



Signs of aging in midlife: physical function and sex differences in microbiota

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Abstract Microbiota composition has been linked to physical activity, health measures, and biological age, but a shared profile has yet to be shown. The aim of this study was to examine the associations between microbiota composition and measures of function, such as a composite measure of physical capacity, and biological age in midlife, prior to onset of age-related diseases. Seventy healthy midlife individuals (age 44.58 ± 0.18) were examined cross-sectionally, and their gut-microbiota profile was characterized from stool samples using 16SrRNA gene sequencing. Biological age was measured using the Klemmera-Doubal method and a composition of blood and physiological biomarkers. Physical capacity was calculated based

on sex-standardized functional tests. We demonstrate that the women had significantly richer microbiota, $p=0.025$; however, microbiota diversity was not linked with chronological age, biological age, or physical capacity for either women or men. Men had slightly greater β -diversity; however, β -diversity was positively associated with biological age and with physical capacity for women only ($p=0.01$ and $p=0.04$; respectively). For women, an increase in abundance of *Roseburia faecis* and *Collinsella aerofaciens*, as well as genus *Ruminococcus* and *Dorea*, was significantly associated with higher biological age and lower physical capacity; an increase in abundance of *Akkermansia muciniphila* and genera *Bacteroides* and *Alistipes* was associated with younger biological age and increased physical capacity. Differentially abundant taxa were also associated with non-communicable diseases. These findings suggest that microbiota composition is a potential mechanism linking physical capacity and health status; personalized probiotics may serve as a new means to support health-promoting interventions in midlife. Investigating additional factors underlying this link may facilitate the development of a more accurate method to estimate the rate of aging.

Omry Koren and Maayan Agmon contributed equally to this work.

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Introduction

Aging is a major risk factor for chronic diseases and decline in function; however, the aging process is highly diverse [1]. This diversity is seen both interpersonally and between sexes; women have longer lifespans but have a lower proportion of life expectancy in good health, identified as the health-survival paradox [2]. In this vein, geroscientists are searching for an accurate and feasible set of markers to represent or estimate the rate of aging, support predictions of health outcomes, and identify potential aging mechanisms [3].

The concept of biological age (BA), which captures aging diversity by estimating the overall state of the body, was found to be associated with health and mortality measures [4]. Accepted methods for BA assessment rely on physiologically-based analyses (e.g., level of DNA methylation [5], different cells' telomere lengths [4], composition of physiological markers [6]), or behavioral ones (e.g., measures of function [7]). Each method sheds light on a unique representation of the aging process and offers a possible avenue to support interventions promoting healthier aging; thus, no single gold standard exists [8]. In recent years, the gut microbiota, which plays a role in a large variety of physiological pathways, has offered a new avenue to understanding aging processes [9, 10], as it makes a unique contribution to many physiological processes and is associated with both physiological and behavioral markers of aging, such as physical capacity [11].

Engaging with physical activity on a regular basis leads to improved physical fitness [12] or physical capacity, an umbrella term for the ability to perform physical function [13], and it has the potential to attenuate many negative age-related health changes (e.g., skeletal muscle changes, vascular system adaptation, metabolic processes) [14, 15]. Physical capacity can be used as a behavioral marker of aging, and as such, it is associated with BA based on physiological markers of aging [6, 16]. Additionally, physical activity is widely investigated in the context of microbiota, showing its contribution to changes in microbiota composition [11, 17].

Our microbiota is a collection of bacteria and other species living in our gastrointestinal tract that changes as we age; these changes can be studied in the context of health and longevity [9]. Described as “an organ

inside another organ,” our microbiota plays a complex and intricate role in regulating human physiology [18], and accumulation of negative alterations in its composition is associated with impaired health and can be regarded as a hallmark of aging [19, 20]. In contrast, improving microbiota composition (e.g., increasing its diversity or changing abundance of specific taxa) may serve as a means to ameliorate age-related disease prevalence [21]. Moreover, microbiota composition is also associated with non-communicable disease development [22], here investigated as age-related diseases [23], further supporting the need to investigate microbiota composition metrics as promising markers to identify negative aging trajectories. One shared factor that has the potential to improve health and consequently both the microbiota composition and BA is performing regular physical activity [11, 15].

