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Enhancing Quercetin Bioavailability Attenuates Aging Phenotypes via the Gut Microbiota–Intestinal Barrier Axis in Aged Mice

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Abstract

Aging is characterized by a progressive decline in physiological functions driven by cellular senescence, chronic inflammation, and alterations in the gut microbiota. Quercetin is a potential anti-aging compound; however, its clinical application is limited by low bioavailability. In this study, we investigated whether enhancing quercetin bioavailability using EubioQuercetin (EQN) improves aging phenotypes by modulating the gut microbiota–intestinal axis. Male C57BL/6J mice were treated with EQN or conventional quercetin for 12 weeks. Aging phenotypes were assessed using a composite aging score based on hair glossiness, hair loss, and the presence of white hair. Gut microbiota composition was analyzed via 16S rRNA sequencing with centered log-ratio transformation, and intestinal gene expression was evaluated via quantitative reverse transcription-polymerase chain reaction. Notably, EQN significantly reduced the aging score compared to the control (median = 4.5 vs. 8; $p < 0.01$), with greater efficacy than conventional quercetin. Microbiota analysis identified taxa that were positively (*Lactobacillus, Romboutsia, Desulfovibrio,* and *Lachnoclostridium*) and negatively (*Akkermansia* and *Christensenellaceae*) associated with aging. EQN selectively suppressed aging-associated taxa and increased the abundance of beneficial bacteria. It also downregulated the expression levels of senescence-related genes (*p21, proliferating cell nuclear antigen,* and leucine-rich repeat-containing G protein-coupled receptor 5) and upregulated those of tight junction genes (*claudin-1* and *-6*), indicating improved intestinal barrier function. No significant associations were observed between the aging score and levels of short-chain fatty acids or most circulating proteins. Overall, these findings suggest that enhancing quercetin bioavailability amplifies its anti-aging effects through the coordinated modulation of gut microbiota and intestinal barrier function. Therefore, targeting the gut microbiota–intestinal axis via bioavailable dietary polyphenols represents a promising strategy for promoting healthy aging.

Keywords: aging; bioavailability; quercetin; gut microbiota; senescence

1. Introduction

Aging is a complex biological process characterized by the progressive accumulation of cellular and molecular damage, leading to a decline in physiological functions, increased disease susceptibility, and ultimately mortality. The World Health Organization defines aging as a gradual loss of intrinsic capacity over time. In recent years, the emerging field of geroscience has focused on identifying the fundamental mechanisms that drive aging and developing interventions that can simultaneously delay or prevent multiple age-related diseases.

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Among the key hallmarks of aging, chronic low-grade inflammation, often referred to as "inflammaging," and gut microbiota alterations are central contributors [1]. The gut–microbiota axis possibly plays a pivotal role in regulating systemic aging processes by interacting with host metabolism, immune function, and epithelial homeostasis. Specific microbial taxa, including *Akkermansia muciniphila* and members of the *Christensenellaceae* family, are associated with healthy aging and metabolic resilience [2].

Cellular senescence is another fundamental driver of aging and age-related diseases. Senolytic therapies that selectively eliminate senescent cells have yielded promising results in preclinical models [3]. Particularly, the combination of dasatinib and quercetin has been demonstrated to reduce tissue inflammation, improve metabolic function, and modulate immune responses in aged animals [4–6]. Recent studies have also shown that senolytic interventions alleviate intestinal senescence and inflammation while reshaping the gut microbiota composition [7]. These findings highlight the close interplay among cellular senescence, intestinal homeostasis, and the microbiome in the regulation of aging.

Quercetin, a widely distributed dietary polyphenol, has attracted considerable attention owing to its anti-inflammatory, antioxidant, and senolytic properties [8]. However, its clinical application remains limited by low water solubility and bioavailability, resulting in insufficient systemic exposure when administered orally. To overcome these limitations, EubioQuercetin (EQN), a highly water-soluble quercetin derivative, has been developed [9,10]. Pharmacokinetic and microbiological studies have demonstrated that EQN exhibits superior absorption and bioavailability compared to conventional quercetin (CQN), suggesting that it exerts enhanced biological effects [10].

