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Review Article

# Food matrix and the microbiome: considerations for preclinical chronic disease studies

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ABSTRACT

Animal models of chronic disease are continuously being refined and have evolved with the goal of increasing the translation of results to human populations. Examples of this progress include transgenic models and germ-free animals conventionalized with human microbiota. The gut microbiome is involved in the etiology of several chronic diseases. Therefore, consideration of the experimental conditions that may affect the gut microbiome in preclinical disease is very important. Of note, diet plays a large role in shaping the gut microbiome and can be a source of variation between animal models and human populations. Traditionally, nutrition researchers have focused on manipulating the macronutrient profile of experimental diets to model diseases such as metabolic syndrome. However, other dietary components found in human foods, but not in animal diets, can have sizable effects on the composition and metabolic capacity of the gut microbiome and, as a consequence, manifestation of the chronic disease being modeled. The purpose of this review is to describe how food matrix food components, including diverse fiber sources, oxidation products from cooking, and dietary fat emulsifiers, shape the composition of the gut microbiome and influence gut health.

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Abbreviations: AI, adequate intake; AV, anisidine value; CMC, carboxymethyl cellulose; CML, N-carboxymethyl-lysine; IOM, Institute of Medicine; MRP, Maillard reaction product; NHANES, National Health and Nutrition Examination Survey; P80, polysorbate 80; PUFA, polyunsaturated fatty acid; PV, peroxide value; SCFA, short-chain fatty acid; T2D, type 2 diabetes.

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## 1. Introduction

The role of the gut microbiome in health and disease has received considerable attention over the last decade. The gut microbiome is the most diverse and complex community of microorganisms in the body, consisting of more than a thousand bacterial species [1]. The relationships between these bacteria and the host are generally commensal or symbiotic in nature. For instance, it is estimated that humans obtain approximately 10% of their daily energy intake from short-chain fatty acids (SCFAs) derived from microbial fermentation [2]. Alternatively, many studies have been performed examining the relationship between the gut microbiome and health, and it is now known that dysbiosis of the gut microbiome is associated with numerous diseases, including metabolic syndrome, inflammatory bowel syndrome, and colorectal cancer [3–5]. Much of this pioneering work has been performed using preclinical models. Rodent models of human disease have been invaluable for mechanistic studies, in part because experimental variation can be tightly controlled among investigations. The use of inbred rodent strains, standardized environments, and semipurified diets has helped reduce experimental variability and allowed for investigations from different laboratories to be compared and replicated. Moreover, gnotobiotic or antibiotic depletion protocols can be used along with microbiota transfer to investigate whether the gut microbiome is correlative or causative in disease models.

An assumption in the use of animal models in biomedical and nutrition research is that the results will be of value to human well-being [6]. Yet, it is estimated that up to 80% of therapeutics fail in humans after being previously shown to be safe and (or) effective in rodents [7]. Two possible explanations for this discordance were suggested by Ioannidis [6]. The first may lie in fundamental physiological differences between the animal model and humans, both in healthy and in diseased conditions. The second reason interventions may work in one species but not in another may be a result of study design and/or publishing bias toward negative results. In terms of study design, factors such as dose and duration may differ significantly between species. Brown et al suggested that a 4-week study in rats with a lifespan of 2.5 years would be similar to a 2-year study in humans due to lifespan differences [14]. Because most nutrition intervention studies in the literature are at least 4 weeks in length, study duration does not seem to be a limiting factor.

Since 2015, the utility of laboratory mice for investigations on human microbiota has been subject to critical review in 3 articles [8–10]. In documenting advantages and disadvantages of mice compared to humans, Nguyen et al [9] list several advantages: the model allows for invasive intervention to investigate causal relationships; genetic information is robust and well curated; the model has a relatively short life span; mice are omnivorous; and the overall structure of the gastrointestinal tract is somewhat similar to humans. Moreover, the use of inbred mice strains reduces interindividual variation in experimental groups, and environmental conditions can be controlled. Conversely, although the overall gastrointestinal tract is similar, the relative size of its organ

components differs, as does villus architecture. Unlike humans, rodents ferment indigestible substrates in the cecum, whereas fermentation in humans happens primarily in the colon. Humans and rodents also differ in stool consistency; rodent stool is typically drier than human, suggesting differential osmotic regulation in the colon. Importantly, the cross talk between the microbiota and host is host specific, and thus, a human microbiome may not be recapitulated in total in mice [9]. In a second review, Hugenholtz and de Vos point out that, although many genera are shared between rodents and humans, only 4% of the bacterial genes share considerable identity [8]. It is likely that basal rodent diets account for some of these differences between mice and human microbiomes. Compounding this problem, inbred mice have been shown to have a different and less resilient microbiome compared to wild mice. Interestingly, in a study by Rosshart et al [11], C57BL/6 mouse embryos were transferred into wild mice to recapitulate the wild mouse microbiome in a laboratory setting. In subsequent studies, the resulting mice more closely resembled humans in terms of immune phenotype compared to conventional mice.