To the best of our knowledge, to date, the link between BA, physical capacity, and microbiota composition was not investigated; exploring this link in midlife, prior to the onset of age-related diseases, may help identify possible aging mechanisms and highlight feasible modifiable markers of aging applicable for population-wide studies [24]. Thus, the aim of this study was to identify common microbiota composition characteristics linking physical capacity and BA, measured using a combination of physiological biomarkers while considering possible sex-related differences. We hypothesized that specific microbiota characteristics, namely, diversity metrics and specific taxa, such as the genus *Roseburia* [25] and species *Akkermansia muciniphila* [26], will be associated with both younger BA and higher physical capacity as well as with non-communicable diseases [22], as non-communicable diseases may represent age-related negative health status. Identified characteristics may support the future development of a robust measure for BA and facilitate potential interventions for healthier aging.

Study sample

This study comprises a subset of adults from the Jerusalem Perinatal Study (JPS), which includes all births between 1974 to 1976 in West Jerusalem [27]. The current sub-set is of individuals who participated in the most recent follow-up examination at age 42–45

(between 2016 and 2021), were willing to take part in this extension of the study, and were inclined to provide a stool sample. The Ethics Committee of the Faculty of Social Welfare and Health Sciences at the University of Haifa (#407/20) and Hadassah Hospital Institutional Review Board (#10–01.04.05) provided ethical approval for the recent extension of the JPS follow-up. All study participants provided an informed consent prior to any study procedure. In short, included participants were healthy midlife adults with no physical or other health limitations prohibiting participation in leisure physical activity.

Biological age estimation and physical capacity measurements

We estimated BA using the Klemera-Doubal method (KDM) [28] and a set of fasting blood markers with additional physiological measures collected during the most recent JPS follow-up at approximate chronological age 44 (Fig. 1). This collection of biomarkers focuses on cardio-metabolic health, here used as an estimated measure of biological aging. The BioAge R package [29] was used to establish BA; additional information on the used methods is given in the supplementary data, eTable 1.

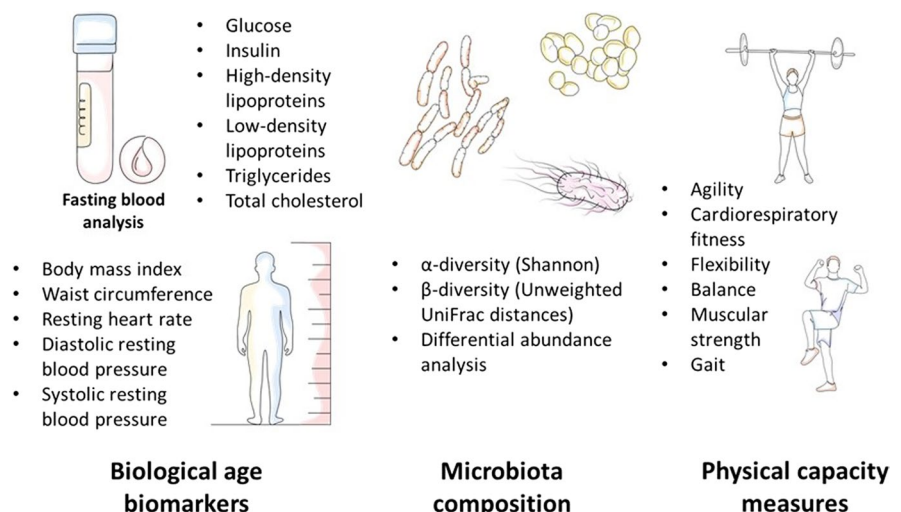
We focused on physical capacity metrics to estimate individuals’ engagement in physical activity, obtaining immediate quantity estimation for objective function across multiple physiological systems; lower physical capacity reflects lower participation in regular physical activity. Moreover, these measures

provide a feasible behavioral goal for positive lifestyle modification. Measures of physical capacity were based on a comprehensive testing battery of functional performance, including five different physical fitness domains associated with health as well as gait analysis under normal and dual-task conditions [16] (Fig. 1). Each domain included several tests, the results of which were standardized using percentiles of total cohort measures to obtain a composite score ranging from 0 (minimum score, very low physical capacity) to 6 (maximum score). These composite scores were calculated separately for each sex group, to avoid any sex-related differences in performance. A complete list of conducted tests is provided in the supplementary data, eTable 1.

