Healthy ageing in older adults: an exploration of interactions between lifestyle, immune, metabolic, and gut microbial health

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CHAPTER 7

General Discussion

7.1 Summary

Ageing populations worldwide face an increasing gap between lifespan and healthspan, with many individuals spending the final decades of life in poor health despite substantial gains in longevity (1,2). Addressing this imbalance requires identifying and targeting modifiable lifestyle factors that can preserve physical and metabolic function and delay biological ageing (3). The overarching aim of this thesis was to elucidate the effects of modifiable lifestyle factors on three health domains relevant to ageing, namely muscle, immune-metabolic, and gut health, to eventually promote healthy ageing. The thesis originally centered on the design, execution and analysis of a novel multimodal intervention (VOILA) to enhance these domains in older adults. The VOILA study was designed and executed but pandemic-related delays postponed the data collection and analysis of the study beyond the time available for completing this thesis. This required expanding the scope of our studies to the analyses of lifestyle factors affecting muscle and gut health in large observational cohort studies such as UK Biobank and Lifelines, respectively. In addition, we explored modifiable lifestyle factors in experimental studies: in a systematic review the relation between sleep disruption and gut and muscle health, and in the PERSON study the relation between dietary intervention responses and immune-metabolic health (quantified using the MetaboHealth score) as baseline stratifier and as outcome. The MetaboHealth score was central to the VOILA intervention study, the algorithm was generated from easily affordable and scalable metabolomic markers to indicate compromised immune-metabolic health and disease risk among older adults. This integrative framework allows for a nuanced understanding of associative, mechanistic, and interventional dimensions of healthy ageing.

The VOILA study (Chapter 2) investigated a multimodal lifestyle intervention that integrated supervised high-intensity resistance training with fortified high-protein supplements in community - dwelling older adults, aiming to counteract age-related physiological decline. To our knowledge, this is one of the first interventions to target multiple physiological outcomes within a single intervention. This innovative 12-week program was designed to concurrently target three interdependent systems central to healthy ageing: muscle function, immune-metabolic regulation, and gut health. The focus of VOILA also lies on studying diversity in intervention effects given baseline variation represented by mobility and metabolically compromised older adults. Within the Leiden arm, for which I was responsible, participants were classified according to their immune-metabolic health, indicated by the MetaboHealth score. Based on this score we stratified metabolically-compromised and – healthy individuals to assess whether metabolic status improves by the multimodal intervention and how baseline status influences responsiveness. Preliminary analyses suggest that on average the MetaboHealth score does not change due to the intervention but individuals with poor immune - metabolic health scores benefited most, exhibiting greater gains in muscle mass, strength, and immune-metabolic markers than their healthier counterparts (personalcommunication with F. Bogaards). As the core practical and experimental component of this thesis, the VOILA study lays the groundwork for biomarker-

guided strategies to promote healthy ageing and extend healthspan in older adults, for example at general practitioners and lifestyle centers for targeted, evidence-based lifestyle interventions.

The UK Biobank (Chapter 3) analysis provided extensive cross-sectional evidence linking lifestyle behaviors to key muscle-related outcomes across adulthood in a large general population sample. By examining a wide array of phenotypic and lifestyle data from individuals aged 40–70 years, the study illustrated how nutrition, physical activity, and sleep collectively influence muscle strength and fat-free mass. Dietary fiber, alongside greater levels of physical activity, were consistently associated with increased handgrip strength and fat-free mass, reinforcing their crucial roles in sustaining musculoskeletal health during ageing. The specificity of the fiber–muscle association, independent of protein intake, underscores the novelty and strength of this observation and suggests that gut-mediated mechanisms may play a previously underappreciated role in maintaining muscle health. These findings reveal a set of modifiable lifestyle behaviors highly relevant to muscle health and provide a robust, population-based foundation supporting targeted, personalized interventions like VOILA to promote healthy ageing.

The Lifelines analysis (Chapter 4) examined associations between different intensities of physical activity and gut microbiome composition and function in a large, population-based cohort. This represents one of the most comprehensive analyses to date linking subjectively measured physical activity intensity to detailed microbiome functional profiles. The findings revealed that higher levels of moderate-to-vigorous physical activity were linked to greater abundance of bacterial species and metabolic pathways associated with anti-inflammatory and metabolically beneficial functions, such as butyrate-producing taxa including Roseburia and Faecalibacterium. These results suggest that physical activity may foster a gut microbial environment that could support metabolic and immune health. However, these associations likely reflect broader lifestyle patterns encompassing diet quality and reduced sedentary behavior, highlighting the importance of accounting for lifestyle clustering in microbiome studies. Overall, Lifelines provides strong observational evidence positioning physical activity as a key determinant of gut microbiome diversity and function, offering a compelling rationale to experimentally test causal effects within integrated lifestyle interventions like VOILA.