One factor driving interest in the microbiota and health is a series of seminal studies that showed an obese phenotype could be induced in a germ-free reared mouse via transplantation of microbiota from obese individuals [12–14]. This work led to the characterization of an “obese microbiome” typified by an increased Firmicutes to Bacteroidetes ratio, reduced diversity, and an increased metabolic capacity to extract energy from the diet [14]. Recently, Dalby et al showed that obesity in high-fat-fed mice is driven primarily by the fat in the diets, not by increased energy harvest due to changes in the microbiome and cecal fermentation [15]. Although many factors have been shown to affect the composition of the microbiota, a recent study by our group found that diet is a primary factor [16]. In the study, the native microbiome of mice was depleted using a cocktail of broad-spectrum antibiotics. Mice then were inoculated with human microbiota from either lean or obese donors. Mice were fed either the standard AIN93G diet; the total Western diet, which is a basal diet that models typical micro- and macronutrient intakes based on National Health and Nutrition Examination Survey (NHANES) data [17]; or a diet-induced obesity diet (diet with 45% of energy as fat). Interestingly, after 22 weeks, diet had the largest impact on the microbiota composition and body weight gain, although some notable, significant differences remained among mice that received the microbiota of different donor types.

Collectively, these data suggest that the choice of basal diet used in preclinical studies to investigate connections between the gut microbiome and systemic health or disease deserves careful consideration. Moreover, to improve translatability of preclinical studies, it is also critical to use basal diets that are relevant to patterns of human nutrition. We performed a literature search to identify preclinical studies that examined the impact of food matrix components including fiber, protein, lipid oxidation products, and emulsions and their effects on gut and metabolic health. In this review, sources of variation between human diets and basal diets used in rodent studies and the possible implications of

these components to the translatability of preclinical models to humans will be examined.

## 2. Basal rodent diets and fiber

The composition and metabolic activity of the gut microbiota influence many aspects of health. Consequently, there is interest in dietary strategies to improve health via modulating the microbiota. Mice are the most common model organisms used to study the microbiota, yet the translational relevance to humans has been questioned [8–10]. Dietary fiber has a large impact on the microbiota composition and metabolic activity via provision of fermentable substrates [18]; yet, to date, there has been very little attention paid to modeling the fiber composition of rodent diets to that of humans. Thus, there is a critical need to establish how the microbiota of mice responds to the range of human dietary fiber intakes and whether the changes are consistent with health benefits provided by dietary fiber in humans.

According to data from NHANES, the average American diet contains 60% of the Adequate Intake (AI) of dietary fiber [19,20] recommended by the Institute of Medicine (IOM). The AI for fiber is scaled to energy and is 14 g per 4184 kJ, which often is simplified to 25 g/d for women and 38 g/d for men. According to *What We Eat in America*, the 2015–2016 NHANES, Americans eat an average of 16.7 g of dietary fiber per 8807 kJ (~8 g/4184 kJ [21]. In 2001, the Food and Nutrition Board of the Institute of Medicine set the AI for total fiber at 14 g/4184 kJ [22], which was based on a number of prospective cohort studies that showed significant reductions in the risk for coronary heart disease [23–25] and type 2 diabetes [26,27] in individuals consuming the most fiber. It is estimated that only ~5% of Americans consume the AI for fiber [28], whereas the average intake (from NHANES) is only 60% of the recommended level. Fiber intakes have increased about 20% since the 2001–2002 NHANES survey yet are still far less than levels shown in prospective cohort studies to reduce the risk of heart disease and diabetes. Evidence from prospective cohort studies and randomized clinical trials suggests that increasing fiber intake reduces gut and systemic inflammation and provides protection against the development of coronary heart disease and type 2 diabetes (T2D) [29–31]. In a recent meta-analysis, the risk for a number of clinical outcomes, such as coronary heart disease and T2D, was reduced when the fiber intake was between 25 and 29 g/d [32]. Interestingly, dose-response curves from this study indicated that higher intakes might even provide more protection.