Microbiome sample collection and analysis

Fixed protocols were followed for the collection and processing of stool samples [30] (supplementary data, eTable1). Briefly, following DNA extraction, the V4 region of the 16S rRNA gene was amplified, and amplicons were sequenced using the Illumina MiSeq platform. Sequences were processed in the QIIME2 pipeline [31] (version qiime2-2020.8), and then data was exported for further analyses in R software. Shannon diversity [32] was used as a measure of α -diversity (within-individuals diversity), and the unweighted UniFrac distances [33] were used for β -diversity (between-individuals diversity). α - and β -diversity were compared between males and females in the context of BA, chronological age, and

Fig. 1 Measured biomarkers, physical capacity domains, and microbiota variables



physical capacity, and differential abundance analysis was performed. Associations between microbiota composition, BA, and physical capacity were assessed, and correlation between BA and physical capacity was examined using Pearson's correlation to confirm the expected association between lower BA and higher physical capacity [16].

To further investigate the association between identified bacteria and aging, we focused on the link between specific microbial taxa and non-communicable diseases or risk factors associated with non-communicable diseases, as those are recognized as being linked with aging. We then conducted a focused literature search (<https://disbiome.ugent.be/home> and <https://scholar.google.com>) to highlight possible pathways linking these taxa to health.

Results

Seventy midlife individuals provided samples and were included in the analysis (mean age 44.58 ± 0.18 , see Table 1). One participant did not provide a blood sample, and four participants did not complete the physical capacity testing phase. For included participants, physical capacity was negatively associated with BA adjusting for chronological age (partial Pearson correlation $r = -0.42$, $p = 0.001$), demonstrating that higher physical capacity was linked with better health represented by lower BA.

Within-person and between-person microbiome composition comparisons (α -diversity, β -diversity)

No significant links between α -diversity and chronological age, BA, or physical capacity were found. A

comparison of α -diversity between men and women revealed that the women had significantly richer microbiota (Shannon α -diversity, Kruskal–Wallis test, $p = 0.025$, see supplementary eTable 2: Results—figures). However, no sex-specific links with chronological age, BA, or physical capacity were found.

Similarly, no associations between β -diversity (unweighted UniFrac distances) and chronological age, BA, or physical capacity were found when using PERMANOVA for the entire cohort. Again, there was a marginal effect of sex on microbiota diversity (PERMANOVA, $p = 0.085$) showing that men have slightly greater β -diversity. Examination of microbiota in the context of chronological age, BA, and physical capacity in men only revealed a trend toward a significant positive association between microbiota β -diversity and chronological age (PERMANOVA, $p = 0.052$). However, the associations between their β -diversity and BA and physical capacity were statistically insignificant. For women, no significant association was found for chronological age; there was a positive association of β -diversity with BA (PERMANOVA, $p = 0.01$) and with physical capacity (PERMANOVA, $p = 0.04$).

Differentially abundant taxa

We identified specific taxa significantly associated with BA and physical capacity, linking microbiota composition to early manifestation of aging. Table 2 lists taxa that were significantly differentially abundant across BA, and physical capacity measures and presents possible associated non-communicable diseases or risk factors reported in previous publications. Figure 2 provides a graphic summary of the main findings; the full list of taxa significantly differentially

Table 1 Main variables

	Men	Women
Chronological age ¹ (N=70, 38 men)	44.55 ± 0.25 (41.78–46.82)	44.62 ± 0.26 (41.83–46.49)
BA ¹ (N=69, 38 men)	44.71 ± 1.37 (30.06–71.41)	42.53 ± 1.19 (26.27–56.52)
Physical capacity ^{1,2} (N=66, 34 men)	3.18 ± 0.22 (0.85–5.05)	3.16 ± 0.19 (1–5.05)
BMI ^{1,3} (N=70, 38 men)	27.82 ± 0.69 (21.33–41.83)	26.03 ± 0.72 (17.93–35.19)

¹Differences between men and women were examined using *T*-test and found to be insignificant for all reported measures ($p = 0.1$ to 0.97)