Bioavailable flavonoids exert protective effects against age-related dysfunctions [11], including cognitive decline [9], supporting the potential of improved polyphenol formulations for aging interventions. Based on these considerations, we hypothesized that EQN exerts stronger anti-aging effects than CQN via enhanced modulation of the gut microbiota–intestinal axis. To verify this, the present study aimed to evaluate the effects of EQN on external aging phenotypes in aged mice and elucidate the underlying mechanisms via integrated analyses of the gut microbiota composition, intestinal gene expression, and systemic proteomics.

2. Materials and Methods

2.1. Animals and Experimental Design

Male C57BL/6J mice (age: 48 weeks; Oriental Yeast Co., Ltd., Tokyo, Japan) were used in this study. After a two-week acclimation period, the mice were randomly assigned to three groups (n = 10/group) using a computerized randomization program (IBUKI, Japan Bio Research Center, Gifu, Japan) to ensure comparable mean body weight and variance among groups. All animals were housed in a controlled environment under the following conditions: 20.0–26.0°C temperature, 40.0–70.0% humidity, a 12 h light/dark cycle (lights on from 6:00 to 18:00), and ventilation at 12 air changes per hour. The animals were monitored daily for general health status, and body weight was measured weekly. The control (CON) group received a standard AIN93G diet. The EQN group received AIN93G supplemented with EQN (ALPS Pharmaceutical Industry Co., Ltd., Gifu, Japan), whereas the CQN group received AIN93G supplemented with quercetin (ALPS Pharmaceutical Industry Co., Ltd.). All diets were provided ad libitum for 12 weeks. Food intake was measured weekly throughout the study.

The experiments were conducted in accordance with the NIH guidelines for the use of animals in research. Kyoto Prefectural University of Medicine Animal Care Committee approved all experimental protocols [permission numbers M2022-39].

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2.2. Dose Calculation

The dose of EQN was set at 150 mg/kg/d based on previous studies (3, 4). Assuming that each mouse consumed approximately 5 g of diet per day, with 70% ingested and 30% lost, the dietary concentration of EQN was adjusted to 0.12% (1.2 g/kg diet). This resulted in an estimated daily intake of 4.2 mg per mouse, corresponding to 150 mg/kg/d for a 28 g mouse. To ensure molar equivalence, the concentration of CQN (molecular weight: 338) was adjusted to 0.033% based on the molecular weight of rutin (molecular weight: 664). The human equivalent dose, calculated using a conversion factor of 12.3, was approximately 800 mg/d.

2.3. Aging Score Assessment

Aging phenotypes were evaluated based on external physical characteristics using a standardized scoring system. Digital images of the face (frontal view) and dorsum were obtained using a digital camera at baseline and on days 27, 55, and 83 after treatment initiation. For facial imaging, the mice were gently restrained in a cylindrical holder to ensure consistent positioning. Three parameters were evaluated: Hair glossiness, hair loss, and the presence of white hair. Each parameter was scored independently for the face and dorsum using the following criteria:

Hair Glossiness

- Score 0: Clearly glossy (normal condition)
- Score 1: Slightly reduced gloss
- Score 2: Clearly reduced gloss
- Score 3: Markedly reduced gloss
- Score 4: Severely deteriorated and dirty appearance

Hair Loss

- Score 0: No hair loss
- Score 1: Hairless area < 25% and thinning < 50%
- Score 2: Hairless area < 25% and thinning ≥ 50%
- Score 3: Hairless area = 25–50%
- Score 4: Hairless area > 50%

The Presence of White Hair

- Score 0: No white hair
- Score 1: White hair detectable upon close inspection
- Score 2: Clearly visible white hair
- Score 3: Diffuse white hair

Scoring was performed by an independent evaluator blinded to the treatment groups. The total aging score was calculated as the sum of the individual scores.

2.4. Functional Assessments

Rotarod Test

Motor coordination was assessed using a rotarod apparatus (UGO BASILE, Italy), with the rotation speed accelerating from 5 to 40 rpm over 5 min. The latency to fall was recorded, with a maximum cut-off time of 300 s. Each mouse was tested thrice at intervals of at least 30 min.

Wire Hang Test

The mice were placed on an inverted wire grid, and the latency to fall was recorded, with a maximum cut-off time of 600 s. Each mouse underwent two trials at intervals of at least 60 min.

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Y-Maze Test

Spontaneous alternation behavior was assessed using the Y-maze apparatus. The mice were allowed to explore freely for 8 min. Subsequently, the total number of arm entries and spontaneous alternation percentage were determined.

