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Nutritional Immunology

Aging Modulates the Effect of Dietary Glycemic Index on Gut Microbiota Composition in Mice

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A B S T R A C T

Background: Gut microbiome composition profoundly impacts host physiology and is modulated by several environmental factors, most prominently diet. The composition of gut microbiota changes over the lifespan, particularly during the earliest and latest stages. However, we know less about diet-aging interactions on the gut microbiome. We previously showed that diets with different glycemic indices, based on the ratio of rapidly digested amylopectin to slowly digested amylose, led to altered composition of gut microbiota in male C57BL/6J mice.

Objectives: Here, we examined the role of aging in influencing dietary effects on gut microbiota composition and aimed to identify gut bacterial taxa that respond to diet and aging.

Methods: We studied 3 age groups of male C57BL/6J wild-type mice: young (4 mo), middle-aged (13.5 mo), and old (22 mo), all fed either high glycemic (HG) or low glycemic (LG) diets matched for caloric content and macronutrient composition. Fecal microbiome composition was determined by 16S rDNA metagenomic sequencing and was evaluated for changes in α- and β-diversity and bacterial taxa that change by age, diet, or both.

Results: Young mice displayed lower α-diversity scores than middle-aged counterparts but exhibited more pronounced differences in β-diversity between diets. In contrast, old mice had slightly lower α-diversity scores than middle-aged mice, with significantly higher β-diversity distances. Within-group variance was lowest in young, LG-fed mice and highest in old, HG-fed mice. Differential abundance analysis revealed taxa associated with both aging and diet. Most differential taxa demonstrated significant interactions between diet and aging. Notably, several members of the *Lachnospiraceae* family increased with aging and HG diet, whereas taxa from the *Bacteroides_H* genus increased with the LG diet. *Akkermansia muciniphila* decreased with aging.

Conclusions: These findings illustrate the complex interplay between diet and aging in shaping the gut microbiota, potentially contributing to age-related disease.

Keywords: aging, microbiome, diet, glycemic index, resistant starch

Introduction

The gut microbiome has been shown to influence a wide array of host physiology, with studies showing profound effects on metabolism, immunology, and disease [1–3]. In parallel, the composition and functionality of the gut microbiome are regulated by factors such as diet, sex, geolocation, medication, and genetics, to name a few [4,5].

Recent attention has been drawn to age-associated microbial changes and the potential causal or consequential influence these may have on healthspan or lifespan [6–10]. Although there is no single "healthy microbiome," compositional patterns within the gut microbiome have been found to reflect healthy aging and predict survival rates in mice and humans [6,11–13]. Conversely, gut microbial dysbiosis, as demonstrated either by functionally relevant increases in pathobionts, decreases in

Abbreviations: ASV, amplicon sequence variant; HG, high glycemic; LG, low glycemic; PCoA, Principal coordinate analysis; PERMANOVA, permutational multivariate analysis of variance.

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commensal microbiota, or loss of microbial diversity, has been suggested to be a major driver of the aging process [8,14]. Considering gut microbiome alterations encompass changes associated with aging overall and those linked to age-related health decline, the gut microbiome has been proposed as a potential modulator of the aging process [6].

One current emphasis in aging research is on understanding the discrepancies between chronological age and biological age, with gut microbial differences proposed to be one such discrepancy [6,7,15,16]. For example, Jackson et al. [17] noted robust associations between gut microbial diversity and frailty in a large cohort of younger female twins, even after adjusting for potential confounding factors such as diet and shared genetic and environmental factors. Similarly, observations in a mouse study revealed associations between age and frailty indices within the gut microbiomes of mice [14].

Aging has additionally been proposed to decrease the diversity and compositional uniqueness of gut bacteria, although the extent of decline is less pronounced in healthier individuals [16]. In a longitudinal study by Wilmanski et al. [11], Bray Curtis measures of uniqueness were tested against multiple clinical variables in over 9000 adults between the ages of 18 and 101 y from 3 individual cohorts. Factors that were found to be significantly associated with a microbial drift toward a more unique compositional state included age, prescription medication, blood lipid profiles, antibiotic usage, and sex. Aging, however, was the strongest predictor of gut microbial uniqueness. Interestingly, this study identified an association between lower bacterial uniqueness and an increase in all-cause mortality in individuals $\geq$ 85 y but not in younger individuals, again highlighting the importance of including age as a contextual factor when interpreting microbial communities and potential health outcomes [11].

The interrelation between diet, nutrition, and the gut microbiome has a profound impact on host health and aging [7,18]. Diet is a major driver of the gut microbiome and, in contrast to other influential factors that shape the gut microbiome, is often a comparatively easier component to modify. For this reason, studying the therapeutic utility of dietary-induced microbial composition shifts and subsequent health outcomes is of particular interest. Dietary interventions, such as whole-food, high--fiber, or the Mediterranean diet, have proven effective in retaining gut microbial compositions with higher abundances of health-associated bacteria in humans [19,20].