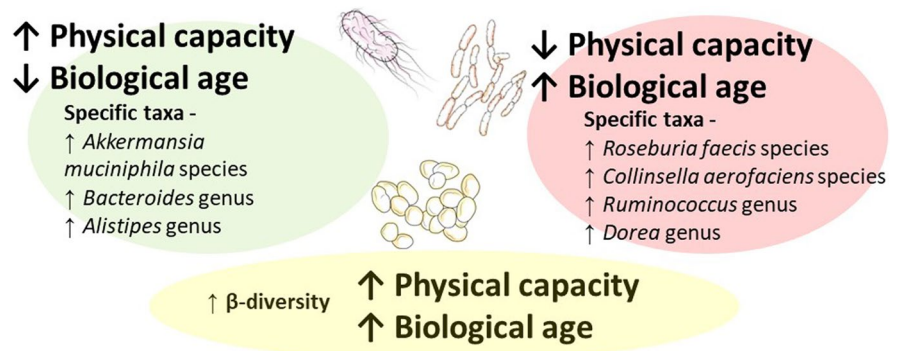
²Composite score based on various tests, range 0–6; higher score is considered better physical capacity

³BMI was used as a biomarker for calculating BA and provided here as a measure for describing the cohort population

Table 2 Taxa, linked variables, and non-communicable diseases

Identified taxa	Current findings	Possible link to non-communicable disease or risk factors
<i>Roseburia_faecis</i>	↑BA	1. Decreased with liver cirrhosis ³⁴
	↑BA (women)	2. Decreased with rheumatoid arthritis ³⁵
	↓Physical capacity (women)	3. Decreased with obesity ³⁶ 4. Decreased with cardiovascular diseases ²⁵ 5. Decreased with lung cancer ³⁷
<i>Ruminococcus</i>	↑BA (women)	1. Increased with non-alcoholic fatty liver disease ^{38,39}
	↓Physical capacity (women)	2. Increased with breast cancer ⁴⁰
<i>Bacteroides</i>	↓BA (women)	1. Decreased with coronary artery disease ^{41,42}
	↑Physical capacity (women)	2. Decreased with atherosclerosis ⁴³ 3. Decreased with non-alcoholic fatty liver disease ^{38,39} 4. Decreased with colorectal cancer ⁴⁴
<i>Collinsella_aerofaciens</i>	↑BA (women)	1. Increased with coronary artery disease ^{41,42}
	↓Physical capacity (women)	2. Increased with pulmonary hypertension ⁴⁵
<i>Akkermansia_muciniphila</i>	↓BA (women)	1. Decreased with type 2 diabetes melitus ²⁶
	↑Physical capacity	2. Decreased with obesity ⁴⁶
<i>Alistipes</i>	↓BA (women)	1. Decreased with obesity ⁴⁷
	↑Physical capacity (women)	2. Decreased with non-alcoholic fatty liver disease ⁴⁸ 3. Decreased with colorectal cancer ⁴⁹
<i>Dorea</i>	↑BA (women)	1. Increased with hypertension ⁵⁰
	↓Physical capacity (women)	2. Increased with non-alcoholic fatty liver disease ⁵¹ 3. Increased with type 2 diabetes melitus ⁵²

Fig. 2 Significant microbiota composition variables associated with both physical capacity and BA



abundant with the study variables (i.e., chronological age, BA, and physical capacity) is in the supplementary data, eTable 2. Figures 3 and 4 present the top 20

abundant genera and observed correlations with BA and physical capacity.

Discussion

In line with our hypothesis, the findings demonstrated that physical capacity and estimated BA share microbial characteristics; however, these results were mainly significant for women. In contrast to our expectation, α -diversity was not correlated with either BA or physical capacity; between-person diversity was positively correlated with physical capacity and BA for women only; thus, increased between-person diversity (β -diversity) was associated with accelerated aging status as well as with higher physical function levels. An increase in abundance of *Roseburia faecis* and *Collinsella aerofaciens*, as well as genus *Ruminococcus* and *Dorea*, was significantly associated with higher BA and lower physical capacity. An increase in abundance of *Akkermansia muciniphila*, as well as genus *Bacteroides* and *Alistipes*, was associated with lower BA and increased physical capacity (see Fig. 2). Additionally, a composite physical capacity score, as a quantitative measure of function, was negatively associated with BA in midlife healthy adults, supporting the link between increased physical capacity and a positive health status represented by younger BA.

