Ageing Research Reviews

Aging in Women – the Microbiome Perspective

--Manuscript Draft--

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Abstract:	Summary Menopause represents a hallmark of women's aging, frequently portrayed as a medical issue. It also comprises social and biological aspects often neglected and not well-understood, leaving women with insufficient support and attention. With the decline in estrogen levels starting years before menopause is fully established, women experience various physical symptoms, and the risk of many age-related diseases increases sharply soon after these hormonal changes occur. Notably, these hormonal shifts also induce significant changes in the vaginal and gut microbiome, contributing to dysbiosis influencing the onset and progression of diseases. Here we examined the complex and dynamic relationship between aging, menopause, and microbiome changes with a particular focus on the vaginal and gut ecosystems. Emerging research emphasizes diet as a potential modulator for maintaining microbiome health throughout menopause. A deeper understanding of microbiome changes across life stages suggests the potential of microbiome-targeted strategies to support well-aging in women.
Opposed Reviewers:	
Response to Reviewers:	

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To the Editor of:
Ageing Research Reviews

Date: December 10th, 2024

Subject: Review submission entitled 'Aging in Women – the Microbiome Perspective'

Dear Editor,

I am writing to submit our Review manuscript entitled "**Aging in Women – the Microbiome Perspective"** for consideration in *Ageing Research Reviews* journal.

Women's health has become a central focus in the field of medicine and biomedical research, as most previous research was predominantly centered on men, sometimes leaving a gap in women underdiagnosed and undertreated for many diseases. Given the biological, hormonal, and physiological differences between the sexes, a gender-specific approach to medicine and research is necessary to address related health conditions at different life stages, including aging.

In our Review, we focus on the aging process in women, starting from the menopause period. Menopause is, in fact, a key stage in women's aging and is often viewed merely as a medical issue. However, it embraces social and biological aspects frequently overlooked. We thoroughly described biological changes occurring in the vaginal and gut microbiomes and discussed their impact on post-menopause syndrome as well as age-related diseases such as osteoporosis and cardiovascular diseases. Diet represents a great opportunity for intervention, either to support medical therapy or as a stand-alone approach. To this end, we have explored which diets and dietary components adapted to women were reported to promote well-aging through microbial modulation.

Our manuscript counts about 5,500 words and includes 5 Figures and 1 Supplementary Table. To facilitate the reader's reading, we also included a Panel describing key concepts and technical words in detail. If the manuscript is of interest but the text limit is a concern, we will be delighted to revise its length as needed.

We recognize *Ageing Research Reviews* strong interest in women's health and the role of microbiota in aging process. We therefore hope that our Review aligns with the topics of the journal and expect it to be of interest to your readers.

Thank you in advance for considering our work. Please do not hesitate to contact us if you have any questions. We look forward to hearing from you.

Yours sincerely on behalf of all co-authors,
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To the Editor of:
Ageing Research Reviews

Date: November 18th, 2025

Subject: Submission of revised manuscript entitled 'Aging in Women – the Microbiome Perspective'

Dear Editor,

Thank you very much for giving us the opportunity to revise our Review manuscript entitled "**Aging in Women – the Microbiome Perspective**".

We are extremely grateful to the reviewers for their constructive comments. We have addressed all the concerns raised, and we believe the manuscript has substantially improved as a result. The entire text has been carefully revised and proofread; we apologize for any errors present in the previous version. Figures have been updated, minor typos corrected, and a new publication license has been uploaded with the revised manuscript.

Moreover, we have generated a Graphical Abstract that could be potentially included in the manuscript, describing all aspects that have been discussed in the review. The short legend could be the following:

"The graphical abstract illustrates the different aspects of aging in women that are discussed in the review."

We hope that our Review is now suitable for publication. Please do not hesitate to contact us if you have any questions. We look forward to hearing from you.

Yours sincerely on behalf of all co-authors,

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RESPONSE LETTER – FERRANDO ARR-D-24-02068

Reviewer #1: Dear Authors,
Based on a careful review of the manuscript titled "Aging in Women - the Microbiome Perspective", I found it to be a well-structured and comprehensive work that explores the impact of menopause on the vaginal and gut microbiomes. The review places particular emphasis on the interplay between estrogen decline, diet, and microbial dysbiosis in shaping women's health during aging. The manuscript is well aligned with the scope of Ageing Research Reviews. However, to further improve clarity, depth, and critical evaluation, there are some minor points to address before a possible publication.
The manuscript focused on women needs in light of the growing recognition within the scientific community to better understand sex-specific mechanisms to ensure equitable healthcare for both genders. The review also consider the influence of diet on women's health and aging by studying the role of bioactive compounds. To support, a well-integrated Figures clarify complex interactions like the gut-vaginal axis and estrogen recirculation.
The methodology used for the literature selection is clearly described, including search terms, inclusion/exclusion criteria, and databases (PubMed, Scopus). which adds transparency and reproducibility to the review process.

We thank the reviewer for the appreciation of our work.

Some minor points to improve:
Q1. In the paragraph: The menopausal transition period presents potential risks for women's health.
I suggest the Authors to add the full definition for STRAW (line 6)
***A1**. We have now included the full name of the acronym STRAW. Thank you for pointing this out.*

Q2. Line 34: "metabolism metabolism disorders" please modify "metabolic disorders"
***A2.** We have fixed this typo, thank you.*

Q3. In the paragraph: The gut microbiome and its connection to aging and women's health
Line 50: Please eliminate the word "studies" present twice.
***A3.** We replaced the sentence*
“The rapid expansion of metagenomic studies to population studies and disease cohorts has consistently highlighted the association between gut microbiome dysbiosis and many diseases” with the following:
“The rapid expansion of metagenomic studies to large-scale population-based and disease cohorts has consistently highlighted the association between gut microbiome dysbiosis and many diseases.”

Q4. In the paragraph: Diet as a potential modulator of microbiome toward healthy aging in women
Lines 44-46: The influence of the matrix is very important, as shown in the detailed differences on the effect of isoflavone extracted from red clover and the synthetic ipriflavone.
Do the Authors have other evidence of different bioactive compounds compared to some synthetic molecules?