2.5. Sample Collection

At the end of the 12-week treatment period (day 84), blood samples were collected from the inferior vena cava under isoflurane anesthesia using ethylenediaminetetraacetic acid-coated syringes. Plasma was separated via centrifugation (4 °C, 2200 × *g*, 15 min) and stored at −80°C. After exsanguination, the small intestine, cecal contents, and skeletal muscles were collected. Intestinal samples were cleaned with saline, and cecal contents were stored for microbiota and metabolite analyses.

2.6. Gut Microbiota Analysis

Gut microbial DNA extraction, library preparation, and MiSeq sequencing were performed as previously described [12]. Briefly, microbial DNA was extracted and purified from 25 mg of cecal contents using the QuickGene DNA Tissue Kit SII (KURABO, Osaka, Japan). This microbial DNA was used to amplify the V3–V4 region of *16S rRNA* using the primer sets 341F (5′-CCTACGGGNGGCWGCAG-3′) and 805R (5′-GACTACHVGGGTATCTAATCC-3′) [13]. Polymerase chain reaction (PCR) was conducted according to the following program: Initial denaturation at 95 °C for 3 min, followed by 25 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, and a final extension step at 72 °C for 5 min. Amplicons were purified using NucleoFast96 PCR plates (TaKaRa Bio, Kusatsu, Japan) and subjected to a second PCR with unique dual-index primer sets for MiSeq sequencing. The resulting amplicons were purified using the SequalPrep Normalization Plate Kit (Life Technologies, Tokyo, Japan) and AMPure XP beads (Beckman-Coulter, Brea, CA, USA) and pooled, followed by 285 bp paired-end sequencing on the Illumina MiSeq platform (Illumina, San Diego, CA, USA) with MiSeq Reagent Kit v3. Raw sequences have been deposited in the National Center for Biotechnology Information Sequence Read Archive under the BioProject ID PRJNA1256317 (available from May 1, 2026).

Data obtained from MiSeq sequencing were analyzed using QIIME2 version 2022.2 [14]. To construct amplicon sequence variants (ASVs), paired-end reads were denoised using DADA2 with the q2-dada2 plugin [15]. Taxonomic classification of ASVs was performed using the Naive Bayes classifier with the q2-classifier sklearn plugin against the SILVA 138 99% reference dataset. Singletons and ASVs assigned to mitochondria and chloroplasts were removed from downstream analyses. A phylogenetic tree was constructed via SATé-enabled phylogenetic placement [16]. Alpha diversity indices were calculated using QIIME2 by setting the sampling depth to 5000. Outputs from QIIME2 were further analyzed using the R program with the Bioconductor packages Phyloseq [17] and MicrobiotaProcess [18]. Beta diversity was calculated based on weighted and unweighted UniFrac distances. Microbiota data were analyzed at the genus level after CLR transformation with zero replacement, followed by Z-score standardization. Statistical significance was assessed via Spearman's rank correlation analysis, with FDR correction using the Benjamini–Hochberg method.

2.7. Measurement of Organic Acid Levels in Cecal Contents

To measure the levels of fecal organic acids, including acetate, propionate, iso-butyrate, butyrate, iso-valerate, valerate, succinate, lactate, and formate, 0.3 g of feces was mixed with 600 μL of distilled water and 90 μL of 14% perchloric acid and centrifuged at 13,000 × *g* for 10 min at 4 °C. The supernatant was filtered through a 0.45-μm cellulose acetate membrane filter (Cosmonice Filter W; Nakalai Tesque, Kyoto, Japan) and degassed under vacuum. The resulting supernatant was subjected to organic acid measurement using a high-performance liquid chromatography apparatus with the SIL-10 autoinjector (Shimadzu, Kyoto, Japan), as previously described [19].

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2.8. mRNA Expression Analysis

Total RNA was isolated using the acid guanidinium phenol–chloroform method with TRIzol reagent (Thermo Fisher Scientific, USA). The resultant cDNA was subjected to quantitative reverse transcription (qRT)-PCR using specific primers for six adhesion molecules (zona occludens-1, occludin, and claudin-1, -6, -7, and -8), three mucins (Muc2, Muc3, and Muc4), growth factors, and cell cycle proteins (Ki67, leucine-rich repeat-containing G protein-coupled receptor 5 [Lgr5], Bmi1, musashi RNA-binding protein 1, Hopx, proliferating cell nuclear antigen [PCNA], p57, p16, p21, and cyclin D1). All forward and reverse primers are listed in Supplementary Table 1. PCR was performed using the PowerUp SYBR Green PCR Master Mix and QuantStudio 6 Pro Real-Time PCR System (Thermo Fisher Scientific). The PCR conditions included 40 cycles of 95 °C for 15 s and primer annealing at 60 °C for 1 min, followed by a melting curve analysis in which the temperature was increased from 60 to 95 °C. Gene expression levels were calculated from the qRT-PCR data relative to those of glyceraldehyde-3-phosphate dehydrogenase.