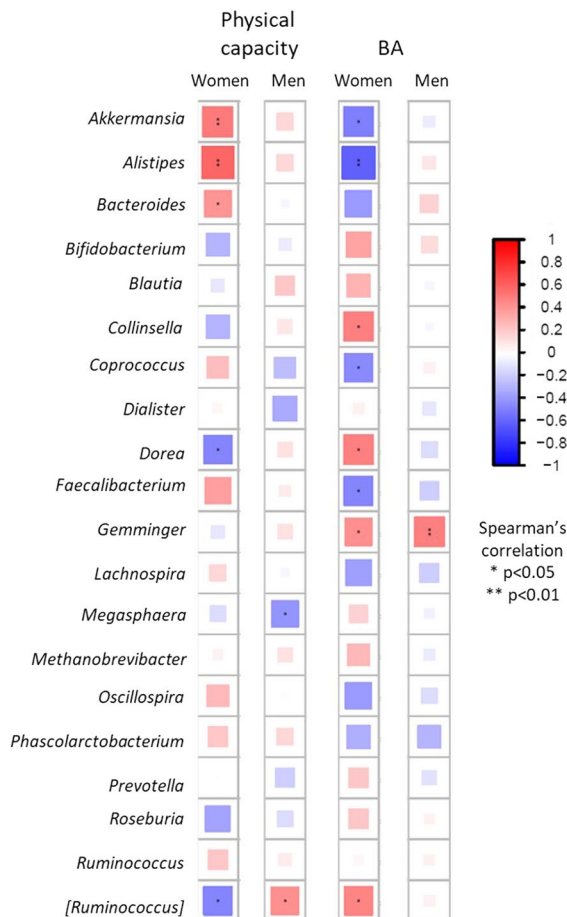
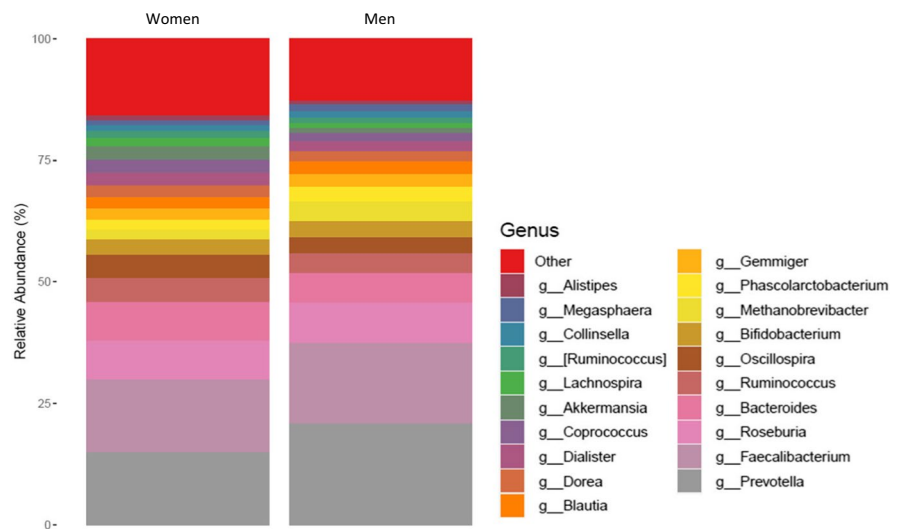


Fig. 3 Top 20 abundant genera correlations with physical capacity and BA

Microbiota diversity in midlife

For the entire cohort of healthy midlife individuals, we did not find an increase in microbiota α - or β -diversity in participants evaluated as biologically younger or with better physical capacity. Considering

Fig. 4 Top 20 abundant genera by sex group

previous reports [53], increased α -diversity was expected to be linked with higher physical capacity and lower BA, representing healthier, younger physiological status [9]. Microbial α -diversity measures the richness of our gut ecosystem [54]; in general, increased α -diversity contributes to higher resilience of the microbiota, supporting its ability to withstand dietary challenges, alcohol, or pathogens [55]. Higher microbial α -diversity may be linked with increased physical activity levels [56] and is widely reported for its association with better health metrics [55, 57]. Physical activity leads to widespread perturbations in numerous cells, tissues, and organs, resulting in multiple health-promoting benefits [58] as well as the mainly positive alteration in microbial α -diversity. Although differences in β -diversity between individuals with different lifestyles or chronological age may be observed [9], and a previous report linked β -diversity to higher levels of physical activity [59], we identified a significant, positive association between β -diversity and physical capacity as well as with BA in the women sub-cohort only. This finding opposes our hypothesis linking microbiota to both slower aging state and higher physical ability.