***A4.** In the case of isoflavone, the differential effect may be due to differences in the absorption and bioavailability of the various compounds. To highlight this, we have added the following short sentence (line 4 pg 14 - ‘This effect may be explained by differences in the absorption and bioavailability of the various compounds, which should be assessed on a case-by-case basis.’*

as well as two new citations about this subject:

84 'Alexandersen P, Toussaint A, Christiansen C, et al. Ipriflavone in the Treatment of Postmenopausal OsteoporosisA Randomized Controlled Trial. JAMA 2001; ***285****: 1482–8.*

and

85 'Tanida S, Kurokawa T, Sato H, Kadota K, Tozuka Y. Evaluation of the Micellization Mechanism of an Amphipathic Graft Copolymer with Enhanced Solubility of Ipriflavone. Chem Pharm Bull (Tokyo) 2016; ***64****: 68–72.*

Furthermore, we searched for existing evidence comparing the effects of natural and synthetic compounds, as well as isoflavones, with a particular focus on molecules that have potential estrogenic activity and relevance to menopause. We only found examples suggesting that natural compounds may exert enhanced effects when used in combination (Baudin et al., Food Funct., 2024, 10.1039/d3fo05122f), or when chemically modified into synthetic derivatives (Wang J. et al., Pharmacol. Sci., 2007, 10.1254/jphs.fp0061549). It is thus unclear whether this differential effect between natural and synthetic isoflavones is specific to isoflavone.

Q5. Same paragraph, page 12, line 19. Please modify the name "Lactobacillus" in Italic
A5. *We have checked that all Lactobacillus instances are now in italic. Thank you.*

Q6. Occasional spacing issues;
A6. *We have reviewed the manuscript and made the necessary corrections. Thank you.*

Q7. Figure 4, line 14, "ana healthy" should be corrected.
A7. *This has been fixed, thank you.*

Q8. Reference Redundancy:
Harlow et al. (2012) appears cited 2 times (Refs 3, 9) with the same title, the same year, but in a different Journal. Ensure consistency in the reference.
A8. *Thanks for pointing this out. Being the two articles extremely similar, we have deleted the article 1)* 'Harlow, S. D., et al (2012). *Menopause (New York, N.y.)*, *19*(4), 387–395.' and kept only 2) ' Harlow, S. D., et al. (2012).. *The Journal of Clinical Endocrinology and Metabolism*, *97*(4), 1159–1168.

Reviewer #2:
In this review, Ferrando, et al. explore how menopause reshape the vaginal and gut microbiomes in women and how these changes may contribute to age-related health conditions. They describe how the menopausal transition is associated with shifts in microbial diversity and community structure, which may influence inflammation, metabolism, and health. The review highlights that declining sex hormones, particularly estrogen, play a central role in driving these microbial and physiological changes. The authors also discuss dietary and probiotic strategies, such as the use of phytoestrogens and nutrient-rich diets, as potential means to restore microbial balance and promote healthy aging. This review provides a well-structured summary of emerging evidence linking menopause, microbiome changes, and women's health. Below are comments focusing on the manuscript's clarity, structure, and scope.

We thank the reviewer for their appreciation and positive evaluation of our work.

<Comments>

Q1. Throughout the manuscript, the term "gender" is often used in contexts that describe biological processes, hormonal changes, and physiological outcomes. These should be referred to as sex differences, not gender differences. The term "Gender" should be reserved for sociocultural factors that influence health or behavior. Given that this review primarily focuses on biological aging, menopause, and hormone-driven effects, all instances need to be revised to accurately reflect sex-based differences.

***A1**. We thank the reviewer for the clarification. In some previous publications, the distinction between “sex” and “gender” was not always explicit, which may have contributed to our initial wording. We appreciate the reviewer’s comment for helping us align our terminology with current best practices.*
The manuscript has been carefully revised to ensure consistent and accurate use of these terms throughout.

Q2. The introduction would benefit from a concise explanation of the biological basis of menopause, specifically, how depletion of the ovarian follicle reserve leads to the cessation of reproductive function and subsequent hormonal decline. Presenting this context upfront would make later sections on downstream metabolic, microbiome, and disease effects more cohesive and grounded in reproductive physiology.

***A2**. We have now added an explanation of the biological bases of menopause in the first paragraph entitled “The menopausal transition period presents potential risks for women’s health.”*

The text now reads as follows:

*‘Menopause represents a key milestone of women’s aging, marking the transition to a new phase of life. Despite its profound impact on health, it remains an often-overlooked aspect of women’s wellbeing. Biologically, it is defined by the permanent cessation of ovarian follicular activity due to the depletion of ovarian follicular reserve. As the number of functional follicles declines, the production of gonadal steroids decreases, ultimately leading to the end of monthly menstruation, and of reproductive capacity 3 “During this phase, the number of granulosa cells within the ovarian follicles declines (ovarian aging), leading to reduced production of estradiol (and inhibin B) and consequently lower circulating estrogen levels (Harlow, Gass, Hall, Lobo, Maki, Rebar, Sherman, Sluss, de Villiers, et al., 2012). Without the inhibitory effect of estrogen on gonadotropins, the pituitary gland increases the production of follicle-stimulating hormone (FSH ≥ 25 mIU/mL), resulting in the cessation of the menstrual cycle (**Figure 1_A**).’*

Q3. In the introduction, the authors state that the review will examine the dynamic relationship between vaginal and gut microbiomes in the context of women's aging. However, in the main text, the vaginal and gut microbiome sections are presented as largely independent summaries, with minimal discussion of how these systems interact. I would suggest that (i) the vaginal and gut sections be their own with subsections for each, instead of multiple sections on vaginal and on gut, and (ii) that the authors include a section, even if short, parsing existing evidence on potential interactions and communications between the compartments. This is all the more important because of studies highlighting the potential benefits of oral pre- and probiotics, which must pass through the gut, on the vaginal microbiome ecosystem. The manuscript would be significantly strengthened by explicitly addressing the mechanistic and physiological links between them. In addition, highlighting gaps in knowledge at that level would also tremendously benefit the field.