The systematic review (Chapter 5) comprehensively synthesized current evidence on the relationship between sleep and the gut microbiome throughout the lifespan. This synthesis is among the first to integrate findings across age groups, highlighting lifespan patterns in sleep–microbiome interactions. The reviewed studies consistently demonstrated that poor sleep -characterized by short duration, irregular timing, or low quality- is linked to alterations in microbial diversity and shifts in specific bacterial taxa involved in metabolic and immune functions. These microbial changes may contribute to systemic inflammation, insulin resistance, and impaired metabolic regulation, indicating that sleep can indirectly influence overall health through gut-mediated mechanisms. However, most of the existing evidence

remains correlational, with limited experimental data to firmly establish causality or the direction of effects. Despite these limitations, the findings highlight sleep as an important yet underexplored factor in gut and metabolic health, strengthening the rationale for including sleep assessments in comprehensive lifestyle interventions like VOILA. Further research, particularly controlled mechanistic studies, is needed to clarify these complex interactions and optimize intervention strategies incorporating sleep modulation.

The PERSON study (Chapter 6) evaluated the efficacy of tissue-specific dietary interventions tailored to the stratification of participants in two Metabotypes exhibiting either muscle or liver insulin resistance, assigning them to a high-monounsaturated fat diet or a low-fat, high-protein diet. Both dietary approaches resulted in improvements in insulin resistance and multiple metabolic biomarkers, confirming the value of phenotype-matched nutritional strategies. Additional stratification using the MetaboHealth score revealed substantial inter-individual variability in response to the intervention between Metabotype groups, with participants in poor metabolic health MetaboHealth tertiles exhibiting greater health gains, underscoring the utility of MetaboHealth as a health indicator in identifying those most likely to benefit from tailored interventions. This consistent and biologically plausible pattern across independent measures strengthens the credibility of MetaboHealth as a novel tool for stratification of metabolically-compromised individuals that would benefit most from lifestyle interventions. However, the MetaboHealth score itself did not change significantly over the 12-week intervention, indicating that short-term dietary modifications alone may be insufficient to shift integrated immune-metabolomic profiles. Collectively, the PERSON study highlights the promise of metabolomics-guided stratification for refining precision nutrition approaches, while emphasizing the need for longer-term and/or multimodal lifestyle interventions to achieve sustained improvements in systemic metabolic health.

7.2. VOILA: Integrating observational and mechanistic evidence to advance understanding of lifestyle factors on muscle, gut, and immune-metabolic health in aging

7.2.1 Dietary protein, fibre, and resistance exercise are fundamental approaches to maintain muscle health

The UK Biobank and VOILA studies together offer complementary perspectives on the critical role of nutrition in preserving muscle health in older adults. The UK Biobank analysis, through a large-scale cross-sectional approach, identified higher protein and fibre intake as strongly associated with greater muscle mass and strength across the general population. This is consistent with recent findings showing that higher dietary fiber intake independently correlates with increased lean mass and handgrip strength, likely due to fiber's beneficial effects on metabolic health and inflammation reduction (4). Together with physical activity, fibre intake should therefore receive greater attention when assessing dietary quality for muscle preservation in aging, aligning with broader evidence on the integral role of lifestyle factors in musculoskeletal health maintenance (5,6). Building on these observational insights, the VOILA study provided causal evidence by implementing a multimodal intervention

combining high-intensity resistance exercise with a protein- and prebiotic fibre-enriched supplement supplying 1.2 g/kg body weight of protein daily, going beyond the current recommendations of 0.8 g/kg for older adults (7). Preliminary findings from VOILA revealed improvements in lean mass and overall muscle strength across diverse participant groups, including metabolically compromised, mobility-impaired, and post–knee replacement individuals (personal communication with A. Monsegue). Notably, mobility-compromised participants showed exceptional gains in functional performance such as chair-stand ability, whereas post–knee replacement and metabolically compromised groups exhibited pronounced improvements in maximal strength across multiple exercises. Together, these studies highlight the combination of dietary protein, fibre intake and resistance exercise for muscle health while demonstrating that integrated, multimodal interventions effectively enhance both functional and strength outcomes in older adults, with varying benefits influenced by baseline health status.

7.2.2 Physical activity and interventions support gut and metabolic health in older adults

Physical activity is increasingly recognized as a key modulator of gut microbiome composition, with the effects depending on both the type and intensity of activity (8–11). Observational evidence from large population-based cohorts, including Lifelines, demonstrated that higher levels of moderate-to-vigorous physical activity were associated with increased abundance of bacterial species and metabolic pathways linked to anti-inflammatory and metabolically beneficial functions, such as butyrate-producing taxa including Roseburia, Faecalibacterium, and Butyricicoccus (12–18). These associations suggest that physical activity fosters a gut microbial environment supportive of metabolic and immune health. However, these findings are also influenced by broader lifestyle factors such as diet quality and sedentary behavior, requiring careful consideration of lifestyle clustering in microbiome research (19–25). In contrast, the VOILA study implemented a structured resistance training intervention alongside protein supplements enriched with prebiotics, vitamin D, and calcium to directly test causal effects on the gut microbiome in older adults. While VOILA's microbiome data are still being analyzed, keeping in mind baseline comparisons with Lifelines' microbial markers provides a valuable reference. VOILA will also test whether targeted resistance exercise combined with enriched protein supplementation induces distinct changes in microbial composition and function compared to the broader physical activity patterns observed in Lifelines. Together, these studies advance understanding of how different forms of exercise influence the gut microbiome in ageing populations, supporting development of targeted, evidence-based lifestyle interventions to optimize gut and metabolic health. This focus resonates with broader research showing that regular exercise in older adults contributes to increased microbial diversity and enrichment of SCFA-producing taxa, supporting metabolic resilience and reduced inflammation associated with ageing. However, systematic reviews emphasize that findings remain heterogeneous and likely depend on exercise intensity, duration, and concurrent dietary patterns, underscoring the need for well-controlled trials such as VOILA to clarify causal mechanisms (26–29).