Although sex differences in BA and physical capacity were insignificant, we observed sex-related differences in microbiota diversity (women had significantly higher α -diversity and men had higher β -diversity), suggesting microbiota diversity may

not be an accurate measure for assessing the health status of otherwise comparable men and women. Although relying on microbiota diversity as a health marker is plausible, it is a limited measure [60] as it may change rapidly. Lack of significant association between higher microbiota diversity and better BA or physical capacity could partially be explained by the relatively small sample size or by dietary differences that were not accounted for [61, 62]. Future studies should closely monitor dietary patterns to ensure the accuracy of microbiota diversity measures and offer a more robust assessment of its association with physical capacity and BA.

Contribution of specific taxon abundance to the link between physical capacity, biological age, and non-communicable diseases

We identified seven distinct taxa (four genera: *Ruminococcus*, *Bacteroides*, *Dorea*, and *Alistipes*; three species: *Roseburia faecis*, *Collinsella aerofaciens*, and *Akkermansia muciniphila*) associated with both BA and physical capacity (see Table 2 and Fig. 2). Identified taxa support the hypothesis that lower BA (being younger biologically) and higher physical capacity (being more physically fit), or vice versa, share a link with specific microbiota characteristics. Moreover, six taxa were also identified as being associated with non-communicable diseases. An increase

in one of the identified species (*Roseburia faecis*) was significantly associated with being biologically older and having lower physical capacity as expected; however, available findings suggest that lower abundance of this species is associated with a variety of non-communicable diseases, such as cardiovascular disease [25] or health-related risk factors, such as obesity [36], while higher abundance is linked with beneficial production of short-chain fatty acids, specifically in older women [63] (therefore, colored differently in Table 2). Keeping in mind that there are many other short-chain fatty acid-producing bacteria at play and that here we focus only on bacteria associated with physical capacity and BA, *Roseburia faecis* could be associated with additional metabolic pathways that are not necessarily beneficial. All identified taxa represent one possible microbiota phenotype associated with an active lifestyle and a healthy younger physiological aging status.

Previous publications suggest that an active lifestyle supports microbial changes that result in a health-supporting microbiota phenotype [64]; in contrast, a sedentary lifestyle promotes dysbiosis, loss of bacteria promoting positive metabolic activity, an increase in opportunistic species, and reduced diversity [65], thus contributing to accelerated aging state and non-communicable disease development [66] (see Fig. 5). As presented in the conceptual model in Fig. 5, microbiota composition changes present a possible mechanism linking lifestyle with pace of aging and non-communicable disease prevalence. Keeping

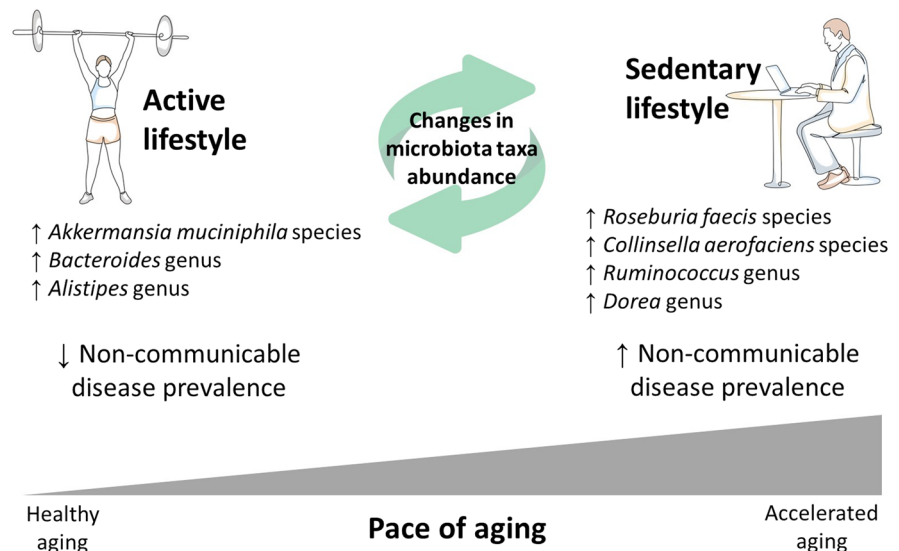
in mind that bacteria work as communities, interacting with each other and with their host [67], these specific taxa represent only one possible microbiota-based phenotype linked with increased physical capacity and better health.