Although the evidence that dietary fiber promotes gut and metabolic health is well documented in humans, the mechanisms via which this is done are not well known. The difference between recommended and actual intakes of fiber has been called the *fiber gap* [33]. Evidence-based strategies to address the fiber gap are needed. It has been suggested this may be achieved via increased consumption of foods with high intrinsic fiber levels, as well as consumption of processed foods where fiber is increased via supplementation of natural or synthetic fibers [34]. It has been shown in humans that diet rapidly changes the structure and activity of the microbiota [35]. Compared to a diet primarily composed of

animal products, a diet rich in plant polysaccharides increased the abundance of carbohydrate-metabolizing microbes including *Rosburia*, *Eubacterium rectale*, and *Ruminococcus bromii*. In humans, dietary fiber at the AI of the IOM has been shown to alleviate T2D via a mechanism involving the microbiota [36]. Zhao et al fed type 2 diabetic patients diets that contained either 15 or 37 g/d dietary fiber for 12 weeks. The high-fiber diet caused a selective increase in microbes that produce SCFAs and was associated with lower hemoglobin A1c and increased glucagon-like peptide-1 production. For mice to be valuable surrogates for human microbiota research, a similar phenotype should result from feeding mice diets with fiber levels at the IOM AI.

A shortcoming of semipurified diets for chronic-disease and microbiome research is that the fiber source is cellulose. Semipurified rodent diets do not contain an adequate diversity of fermentable substrates, and over multiple generations, the microbiomes of mice fed these diets progressively lose diversity [18]. In 1980, Wise and Gilbert noted that semipurified rodent diets are analogous to Western diets, whereas chow is more like an unrefined African diet [37]. Interestingly, in 2017, De Filippo et al compared the microbiomes of children from the African nation of Burkina Faso who consumed traditional diets rich in cereals, legumes, and vegetables to Italian children who consumed Western diets [38]. The fecal SCFAs were roughly 3-fold higher in the African children consuming traditional diets compared to the urban Italian children. Using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States, they inferred functional differences in the microbiomes between the children and found that the rural African microbiomes were enriched in genes for complex carbohydrate metabolism. A possible link between low fiber intakes, gut carbohydrate metabolism, and gut inflammation was revealed in gnotobiotic mice by inoculating them with a defined human microbiota of 16 fully sequenced species [39]. Mice were fed either a chow diet with ~50 g fiber/1000 kcal or a semipurified diet that contained ~20 g cellulose/1000 kcal. Compared to the microbiome of mice fed the fiber-rich diet, the microbiome of mice fed the diet with only cellulose was enriched in the metabolic capacity to consume host-derived mucins. This change was also associated with a degraded mucin layer and closer proximity of the luminal microbes to the intestinal epithelium. Mice with degraded mucin layers also had increased fecal lipocalin, a neutrophil protein associated with low-grade inflammation and decreased colon length. Others have also shown that defined diets containing cellulose cause a loss of cecal and colonic mass as compared to chow diets and that this effect can be ameliorated by replacing cellulose with inulin [40].

The use of different fiber types in formulating experimental diets for preclinical models of chronic disease often leads to changes in the disease phenotype and the composition and/or function of the intestinal microbiome. Moen et al [41] fed APC<sup>Min/+</sup> mice the AIN93M diet with cellulose, inulin, or brewers spent grain as the fiber source at either 5 or 15% wt/wt of the diet. Mice fed inulin as the fiber source had a lowered colonic tumor burden and decreased cecal  $\alpha$  diversity compared to the other treatments. Moreover, the fiber type appeared to drive distinction of the gut microbiomes and

explained 28.8% of the taxa variation among treatment groups. Dietary fiber has also been studied in relation to colitis. Mice challenged with dextran sodium sulfate and fed a semipurified diet had increased colitis severity compared to chow-fed controls, and this phenotype could not be rescued by replacing cellulose in the semipurified diet with inulin [42]. Using an IL-10 receptor antibody colitis model, Singh et al [43] fed mice either diets containing exclusively cellulose or diets supplemented with inulin or pectin. They reported that pectin, but not inulin, suppressed colitis in this model, and this effect was mediated through decreased butyrate production. Mice fed the inulin-containing diet had increased butyrate-producing bacteria, including *γ-Proteobacteria*.

Dietary fiber manipulations have also been used to study effects on obesity in the context of high-fat diets. In a study that compared inulin, cellulose, and guar gum as fiber sources in high-fat diets, Weitkumat et al [44] reported that inulin protected against weight gain compared to the cellulose and guar gum treatments. The fiber manipulations also caused differences in the gut microbiomes. Mice fed the inulin diet had increased *Bifidobacterium animalis*, whereas the guar gum-fed mice had increased *Bifidobacterium pseudolongum*. Drew et al [45] compared a traditional high-fat diet with cellulose as the fiber source to high-fat diets with 5% of the cellulose and 5% of cornstarch replaced with  $\beta$ -glucan, pectin, inulin, or 3 different inulin SCFA esters (inulin acetate ester, inulin propionate ester, inulin butyrate ester or a combination of inulin propionate ester and inulin butyrate). Mice were fed the experimental diets for 8 weeks. Mice fed the traditional high-fat diet gained more weight and had increased fat mass compared to all of the high-fat treatments with added fiber, suggesting a protective effect of inulin,  $\beta$ -glucan, pectin, and inulin SCFA esters against obesity. However, despite having similar effects in terms of obesity prevention, all pairwise  $\beta$  diversity comparisons among inulin, pectin, and  $\beta$ -glucan treatments revealed distinct microbiomes, suggesting that the antiobesity properties of these diets were independent of the microbiome composition.