Isolating the specific effects of diet on gut microbial communities and human health in human studies is a particularly challenging endeavor, as a range of confounders, such as genetics, lifestyle factors, and ethical considerations, are often unavoidable [21]. As such, preclinical rodent models have long served a crucial role in biomedical research, enabling the elucidation of the intricate relationship between the gut microbiome, age-related dysfunction, and diet while simultaneously facilitating the development of effective therapeutic interventions [22–24]. The isolated effects of individual food groups, micronutrient and macronutrient intakes, and dietary patterns on the gut microbiome and health metrics of mice have been well documented [25,26]. It should be noted, however, that the majority of the murine models used in nutritional research make use of young adult mice, usually between the ages of 8 to 12 wk [27]. This is problematic, as older animals may respond differently to research interventions compared with their younger counterparts, thus limiting the generalizability and predictive accuracy of preclinical research when applied to various age groups. Therefore, there is a clear necessity for studies that delve into the nutritional impact on the gut microbiome and health outcomes in aging murine models.

Prior studies have identified digestive and metabolic differences between aged and young mice [15,28]. The results from a recent meta-analysis indicated significant alterations in the carbohydrate metabolism of the gut microbiome in response to aging, again highlighting the potential role of gut microbiota in aging and age-related diseases [29]. Evidence has also demonstrated the association of the gut microbiota with diabetes and plasma glucose levels [30]. Given the rising rates of type 2 diabetes, obesity, and other metabolic disorders, research focused on utilizing the gut microbiome as a target for intervention strategies has become an important area of exploration. A pivotal step in this process, however, is understanding how these gut microbial influences differ across age spans [27].

We have previously studied the role of the dietary glycemic index, a measure of carbohydrate quality, in the context of aged mice. We found that a low glycemic (LG) index diet, achieved by the addition of slowly digested high-amylose corn starch, was associated with an altered gut microbiome relative to mice fed a HG index diet [31,32]. These alterations in gut microbiota were associated with protection from age-related retinopathy only in mice fed LG index diets.

Here, we evaluated the impact of both HG and LG index diets on the gut microbiomes of mice belonging to 3 different age categories (young, middle-aged, and old). Overall diversity changes, as well as changes to individual taxonomy abundances in response to diet allocations, were compared across age groups. We found that diet-age interactions dominated gut microbiota composition and diversity.

Methods

Study design and animal care

Animal work was performed at the Tufts University Human Nutrition Research Center on Aging and approved by the Tufts University Institutional Animal Care & Use Committee in adherence with the NIH guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). Two cohorts of mice were studied. For young mice, male C57BL/6J mice aged 8–9 wk were obtained (Jackson Laboratories). Mice were individually housed in plastic micro-isolator cages with ad libitum water and were acclimated to the facility for 2 wk prior to treatment and provided standard unpurified diet during this time (Teklad 2916 irradiated diet). At 2.5 mo of age, mice were randomly assigned to a HG diet or LG diet for 6 wk (n = 12/group). For middle-aged and old mice, male C57BL/6J retired breeder mice were obtained at 11 mo of age (Jackson Laboratories, Bar Harbor) and individually housed in plastic micro-isolator cages with ad libitum water. Mice were acclimated to the facility for 1 mo prior to treatment and provided standard unpurified diet during this time (Teklad 2916 irradiated diet). At 12 mo of age, mice were randomly assigned to a HG or LG diet (n = 16/group).

Diets, feeding, and metabolic phenotyping

All diets have been previously published by our group and others [31,33]. The HG and LG diets are isocaloric and identical apart from the starch composition, which is 100% rapidly digested amylopectin (Amioca starch, Ingredion Inc.) in the HG diet and 70% slowly digested amylose and 30% amylopectin in the LG diet (Hylon VII starch, Ingredion Inc.). Diet composition was 542 g/kg starch, 200 g/kg casein, 85 g/kg sucrose, 56 g/kg soybean oil, 50 g/kg wheat bran, 2 g/kg DL-methionine, 10 g/kg AIN-93G vitamin mix, and 35 g/kg AIN-93G mineral mix. Macronutrient energy percentages were 65% carbohydrate, 21% protein, and 14% fat for both HG and LG diets. All diets were formulated by Bio-Serv.

Young mice were fed ad libitum, and food intake was measured and determined to be equivalent between groups (HG intake: 2.60 g/d +/− 0.09 g; LG intake: 2.60 g/d +/− 0.13 g; $P = 0.97$). To ensure equal consumption of diets in middle-aged and older mice, the mice were group pair-fed. Middle-aged and old mice consumed 4.25 g/d.

All mice were weighed weekly. Body composition was determined by MRI using the EchoMRI system. For the young mouse cohort, MRIs were performed at 5 wk. For the old mouse cohort, MRIs were performed at 34 wk of feeding.

Intraperitoneal glucose tolerance tests were performed by first fasting the mice for 6 h, followed by intraperitoneal administration of 1 mg/kg glucose in sterile saline solution. Blood glucose was measured at baseline, prior to glucose injection, and at 15-min, 30-min, 60-min, and 120-min intervals from tail vein blood, measured via glucometer (OneTouch Ultra). Glucose tolerance tests were performed at 4 wk of feeding for young mice and 37 wk of feeding for old mice.