To evaluate global patterns of intestinal gene expression, principal component analysis (PCA) was performed using the mRNA expression data of genes related to barrier function, cell proliferation, and aging. Before PCA, gene expression values were standardized via Z-score transformation to ensure comparability across genes. PCA was conducted using Python (version 3.10) with the scikit-learn package. The first two principal components (PC1 and PC2) were used to visualize clustering patterns among samples. To illustrate group-wise data distribution, 95% confidence ellipses were constructed for each group based on the covariance matrix of PC1 and PC2 scores. The ellipses were calculated assuming a multivariate normal distribution and scaled using the chi-square distribution with two degrees of freedom ($\chi^2 = 5.991$), corresponding to the 95% confidence region.

2.9. Blood Biochemistry and Proteomics

Standard biochemical parameters were measured, and plasma proteomic analysis was performed using the Olink Target 48 panel.

2.10. Statistical Analyses

All statistical analyses were conducted using Python (version 3.10; Python Software Foundation, Wilmington, DE, USA), unless otherwise specified. Data are represented as the mean ± standard deviation or median (interquartile range), as appropriate. Comparisons among the three groups (CON, EQN, and CQN) were performed using one-way analysis of variance followed by post-hoc Tukey's multiple comparison test for normally distributed variables. For non-normally distributed data, the Kruskal–Wallis test followed by Dunn's post-hoc test was applied. Spearman's rank correlation analysis was used to evaluate the associations between aging score and microbiota abundance, gene expression levels, organic acid levels, and proteomic marker levels. To account for multiple comparisons, false discovery rate correction (FDR) was performed using the Benjamini–Hochberg method. For microbiota data, relative abundances at the genus level were transformed via centered log-ratio transformation after zero replacement (1×10^{-6}), followed by Z-score standardization before statistical analysis. For gene expression analysis, relative mRNA expression levels were normalized to glyceraldehyde-3-phosphate dehydrogenase levels and analyzed using appropriate parametric or non-parametric tests, as described above. Statistical significance was set at $p < 0.05$, unless otherwise specified.

3. Results

3.1. Body Weight and Food Intake

During the experimental period, two mice in the EQN group died on days 4 and 9, respectively, after treatment initiation. Necropsy was performed; however, the cause of death could not be

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determined. No abnormal findings were observed in the remaining EQN group animals throughout the study period. Therefore, the final number of animals analyzed in the EQN group was reduced to $n = 8$, whereas the CON and CQN groups each consisted of $n = 10$ animals. No significant differences in food consumption and body weight were observed among the three groups throughout the study period (Table 1).

3.1. Aging Score

Temporal changes in external aging parameters, including hair glossiness, hair loss, and the presence of white hair, are shown in Figure 1. In the CON group, the hair glossiness score on the dorsum increased markedly by day 27 and remained elevated until day 83. This increase was significantly suppressed in the EQN group. The hair loss score increased more prominently on the face than on the dorsum in the CON group; however, this increase was attenuated in both the EQN and CQN groups. The white hair score showed modest changes without significant differences among groups. The composite aging score, calculated as the sum of hair glossiness, hair loss, and white hair scores, is shown in Figure 2ab. Compared to that in the CON group (median = 8), the aging score was significantly reduced in both the CQN (median = 5; $p < 0.05$) and EQN (median = 4.5; $p < 0.01$) groups, with a greater reduction observed in the EQN group.

Figure 1. Temporal changes in external aging parameters. Changes in hair glossiness, hair loss, and the presence of white hair scores on the face and dorsum were evaluated during the experimental period. Data are represented as the mean ± standard deviation (SD). *p<0.05 vs the CON group, #p<0.05 vs the EQN group.