***A3**. We briefly mentioned the potential interaction between the gut and the vagina at the end of the Gut microbiome section (the estrobolome systems should be the connection between the two organs, though it involves sex hormone metabolism). However, we agree with the reviewer that a separate section with more in-depth elaboration of the current evidence for a gut-vagina axis will enhance the review perspectives. We have now restructured the manuscript and added this section. There, we discuss current evidence on the bidirectional interactions between the gut and vaginal microbiomes, the potential mechanisms underlying the effects of diet and oral probiotics on the vaginal ecosystem, and the remaining knowledge gaps in this area.*

Q4. The figures are detailed and informative, but several elements are not clearly discussed or referenced in the main text. Providing more direct connections between the figures and the narrative would help readers better understand and appreciate their relevance.
***A3**. We have expanded the discussion in the main text of the elements presented in the Figures. We also revised figures and fixed minor typos. A new publication license will be attached to the revised version.*

Q5. There are several typographical issues (e.g., line 27: "In addition, they hypoestrogenic state flowing…" should read "the hypoestrogenic state following…"). A thorough proofread is needed to correct similar minor errors throughout the manuscript.
***A5**. The manuscript has been carefully revised and proofread; we apologize for any errors that may have been present in the previous version.*

Highlights

- The decline of menopause-related estrogen affects the vaginal and gut microbiota, likely influencing onset and progression of age-related conditions
- In most postmenopausal women (60-70%), the vaginal microbiome presents anaerobic communities and greater microbial diversity.
- Aging during menopause is linked to decreased gut bacterial diversity, with women's microbiota being more similar to men's microbiota.
- Key taxa shifts include reductions in SCFA-producers, estrobolome microbes, and beneficial bacteria and increase of Bacteroides and some pathobionts
- The gut-vaginal axis represents a bidirectional relationship between the gut microbiome and the vaginal microbiome, where microbial communities in these two regions interact and influence each other.
- Diet represents a great opportunity for intervention, either to support medical therapy or as a stand-alone approach.

***Title**:*

Aging in Women – the Microbiome Perspective

Authors:

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Summary:

Menopause represents a hallmark of women's aging, frequently portrayed as a medical issue. It also comprises social and biological aspects often neglected and not well-understood, leaving women with insufficient support and attention. With the decline in estrogen levels starting years before menopause is fully established, women experience various physical symptoms, and the risk of many age-related diseases increases sharply soon after these hormonal changes occur. Notably, these hormonal shifts also induce significant changes in the vaginal and gut microbiome, contributing to dysbiosis influencing the onset and progression of diseases. Here we examined the complex and dynamic relationship between aging, menopause, and microbiome changes with a particular focus on the vaginal and gut ecosystems. Emerging research emphasizes diet as a potential modulator for maintaining microbiome health throughout menopause. A deeper understanding of microbiome changes across life stages suggests the potential of microbiome-targeted strategies to support well-aging in women.

Panel

Estrogens: Estradiol, known also as 17β-estradiol or estrogen (**E2**), is the most common and important form of circulating estrogens. In premenopausal women, **E2** reaches the highest amount before ovulation (110–410 pg/mL), and approx. 19–150 pg/mL during the follicular and luteal phases, whereas in postmenopausal women is <35 pg/mL. Two other less abundant forms include estrone (**E1**) and estriol (**E3**); the latter is derived from E1 through the 16α-hydroxylation process. Their roles become more critical during pregnancy when the placenta produces them in large quantities.

Microbiota: the microbial biomass community residing in the host's organs, including bacteria, archaea, fungi, and viruses

Microbiome: the whole collection of microbiota genomes and their products

Microbial diversity: the variety and abundance of taxa in the microbiota

16S rRNA amplicon sequencing: the targeted sequencing of 16S ribosomal RNA (16S rRNA)

Shotgun sequencing: the untargeted sequencing of all microbial genomes (metagenomes)

Dysbiosis: alteration in the microbiome's composition and/or function that can lead to losing its supportive function in host health.

Pathobionts: normally harmless microorganisms that can become pathogenic under certain conditions, often associated with dysbiosis or immune dysfunction. In the vaginal microbiota, pathobionts include *Prevotella*, *Streptococcus*, *Gardnerella*, and *Atopobium.* In the intestinal microbiota, they include *Bilophila*, *Desulfovibrio*, *Eggerthella*, all *Enterobacteriaceae*, *Campylobacter*, *Fusobacterium*, *Streptococcus*, *Bacteroides fragilis*, *Actinomyces*, *Corynebacterium*, *Staphylococcus*, *Parvimonas*, *Porphyromonas*, *Ruminococcus torques*, *Clostridium* species such as *C. difficile*.

Short-chain fatty acids (**SCFAs**): fatty acids with 1 to 6 carbon atoms produced through the saccharolytic fermentation of undigested dietary fibers. The main SCFAs—acetic acid (acetate), propionic acid (propionate), and butyric acid (butyrate)—are typically present in a ratio of 60:20:20. *Clostridiales* members are key SCFAs-producers with the families *Ruminococcaceae* (e.g., *Faecalibacterium*, *Ruminococcus*, *Oscillibacter*), *Lachnospiraceae* (i.e., *Roseburia*, *Anaerostipes*, *Blautia*, *Coprococcus, Butyrivibrium, Dorea*) and *Eubacteriaceae* being particularly efficient butyrate-producers. Other bacteria, such as *Bifidobacterium*, can produce non-butyrate metabolites (i.e., acetate, succinate, and lactate) used by butyrogenic-producer bacteria.

Trimethylamine N-oxide (**TMAO**): class of amine oxide produced by gut microbes through the metabolism of dietary nutrients, primarily choline, carnitine, and betaine, and its levels increase with the consumption of animal protein.

Estrobolome: collection of bacterial genes involved in the reactivation of sex hormones (e.g., estrogens), encoding glycoside hydrolase 2 (GH2) family enzymes, which includes gut microbial β-glucuronidases (gmGUS), β-galactosidases, and β-mannosidases. GUS genes are present in Bacteroidetes (52%, e.g., *Bacteroides Prevotella, Alistipes, etc.*), Firmicutes (43%, e.g., *Faecalibacterium, Lactobacillus*, *Streptococcus, Eubacterium, Ruminococcus, Roseburia, Romboutsia, some Clostridium, and Coprococcus*), Actinobacteria (1.6% e.g., *Bifidobacterium*) Verrucomicrobia (1.5% e.g., *Akkermansia*) and Proteobacteria (1.3%, e.g., *Escherichia*).