Taken together, these studies suggest that fiber is not an inert dietary component and can have large effects on disease endpoints commonly assessed in preclinical studies. Dietary fiber also plays a major role in shaping the microbiome. These studies also suggest that semipurified diets that contain cellulose such as the AIN93G series may exacerbate chronic disease. Therefore, care must be taken in interpreting the microbiome and disease endpoint results of preclinical studies that use different fiber sources, that is, chow diets vs semipurified diets containing cellulose.

The AI for fiber set by the IOM does not specify specific fiber types, and thus, it is not clear how to model human fiber intakes in rodent semipurified diets [19]. However, one strategy to address this would be to determine the fiber content of a recommended 1-day meal plan. Table 1 shows a 1-day meal plan included in the IOM report that provides 38 g of dietary fiber. In 1997, Marlett and Cheung [46] published a fiber profile for 228 commonly consumed foods. For each food, the soluble and insoluble fiber content was determined. For soluble fiber, further chemical tests were run for hemicellulose, pectin, and  $\beta$ -glucan. For the insoluble fiber, the cellulose, hemicellulose, pectin, and lignin contents were

**Table 1 – Example of a high-fiber diet suggested by the IOM<sup>a</sup>**

Meal	Foods eaten	Energy (kj)	Total fiber (g)	
Breakfast	Grapefruit (1/2 medium)	159	1.4	
	Banana (1 medium)	456	2.8	
	Cereal, shredded oats (1 cup)	469	3	
	English muffin (white, 1 whole)	561	1.5	
	Margarine (2 tsp)	285	0	
Snack	Milk, 1% (1 cup)	427	0	
	Crackers, whole wheat (6 each)	456	0.9	
	Cheddar cheese (1.5 oz)	716	0	
Lunch	Juice (3/4 cup)	326	0.4	
	Tossed salad (1 cup)	67	1.5	
	Salad dressing (1 tbs)	276	0	
	Chili with beans and beef (1 cup)	1142	6.5	
	Cornbread (1 piece)	724	1.3	
	Grapes (1/2 cup)	142	0.8	
	Fig bar cookies (2)	238	1.5	
	Milk, 1% (1 cup)	427	0	
	Dinner	Salmon in soy sauce (3.5 oz)	707	0.2
		Rice with vegetables (3/4 cup)	699	1.4
Broccoli (1-1/2 cup)		167	4.4	
Roll, whole wheat (2 medium)		740	5	
Ice cream (1/2 cup)		410	0.3	
Snack	Carrots, raw (12 medium baby)	213	3.6	
	Spinach dip (2 tbs)	243	0.4	
	Turkey sandwich	1435	1.2	
	Cola	640	0	
	Total	12878	38.1	

<sup>a</sup> Adapted from [19].

evaluated. Using this database and the food list in Table 1, we determined that the fiber profile in the 1-day diet is approximately 80% insoluble and 20% soluble. The fiber in this proposed diet was approximately 4%  $\beta$ -glucan, 15% pectin, 37% hemicellulose, 29% cellulose, and 15% lignin. Although cellulose, pectin, and oat-derived  $\beta$ -glucan are commercially available, there are no purified sources of hemicellulose or lignin. However, there are reports of an oat fiber product composed primarily of cellulose (70%), hemicellulose (25%), and lignin (5%) [47]. Although it is not currently possible to precisely model the recommended IOM intake for mice currently, a mix of pectin,  $\beta$ -glucan, and the oat fiber listed above should be an improvement on cellulose alone.

### 3. Food processing and the microbiome

In terms of providing substrates to the gut microbiome, human diets are much more complicated than the sum of their macro- and micronutrient components. Cooking and processing of food can lead to the introduction of protein and lipid oxidation products into the diet. Moreover, dietary

emulsifiers are commonly added to foods to improve sensory aspects of processed foods. All of these food matrix factors can independently affect health and the composition of the microbiome but are rarely considered in preclinical disease models despite being common components in human diets. In a recent investigation by Johnson et al, human volunteers kept 24-hour food records, and their gut microbiomes were tracked for 17 days. The dietary records were then used to predict changes to the microbiome. They reported that conventional methods of describing foods based on nutrients were a poor predictor of the microbiome composition compared to a whole food-based, hierarchical tree of foods developed for the study [48]. This observation suggests that the food matrix is more important for shaping the microbiome than the micro- and macronutrient content of the diet. Aside from the nutrient content, varying methods of food processing or preparation may also impact the composition and/or function of the gut microbiome. For example, one study explored the impact of consumption of raw or cooked meat and tubers on the gut microbiome in mice [49]. Researchers noted that differences in the mice microbiomes were evident when comparing raw vs cooked tubers but not raw vs cooked meat. Also,  $\alpha$  diversity was lower in mice fed raw tubers compared to cooked tubers, whereas Bacteroidetes were increased [49].