Microbiome collection and extraction

After 1.5 mo (young and middle-aged) or 10 mo (old) of feeding, fecal pellets were collected from empty sterile cages and stored at −80°C prior to gut microbiota sequencing (n = 10/group). Microbial DNA extraction and sequencing were performed by the Phoenix Laboratory, a core facility at Tufts University. All sample processing and sequencing were performed in a single batch. DNA was isolated using the QiaAMP PowerFecalPro DNA kit (QIAGEN). 16S rRNA libraries were prepared using the Earth Microbiome Project protocol with the V4–V5 primer region as previously described [34].

Bioinformatics

Demultiplexed paired end reads were imported into QIIME2 (v2021.11) for quality control, feature table construction, and computation and significance testing of α- and β-diversity [35–37]. The DADA2 pipeline was used for quality filtering and table construction [38]. As the quality scores were consistently high across the length of the sequence, sequences were not trimmed. Unassigned chimeric and sequences of chloroplast and mitochondria were excluded. Prior to diversity analyses, samples were rarefied to the minimum read count across all samples, which was ~34,000 in our sample set. The filtered sequences were taxonomically assigned to Amplicon sequence variants (ASVs) by training a Naïve Bayes Classifier [39] against Greengenes2 [40]. For α-diversity, observed features and Shannon index were calculated. For β-diversity, principal coordinate analysis (PCoA) was performed based on Bray Curtis and weighted UniFrac distances.

Differential abundance of taxa and ASVs was determined using the MaAsLin2 package (v1.10.0) in R (v 2023.03.0+386) [41]. Briefly, a matrix of annotated and unfiltered raw count data was imported into R and evaluated using the following MaAsLin2 settings, including filtering out taxa/ASVs with a feature count abundance of < 200, total-sum-scaling normalization, log transformation, and the linear model analysis method. The LG, 4-mo group served as the reference for differential abundance. Features were considered significantly different if the false discovery rate corrected q value was < 0.25.

Statistical analysis

Group comparisons of continuous variables were performed by first checking the assumptions for parametric tests, followed by the appropriate analysis. Nonparametric data were analyzed via Kruskal-Wallis followed by Wilcoxon post hoc analysis. Permutational multivariate ANOVA (PERMANOVA) test was used for β-diversity to determine overall microbial composition and structure differences among groups. Linear regression was performed on differential taxa for aging effect analysis using R with Benjamini-Hochberg correction. All the statistical analyses was performed in R. Boxplots were prepared using the ggplot2 package (v3.4.2). Heatmaps were prepared using the corrplot package (v0.92).

Results

In order to understand the role of age and aging on mouse gut microbiome composition in response to dietary intervention, we designed a study to compare the gut microbiome composition in young mice (4 mo) to middle-aged mice (13.5 mo) and old mice (22 mo), as outlined in Figure 1. In all comparisons, mice were fed HG and LG diets, which are macronutrient and calorie-matched diets that vary only in starch composition. Young and middle-aged mice were fed HG and LG diets for 1.5 mo, whereas old mice were fed HG and LG diets for 10 mo.

Consistent with previous studies [31,33], HG diet led to increased body mass and adiposity in young and old mice (Figure 2A–D). In young mice, HG diet led to increased fasting blood increase, but normal insulin sensitivity (Figure 2E, G), whereas in old mice, HG diet led to insulin resistance (Figure 2F, H).

We collected feces from young, middle-aged, and old mice and performed 16S rRNA sequencing to determine microbiome composition. First, we assessed α-diversity, which is a within-sample measure of microbial richness. We obtained similar results using 2 different measures of α-diversity—observed features and the Shannon index, which measures both richness and evenness (Figure 3A, B). When comparing diet effects on α-diversity, we observed statistically significant increases in α-diversity in HG samples compared with LG samples across some age groups. Diet effects were most prominent in young mice, which showed statistically significant differences in α-diversity measures. Shannon index was statistically significant between diets only in young mice. When comparing age effects on α-diversity, we observed that α-diversity was lowest in the young mice, highest in the middle-aged mice, and then lower in the

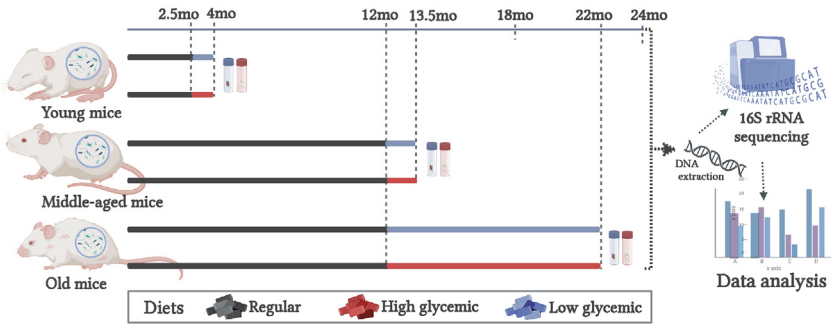

FIGURE 1. Study design for aging/diet study. Male C57BL/6J mice were initially fed regular diets, randomized to HG or LG diets, and aged to 4 mo, 13.5 mo, or 22 mo. Feces were then collected and analyzed for gut microbiome composition. Created with Biorender.com.