Figure 2. Comparison of the aging score among experimental groups. (a) Representative images of mice in each group. (b) The aging score, calculated as the sum of hair glossiness, hair loss, and the presence of white hair scores, was compared among the control (CON), conventional quercetin (CQN), and EubioQuercetin (EQN) groups. Data are represented as individual values. Statistical analysis was conducted using the non-parametric Dunn's test. *$p < 0.05$ and **$p < 0.01$ vs the CON group.

3.3. Muscle and Cognitive Functions

No significant differences in muscle strength measured by the rotarod and wire hang tests, muscle mass measured by cross-sectional area, or cognitive function measured by the Y-maze test were observed among groups (Table 1).

3.4. Gut Microbiota

Spearman's rank correlation analysis identified nine bacterial taxa significantly associated with the aging score (Figure 3b). Among these, *Lactobacillus, Romboutsia, Desulfovibrio,* and *Lachnoclostridium* were significantly positively correlated, whereas members of the *Lachnospiraceae* family (uncultured genus), *Lactococcus, Christensenellaceae* (uncultured genus), *Eubacterium coprostanoligenes group*, and *Akkermansia* were negatively correlated with the aging score. Notably, *Lactobacillus* demonstrated the strongest positive correlation with the aging score (Figure 3a, Spearman's $\rho = 0.628$; $p < 0.001$; false discovery rate = 0.034), suggesting robust associations between this genus and external aging phenotypes.

Figure 3. Correlation between gut microbiota abundance and the aging score. (a) Representative scatter plot showing the association between the aging score and *Lactobacillus* abundance (centered log-ratio [CLR]-transformed Z-score). The solid line indicates the linear regression fit. Spearman's $\rho = 0.628$; $p < 0.001$; false discovery rate (FDR) = 0.034. (b) Spearman's rank correlation analysis between the aging score and gut microbial taxa. Nine taxa with $p < 0.05$ were identified. Spearman's ρ, p-values, and FDR-adjusted values are shown.

To further investigate the impacts of interventions on aging-associated microbiota, the relative abundances of the nine selected taxa were compared among the three groups (CON, CQN, and EQN; Figure 4). Among the taxa positively associated with aging, *Lactobacillus* abundance was significantly

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reduced in both the CQN and EQN groups compared to that in the CON group. Similarly, *Desulfovibrio* abundance was significantly decreased in the EQN group. Similarly, the abundances of *Romboutsia* and *Lachnoclostridium* exhibited a trend toward reduction, with more pronounced decreases observed in the EQN group. In contrast, among the taxa negatively associated with aging, *Lactococcus* abundance was significantly increased in both the CQN and EQN groups, whereas *Akkermansia* and *Eubacterium coprostanoligenes group* abundances tended to be elevated, although these changes did not reach statistical significance. Members of the *Lachnospiraceae* and *Christensenellaceae* families showed a modest increase in abundance without significant differences among groups.

Figure 4. Comparison of aging-associated gut microbiota among experimental groups. Relative abundances of nine selected bacterial taxa significantly associated with the aging score were compared among the CON, CQN, and EQN groups. Data are represented as individual values (CLR-transformed Z-scores). Statistical analysis was conducted using the non-parametric Dunn's test. *$p < 0.05$ and **$\boldsymbol{p} < 0.01$ vs the CON group. CON, control; CQN, conventional quercetin; EQN, EubioQuercetin; CLR, centered log-ratio.

3.5. Organic Acid Levels in Cecal Contents

The concentrations of nine organic acids in cecal contents were measured. Notably, no organic acid showed a significant correlation with the aging score. Additionally, no significant differences in organic acid concentrations were observed among the three groups (Supplementary Table 2).

3.6. mRNA Expression Levels of Intestinal Genes

The mRNA expression levels of 20 genes related to intestinal barrier function, cell proliferation, and aging were quantified via RT-PCR. Spearman's rank correlation analysis identified several intestinal genes associated with the aging score. Among these, *p21, PCNA, Lgr5,* and *Ki67* were positively correlated, whereas *claudin-1, claudin-6,* and *p16INK4a* were negatively correlated with the aging score (Supplementary Table 3). Notably, *claudin-1* expression showed a significant inverse correlation with the aging score ($R^2 = 0.30$; $p < 0.01$), indicating strong associations between intestinal barrier integrity and external aging phenotypes. In contrast, *p21* expression exhibited a positive correlation trend with the aging score ($R^2 = 0.13$; $p = 0.06$; Figure 5a and b). Comparisons among groups revealed that *p21* levels were significantly reduced in both the CQN and EQN groups compared to that in the CON group. Conversely, *claudin-1* levels were significantly upregulated in the EQN and CON groups ($p < 0.01$ and $p < 0.05$, respectively; Figure 5c and d).

