73(5):951–64.

60. Séralini GE, Clair E, Mesnage R, Gress S, Defarge N, Malatesta M, Hennequin D, de Vendômois JS. Republished study: long-term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. Environ Sci Eur 2014;26(1):1–17.

61. Schecter AJ, Olson J, Papke O. Exposure of laboratory animals to polychlorinated dibenzodioxins and polychlorinated dibenzofurans from commerical rodent chow. Chemosphere 1996;32(3):501–8.

62. Zhang L, Nichols RG, Patterson AD. The aryl hydrocarbon receptor as a moderator of host-microbiota communication. Curr Opin Toxicol 2017;2:30–5.

63. Ito S, Chen C, Satoh J, Yim S, Gonzalez FJ. Dietary phytochemicals regulate whole-body CYP1A1 expression through an arylhydrocarbon receptor nuclear translocator-dependent system in gut. J Clin Invest 2007;117(7):1940–50.

64. Li Y, Innocentin S, Withers DR, Roberts NA, Gallagher AR, Grigorieva EF, Wilhelm C, Veldhoen M. Exogenous stimuli maintain intraepithelial lymphocytes via aryl hydrocarbon receptor activation. Cell 2011;147(3):629–40.

65. Stevenson L, Phillips F, O'Sullivan K, Walton J. Wheat bran: Its composition and benefits to health, a European perspective. Int J Food Sci Nutr 2012;63(8):1001–13.

66. Vitaglione P, Napolitano A, Fogliano V. Cereal dietary fibre: a natural functional ingredient to deliver phenolic compounds into the gut. Trends Food Sci Technol 2008;19(9):451–63.

67. den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud D-J, Bakker BM. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. J Lipid Res 2013;54(9):2325–40.

68. Ericsson AC, Gagliardi J, Bouhan D, Spollen WG, Givan SA, Franklin CL. The influence of caging, bedding, and diet on the composition of the microbiota in different regions of the mouse gut. Sci Rep 2018;8(1):1–13.

69. Jovel J, Patterson J, Wang W, Hotte N, O'Keefe S, Mitchel T, Perry T, Kao D, Mason AL, Madsen KL, et al. Characterization of the gut microbiome using 16S or shotgun metagenomics. Front Microbiol 2016;7:1–17.

70. Dalby MJ, Ross AW, Walker AW, Morgan PJ. Dietary uncoupling of gut microbiota and energy harvesting from obesity and glucose tolerance in mice. Cell Rep 2017;21:1521–33.

71. Chassaing B, Miles-Brown J, Pellizzon M, Ulman E, Ricci M, Zhang L, Patterson AD, Kumar MV, Gewirtz AT. Lack of soluble fiber drives diet-induced adiposity in mice. Am J Physiol Gastrointest Liver Physiol 2015;309:G528–41.

72. Hwang IS, Ho H, Hoffman BB, Reaven GM. Fructose-induced insulin resistance and hypertension in rats. Hypertension 1987;10(5):512–6 .

73. Pagliassotti MJ, Prach PA. Quantity of sucrose alters the tissue pattern and time course of insulin resistance in young rats. Am J Physiol 1995;269(3 Pt 2):R641–6.

74. Pagliassotti MJ, Prach PA, Koppenhafer TA, Pan DA. Changes in insulin action, triglycerides, and lipid composition during sucrose feeding in rats. Am J Physiol 1996;271(5 Pt 2):R1319–26.

75. Mattson DL, Kunert MP, Kaldunski ML, Greene AS, Roman RJ, Jacob HJ, Cowley AW. Influence of diet and genetics on hypertension and renal disease in Dahl salt-sensitive rats. Physiol Genomics 2004;16(2):194–203.

76. Fullerton FR, Greenman DL, Bucci TJ. Effects of diet type on incidence of spontaneous and acetylaminofluorene-induced liver and bladder tumors in Balb/c mice fed AIN-76A versus NIH-07 diet. Fundam Appl Toxicol 1992;18:193–9.

77. Lien EL, Boyle FG, Wrenn JM, Perry RW, Thompson CA, Borzelleca JF. Comparison of AIN-76A and AIN-93 G diets: a 13-week study in rats. Food Chem Toxicol 2001;39(4):385–92.

78. Reeves PG, Rossow KL, Lindlauf J. Development and testing of the AIN-93 purified diets for rodents: results on growth, kidney calcification and bone mineralization in rats and mice. J Nutr 1993;123:1923–31.

79. Delzenne NM, Daubioul C, Neyrinck A, Lasa M TH. Inulin and oligofructose modulate lipid metabolism in animals: review of biochemical events and future prospects. Br J Nutr 2002;87(Suppl 2):S255–9.

80. Delzenne NM, Cani PD, Neyrinck AM. Modulation of glucagon-like peptide 1 and energy metabolism by inulin and oligofructose: experimental data. J Nutr 2007;137(11 Suppl):2547S–51S.

81. Zou J, Chassaing B, Singh V, Pellizzon M, Ricci M, Fythe MD, Kumar MV, Gewirtz AT. Fiber-mediated nourishment of gut microbiota protects against diet-induced obesity by restoring IL-22-mediated colonic health. Cell Host Microbe 2018;23:1–13.

82. Chassaing B, Vijay-Kumar M, Gewirtz AT. How diet can impact gut microbiota to promote or endanger health. Curr Opin Gastroenterol 2017;33(6):417–21.