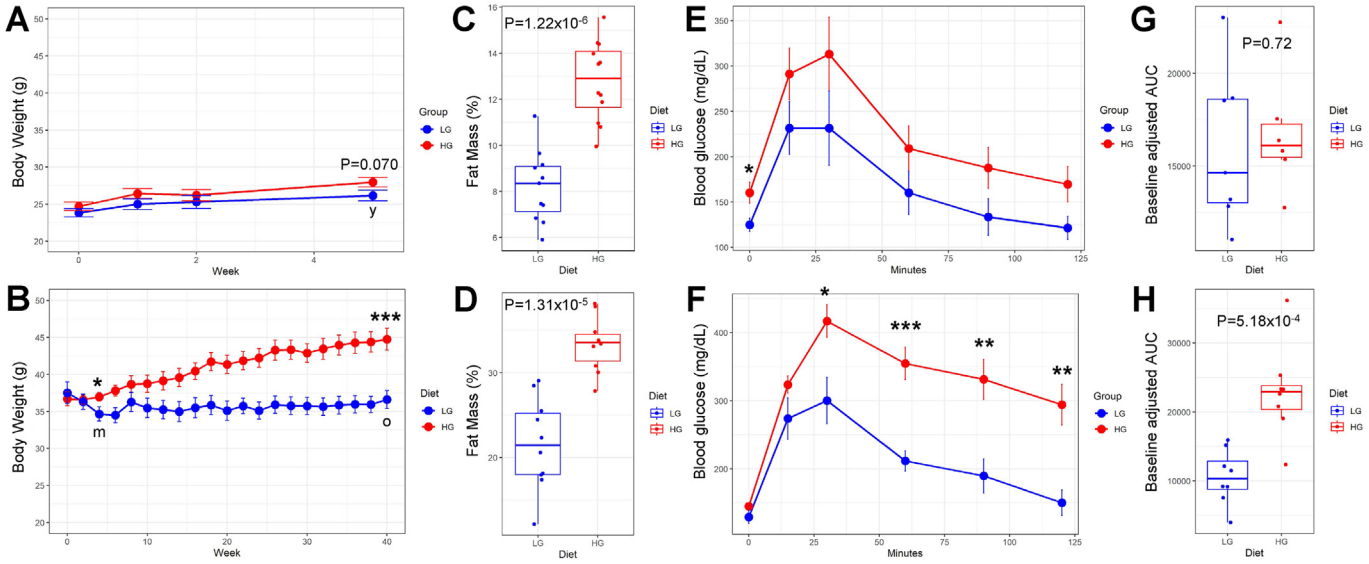

FIGURE 2. Metabolic effects of diets. Top panels show results for the cohort of young mice (A, C, E, G), and bottom panels show results for the middle-aged/old mice (B, D, F, H). (A, B) Body weight of mice during feeding. (C, D) Percentage of fat mass was determined by MRI. (E, F) Intraperitoneal glucose tolerance test. (G, H) AUC of glucose tolerance test, adjusting for differences in fasting blood glucose levels. Statistics: Student's T-test. Significance is indicated by *P* value or by asterisks: * $P < 0.05$; ** $P < 0.01$; ***$P < 0.001$. For body weight data, statistical testing was performed at feeding times corresponding to microbiome collection for young mice (y), middle-aged mice (m), or old mice (o), as indicated in (A, B). Error bars indicate standard error of the mean.

older mice. The largest change was the increase between young and middle-aged mice, which was diet-independent and statistically significant.

We next performed β-diversity analysis to compare microbiota composition between samples using principal coordinate analysis (PCoA) of Bray Curtis dissimilarity (Figure 3C–F). When comparing samples by diet, we found the largest differences in young samples, with smaller differences in older samples (Figure 3C). We also evaluated within-group distances, which indicates the similarity within the group. Young mice fed LG diets showed the smallest within-group variance, whereas old mice fed HG diets showed the largest within-group variance (Figure 3D). Middle-aged mice did not show diet-dependent differences in within-group variance.

When comparing β-diversity by age, we observed significant diet-independent changes in β-diversity across aging (Figure 3C). These age dependent differences were reflected by the distance between diet groups along the PC1 axis, which increased with increasing age (Figure 3E). In contrast, the distance between diet groups along the PC2 axis was high only in young mice (Figure 3F). Similar β-diversity results were observed using a different diversity measure, weighted UniFrac, although an aging effect between diets on the PC1 axis was not observed (Supplemental Figure 1).

In order to determine which bacterial taxa were responsible for the aging and diet effects we observed in the diversity analyses, we performed differential abundance analysis using MaAsLin2. We considered taxa that contributed to changes during aging, by diet, or that showed an age-diet interaction and identified 30 different taxa at the genus level that were significantly altered (Figure 4A). Approximately half of the taxa were significantly altered by diet (16/30), and most taxa showed differential abundance during aging (26/30).