7.2.3 Improved sleep as a strategy to enhance microbial, metabolic, and muscle health

Sleep is increasingly recognized as a critical determinant of gut microbiome composition and metabolic health. Chapter 4 and posterior systematic reviews have synthesized growing evidence that sleep disturbances-whether in duration, quality, or timing-consistently associate with in lower microbial diversity and composition, often implicating a lower abundance of key taxa such as Faecalibacterium prausnitzii and Bacteroides species linked to metabolic and immune function (30,31). These microbial shifts may contribute to systemic inflammation, insulin resistance, and dysregulation of neuro-metabolic pathways through the gut-brain axis, potentially compromising muscle health via the gut-muscle axis (32–39). Building on this foundation, the VOILA study collects detailed sleep data via the PSQI questionnaire alongside gut microbiome samples at baseline and endline, offering a unique opportunity to explore whether sleep quality categories-poor, average, and good sleepers-correlate with distinct microbial profiles and whether changes in sleep metrics correspond to microbiome alterations in an older population (31). This integration allows VOILA to move beyond association, facilitating investigation into potential causal pathways suggested by prior observational research and clarifying the interplay between sleep, the microbiome, and healthy ageing. Such insight is important for informing the development of targeted lifestyle interventions that incorporate sleep optimization for gut and metabolic health benefits.

7.2.4 MetaboHealth enables personalized strategies to enhance muscle and metabolic health

Both the PERSON and VOILA studies effectively utilized biomarker-based stratification to tailor interventions and understand variability in individual responses, reflecting a broader trend toward precision medicine approaches that leverage molecular and physiological markers to guide personalized interventions across fields ranging from oncology and neurodegeneration to metabolic health (40–46). PERSON showed that apart from Metabotypes, baseline MetaboHealth tertiles and tissue-specific insulin resistance predict dietary responsiveness. However, the MetaboHealth score itself remained largely unchanged after three months of dietary changes alone (without increased physical exercise). Similarly, VOILA stratifies participants using MetaboHealth and applies a multimodal intervention combining resistance exercise with fortified protein and fibre supplementation. While muscle mass and strength improved across all groups in PERSON, the overall MetaboHealth score did not shift significantly. The intervention enhanced vitamin D levels in metabolically compromised participants and reduced trunk fat, indicating broader metabolic benefits captured by the MetaboHealth score. These findings suggest that shifting integrated metabolomic profiles may require interventions longer than three months (likely six months or more) to detect significant measurable changes in the score itself, despite achieving substantial functional and metabolic improvements. Overall, these studies highlight MetaboHealth's promise as a global health estimator in the population linked to stratified lifestyle interventions, with VOILA providing a platform to evaluate its utility in a multimodal context.

7.3. Strengths and limitations

This thesis combines multiple complementary studies to provide a comprehensive perspective on the interactions between lifestyle, metabolism, muscle, and gut microbiomes. Key strengths include the use of large, well-characterized cohorts such as Lifelines and UK Biobank, which provided high-resolution microbiome data, extensive phenotyping, and rigorous statistical adjustment for multiple confounders, including diet, BMI, and stool consistency. Clustering of these factors hamper an estimation of independent effect of these variables in smaller studies. The PERSON study represents a unique, randomized, tissue-specific dietary intervention, allowing exploration of personalized nutrition strategies guided by muscle and liver insulin resistance phenotypes. Similarly, the VOILA study contributed detailed clinical and functional assessments, enhancing mechanistic insight into metabolic health in older adults. Across studies, analytical approaches were robust, including mixed-effects modeling and false discovery rate corrections, ensuring transparency and reliability of the reported associations.

Nevertheless, several limitations temper the interpretation of these findings. Lifelines and UK Biobank are cross-sectional, precluding causal inference and raising the possibility of reverse causation or residual confounding. In the PERSON intervention, the relatively short 12-week duration and small subgroup sizes limited the magnitude of detectable changes in MetaboHealth scores and statistical power for higher-order interactions. Self-reported measures in Lifelines and VOILA, including physical activity, dietary intake, and sleep, introduce potential measurement error, while the exclusion of participants with incomplete data may reduce generalizability. Exploratory associations, such as links between vigorous physical activity and microbial functional pathways or between sleep, gut microbiota, and sarcopenia, remain largely hypothesis-generating and could be influenced by unmeasured factors, including age-related inflammation or lifestyle variability. Finally, the selective inclusion of relatively healthy participants in Lifelines and PERSON constrains extrapolation to populations with chronic disease.