The Maillard reaction, which is also known as *nonenzymatic browning*, is the result of the reaction between a reducing sugar and a free amino group of a protein, or nucleic acid. The Maillard reaction is very important in foods because it contributes to the formation of flavors and golden-brown color. However, it is a very complicated reaction, and some of the products may be toxic. In addition, the Maillard reaction may reduce the nutritional value of foods by destroying essential amino acids, such as lysine. Maillard reaction products (MRPs) usually are classified into 3 groups: early MRPs, advanced MRPs, and melanoidins [50].

N-carboxymethyl-lysine (CML) is an advanced MRP that is typically measured in food, and the overall load of dietary MRPs is often inferred from the CML content. Animal studies have shown that high intakes of MRPs result in increased plasma levels of CML, and up to 30% of dietary CML is absorbed from the gut [51]. Since 2002, a number of published studies in mice and rats have shown that diets high in MRPs (as specifically measured by CML) may promote many of the chronic metabolic diseases common in the United States such as diabetes and nonalcoholic fatty liver and cardiovascular diseases [52]. Diets with high CML loads have been shown to promote glucose intolerance [53–55], liver inflammation [54,56,57], and cardiac inflammation [58] when compared to diets lower in CML. In addition, a recent study found that a diet high in MRPs was associated with negative changes in gut health including an increase in inflammation, a reduction in fecal SCFAs, and a reduction in the diversity of the fecal microbiome [59]. In that study, rats were fed an AIN93G-based diet containing either 2.79 or 14.43 mg CML/kg diet for 18 weeks. Rats fed the high-CML diets had decreased  $\alpha$  diversity and distinct cecal microbiomes when compared using  $\beta$  diversity analysis. Specifically, the high-CML group had increased Proteobacteria, *Allobaculum*, *Bacteroides* and decreased *Alloprevotella* and Ruminococcaceae. The high-CML-fed rats also had higher levels of cecal ammonia

suggesting increased protein fermentation. Yet, other studies with MRPs have suggested they may have at least some potential to improve gut health. Anton et al [60] used a colitis model in mice and provided them with a control diet and experimental diets with MRPs produced via 2 methods. Mildly heated pellets were autoclaved at 120°C for 30 minutes, and the highly heated pellets were baked at 150°C for 15 minutes after pellets were rehydrated with 30% (wt/wt) water. Both the mild- and high-heat pellets had significantly more CML than the control pellets, and the high-heat pellets had more soluble melanoidins than both the mild-heat and control pellets. Mice were provided with 3% DSS in the drinking water for 8 days, and the inflammatory response was subsequently measured in the colon. Interestingly, the high-heat pellets, rich in melanoidins, reduced the macroscopic damage score and colon shortening and decreased myeloperoxidase activity.

A few small, randomized, crossover studies with dietary MRPs have been conducted in humans, and 3 meta-analyses have compiled the data in an effort to distill the relevant effects on health [61–63]. Overall, the main findings were that increasing MRPs in the diet is associated with mild yet significant systemic inflammation (as measured by plasma tumor necrosis factor- $\alpha$ ) as well as elevated oxidative stress (as measured by plasma 8-isoprostanes). Seiquer et al [64] fed 20 male adolescents diets containing either 6.62 or 15.72 mg CML/100 g protein and rats a high- or low-CML diet (2.2 vs 12.46 mg CML/kg diet). Lactobacilli were lower in the high-CML diets for both humans and rats and were the only taxon that changed in the same direction in response to CML in both humans and rats. Taken together, the rodent and human studies listed above suggest that it would be beneficial to reduce intake of MRPs, yet there is a significant methodological flaw across these studies that limits our ability to specifically implicate MRPs. This flaw is due to the method by which the MRPs were introduced into the diet. For example, in all the rodent studies, MRPs were produced in the diet by heating the diet pellets at elevated temperatures (125–160°C) for specific time periods (30 minutes to 3 hours), and the control diets were unheated diet pellets. In addition to inducing MRP formation, these time-temperature treatments also induce thermal destruction of vitamins and oxidation of the fat in the diet. Consequently, it is currently unclear to what extent the metabolic dysregulation in these studies can be attributed to MRPs specifically vs the combination with oxidized lipids and decreased vitamin intakes.