Consistent with our diversity analyses, we observed that most taxa increased in abundance during aging. One such example is the genus *14-2* in the family of *Lachnospiraceae* (Figure 4B). *14-2* abundance increased most dramatically between the young and middle-aged timepoints and was largely unchanged between

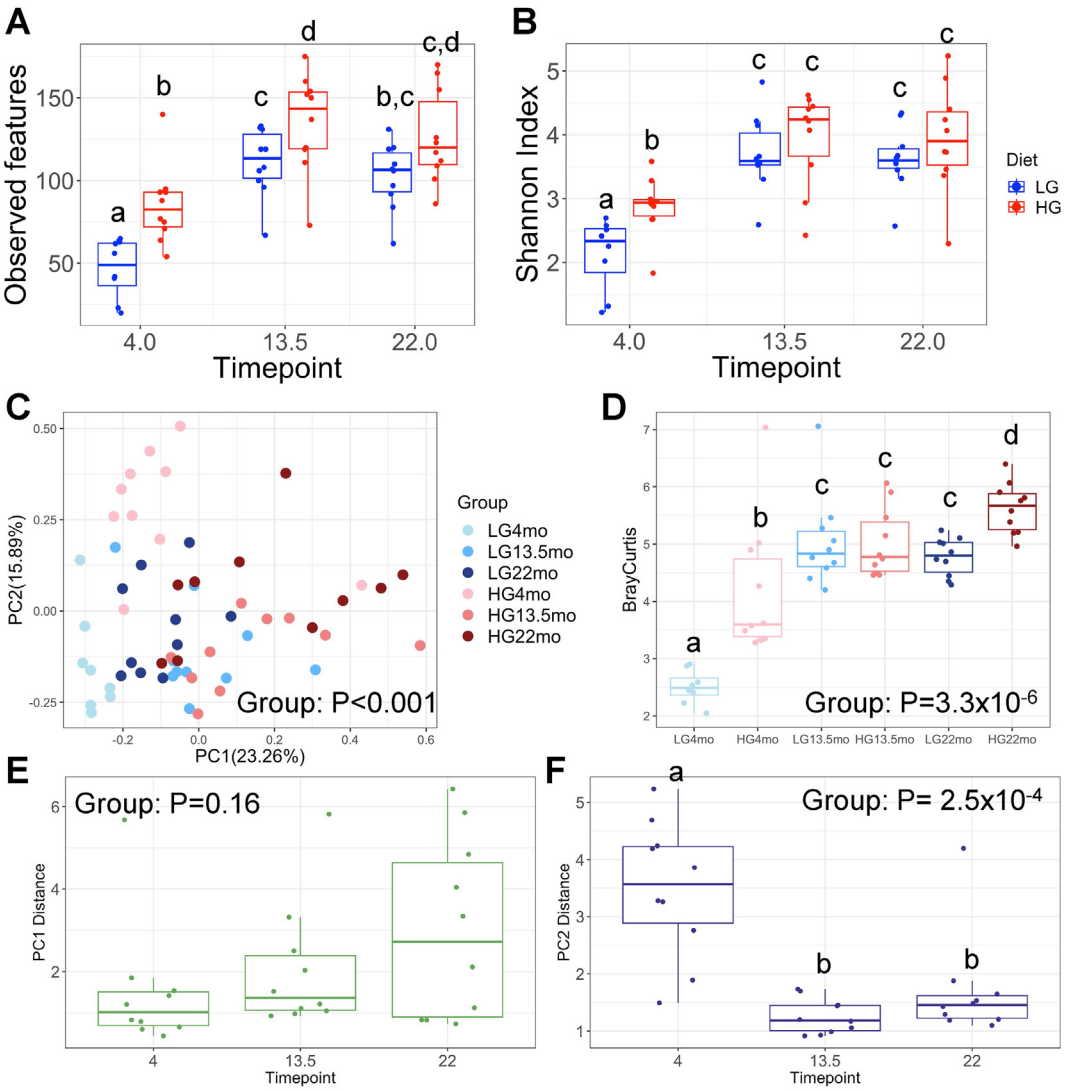

FIGURE 3. Gut microbiome α- and β-diversity is affected by diet and age. (A, B) α-Ddiversity was determined by number of observed features (A) or Shannon index (B) in mice fed LG diets (blue datapoints) or HG diets (red datapoints). (C–F) β-Diversity was determined by Bray Curtis distance (C) and plotted as within-group centered distance (D) or distance between dietary groups along the PC1 axis (E) or PC2 axis (F) by timepoint. Statistics: Kruskal-Wallis with post hoc analysis by Wilcoxon in (A, B, D–F); PERMANOVA in (C). Groups with different letters show $P < 0.05$ following post hoc analysis.

middle-aged and old timepoints. Of the different genera that increased during aging, 14 belonged to the *Lachnospiraceae* family, which showed robust diet-aging associations. Only one genus, *Akkermansia*, was significantly decreased between young and middle-aged or old timepoints (Figure 4C). However, at the ASV level, which can differentiate species at the same genus level, we identified several ASVs that decreased significantly with aging, such as ASV20, a member of the genus *Parasutterella* (Supplemental Figure 2). These findings indicate that small numbers of abundant taxa decline with aging, whereas large numbers of less abundant taxa increase with aging and explain the large increase in α-diversity observed between young mice and middle-aged mice or old mice.