7.4. Future perspectives

7.4.1 Defining a healthy microbiome

The collective findings presented in this thesis from Lifelines, VOILA, and the systematic review highlight a critical question in microbiome science: what defines a “healthy” gut microbiome, particularly in ageing populations? Evidence increasingly suggests that there is no single microbial signature of health (47,48). Instead, health may be best defined by a dynamic balance of microbial diversity and functionality that supports key metabolic, immune, and physiological processes. In both Lifelines and VOILA, physical activity and dietary interventions were associated with specific microbial pathways, including energy metabolism and purine degradation, suggesting that microbial composition and functional output are closely linked to lifestyle behaviors. Future studies should focus on integrating compositional data with functional outcomes, such as metabolomic profiles or MetaboHealth

scores, to establish mechanistic links between microbial ecology and health resilience. Multiomics approaches are increasingly used to combine metagenomic, metabolomic, and fluxbalance data to uncover causal pathways connecting microbial activity to host physiology. Recent studies in metabolic disorders, inflammatory bowel disease, and exercise interventions demonstrate how networkbased and statistical integration models reveal metabolite–microbe relationships shaping immune and energy regulation (49–55). Additionally, longitudinal studies are needed to capture the temporal stability and adaptability of the microbiome, enabling the development of operational definitions of "healthy" microbiomes that can guide targeted interventions in older adults (56). Defining the stability and adaptability will be essential to translate microbiome research into meaningful, personalized strategies for maintaining metabolic and functional health with ageing.

7.4.2 Clinical translation of MetaboHealth

The MetaboHealth score emerges as a promising biomarker for stratifying inflammatory and metabolic risk in older adults. Its potential lies not only in research but also in practical application: by integrating MetaboHealth into routine health assessments, general practitioners and lifestyle physicians in the Netherlands could identify individuals at heightened risk of metabolic deterioration before clinical symptoms arise. This early stratification would enable precision guidance in lifestyle interventions, such as diet optimization or structured exercise programs (57,58). Moreover, collaboration with health insurance providers could support implementation within preventive care frameworks, promoting accessibility and adherence while reducing long-term health costs. Importantly, future work should explore the real-world feasibility of integrating MetaboHealth into routine clinical workflows, including cost-effectiveness, patient acceptability, and the capacity of healthcare systems to respond to identified risks. By bridging research findings and clinical practice, MetaboHealth could become a cornerstone for precision health strategies aimed at preventing age-related metabolic decline. Many steps of analysis would have to be executed, though: making a personalized version of the score; explore prediction of healthspan based on changes in interventions; studying the changes of the score across diverse interventions; explore whether older adults wish to be informed of their scores and more (59–62). Ultimately, establishing MetaboHealth as a standardized biomarker could bridge the gap between molecular aging research and preventive healthcare, enabling earlier, more personalized interventions that promote healthy aging trajectories.

7.4.3 Scaling and implementation of VOILA

While VOILA provides proof-of-concept for structured lifestyle interventions in older adults, scaling such programs presents practical and logistical challenges (63–65). In the VOILA intervention, recruitment proved challenging as many older adults declined participation due to mobility issues, health limitations, or discomfort with structured exercise routines. Such difficulties are typical across lifestyle and aging trials, where physical constraints, time demands, or lack of familiarity with program formats often hinder enrollment. Similar

recruitment barriers have been documented in other interventions, emphasizing the need for age-friendly communication, flexible participation formats, and trust-building strategies involving healthcare providers and local networks (66–72). If VOILA demonstrates measurable benefits for muscle, immune-metabolic, and gut health, broader implementation will require careful planning, including the development of supportive infrastructure, flexible program delivery models, and collaboration with local community, peer groups and lifestyle centers. Ensuring adherence will be critical, which may be facilitated through digital support tools, group-based activities, or personalized coaching (73–75). Additionally, effective stakeholder engagement, including healthcare providers, policymakers, and insurers, will be necessary to sustain implementation and ensure equity of access (76,77). Ultimately, translating VOILA from a research study into routine practice will depend on creating an environment that supports participation, monitors outcomes, and integrates interventions seamlessly into the daily lives of older adults.

7.5 Conclusion

This thesis addresses the growing gap between lifespan and healthspan by focusing on muscle, metabolic, and gut health in ageing populations - key domains whose decline drives functional impairment and multiple chronic diseases. By integrating complementary approaches, we captured both population-level associations and individual responses of modifiable lifestyle factors and health benefits. Large cohorts such as UK Biobank and Lifelines revealed strong links between lifestyle factors (physical activity, diet, sleep), gut microbiome, metabolism, and muscle function. Evidence synthesis clarified pathways from sleep and microbiome to sarcopenia and metabolic health. Targeted interventions in PERSON and VOILA demonstrated the feasibility of metabolic phenotyping and the MetaboHealth score to guide precision lifestyle changes. This cumulative evidence supports biomarker-guided, multimodal interventions to enhance metabolic resilience, preserve muscle, and optimize gut health. Critical next steps include confirming causality in long-term trials, scaling VOILA-like programs, and embedding MetaboHealth in clinical care, advancing a precision health framework to extend healthspan alongside lifespan.

REFERENCES

1. Garmany A, Terzic A. Healthspan-lifespan gap differs in magnitude and disease contribution across world regions. Communications Medicine . 2025 Dec 1 [cited 2025 Oct 20];5(1):1–9.

2. Lu WH. Effect of Modifiable Lifestyle Factors on Biological Aging. Journal of Aging Research and Lifestyle . 2024 Jan 1 [cited 2025 Oct 20];13:88–92.