Figure 5. Association between intestinal gene expression and the aging score, and comparison among experimental groups. Upper panels (a and b) show correlations between the aging score and intestinal mRNA expression levels of *claudin-1* and *p21*. Linear regression lines are shown. *Claudin-1 expression* exhibited a significant inverse correlation, whereas *p21* showed a positive correlation trend with the aging score. Lower panels (c and d) show group comparisons of mRNA expression levels among the CON, CQN, and EQN groups. Data are represented as individual values and median. Statistical analysis was conducted using the non-parametric Dunn's test. *$p < 0.05$ and **$\boldsymbol{p} < 0.01$ vs the CON group.

To determine whether intestinal gene expression profiles differed globally, PCA was performed using mRNA expression data. As shown in Figure 6, samples from the three groups exhibited distinct clustering patterns in the PC1–PC2 space. Notably, the EQN group was clearly separated from the CON group, indicating that EQN induced a distinct intestinal gene expression profile. The CQN group showed partial overlap with the CON group but also exhibited a tendency toward separation, indicating intermediate effects. These findings suggest that EQN both affects individual genes and globally remodels intestinal gene expression patterns. Furthermore, the separation of groups in PCA supports the notion that the coordinated regulation of barrier-related and senescence-associated genes underlies the observed anti-aging effects.

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Figure 6. Principal component analysis (PCA) of intestinal mRNA expression with 95% confidence ellipses. Principal component analysis (PCA) was performed using intestinal mRNA expression data. Each point represents an individual mouse, and the circle, triangle, and square indicates the CON, CQN, and EQN experimental groups, respectively. Ellipses represent the 95% confidence intervals based on the covariance of each group. The first two principal components (PC1 and PC2) are shown, with the percentage of variance explained indicated on each axis. Samples from the EQN group were clearly separated from those from the CON group, indicating distinct gene expression profiles. The CQN group showed partial separation, suggesting intermediate effects.

3.7. Blood Biochemistry and Proteomics

Notably, no abnormalities were observed in blood biochemical parameters. Proteomic analysis revealed no significant associations between most protein levels and the aging score. Only hepatocyte growth factor (HGF) levels showed a trend toward a negative correlation ($r = -0.370$; $p = 0.052$) and tended to increase in the CQN group.

4. Discussion

In this study, we found that EQN, a highly bioavailable quercetin formulation, significantly attenuated external aging phenotypes in aged mice, with greater efficacy than CQN. Its effects were accompanied by coordinated alterations in the gut microbiota composition and intestinal gene expression, suggesting that the enhancement of quercetin bioavailability improves aging phenotypes by modulating the gut microbiota–intestinal barrier axis. To the best of our knowledge, this study provides novel evidence that improving the bioavailability of a dietary polyphenol, especially quercetin, amplifies its anti-aging effects via gut-centered mechanisms.

Cellular senescence is a central hallmark of aging and a major therapeutic target in geroscience. Senolytic interventions, particularly the combination of dasatinib and quercetin, selectively eliminate senescent cells and ameliorate age-related dysfunction across multiple tissues, including adipose tissue, the immune system, and the intestine [4,5,7]. Additionally, senolytic treatments reduce intestinal inflammation and reshape the gut microbiota composition in aged animals. Consistently, this study found that EQN significantly suppressed the expression of *p21*, a key cellular senescence marker, in intestinal tissues. This suggests that EQN exerts senescence-modulating effects even in the absence of combination therapy, potentially via enhanced tissue exposure resulting from improved bioavailability.

A major limitation of CQN is its low solubility and bioavailability, which restrict its clinical applicability. A pharmacokinetic study has demonstrated that EQN, a water-soluble quercetin derivative, exhibits superior absorption compared to CQN [10]. The greater efficacy in the EQN group observed in this study further supports that bioavailability is a critical determinant of the biological activity of polyphenols. This finding is consistent with emerging reports that formulation strategies, including the development of water-soluble flavonoid derivatives, can significantly enhance the therapeutic potential of dietary compounds. Similar approaches using other water-soluble flavonoids, such as rutin derivatives, have also exerted protective effects against age-related conditions, including cognitive dysfunction [9].