Dietary differences were frequently observed alongside aging effects, with increased abundance of differential taxa in HG groups compared with LG groups. Some taxa, such as *Porcincola*, showed increased abundance in HG groups at all timepoints and dramatic increases in abundance in LG between young and middle-aged or older timepoints (Figure 4D). We also observed taxa, such as *Oscillospiraceae_88309*, that only showed significant dietary differences in young mice while also increasing in abundance with aging (Figure 4E). These taxa explain the decreasing distance between LG and HG samples with increasing age in principle coordinate analyses. We were also able to identify taxa that were increased in LG samples compared to HG samples, but only at the ASV level. An example is ASV17, a member of the genus *Bacteroides_H*, which was increased in LG samples, particularly in young mice (Supplemental Figure 2). Another member of the genus *Bacteroides_H*, ASV1, also showed LG enrichment in young mice. However, at the genus level, *Bacteroides_H* was not significantly associated with diet or aging.

Taxa that were different between HG and LG samples almost always (15/16) had significant diet-aging interactions (Figure 4A). The nature of the diet-age interaction varied, but usually involved attenuation of dietary effects with aging, as seen for *Porcincola* (Figure 4D). We identified taxa that showed

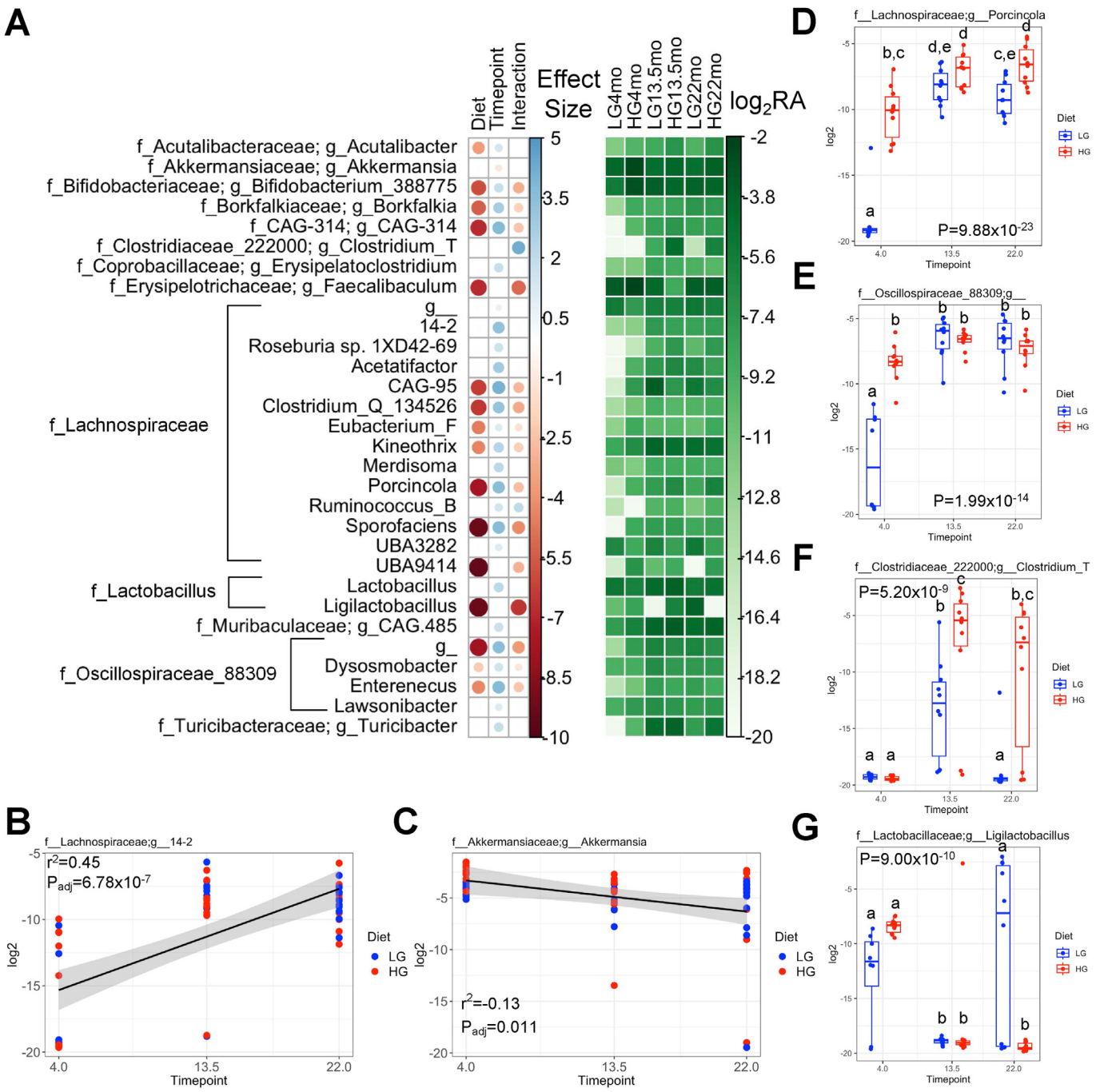

FIGURE 4. Identification of taxa that change by diet or age. (A) Heatmap of differential genera that associate with diet, timepoint, or have significant age-diet interaction. For each genus, heatmaps are shown corresponding to $\log_2$ transformation of average relative abundance (RA). (B, C) Plots of RA of *14-2* (B) and *Akkermansia* (C) against timepoint with datapoints colored by diet, as indicated. (D–G) Box plots of RA of *Porcincola* (D), *Oscillospiraceae_88309*;g_ (E), *Clostridium_T* (F), or *Ligilactobacillus* (G) for each diet and timepoint, as indicated. Statistics: Linear regression with *P* value adjusted for multiple hypothesis testing using Benjamini-Hochberg False Discovery Rate in (B–C); Kruskal-Wallis with post hoc analysis by Wilcoxon in (D–G). Groups with different letters show $P < 0.05$ following post hoc analysis.