3. Li Y, Huang Q, Jiang J, Du X, Xiang W, Zhang S, et al. Large language model-based biological age prediction in large-scale populations. Nat Med . 2025 Sep 1 [cited 2025 Oct 20];31(9):2977–90.

4. Frampton J, Murphy KG, Frost G, Chambers ES. Higher dietary fibre intake is associated with increased skeletal muscle mass and strength in adults aged 40 years and older. J Cachexia Sarcopenia Muscle . 2021 Dec 1 [cited 2025 Oct 26];12(6):2134.

5. Alahmari LA. Dietary fiber influence on overall health, with an emphasis on CVD, diabetes, obesity, colon cancer, and inflammation. Front Nutr . 2024 [cited 2025 Oct 26];11:1510564.

6. Bulsiewicz WJ. The Importance of Dietary Fiber for Metabolic Health. Am J Lifestyle Med . 2023 Sep 1 [cited 2025 Oct 26];17(5):639.

7. Antonio J, Evans C, Ferrando AA, Stout JR, Antonio B, Cinteo H, et al. Common questions and misconceptions about protein supplementation: what does the scientific evidence really show? J Int Soc Sports Nutr . 2024 [cited 2025 Oct 20];21(1):2341903.

8. Yang J, Zhang W, Dong C. Gut Microbiota Alteration with Moderate-to-Vigorous-Intensity Exercise in Middle School Female Football Athletes. Biology (Basel) . 2025 Feb 1 [cited 2025 Oct 26];14(2):211.

9. Varghese S, Rao S, Khattak A, Zamir F, Chaari A. Physical Exercise and the Gut Microbiome: A Bidirectional Relationship Influencing Health and Performance. Nutrients . 2024 Nov 1 [cited 2025 Oct 26];16(21).

10. Varghese S, Rao S, Khattak A, Zamir F, Chaari A. Physical Exercise and the Gut Microbiome: A Bidirectional Relationship Influencing Health and Performance. Nutrients . 2024 Nov 1 [cited 2025 Oct 26];16(21):3663.

11. Urban S, Chmura O, Wątor J, Panek P, Zapała B. The intensive physical activity causes changes in the composition of gut and oral microbiota. Sci Rep . 2024 Dec 1 [cited 2025 Oct 26];14(1):1–15.

12. Zhernakova D V., Wang D, Liu L, Andreu-Sánchez S, Zhang Y, Ruiz-Moreno AJ, et al. Host genetic regulation of human gut microbial structural variation. Nature . 2024 Jan 25 [cited 2025 Oct 20];625(7996):813–21.

13. Torquati L, Gajanand T, Cox ER, Willis CRG, Zaugg J, Keating SE, et al. Effects of exercise intensity on gut microbiome composition and function in people with type 2 diabetes. Eur J Sport Sci . 2023 Apr 3 [cited 2025 Oct 26];23(4):530–41.

14. Aya V, Flórez A, Perez L, Ramírez JD. Association between physical activity and changes in intestinal microbiota composition: A systematic review. PLoS One . 2021 Feb 1 [cited 2025 Oct 26];16(2):e0247039.

15. Yun S, Seo Y, Lee Y, Lee DT. Gut microbiome related to metabolic diseases after moderate-to-vigorous intensity exercise. J Exerc Sci Fit . 2024 Oct 1 [cited 2025 Oct 26];22(4):375.

16. Yun S, Seo Y, Lee Y, Lee DT. Gut microbiome related to metabolic diseases after moderate-to-vigorous intensity exercise. J Exerc Sci Fit . 2024 Oct 1 [cited 2025 Oct 26];22(4):375.

17. Clarke SF, Murphy EF, O'Sullivan O, Lucey AJ, Humphreys M, Hogan A, et al. Exercise and associated dietary extremes impact on gut microbial diversity. Gut . 2014 Dec 1 [cited 2025 Oct 26];63(12):1913–20.

18. Estaki M, Pither J, Baumeister P, Little JP, Gill SK, Ghosh S, et al. Cardiorespiratory fitness as a predictor of intestinal microbial diversity and distinct metagenomic functions. Microbiome . 2016 [cited 2025 Oct 26];4:42.

19. Pérez-Prieto I, Plaza-Florido A, Ubago-Guisado E, Ortega FB, Altmäe S. Physical Activity, Sedentary Behavior and Microbiome: A Systematic Review and Meta-analysis. J Sci Med Sport . 2024 Nov 1 [cited 2025 Oct 26];27(11):793.

20. Boelius HM, Aatsinki AK, Heiskanen MA, Haapala EA, Munukka E, Mykkänen J, et al. Association of leisure time physical activity with gut

microbiota composition in early adulthood. Sci Rep . 2025 Dec 1 [cited 2025 Oct 26];15(1):1–12.

21. García-Gavilán JF, Atzeni A, Babio N, Liang L, Belzer C, Vioque J, et al. Effect of 1-year lifestyle intervention with energy-reduced Mediterranean diet and physical activity promotion on the gut metabolome and microbiota: a randomized clinical trial. Am J Clin Nutr . 2024 May 1 [cited 2025 Oct 26];119(5):1143–54.