A key finding of this study was the selective modulation of gut microbiota associated with aging. Several bacterial taxa, including *Lactobacillus, Romboutsia, Desulfovibrio,* and *Lachnoclostridium*, were positively correlated with the aging score, whereas *Akkermansia* and members of the *Christensenellaceae* family exhibited negative correlations. Notably, EQN suppressed aging-associated taxa while promoting bacteria associated with healthy aging, indicating targeted remodeling of the gut microbiome. Although *Lactobacillus* is generally regarded as a beneficial genus, its expansion in this context may reflect age-related dysbiosis or strain-specific effects, highlighting the complexity of host–microbe interactions during aging. These findings are consistent with a previous report that senolytic interventions reshape the gut microbiota composition and improve intestinal homeostasis in aged animals [7].

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Interestingly, despite the observed microbiota changes, no significant associations were observed between short-chain fatty acid levels and the aging score. This suggests that the anti-aging effects of EQN are not primarily mediated by global changes in microbial metabolite production. Specific microbial taxa and localized host–microbe interactions may play a more critical role than bulk metabolite levels. Microenvironmental signaling in the intestinal mucosa, rather than circulating metabolite levels, may be a key determinant of age-related changes.

At the molecular level, EQN exerted distinct effects on intestinal gene expression, particularly in pathways related to epithelial senescence and barrier function. The levels of genes associated with cell proliferation and senescence, including *p21*, *PCNA*, and *Lgr5*, were positively correlated with the aging score and significantly decreased by EQN. In contrast, the levels of tight junction-related genes, such as *claudin-1* and *-6*, were negatively correlated with the aging score and upregulated, especially in the EQN group. The inverse relationship between *claudin-1* expression and the aging score, together with its upregulation by EQN, suggests that preservation of epithelial barrier integrity is a key mechanism underlying the attenuation of aging phenotypes.

The opposing regulation of *p21* and *claudin* genes is particularly noteworthy. Increased *p21* expression reflects cellular senescence and dysregulated epithelial turnover, whereas increased *claudin* expression indicates improved barrier integrity. The simultaneous suppression of *p21* expression and enhancement of *claudin* expression by EQN suggests a coordinated restoration of intestinal homeostasis. PCA further demonstrated that EQN induces a distinct global intestinal gene expression profile, supporting coordinated regulation of barrier integrity and cellular senescence pathways. These findings support the concept of "gut frailty," in which disrupted intestinal barrier function and increased epithelial senescence contribute to systemic aging processes [20]. Collectively, our results provide experimental evidence linking intestinal barrier integrity to external aging phenotypes.

In contrast to the pronounced intestinal effects, systemic proteomic analysis revealed minimal associations between circulating protein levels and the aging score. Only HGF levels showed a trend toward a negative correlation. As HGF is involved in tissue repair and regeneration, this finding possibly reflects a compensatory response rather than a primary driver of aging. The overall lack of systemic proteomic changes suggests that the anti-aging effects of EQN are predominantly localized in the gut environment rather than being mediated via circulating inflammatory factors.

The main strengths of this study include the use of aged animals, a comprehensive multi-omics approach integrating microbiota, gene expression, and proteomics, and direct comparisons with CQN. However, this study also has several limitations. First, causal relationships between microbiota alterations and aging phenotypes were not directly assessed. Second, this study used a single animal model, necessitating further studies for translation to human physiology. Third, species- or strain-level resolution of microbiota was not evaluated, which may have provided additional mechanistic insights. Future studies should incorporate microbiota transplantation, metabolomic profiling, and clinical trials to validate our findings and further elucidate the underlying mechanisms.

In conclusion, EQN effectively attenuated external aging phenotypes in aged mice, with greater efficacy than CQN. Its effects were associated with the coordinated modulation of the gut microbiota composition and intestinal barrier function, highlighting the gut microbiota–intestinal axis as a central pathway in aging regulation. Overall, our findings suggest that enhancing the bioavailability of dietary polyphenols is a promising strategy to promote healthy aging.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Table S1: Primers used for qRT-PCR assay; Table S2: Organic acids levels in cecal content after the treatment for 12 weeks among the control (CON), conventional quercetin (CQN), and EubioQuercetin (EQN) groups; Table S3: Intestinal gene expression associated with aging score after the treatment for 12 weeks.

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