complex diet-age interactions. For example, the *Clostridium_T* genus only showed dietary effects in middle-aged and old mice and was only present in LG samples in middle-aged mice (Figure 4F). Another genus, *Ligilactobacillus*, was present in both dietary groups in young mice, absent in middle-aged mice, and present in old mice only in the LG group (Figure 4G). These age-diet interactions underlie the diversity differences across aging.

Discussion

In this study, the impact of HG and LG diets on the gut microbial responses was examined in male mice at young, middle, and older age. Determining how diets alter gut microbial communities could improve age-related health outcomes and has appealing relevance, particularly for populations with expanding elderly proportions [3,42]. Upon subsequent analyses of 16S

rRNA sequencing, it was observed that the gut microbial composition varied between the dietary groups. This was expected, as multiple studies have now confirmed dietary factors as a major determinant of gut microbial variation [25,26]. Indeed, dietary patterns are predicted to account for as much as 50% of microbial structural variations in mice and 20% in humans [43,44]. However, the extent to which dietary-induced variations in microbial structure are affected by age necessitates further investigation.

The results from this study indicated a decline in susceptibility to dietary-induced gut microbial changes with age. In both dietary groups, bacterial α-diversity was lowest in young mice, highest in middle-aged mice, and began declining in the aged group of mice. Presumably, a further decline of gut microbial diversity would have been observed should the study have continued [9]. α-Diversity of gut microbes in humans is reported to be lowest in infancy, rising until plateaued in middle-aged adults, and gradually changing in composition as individuals age, with some, but not all studies, reporting a decline in α-diversity at older ages [6,9,45,46]. Although our findings do not inherently suggest age-related dysbiosis, the alterations in diversity observed between our middle-aged and old mice might signify initial changes that could become more evident toward the end of life.

The association between gut microbial α-diversity levels and healthy aging has been somewhat controversial, with its utility in predicting healthy outcomes still debated [9]. In this study, mice fed the HG diet displayed higher overall levels of gut bacterial α-diversity than those fed an LG diet across all age groups. Given that the LG diet was primarily composed of a single prebiotic (amylose), it was not unexpected that the emergence of a more diverse microbial community was restricted [47]. Similar findings have been reported in mice and pigs fed high resistant corn starch or potato starch diets [31,48,49]. This observation reinforces the importance of interpreting gut microbial diversity assessments considering the study design framework and diligently addressing potential confounding factors to ensure accurate meaning from findings.

The analysis revealed that clustering patterns in the microbial composition of HG and LG diets were more distinct in younger mice and reduced with age. These findings suggest that diet has a greater impact on microbial composition in juvenile years. Conversely, middle-aged mice showed very little difference in β-diversity metrics between dietary groups. This is consistent with existing literature, which has revealed younger years to be a more malleable period of gut microbial community development, with adult ages being a more stable retention period of the "core" microbiome [16]. Mice of older ages, however, displayed vastly wider within-group variability among each dietary group. Here again, this increased individual variability might reflect a drift from the "core" microbiome previously reported with aging, less so with healthy aging [6,16]. Because the old HG diet mice displayed greater intergroup gut microbial dispersion, one might speculate this is indicative of a greater drift from the core microbiome, demonstrating a greater loss in functional health than those of the LG diet group [50]. The colonic environment also undergoes extensive age-related changes that do not happen uniformly. Thus, the aging microbiome reflects stochastic events in the host and community. Further investigation is required to gain a comprehensive understanding of the distinctions in alterations to the gut microbiome between LG and HG dietary groups.

An important consideration when interpreting outputs from gut microbial analyses is the metrics and measures used to do so [51]. It was noted that differences in individual taxonomies are better quantifiers of aging than summary metrics (e.g., α-diversity or uniqueness) [16]. Indeed, Ghosh et al. [16] analyzed 21,000 fecal microbiomes from 7 data repositories across 5 continents spanning participant ages 18–107 y and found that aging can more accurately be characterized by a depletion of a coabundant subset of health-associated taxa and a concomitant rise in disease-associated taxa. Given the abundance of studies on the subject, many presenting contradictory findings, further research is needed to fully understand the predictive and diagnostic capabilities of the gut microbiome for age-related health outcomes [3,9].