Abbreviations used for Aged-related diseases in women:
Genitourinary Syndrome of menopause (GSM)
Vulvovaginal atrophy (VVA)
Vasomotor symptoms (VMS)
Genitourinary syndrome of menopause (GSM)
Urinary tract infections (UTI)
Postmenopausal osteoporosis (PMO)
Cardiovascular diseases (CVD)
Metabolic diseases (Met)
Type 2 diabetes mellitus (T2DM)

Introduction

Aging is a natural process that causes significant changes in the physiology of the human body. Over the past century, global life expectancy has greatly increased, and this trend is forecast to continue. Accordingly, the elderly population (over 65) is expected to rise from 761 million in 2021 to 1.6 billion in 2050 [1], posing a worldwide challenge to governments and economies. Women generally live longer than men, partly due to the protective effects of female sex hormones, though the precise reasons for this longevity gap remain unclear[2]. Sex hormone fluctuations and modifications significantly impact women's health, especially those occurring throughout menopause, a major hallmark of aging in women [3]. These hormonal alterations are linked to diverse age-related diseases, such as cardiovascular diseases, metabolic disorders, and osteoporosis [3–5]. In contrast, men undergo a progressive fall in testosterone levels, affecting muscle strength and cognitive functions. However, these changes are generally less abrupt than those observed in women. Aging is also associated with changes in the **microbiome,** the microbial communities inhabiting different organs in the body, as well as their products, essential for metabolic, immune, and neurological functions. While changes in the microbiome during aging are partially attributable to increased use of medications in late adulthood, it is also known that significant alterations in the composition and diversity of the microbiome (**dysbiosis**) contribute to the onset and progression of age-related conditions [6]. Remarkably, in women, the **estrogen** decline during menopause further influences the gut microbiota, as well as the vaginal microbiome [7,8]. This is likely to lead to differential mechanisms of the microbiota-host health relationship in men compared to women, which are largely unknown.

This review examines the dynamic relationship between vaginal-gut microbiomes and age-related conditions in women and explores potential avenues to promote women's well-aging through dietary microbial modulation. Understanding these gender-specific mechanisms, including those related to the microbiome, is crucial to fostering personalized healthy aging.

Search strategy and selection criteria

A search of the scientific literature was conducted by using PubMed and Scopus and looking for the following search terms ((vaginal microbiome OR vaginal microbiota) AND (ageing OR aging OR menopause OR postmenopausal women)) (results **269**); ((gut AND (microbiome OR microbiota) AND women AND (ageing OR menopause OR postmenopausal women)) (results **418**); for the dietary intervention on postmenopausal women a different combination of terms was used: diet OR (*Lactobacillus* OR probiotic) OR (phytoestrogens OR soybean OR isoflavone OR flavonoid) AND (microbiome OR microbiota) AND (menopause OR postmenopausal OR older) AND women (results **649**); (diet OR lactobacillus OR flavonoid) AND (menopause OR postmenopausal OR postmenopausal) AND women AND trials AND (hypertension OR osteoporosis OR vasomotor) (results **484**), food elements were added to the search including red clover, prunes, blueberry, and royal jelly. More specific search combinations for sex hormone changes in menopause were then used, including the terms mentioned above and additional items; for example, (estrogen) OR (sex hormones) AND (menopause OR perimenopausal OR postmenopausal women) AND (microbiota) was used to find articles related to considerations for sex hormones variation in aging. We examined the studies and included those relevant to this review's topic according to the following decision flow chart. **Inclusion criteria:** studies with original data, studies conducted in (premenopausal) OR perimenopausal AND postmenopausal women in healthy status; observational studies including prospective, retrospective, longitudinal, cross-sectional study designs, clinical trials, population cohort studies; a measure of microbiota composition by high-throughput sequencing (16S rRNA or shotgun sequencing). **Exclusion criteria**: studies conducted in women only of reproductive age (18-44 years); commentaries, book chapters, letters, editorials, conference proceedings, case reports, conference, abstracts, or non-peer-reviewed articles, publication in a different language than English; studies conducted in gynecologic cancer patients, with infection, chronic diseases; studies conducted in a cohort lower than **30** postmenopausal women. Molecular methods excluded i) quantitative polymerase chain reaction (qPCR), ii) microbiota measured by non-molecular methods (such as culture and microscopy), no relevant outcomes reported, or when there is a lack of quantifiable outcomes (e.g., not defined abundance of microbiota).

Pertinent original articles and reviews (only used for the introduction paragraph) were peer-reviewed and indexed in PubMed and Scopus. We limited the publication dates from 2010 to 2024 and conducted literature searches between May 2024 and July 2024. **Supplementary Table 1** provides a comprehensive list of publications screened, methods used, and a summary of reported results.

The menopausal transition period presents potential risks for women's health.

Menopause is an important milestone of women's aging, marking a new phase of life but still an overlooked aspect of health. It is defined by a drop in circulating sex hormones, the permanent end of monthly menstruations, and the end of reproductive age [3].