The focused literature search on the identified taxa suggests they may also play a role in non-communicable diseases (e.g., non-alcoholic fatty liver disease, coronary artery disease, type 2 diabetes mellitus). Note that although all participants were in good health, non-communicable diseases develop slowly and are associated with lifestyle choices, such as physical inactivity [68]; thus, there may be microbial precursors to these disorders prior to diagnosis of such conditions in this study population.

Sex difference impact on microbiota composition and estimated BA

Comparisons of women's and men's physiological and physical traits are often significantly different [69], especially when studying aging [2]. In this cohort, studied variables (i.e., chronological age, BA, physical capacity) across sexes were comparable, as both BA and physical capacity were calculated separately for each sex and the cohort used was chronological age homogenous. However, we identified some differences in microbiota diversity measures, suggesting that sex-based microbiota composition differences could not be disregarded. Moreover, specific taxon abundance associations with study

Fig. 5 Conceptual model describing the link between active lifestyle, microbiota composition, and biological aging



variables were mostly observed when investigating women separately from men, limiting our ability to generalize specific taxa roles in aging-related mechanisms. These findings are in-line with current literature on sex contributions to microbiota compositional changes [70] and can potentially explain these results. Sex hormones (e.g., testosterone, estradiol) are correlated with microbiota composition characteristics [71], such as diversity or specific taxon abundance. Presented results suggest that women could be more sensitive to microbiota compositional changes associated with aging mechanisms or physical capacity status, explaining the main findings being observed only in midlife women.

Strengths and limitations

This study utilizes physical capacity, based on an extended testing battery for measuring physical fitness as well as gait, to represent a robust estimation of participants' physical status, thus providing a comprehensive estimation for the physical state. This method offers additional insight on the role behavioral measures play in the aging processes. Additionally, investigating a midlife cohort with relatively homogenous age enables us to capture possible variations in all investigated variables before the onset of any age-related illness. This study has several limitations: (1) our sample size, although acceptable for microbiota studies, is relatively small thus limiting our analysis, particularly when assessing the impact of body weight or additional lifestyle factors. (2) The cross-sectional design does not provide an in-depth understanding of the association between microbiota and physical capacity; although it can be inferred, having a longitudinal comparison demonstrating the impact of changes in physical capacity on microbiota composition would have added another important perspective on these complicated interactions. (3) 16S rRNA analysis mainly allowed us to characterize the microbiota composition. Whole genome shotgun sequencing and metatranscriptomics may bridge this gap by identifying specific bacterial gene involvement and allowing for a comprehensive understanding of the role certain bacteria play [72], supporting the idea that better health represented by lower BA shares similar

microbiota functions with higher physical capacity. To give context to our results, we characterized possible shared pathways using functional analysis of the 16S rRNA data and did not reveal any shared functional link between physical capacity and BA (see supplementary eTable 2: Results—figures).

Conclusions

We show that several taxa in the microbiota were associated with both BA and physical capacity, suggesting microbiota composition plays a role in the association between physical capacity and our health status, in community dwelling midlife individuals. As such, altered microbiota composition may be regarded as an aging mechanism and specific modifications to its composition could be established as a leverage point for interventions addressing accelerated aging status. Additional dimensions of microbiota composition, studied by microbiota diversity metrics (within-person and between-person diversity), were not significantly associated with study variables, possibly due to study participants being healthy or the limited sample size. The rising prevalence of sedentary lifestyle increases the risk of age-related disease and a negative aging trajectory; investigating additional factors underlying the link between physical capacity and BA may facilitate the development of a more accurate method to estimate the rate of aging. Moreover, these findings may assist in the development of new intervention strategies. Future studies should account for microbiota composition as a possible force multiplier, enhancing physical activity interventions' positive contribution to health with additional microbiota alteration methods supporting the same aim.

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Data availability The microbiota data used here is publicly available in the European Bioinformatics Institute (EBI) database, <https://www.ebi.ac.uk/ena/browser/view/PRJEB63185>.

Declarations

Competing interests The authors declare no competing interests.

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