In food systems, lipid oxidation affects food quality and the acceptance by consumers [65]. Deleterious changes in foods caused by lipid oxidation include development of off-flavors, and loss of color, nutrition, and functionality. Oxidation consists of 3 stages: initiation (the formation of radicals), propagation (free-radical chain reactions), and termination (the formation of nonradical products). The production of free-radicals generates cytotoxic compounds and co-oxidizes vitamins. In the food industry, the oxidation status of food fats is typically determined by 2 measurements, peroxide value (PV), and anisidine value (AV). PV measures the first step of lipid oxidation (ie, the presence of lipid hydroperoxides), and AV measures the aldehyde break-down products of the lipid hydroperoxides. As a fat becomes more oxidized, the PV starts to decline, and the AV increases. A high

PV and low AV suggest that an oxidized fat contains primary oxidation products and vice versa.

The primary lipids that become oxidized in foods are the essential polyunsaturated fatty acids (PUFAs), such as linoleic acid and linolenic acid, as well as the long-chain highly unsaturated PUFAs arachidonic and docosahexaenoic acids [66]. The rate of oxidation of these fatty acids depends on the degree of unsaturation, and the more highly unsaturated the fatty acid is, the faster the reaction occurs. Oxidation of PUFA leads to the formation of hydroperoxides (initiation stage), and depending on the conditions, these may undergo fragmentation to produce reactive aldehyde products (promotion stage), which covalently bond with nucleophilic groups in proteins and nucleic acids. This results in a broad range of protein and DNA adducts and promotes oxidative stress [67]. Consequently, depending on its composition and processing history, a food fat may have a different profile of oxidation products. For example, a PUFA-rich oil held at room temperature for a long period will be rich in hydroperoxides (measured by PV) yet low in reactive aldehydes (measured by AV). Conversely, some fats are oxidized at very high temperatures (ie, 175°C) when used to fry foods, and such “thermally abused” oils tend to be low in hydroperoxides and rich in reactive aldehydes. Although the toxicity of the aldehydes produced in the latter stages of lipid oxidation is well characterized, it has been suggested more recently that the lipid hydroperoxides, common in oils oxidized at lower temperatures, may decompose in the digestive tract and promote gut inflammation and dysfunction [68]. In the last century in the United States, dietary intake of linoleic acid (18:2n6; omega-6) has increased from 2.79% of energy to 7.21% of energy, whereas consumption of linolenic acid (18:3n3; omega-3) has increased from 0.39% to 0.72% of energy [69]. Many foods are now supplemented with the long-chain omega-3 PUFA. In addition, many foods in the Western diet are consumed either heated or fried, which also promotes lipid oxidation [66]. Thus, the Western diet contains significant quantities of oxidized fatty acids, oxidized cholesterol, and aldehyde breakdown products [70].

In experimental animal models and humans, consumption of oxidized fats, compared to control diets, has been shown to promote harmful systemic physiological responses such as oxidative stress, endothelial dysfunction, and hypertension [71]. In addition, the oxidation products of dietary fats have been implicated in the etiology of many chronic Western diseases such as cancer and cardiovascular disease [72]. Oxidized dietary fats also promote glucose intolerance via mechanisms involving oxidative stress [73]. Chiang et al found that adding oxidized fats to the diets of mice caused a decrease in glucose tolerance, as measured by an oral glucose tolerance test, and the effect was due to decreased insulin production in the pancreas [74]. Interestingly, increasing the vitamin E content of the diet prevented the oxidative stress and subsequent glucose intolerance. Lastly, oxidized dietary fats also appear to promote intestinal oxidative stress and inflammation, again via an oxidative stress mechanism [71,75].

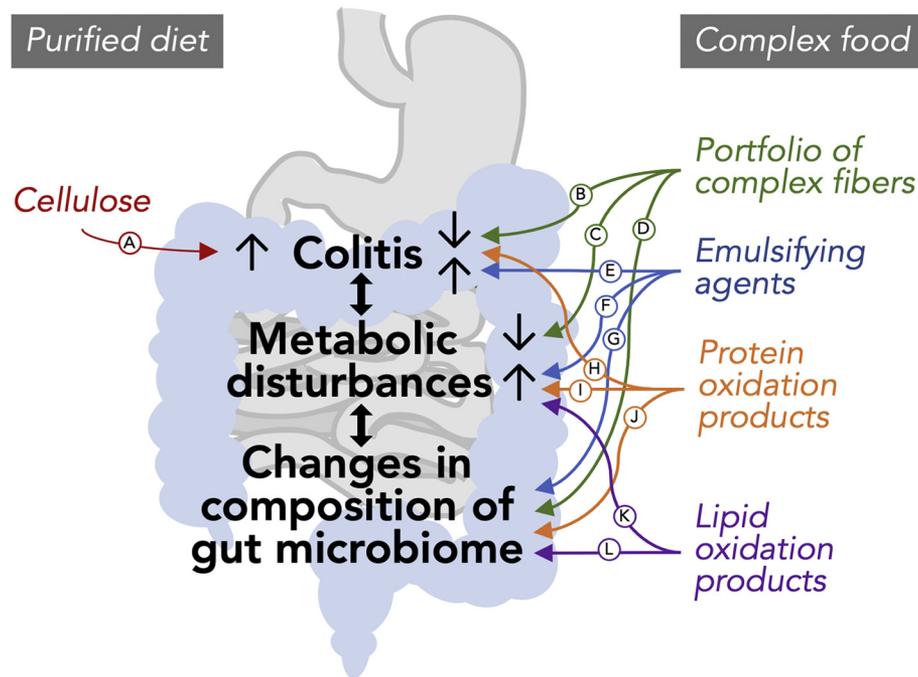
Although endogenous lipid peroxidation is a well-known factor in the development of chronic disease, the designs of the studies cited above involve comparison of oxidized oils to