The menopausal transition (MT) occurs in three stages: perimenopause, menopause, and post-menopause. Each stage is characterized by variable duration and symptoms according to the STRAW staging (**Figure 1A**) [9]. Perimenopause encompasses one year preceding the final menstrual period (FMP) and the subsequent year following menopause. During this period, estrogen and progesterone levels fluctuate (**Figure 1A**), and women may experience some symptoms of the 'postmenopausal syndrome' although ovulatory menstruation can still occur [9]. Vasomotor symptoms (VMS), such as hot flashes, followed by abundant menstruation (dysmenorrhea), are often among the earliest signs of perimenopause [10]. Menopause is recognized when menstruation stops for twelve consecutive months, typically between 45 and 55. During this phase, the ovary reduces the granulosa cells (ovarian aging), which are the principal producers of estradiol (or estrogen) (and inhibin B, resulting in a fall in estrogen levels (<35 pg/mL) [9]. Without the inhibitory effect of estrogen on gonadotropins, the pituitary gland increases the production of follicle-stimulating hormone (FSH ≥25 mIU/mL), resulting in the cessation of the menstrual cycle (**Figure 1A**). Post-menopause, referring to the years following menopause, is characterized by a further decrease in sex hormones, with the adipose tissue becoming the principal producer of estrogens [9]. During post-menopause, a woman's body goes through further changes that might influence many organs, leading to a range of symptoms known as postmenopausal syndrome (**Figure 1B**). Declining estrogen levels primarily impact [11]. Therefore, approximately 45–77% of women suffer from the genitourinary syndrome of menopause (GSM) [11], which includes urinary, genital, and sexual symptoms. Reduced glycogen in vaginal cells raises vaginal pH and promotes bacterial growth, increasing infection risk [12]. While VMS usually improves with time, GSM might deteriorate without adequate therapy. Sex hormonal variations can also impact the nervous system, creating neurological disorders such as brain fog, anxiety, and sleep disturbances [13]. The drop of estrogen can accelerate bone density loss and increase the risk of postmenopausal osteoporosis (PMO).

In addition, the hypoestrogenic state flowing menopause can exacerbate chronic diseases impacting vital systems and accelerate the progression of risk of cardiovascular and metabolic diseases (CMD) - aging in women is associated with a significant increase in blood pressure, body mass index (BMI), obesity, and body fat distribution, as well as an increase in insulin resistance [14]. Noteworthy, epidemiological studies show that women tend to develop heart diseases later in life compared to men, mainly after menopause [4,15]. This delay is attributed to the cardioprotective effects of estrogen before menopause, which include enhanced angiogenesis, improved vasodilation, and reduced oxidative stress and fibrosis [14].

Similarly, the decline of estrogen can influence insulin resistance and glucose metabolism, increasing the risk of metabolism metabolic disorders (e.g., type 2 diabetes mellitus, T2DM) [5].

Furthermore, emerging evidence suggests that substantial alterations can occur in intestinal and vaginal microbiomes, altering bacterial populations and boosting the abundance of pathobionts, ensuing dysbiosis linked to inflammatory diseases such as GSM and PMO.

Vaginal microbiota in premenopausal women

Longitudinal studies have observed that the vaginal microbiome community is overall stable in a short-term period (months), with fluctuations occurring only in a small fraction of the female population[16]. Factors like age, ethnicity, sex hormones, and personal habits influence its composition, and some may introduce substantial changes like vaginal infections [17,18]. Estrogens are also key players in shaping the microbiome by promoting glycogen accumulation in vaginal cells and providing a carbohydrate source for *Lactobacillus,* the main dominant bacteria present in women's vaginal microbiome during reproductive age [18]. These bacteria maintain vaginal health by creating an acidic environment (pH 3.5–4.5) that inhibits pathogens and produce anti-inflammatory compounds that strengthen the vaginal epithelium [19]. The four main vaginal *Lactobacillus* species are *L. crispatus*, *L. gasseri*, *L. iners*, and *L. jensenii* (**Figure 2**). *L. iners* has been observed in both healthy women as well as in women with vaginal infections, and the possibility of strains with potential pathogenic roles is under debate [20]. Similarly, vaginal anaerobes, such as *Gardnerella*, *Prevotella*, *Atopobium*, and *Streptococcus*, have pathogenic potential and can destabilize the vaginal microenvironment. To represent and distinguish the different vaginal microbiome types, community state types (CSTs) classification based on taxa abundance [21] was proposed, with four classes dominated by *Lactobacillus spp* (CST I: *L. crispatus*, CST

II: *L. gasseri*, CST III: *L. iners*, CST V: *L. jensenii*) and one, CST IV, which lacks *Lactobacilli* and has high microbial diversity. CST subtypes have further refined this classification (**Figure 2**) [22]. While the use of CSTs to define microbiota composition is common, there is a broader call for more dynamic, higher-resolution approaches to understanding microbial communities and modeling their interconnected nature and mutual coexistence within the vaginal microenvironment [18].

Vaginal microbiome during and after menopausal transition

Most research on vaginal microbiota focused on women of reproductive age, leaving a critical knowledge gap in our understanding of changes that occur throughout and after MT. However, major changes are expected to exist and are likely to correlate with menopause-related health conditions. One of the pioneer studies tracked over two months the vaginal microbiota in postmenopausal women with and without vulvovaginal atrophy (VVA), observing that women without symptoms had low bacterial diversity dominated by *Lactobacilli*. In contrast, symptomatic women had higher diversity with anaerobes like *Prevotella timonensis*, *Porphyromonas*, *Peptoniphilus*, and *Bacillus* [23]. Moreover, *Lactobacillus spp.* presence was inversely correlated with dryness. Other studies have also observed that a low percentage of postmenopausal women (10-30%) still maintain *Lactobacillus*-dominated communities, while in the majority of postmenopausal women (approx. 60-70%), the vaginal microbiome is more likely to be characterized by anaerobic communities and increased microbial diversity (rather than having one dominant species) and elevated vaginal pH [24,25]. Larger cohort studies have further confirmed that postmenopausal women often exhibit CST IV, with a moderate prevalence of subtypes (CST IV-C0 and CST IV-B), which were also associated with the risk of VVA and GSM biomarkers, though the underlying causal genera were not consistent [26–28]. Intriguingly, the transition from a vaginal microbiome dominated by *Lactobacillus spp.* (CST I, II, III, V) to CST IV appears to be a gradual process triggered by estrogen decrease beginning in perimenopause, with an abundance of *Lactobacillus spp.* progressively declining from premenopausal to postmenopausal stages [29]. Interestingly, Byrne *et al.* found that postmenopausal women with mixed microbiomes lacking *Lactobacillus* exhibited higher levels of proinflammatory cytokines [30], like those in premenopausal with mixed communities dominated by *Prevotella*[31]. Another robust evidence of *Lactobacillus*'s crucial role in maintaining vaginal health also in postmenopausal women comes from the Isala study, a comprehensive study of the vaginal microbiome of over 3,000 women of different ages living in Belgium [18]. In this study, key bacterial modules were linked with measured host factors using network-based statistical approaches and linked with measured host factors. High estrogen levels and a healthy vaginal microbiome were associated with the *Lactobacillus* module, while menopausal status was linked to the *Prevotella* module, confirming at large scale the shift toward a reduction in *Lactobacillus spp* after menopause.