22. Zeng Q, Feng X, Hu Y, Su S. The human gut microbiota is associated with host lifestyle: a comprehensive narrative review. Front Microbiol. 2025 Jun 23;16:1549160.

23. Xu L, Li W, Ling L, Zhang Z, Cui Z, Ge J, et al. A Sedentary Lifestyle Changes the Composition and Predicted Functions of the Gut Bacterial and Fungal Microbiota of Subjects from the Same Company. Curr Microbiol . 2023 Dec 1 [cited 2025 Oct 26];80(12):368.

24. Pandit SS, Meganathan P, Vedagiri H. Harmonizing gut microbiota dysbiosis: Unveiling the influence of diet and lifestyle interventions. Metabol Open . 2025 Sep 1 [cited 2025 Oct 26];27:100384.

25. Varghese S, Rao S, Khattak A, Zamir F, Chaari A. Physical Exercise and the Gut Microbiome: A Bidirectional Relationship Influencing Health and Performance. Nutrients . 2024 Nov 1 [cited 2025 Oct 26];16(21):3663.

26. Aya V, Jimenez P, Muñoz E, Ramírez JD. Effects of exercise and physical activity on gut microbiota composition and function in older adults: a systematic review. BMC Geriatr . 2023 Dec 1 [cited 2025 Oct 26];23(1).

27. Varghese S, Rao S, Khattak A, Zamir F, Chaari A. Physical Exercise and the Gut Microbiome: A Bidirectional Relationship Influencing Health and Performance. Nutrients . 2024 Nov 1 [cited 2025 Oct 26];16(21):3663.

28. Brooks CN, Wight ME, Azeez OE, Bleich RM, Zwetsloot KA. Growing old together: What we know about the influence of diet and exercise on the aging host's gut microbiome. Front Sports Act Living. 2023 Apr 17;5:1168731.

29. Clark JS, Simpson BS, Murphy KJ. The role of a Mediterranean diet and physical activity in decreasing age-related inflammation through modulation of the gut microbiota composition. British Journal of Nutrition . 2022 Oct 14 [cited 2025 Oct 26];128(7):1299–314.

30. Lin Z, Jiang T, Chen M, Ji X, Wang Y. Gut microbiota and sleep: Interaction mechanisms and therapeutic prospects. Open Life Sci . 2024 Jan 1 [cited 2025 Oct 20];19(1).

31. Seong HJ, Baek Y, Lee S, Jin HJ. Gut microbiome and metabolic pathways linked to sleep quality. Front Microbiol. 2024 Jul 31;15:1418773.

32. Caricilli AM, Picardi PK, de Abreu LL, Ueno M, Prada PO, Ropelle ER, et al. Gut Microbiota Is a Key Modulator of Insulin Resistance in TLR 2 Knockout Mice. PLoS Biol . 2011 [cited 2025 Oct 26];9(12):e1001212.

33. Scheithauer TPM, Rampanelli E, Nieuwdorp M, Vallance BA, Verchere CB, van Raalte DH, et al. Gut Microbiota as a Trigger for Metabolic Inflammation in Obesity and Type 2 Diabetes. Front Immunol . 2020 Oct 16 [cited 2025 Oct 26];11:571731.

34. Ji H, Su S, Chen M, Liu S, Liu S, Guo J. The role of gut microbiota in insulin resistance: recent progress. Front Microbiol . 2025 [cited 2025 Oct 26];16:1633029.

35. Takeuchi T, Kubota T, Nakanishi Y, Tsugawa H, Suda W, Kwon ATJ, et al. Gut microbial carbohydrate metabolism contributes to insulin resistance. Nature . 2023 Sep 14 [cited 2025 Oct 26];621(7978):389–95.

36. Semo D, Reinecke H, Godfrey R. Gut microbiome regulates inflammation and insulin resistance: a novel therapeutic target to improve insulin sensitivity. Signal Transduct Target Ther . 2024 Dec 1 [cited 2025 Oct 26];9(1):1–3.

37. Ji H, Su S, Chen M, Liu S, Liu S, Guo J. The role of gut microbiota in insulin resistance: recent progress. Front Microbiol . 2025 [cited 2025 Oct 26];16:1633029.

38. Zhao J, Huang Y, Yu X. A Narrative Review of Gut-Muscle Axis and Sarcopenia: The Potential Role of Gut Microbiota. Int J Gen Med . 2021 [cited 2022 Feb 7];14:1263.

39. Ticinesi A, Lauretani F, Milani C, Nouvenne A, Tana C, Del Rio D, et al. Aging Gut Microbiota at the Cross-Road between Nutrition, Physical Frailty, and Sarcopenia: Is There a Gut-Muscle Axis? Nutrients . 2017 Dec 1 [cited 2024 Sep 16];9(12).

40. Ioannou M, Foiselle M, Mallet J, Stam EL, Godin O, Dubertret C, et al. Towards precision medicine: What are the stratification hypotheses to identify homogeneous inflammatory subgroups. European Neuropsychopharmacology . 2021 Apr 1 [cited 2025 Oct 26];45:108–21.

41. Skov J, Kristiansen K, Jespersen J, Olesen P. Status and perspectives of biomarker validation for diagnosis, stratification, and treatment. Public Health . 2021 Jan 1 [cited 2025 Oct 26];190:173–5.