unheated controls and effects on chronic disease. In many of the animal studies, the oils were subjected to severe thermal treatments, and the levels of oxidation products are probably much higher than would be consumed by humans. Although, in some cases, care was taken to gently oxidize the oils, and significant effects were nonetheless reported [75]. However, deep-fried safflower or olive oil does adversely affect endothelial function in humans [76], and in a Spanish cohort, where olive and sunflower oils are commonly used for deep-frying, consumption of fried foods was not associated with heart disease or all causes of mortality [77].

There have been very few carefully controlled studies examining oxidized fats on the microbiome. In a large-scale population-based study, fried food consumption was correlated with lowered  $\alpha$  diversity, whereas foods such as raw vegetables, eggs, fish, and raw fruit were associated with higher  $\alpha$  diversity [78]. Zhou et al [79] compared the effects of nonheated vs deep-fried canola oil on the microbiomes of rats fed a chow diet. In this study, 1.5 mL of either oil was gavaged daily for 6 weeks. Rats treated with the control or deep-fried oils had distinct microbiomes as assessed by  $\beta$  diversity analysis, but there were no differences in  $\alpha$  diversity. When Linear discriminant analysis effect size (LEfSe) analysis was performed, the top discriminating taxon for the deep-fried oil treatment was an enrichment of *Allobaculum*, *Lactobacillus*, *Bacteroides*, *Oscillospira*, and *Bifidobacterium* spp relative to the control oil. These studies suggest that the oxidation of fat, a common byproduct of thermal processing, affects the microbiome independent from protein oxidation products; however, how these changes to the microbiome might affect chronic disease risk in humans is not known.

Emulsions are heterogeneous systems composed of water and an oil phase stabilized by an emulsifier such as whey protein or polysorbate 80 (P80). Emulsions can be broadly classified into oil-in-water emulsions, where the oil phase is dispersed in the form of droplets in the water phase, and water-in-oil emulsions, where the water phase is dispersed in the continuous oil phase. Examples of oil-in-water food emulsions include dressings, cream, and milk, whereas examples of water-in-oil emulsions include butter, margarines, and spreads. Not only are emulsions used in food systems to allow for the coexistence of 2 immiscible phases, but they are also commonly used to deliver flavors or nutrients. In the case of a liquid emulsion, these compounds can be delivered in a beverage, spread, or dressing.

In foods, emulsifiers are often used with texture modifiers and weighting agents to modulate food structure, and all 3 food additives may affect the microbiome via direct and indirect mechanisms. In a recent review, Halmos et al [80] covered the most common emulsifiers and listed areas of concern as well as gaps in scientific knowledge in this field. According to the review, the 7 most commonly used additive emulsifiers are carboxymethyl cellulose (CMC), P80, lecithin, mono- and diglycerides, stearyl lactylates, sucrose esters, and polyglycerol polyricinoleate. Some of these emulsifiers, such as the mono- and diglycerides, are commonly found in foods and should be digested and absorbed in the small intestine. Others, like CMC, resist digestion.



**Fig. 1 – Effects of food matrix components on the gut microbiome composition, associated health parameters, and implications for preclinical chronic disease models.** (A and B) Cellulose as the only source of fiber in semipurified diets promotes mucin degradation [39,40] and promotes colitis [42]. (C) Moreover, diets with fiber sources other than cellulose can protect against diet-induced obesity and insulin resistance [44,45]. (D) Mice fed semipurified diets containing cellulose have dissimilar microbiomes to mice fed polysaccharides-rich diets in chronic disease models [15], and diets containing only cellulose cause a loss of taxa over generations [18]. Components typically found in human diets but not in basal rodent diets such as emulsifying agents and protein or lipid oxidation products can also affect colitis, chronic disease, and the gut microbiome. Commonly consumed emulsifying agents used in human diets have been demonstrated to (E) increase colitis [83,85,86], (F) promote metabolic syndrome [83], and (G) cause changes to the gut microbiome [83,85–87]. Thermal processing of food can lead to the formation of protein and lipid oxidation products not found in rodent diets. Protein oxidation metabolites have been shown to (H) induce diabetes [53], nonalcoholic fatty liver [54,56,57], and cardiovascular disease [58] but (I) decrease colitis in animal models [60] and (J) can change the gut microbiome composition [59]. Lipid oxidation products have been shown to (K) promote aberrant glucose metabolism [73,74] and (L) change the gut microbiome composition [79].