Based on literature results of these studies, we propose a model of expected vaginal microbiota changes during MT stages and the underlying biological mechanisms that we depicted in **Figure 3**. It would, therefore, be ideal to design prevention and treatment strategies for restoring *Lactobacillus* levels to a minimum beneficial level after menopause.

Considering the impact of sex hormone decline in the vaginal microbiome and its consequences for women preventing and controlling clinical conditions via estrogen intake. Hormone replacement therapy (HRT) restores estrogen levels (30–60 pg/mL) and is available in various forms, including oral tablets, vaginal creams, rings, and transdermal patches [32]. Earlier research has shown that postmenopausal women using vaginal estradiol or oral menopausal hormone therapy are more likely to exhibit *Lactobacillus*-dominated vaginal microbiota and decreased microbial diversity [28,33–35]. While HRT effectively alleviates symptoms of VVA in postmenopausal women, it is not recommended for women with a history of breast cancer and cardiovascular and liver diseases [32]. Additionally, estrogen therapy may cause additional side effects such as vaginal bleeding, breast pain, and perineal pain [32]. Taken together, HRT is not always a suitable treatment for GSM symptoms, and other solutions should be sought.

The gut microbiome and its connection to aging and women's health

The rapid expansion of metagenomic studies to population studies and disease cohorts has consistently highlighted the association between gut microbiome dysbiosis and many diseases [36,37]. Nevertheless, while a clear distinction can be seen between healthy individuals and those diagnosed with diseases, a "universal" healthy microbiome cannot be defined since the microbial composition varies greatly between individuals and populations [38]. The gut microbiota is primarily composed of phyla including Bacteroidetes (e.g., *Bacteroides*, *Prevotella, Alistipes*), Firmicutes (e.g., *Clostridium*, *Lactobacillus*, *Bacillus*, *Enterococcus*, *Ruminococcus, Faecalibacterium, Roseburia, Blautia*), Actinobacteria (e.g., *Bifidobacterium*), Proteobacteria (e.g., *Escherichia*, *Shigella, Helicobacter, Sutterella*), Fusobacteria (*Leptotrichia*, *Fusobacterium*), and Verrucomicrobia (e.g., *Akkermansia*)[39]. In adults, over 90% of the gut microbiota comprises Bacteroidetes and Firmicutes, reflecting their foundational role in gut ecology [39]. Unlike the vaginal ecosystem, where a microbiome dominated by one or few species is preferred, high diversity in the gut microbiome is essential to guarantee resilience to environmental stressors and has been associated with better human health [39]. Not only microbial composition but also the metabolic activity of the different microbes is essential in maintaining human health. Gut microbes metabolize both dietary and endogenous compounds, producing secondary metabolites that serve as energy sources for themselves and signaling molecules for human metabolism and immune function. Short-chain fatty acids (**SCFAs**) and trimethylamine-N-oxide (**TMAO**) are diet-derived metabolites important for their impact on host health. SCFAs are produced by specific Firmicutes taxa during the breakdown of dietary fiber and act by binding to G-protein-coupled receptors (GPCRs) located on various immune and organ cells [40]. They are essential for maintaining intestinal barrier integrity and immunological homeostasis and play a significant role in regulating blood pressure and controlling insulin resistance [40]. In contrast, TMAO, a metabolite produced from dietary phosphatidylcholine and L-carnitine (abundant in meat and processed foods), contributes to atherosclerosis by interfering with cholesterol clearance from the liver, increasing plaque formation and heart disease risk [41].

The gut microbiota ecosystem is dynamic, evolving throughout the human lifespan, shaped by dietary, environmental, and physiological changes. Transitions of the gut microbiome during life do not occur equally in both genders [42]. However, only a few studies have investigated the impact of aging on the gut microbiome, especially regarding women's health and menopause. In women, we expect that the occurring shifts in sex hormones further complicate the impact of aging. While, to our knowledge, no current studies have interrogated simultaneously gut and vaginal microbiomes in population cohorts, emerging research supports the existence of a gut-vaginal axis. This complex and reciprocal interaction could be affected by the shifts in sex hormone levels and potentially impact overall health (**Figure 4A**). Estrogens produced in ovaries are conjugated in the liver and enter the gut, where bacteria from the **estrobolome** can deconjugate estrogen into an active form [43–45]. Dysbiosis and inflammation could alter gmGUS activity, with a consistent increase or decrease of free active estrogens in the bloodstream affecting hormonal balance, metabolic processes, and the risk of developing estrogen-related conditions. Similarly, fluctuations in estrogen levels during menopause can lead to alterations in gut microbial communities, contributing to age-related diseases.

Changes in gut microbiome composition and function in postmenopausal women

Gut microbiome diversity in postmenopausal women

Understanding the differences in gut microbiota diversity between premenopausal and postmenopausal women is complex; confounding factors—such as age-related diseases—can further influence microbiota composition. In this review, we selected studies involving cohorts comprising older women who, while generally healthy, may be at an increased risk for aging-related diseases. Some selected large-scale studies focused on differences in microbial composition across gender and age groups, enabling the identification of gut biodiversity in relation to age and sex [42,46–48]. Generally, younger women exhibit greater microbial diversity than men, but this difference diminishes with age, as middle-aged women display a gut microbiota more similar to men [42,46–48] (**Figure 4B**). In contrast, differences in microbiota composition between younger and older men appear less pronounced [46]. Lifestyle factors—such as alcohol consumption, smoking, drug use, and diet—also contribute to age- and gender-dependent declines in microbiome diversity. However, sex hormones, especially estrogen decline, remain a crucial determinant of gut microbiome specificities in women[46]. Notably, two studies reported that the dissimilarity between women and men during the premenopausal period is not significant when restricting the analysis to obese participants, likely due to lower estrogen levels in obesity, which makes the gut microbiome similar across genders [42,49].