42. Zhang RQ, Shi Z, Chen H, Chung NYF, Yin Z, Li KKW, et al. Biomarker-based prognostic stratification of young adult glioblastoma. Oncotarget . 2015 [cited 2025 Oct 26];7(4):5030.

43. Glaab E, Rauschenberger A, Banzi R, Gerardi C, Garcia P, Demotes J. Biomarker discovery studies for patient stratification using machine learning analysis of omics data: a scoping review. BMJ Open . 2021 Dec 6 [cited 2025 Oct 26];11(12):e053674.

44. Passaro A, Al Bakir M, Hamilton EG, Diehn M, André F, Roy-Chowdhuri S, et al. Cancer Biomarkers - Emerging Trends and Clinical Implications for personalized treatment. Cell . 2024 Mar 28 [cited 2025 Oct 26];187(7):1617.

45. Shah A, Vaccarino V. Heart Rate Variability in the Prediction of Risk for Posttraumatic Stress Disorder. JAMA Psychiatry . 2015 Oct 1 [cited 2025 Oct 26];72(10):964–5.

46. Zhang J, Zhang Y, Wang J, Xia Y, Zhang J, Chen L. Recent advances in Alzheimer's disease: Mechanisms, clinical trials and new drug development strategies. Signal Transduct Target Ther . 2024 Dec 1 [cited 2025 Oct 26];9(1):1–35.

47. Seekatz AM. Straining to define a healthy microbiome. mSphere . 2025 Jul 29 [cited 2025 Oct 20];10(7):e00797-24.

48. Van Hul M, Cani PD, Petitfils C, De Vos WM, Tilg H, El-Omar EM. What defines a healthy gut microbiome? Gut . 2024 Oct 7 [cited 2025 Oct 20];73(11):1893–908.

49. Deek RA, Ma S, Lewis J, Li H. Statistical and computational methods for integrating microbiome, host genomics, and metabolomics data. Elife . 2024 Jun 4 [cited 2025 Oct 26];13.

50. Li Y, Han S. Metabolomic Applications in Gut Microbiota-Host Interactions in Human Diseases. Gastroenterol Clin North Am . 2024 Sep 1 [cited 2025 Oct 26];53(3):383–97.

51. Aya V, Pardo-Rodriguez D, Vega LC, Cala MP, Ramírez JD. Integrating metagenomics and metabolomics to study the gut microbiome and host relationships in sports across different energy systems. Sci Rep . 2025 Dec 1 [cited 2025 Oct 26];15(1).

52. Huang L, Meng J, Lin S, Peng Z, Zhang R, Shen X, et al. Integrating Gut Microbiome and Metabolomics with Magnetic Resonance Enterography to Advance Bowel Damage Prediction in Crohn's Disease. J Inflamm Res . 2025 [cited 2025 Oct 26];18:7631.

53. Mangnier L, Bodein A, Mariaz M, Mathieu A, Scott-Boyer MP, Vashist N, et al. A systematic benchmark of integrative strategies for microbiome-metabolome data. Commun Biol . 2025 Dec 1 [cited 2025 Oct 26];8(1).

54. Shaffer M, Armstrong AJS, Phelan V V., Reisdorph N, Lozupone CA. Microbiome and Metabolome Data Integration Provides Insight Into Health and Disease. Transl Res . 2017 Nov 1 [cited 2025 Oct 26];189:51.

55. Yang SY, Han SM, Lee JY, Kim KS, Lee JE, Lee DW. Advancing Gut Microbiome Research: The Shift from Metagenomics to Multi-Omics and Future Perspectives. J Microbiol Biotechnol . 2025 [cited 2025 Oct 26];35.

56. Armoni R, Borenstein E. Temporal Alignment of Longitudinal Microbiome Data. Front Microbiol . 2022 Jun 22 [cited 2025 Oct 20];13:909313.

57. Bizzarri D, Akker EB van den, Reinders MJT, Pool R, Beekman M, Lakenberg N, et al. Extreme MetaboHealth scores in three cohort studies associate with plasma protein markers for inflammation and cholesterol transport. Immun Ageing . 2025 Sep 9 [cited 2025 Oct 20];22(1):34.

58. Smit AP, Herber GCM, Kuiper LM, Rietman ML, Wesenhagen KEJ, Picavet HSJ, et al. Association between metabolomics-based biomarker scores and 10-year cognitive decline in men and women. The Doetinchem Cohort Study. Age Ageing . 2024 Nov 1 [cited 2025 Oct 20];53(11):afae256.

59. Zonneveld MH, Al Kuhaili N, Mooijaart SP, Slagboom PE, Jukema JW, Noordam R, et al. Increased 1H-NMR metabolomics-based health

score associates with declined cognitive performance and functional independence in older adults at risk of cardiovascular disease. Geroscience . 2025 Apr 1 [cited 2025 Oct 26];47(2):2035–45.

60. van Holstein Y, Mooijaart SP, van Oevelen M, van Deudekom FJ, Vojinovic D, Bizzarri D, et al. The performance of metabolomics-based prediction scores for mortality in older patients with solid tumors. Geroscience . 2024 Dec 1 [cited 2025 Oct 26];46(6):5615.