Recently, the effects of dietary emulsifiers on the gut microbiome and gut health have been investigated. For instance, when obese human subjects consumed beverages containing fat in either an emulsified or nonemulsified form, chylomicron-endotoxemia kinetics differed according to the presence of emulsified fat [81]. In this study, the emulsions were formed directly in milk, and thus, the emulsifying agent was milk protein and not one of the additives described above. Similar to the mono- and diglycerides, the milk proteins which stabilized the emulsions should be digested in the small intestines, and thus, it is not clear how the physical structure of the emulsion was responsible for the differential endotoxin absorption. Within a specific class of emulsifiers, the specific chemical composition may affect the gut barrier and systemic metabolism. For example, Lecomte et al [82] fed mice high-fat diets with emulsions prepared with either soybean lecithin or milk polar lipids. Mice fed the diets with milk-derived polar lipids had an improved gut barrier which was attributed to increased goblet cells. Mice in the soybean lecithin had increased white adipose tissue and adipose tissue inflammation.

Chaissang and colleagues [83] demonstrated that inclusion of emulsifying agents into mouse diets or drinking water had detrimental effects on several parameters associated with metabolic syndrome and unfavorably changed the gut microbiome. Both CMC and P80 treatment reduced  $\alpha$  diversity and increased the relative abundance of several mucolytic OTU including *Ruminococcus gnavus* and *Akkermansia muciniphilia* as well as inflammation-promoting Proteobacteria. Inclusion of P80 to the drinking water at a concentration of 1% wt/vol resulted in intestinal inflammation, a distinct microbiome, and reduced  $\alpha$  diversity compared to control mice. Moreover, mice in the P80 treatment had increased energy intake, fat mass, and impaired glycemic control relative to controls. Interestingly, germ-free mice seeded with bacteria from mice treated with P80 had increased adiposity and impaired glycemic control, suggesting that these effects were mediated through changes in the microbiome. Levels as low as 0.1% of P80 caused observable low-grade inflammation and 0.5% resulted in dysglycemia. In a subsequent study by the same group [84], P80 was again included in drinking water (1% wt/vol) to determine if inclusion exacerbates inflammatory driven colorectal cancer.

Mice in the P80 treatment had increased gut inflammation, a higher load of bioactive lipopolysaccharide, and a 3-fold increase in tumor surface area compared to controls. Interestingly, when germ-free mice were conventionalized with altered Schraedler flora, a defined 8-strain microbiome, CMC and P80 treatment did not affect the microbiome or markers of metabolic syndrome contrary to studies with complex microbiomes. Moreover, when mice were conventionalized with emulsifier treated bacteria from the ex vivo mucosal simulator of the human intestinal microbial ecosystem, recipients gained more weight and had increased fasted glucose and shorter colons compared to mice conventionalized with control mucosal simulator of the human intestinal microbial ecosystem bacteria. These studies suggest that dietary emulsifying agents such as P80 increase intestinal inflammation that, in turn, increases adiposity and insulin resistance and that these changes are mediated via changes to the gut microbiome and not from the emulsifiers acting directly on the gut architecture.

#### 4. Conclusions

Although mice are the most commonly used species for microbiota studies, the translational relevance of preclinical studies using mice as models for studying the intersection of nutrition and the microbiome as pertains to health and disease in humans is unclear. Although individual food matrix components have been tested in rodent models as reviewed herein, the effects of all of these factors combined on the microbiome in preclinical models of chronic disease are unclear, representing a critical knowledge gap. Future research to address this issue could increase the translatability of these models.

Unless mice are provided with diets that reflect actual human intakes of fiber and possibly other components, such as oxidized protein and fat or emulsifiers, and the resulting phenotypes are characterized, translatability of preclinical models in studies focused on diet and the gut microbiome to human populations may be hampered because all of these components can independently affect chronic disease and the microbiome in mouse models (Fig. 1). Semipurified rodent diets only contain cellulose as a source of dietary fiber, which promotes a microbiome that degrades the mucin barrier, promotes intestinal inflammation, and changes to the microbiome. A possible first step to improve the translatability of microbiota/chronic-disease models would be to use a diverse portfolio of fiber in semipurified diets that reflect human intakes instead of cellulose. This approach would address an important source of variability in the gut microbiome between humans and experimental animals.

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