Bacterial taxa associated with age-related diseases in women

Studies in large cohorts have identified significant changes in bacterial diversity and microbial taxa abundance in patients with cardiometabolic diseases [50–52], albeit only a minority of these have examined men and women separately. These studies reported reduced microbiome potential for the biosynthesis of SCFAs and higher levels of pathobionts belonging to Proteobacteria linked to systemic inflammation, high adiposity, obesity, and hypertension (**Figure 4 B-C**).

Similar results were found in a recent study investigating postmenopausal women with CVD risk [53]. The depletion of SCFA-producing bacteria was reported to be associated with CVD risk in postmenopausal women. For instance, in different studies, postmenopausal women had a lower abundance of SFCA-producers (e.g., *Ruminococcus, Eubacterium, Roseburia, Clostridium*) [48,49,54,55] associated with high-density lipoprotein (HDL) cholesterol levels, lower waist circumference, and a reduced risk of metabolic syndrome [47]. Other SCFA-producers, specifically *Parabacteroides* and *Oscillibacter,* were found to be depleted in postmenopausal women and associated with lower HDL levels in feces and plasma [47]. Notably, a recent study revealed that *Oscillibacter* encodes cholesterol-α-glucosyltransferase (CgT), which metabolizes cholesterol into secondary metabolites, regulating serum cholesterol levels and thus remarking its importance for cardiovascular health [56].

The depletion of SCFA metabolites and SCFA-producers has also been associated with a higher incidence of metabolic diseases such as T2DM already in the general population [57]. *A. muciniphila*, which supports butyrate metabolism and regulates insulin resistance, was more abundant in premenopausal than postmenopausal women [57–59]. Intriguingly, this bacterium has been linked to changes in progesterone metabolism [47].

Additionally, other bacterial metabolic pathways were downregulated in postmenopausal individuals, such as the folate pathway [48,49] , due to a significant reduction in *Lactobacillus plantarum*, a key folate producer. Folate participates in the methionine cycle, facilitating the conversion of homocysteine to methionine, and low levels can lead to increased homocysteine accumulation in the serum (hyperhomocysteinemia), which is linked to CVD due to its harmful effects on cardiovascular endothelium [60] (**Figure 4 B-C**).

Sex hormone changes affect gut microbiota composition in post-menopause

Noteworthy, the menopause transition and menopausal symptoms are often accompanied by gastrointestinal complaints, likely attributable to sex hormone fluctuations. Clinical studies ($n < 100$) found that estrogen levels positively correlate with an abundance of beneficial bacteria such as *Clostridiales*, especially *Ruminococcaceae,* and negatively with Bacteroides, regardless of age or BMI [61]. A reduction in estrogen, which decreases the estrobolome's capacity, may lower estrogen reabsorption and also lead to gut dysbiosis. Some gmGUS-encoding microbes (mainly species belonging to *Clostridiales* order) were decreased in obese postmenopausal women [47,49,62], especially those with CDM risk [54]. Notably, Peters *et al*. examined the menopause-microbiome relationship, finding a decrease in gmGUS enzymes (e.g., β-glucuronidase and aryl-sulfatase orthologs) involved in activating sex hormones [47]. In this study, the low abundance of specific bacterial taxa in postmenopausal women strongly correlated with depleted gmGUS enzymes carried by various beneficial bacteria (e.g., *Parabacteroides johnsonii*, *Clostridium lactatifermentans, Escherichia coli, Akkermansia muciniphila*) [47] (**Figure 4 B-C**). However, some bacterial species encoding gmGUS enzymes were also reported to increase in postmenopausal women. Since the discovery of gmGUS is relatively recent, more research is needed to characterize the properties of gmGUS producers.

Bacterial taxa associated with menopause-related disorders

Several studies reported that gut microbiota might have a role in postmenopausal osteoporosis (PMO), as the depletion of SCFAs-producers can affect bone health by altering calcium absorption and metabolism [63–65]. PMO symptoms have been negatively correlated with different species of *Lactobacillus* and *Bifidobacterium,* indicating a possible protective role on bone absorption. These two beneficial species were already observed to decline during perimenopause [66]. Recent studies found that potential pathogens, such as *Klebsiella, Streptococcus parasanguinis,* and *Clostridium perfringens,* were negatively associated with bone mineral density (BMD), suggesting their role in bone loss [64,67,68]. On the contrary, members of *Lachnospiraceae* family showed a positive correlation with higher BMD, suggesting their potential role in supporting bone health [64,68]. A similar association of taxa was found in a study investigating VMS symptoms [69] (**Figure 5 B-C**).

These observations suggest that menopause, through physiological and hormonal changes, significantly impacts the gut microbiota, which plays a multifaceted role in women's health.

Diet as a potential modulator of microbiome toward healthy aging in women

Emerging evidence supports the effect of diet on healthy aging and longevity in the general population, and the gut microbiome may mediate this effect [70]. While using diet to modulate gut microbiome is appealing, targeted modulatory interventions are premature until evidence from multiple studies supports the causal or curative effect of specific bacteria and function in age-related conditions. Furthermore, such intervention studies will have to be personalized, which means that the baseline microbiome composition will have to be

considered to predict the efficiency and likelihood of persistent microbiome change as needed. The field of personalized nutrition on the microbiome is yet in the infancy stage, but we foresee the potential impact of using the diet for preventing or treating different age-related conditions in both men and women.
For example, in postmenopausal women, increased intake of 'anti-aging nutrients' could improve the side effects of estrogen decline (**Figure 5**). Likewise, diet regimes that favor the production of SCFAs-producing bacteria, whose abundance declines with age, might be important for a protective effect on the cardiovascular and skeleton systems. In a murine osteotomy model, propionate and butyrate supplementation enhanced bone density and prevented bone loss by inhibiting osteoclast formation and bone reabsorption [58]. Studies conducted on fiber-rich diets like the Mediterranean diet (MedDiet) showed that a high abundance of SCFAs promotes a healthier cardiovascular system and reduces chronic inflammation [71]. Conversely, a diet with a high amount of animal fats, sweets, refined oils, and processed food exacerbates inflammation, increasing the accumulation of TMAO. In a study on postmenopausal women with and without PMO, those following a diet rich in grains, vegetables, and fruits and low in red meat exhibited normal bone mass and a lower abundance of gut methanogen bacteria responsible for decreasing SCFAs [66,72].