In our study, differential abundance analyses identified a consistent association between genera that increased during aging, particularly between young and middle-aged mice, and genera that associated with the HG diet. These genera, many of which belong to the Firmicutes phylum, contribute to the increased α-diversity and β-diversity distance observed between young and middle-aged mice and between LG and HG mice. For instance, the genus *14-2* in the family *Lachnospiraceae* exhibited a dramatic increase in abundance between young and middle-aged mice, remaining relatively stable in older mice. This finding is consistent with previous studies reporting age-associated changes in the *Lachnospiraceae* family [9,52]. *Lachnospiraceae* are well-known as producers of short-chain fatty acids and are responsive to dietary changes, but as part of a very diverse family, they have been associated with both health and disease in rodent and human studies [53]. Interestingly, many of the *Lachnospiraceae* genera we identified associated with HG diet and aging have minimal or no health-associated literature reports, such as *14-2*, *Clostridium_Q_134526*, *Merdisoma*, *Porcincola*, and *UBA9414*. Fluctuations in abundance of these taxa with age may, therefore, be an influential contributor to the host aging process and worthy of future study. In contrast, the genus *Akkermansia* showed a significant decrease from young to middle-aged and then old mice. These results align with studies highlighting the decline of *Akkermansia*, typically a health-associated bacterium, with aging [9,54,55].

At the ASV level, a decline in certain taxa, such as ASV20 of the genus *Parasutterella*, was also observed with age. *Parasutterella* is considered a core component of both the human and mouse gut microbiota and is correlated with various health outcomes [56]. This shift from a "core microbiome" and increase in intraindividual variability of the gut microbiome has been characterized in humans [11,16]. In this study, this shift was more characterized in mice fed an HG diet, highlighting that age-associated microbial shifts can be modified further by diet. These findings suggest that aging is characterized by a decrease in some abundant taxa, whereas an increase in less abundant taxa contributes to the overall increase in α-diversity. Due to the complex interaction between the gut microbiome and host health, any alteration in microbial α-diversity not only reflects a change in microbial taxonomy but also influences the host's metabolic, immune, and biochemical physiology involved in the aging process [1,43,47].

In this study, mice showed diet-dependent metabolic responses with HG-fed mice developing adiposity and eventually glucose intolerance. Both diet and the gut microbiome impact host metabolic parameters, and the often co-occurring effects are challenging to disentangle. However, a study in mice with and without genetic resistance to obesity observed shifts in gut microbiota following a 1-mo dietary intervention, thereby demonstrating that both host genetics and diet, but not the metabolic phenotype of the host, were independent determinants of the gut microbiome [57]. Moreover, in a murine study investigating the effects of refined high- or low-fat diets in relation to unrefined standard unpurified diet on the gut microbiome and metabolic characteristics, mice consuming the refined diets exhibited substantial gut microbial shifts, despite high-fat group gaining weight and the low-fat group remaining lean [58]. The findings from these studies underscore the microbial susceptibility to dietary changes, irrespective of host weight variations. Thus, although mice in this study displayed diverse metabolic characteristics based on their diets, we posit that the influential factors on gut microbial shifts are primarily attributed to the HG and LG dietary compositions rather than the observed phenotypic alterations.

Strengths of this study include the use of well-defined and macronutrient/micronutrient matched diets, assessment of gut microbiome across a wide range of ages, and minimization of environmental confounding variables. This study also has limitations. For one, this study utilized 16S rRNA sequencing data, which is limited by the taxonomic resolution and coverage of detectable organisms in comparison to metagenomic sequencing methods. Newer metagenomic methods such as MetaPhlAn4 have identified 540 previously unknown species-level genome bins, many of which served as dietary biomarkers in mice [59]. Additionally, the mice in this cohort were only male, limiting the generalizability of the results to female mice. This study was also not strictly longitudinal, with >1 cohort of mice utilized. Furthermore, the study did not progress past 24 mo of age to assess the impact of aging at the end of the lifespan. This warrants further investigation going forward. Lastly, it should be noted that there was a difference in feeding duration between young and middle-aged mice, who were fed for 1.5 mo, and old mice, who were fed for 10 mo before microbiome sampling. Although there may have been a duration-dependent effect of feeding on the results, previous mouse studies have shown that a diet alteration alters the murine gut microbiome most drastically in the initial day or few days following the intervention [60–62]. Thus, we propose that the main effects of HG and LG diets on the gut microbiome would have occurred within the shorter feeding window of the young and middle-aged mice as well as the old mice.

In conclusion, this study provides valuable insights into the complex interplay between age, diet, and gut microbiome composition in male mice. The results highlight the significant impact of dietary choices on microbial diversity, with HG diets leading to higher α-diversity compared to LG diets, especially in young mice. Additionally, the study reveals that the influence of diet on microbial composition is most pronounced during the early stages of life, with the microbial communities of middle-aged mice displaying greater stability. Age-related changes in the gut microbiome are characterized by an increase in the abundance of certain genera, such as those from the *Lachnospiraceae* family, and a decrease in others, like *Akkermansia*. These age-related microbial shifts are further influenced by dietary factors, with HG diets exacerbating the observed changes. Further research is needed to explore the implications of these amplified alterations on host physiological aging processes.

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Author contributions

The authors' responsibilities were as follows – YZ, KMS, and SR designed research; YZ and KMS conducted research; YZ and ENY analyzed data; ENY, ASG, and SR wrote the article. SR had primary responsibility for final content; and all authors: read and approved the final manuscript.

Conflict of interest

The authors report no conflicts of interest.

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Data Sharing

Data described in the manuscript, code book, and analytic code will be made available upon request from the corresponding author.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tjnut.2024.07.014.

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