61. Bizzarri D, Akker EB van den, Reinders MJT, Pool R, Beekman M, Lakenberg N, et al. Extreme MetaboHealth scores in three cohort studies associate with plasma protein markers for inflammation and cholesterol transport. Immun Ageing . 2025 Sep 9 [cited 2025 Oct 26];22(1):34.

62. Panyard DJ, Yu B, Snyder MP. The metabolomics of human aging: Advances, challenges, and opportunities. Sci Adv . 2022 Oct 21 [cited 2025 Oct 26];8(42):6155.

63. van der Laag PJ, Dorhout BG, Heeren AA, Veenhof C, Barten DJJA, Schoonhoven L. Barriers and facilitators for implementation of a combined lifestyle intervention in community-dwelling older adults: a scoping review. Front Public Health. 2023 Oct 11;11:1253267.

64. Shrestha A, Yang L, Demissie GD, Dhital R, Panniyammakal J, Parasuraman G, et al. Scaling up structured lifestyle interventions to improve the management of cardiometabolic diseases in low-income and middle-income countries: a systematic review of strategies, methods and outcomes. BMJ Public Health . 2025 Mar 4 [cited 2025 Oct 26];3(1):e001371.

65. Cano-Ibáñez N, Bueno-Cavanillas A. Lifestyle Interventions in an Aged Population: Challenges and Opportunities from a Public Health Perspective. Nutrients . 2024 Jan 1 [cited 2025 Oct 26];16(1):173.

66. Saleem A, Appannah A, Meyer C, Hutchinson AM, Mills A, Smit DV, et al. A qualitative exploration of challenges recruiting older adults for Being Your Best, a co-designed holistic intervention to manage and reduce frailty: lessons learnt amidst the COVID-19 pandemic in Melbourne, Australia. BMJ Open . 2024 May 1 [cited 2025 Oct 26];14(5):e082618.

67. Anzuoni K, Field TS, Mazor KM, Zhou Y, Garber LD, Kapoor A, et al. Recruitment Challenges for Low-Risk Health System Intervention Trials in Older Adults: A Case Study. J Am Geriatr Soc . 2020 Nov 1 [cited 2025 Oct 26];68(11):2558–64.

68. Mody L, Miller DK, McGloin JM, Freeman M, Marcantonio ER, Magaziner J, et al. Recruitment and Retention of Older Adults in Aging Research. J Am Geriatr Soc . 2008 [cited 2025 Oct 26];56(12):2340.

69. Withall J, Group for the RSR, Greaves CJ, Group for the RSR, Thompson JL, Group for the RSR, et al. The Tribulations of Trials: Lessons Learnt Recruiting 777 Older Adults Into REtirement in ACTion (REACT), a Trial of a Community, Group-Based Active Aging Intervention Targeting Mobility Disability. The Journals of Gerontology: Series A . 2020 Nov 13 [cited 2025 Oct 26];75(12):2387–95.

70. Mohammad Hanipah J, Mat Ludin AF, Singh DKA, Subramaniam P, Shahar S. Motivation, barriers and preferences of lifestyle changes among older adults with frailty and mild cognitive impairments: A scoping review of qualitative analysis. PLoS One . 2025 Jan 1 [cited 2025 Oct 26];20(1):e0314100.

71. Buttgereit T, Palmowski A, Forsat N, Boers M, Witham MD, Rodondi N, et al. Barriers and potential solutions in the recruitment and retention of older patients in clinical trials-lessons learned from six large multicentre randomized controlled trials. Age Ageing . 2021 Nov 1 [cited 2025 Oct 26];50(6):1988–96.

72. Lacey RJ, Wilkie R, Wynne-Jones G, Jordan JL, Wersocki E, Mcbeth J. Evidence for strategies that improve recruitment and retention of adults aged 65 years and over in randomised trials and observational studies: a systematic review. Age Ageing . 2017 Nov 1 [cited 2025 Oct 26];46(6):895–903.

73. Duijvestijn M, van den Berg SW, Wendel-vos GCW. Adhering to the 2017 Dutch Physical Activity Guidelines: A Trend over Time 2001–2018. Int J Environ Res Public Health . 2020 Feb 1 [cited 2025 Oct 20];17(3):681.

74. Sun X, Wang L, Shen X, Huang C, Wei Z, Su L, et al. Correlates of Adherence of Multimodal Non-pharmacological Interventions in Older Adults With Mild Cognitive Impairment: A Cross-Sectional

Study. Front Psychiatry . 2022 Jun 3 [cited 2025 Oct 26];13:833767.

75. Room J, Hannink E, Dawes H, Barker K. What interventions are used to improve exercise adherence in older people and what behavioural techniques are they based on? A systematic review. BMJ Open . 2017 Dec 1 [cited 2025 Oct 26];7(12):e019221.

76. Bardach SH, Schoenberg NE. The role of primary care providers in encouraging older patients to change their lifestyle behaviors. Clin Gerontol . 2017 Aug 8 [cited 2025 Oct 26];41(4):326.

77. Lind Infeld D, Whitelaw N. Policy initiatives to promote healthy aging. Clin Geriatr Med . 2002 Aug [cited 2025 Oct 26];18(3):627–42.