Polyphenols are another important food component with anti-inflammatory and antioxidant properties that help mitigate age-related inflammation. This broad class of compounds also includes phytoestrogens (i.e., isoflavones), and anthocyanins, making them a popular dietary supplement for promoting healthy aging.
Various natural compounds of phytoestrogens, particularly daidzein and genistein, mimic human estrogen and are present in soybeans, legumes, mung beans, green beans, and plants (e.g., red clover). Soybeans are especially rich in phytoestrogens (~ 150 mg/100 g of cooked soybeans). The recommended intake of isoflavones is ~ 40-50 mg/d, whereas, in European countries, the consumption of phytoestrogens is, on average, ~ 0.76 mg/d [73]. GmGUS enzymes metabolize these molecules, and once activated, they can bind to the same estrogen receptors (ERs) as endogenous estrogens, thereby regulating downstream signaling pathways [45]. The advantage of introducing isoflavonoids with diet lies in their minimal side effects compared to HRT, as they show a strong binding affinity to ERβ and a weaker affinity to ERα. While estrogen is a strong agonist, binding effectively to both receptors, the active metabolite of isoflavones selectively targets ERβ. This receptor selectivity provides health benefits and lowers cancer risk, as ERβ is primarily expressed in non-gonadal tissues. Moreover, secondary metabolic products of phytoestrogens degradation, produced by gut microbe metabolism, act as potent metabolites with various beneficial effects, including anti-androgenic and antioxidant activities. For instance, *Eggerthella*, *Adlercreutzia*, *Asaccharobacter*, *Slackia*, and *Lactococcus spp* produce equol, a potent metabolite of soy daidzein [74]. Clinical trial studies showed that this metabolite had been shown to reduce the risk of CVD in postmenopausal women [75,76]. However, only 30–50% of the population is able to convert daidzein into equol, depending on intestinal microbiota composition. For example, obesity in postmenopausal women was associated with a loss of ability to metabolize the soy isoflavone daidzein to O-desmethylangolensin [77]. Furthermore, phytoestrogens are believed to induce an increase in butyrate-producing bacteria (*Faecalibacterium* and members of the *Clostridium* clusters), showing potential antioxidant properties [74]. Studies on postmenopausal women suggested that the treatment with phytoestrogens may help prevent many adverse postmenopausal conditions, including osteoporosis and vasomotor symptoms [78–80]. Recent clinical studies have demonstrated the effectiveness of bioactive isoflavones from red clover in treating osteoporosis with few side effects [81]. The use of phytoestrogens in the treatment of osteoporosis is well-documented, as they reduce tumor necrosis factors (RANKL and TNF-α) activity, which limit bone reabsorption, and enhance growth factors (es RUNX2 and TGF-β), promoting bone formation. However, there is debate on the quantity, origin, and type of phytoestrogens to employ for therapy [82]. For instance, isoflavones have bone benefits regardless of the source (e.g., extracted from red clover or soybeans or consumed in soy foods), while the synthetic ipriflavone did not show a positive effect on bone density [83]. Indeed, some intervention studies administering isoflavonoids did not have a positive effect on BMD [84]. Interestingly, phytoestrogens and probiotics can work synergistically, enhancing their beneficial effects against menopausal comorbidities such as osteoporosis and vasomotor symptoms [80,81]. It has been suggested that probiotics can also restore the impaired activation of phytoestrogens in conditions of gut dysbiosis, as certain strains like *Lactobacillus* and *Bifidobacterium* possess gmGUS enzymes that facilitate phytoestrogen metabolism [80,81].
Royal jelly is another well-studied component of a diet rich in flavonoids (such as chrysin and pinocembrin) with estrogenic activity. Produced by honeybees (*Apis mellifera*), royal jelly has been shown to have multiple therapeutical applications, including immune modulation, anti-fatigue, anti-tumor, anti-hypertensive, and anti-osteoporosis effects [85]. A clinical study studies (n=90 women) showed that the application of a 15% royal jelly

vaginal cream improved GMS in postmenopausal women [86]. Despite its promising benefits, the impact of royal jelly on microbiota in postmenopausal women is still underexplored. So far, only one study on a db/db mouse model (characterized by overeating, obesity, and hyperglycemia) has shown that royal jelly influences the microbiota by the increase of SCFA-producing [87].

Anthocyanins, a class of polyphenols abundantly found in prunes, grapes, blueberries, and black and green tea, are associated with a reduction of CVD risk in both women and men [88,89]. Additionally, they are frequently utilized as an alternative therapy to alleviate menopausal symptoms. The gut microbiota transform them into bioactive, low-molecular-weight phenolic metabolites that modify the host's metabolism [90]. For instance, the conversion of anthocyanins is also related to the enzyme β-glucosidase found in some species of *Lactobacillus, Bifidobacterium, Blautia,* and *Erysipelatoclostridium*. The consumption of anthocyanin-rich fruits in clinical trials conducted on adults has been shown to increase *A. muciniphila, Lactobacillus spp., Roseburia,* and *Ruminococcus* and attenuate host gut inflammation and obesity [91,92]. Pilot intervention studies, particularly those based on polyphenol-rich food (dose > 90 mg/d), appeared to affect postmenopausal women, decreasing hypertension, inflammation, and osteoporosis, but only certain individuals experience these benefits [93–95]. Importantly, postmenopausal women with initial low BMD could benefit *Oscillospiraceae* and *Lachnospiraceae* families, which can metabolize these compounds [95].

In addition to diet interventions, probiotics have emerged as a promising treatment for ameliorating PMO symptoms. Different probiotic Lactobacillus strains have been shown to heighten bone health by increasing calcium absorption by the gut and modulating bone cell activity. Probiotic treatments in PMO murine models boosted higher bone calcium deposition and suppressed osteoclast activity related to bone loss [96]. However, some controlled clinical trials in postmenopausal women have shown no significant effect of probiotics use on bone loss [97,98]. Yet, significant positive effects on bone metabolism were observed when dysbiosis was present, a condition frequently common in obesity and with advanced age [99–101]. Other studies showed that administering *Lactobacillus* probiotics increased the relative abundance of core SCFAs-producers in the gut, which augments butyrate levels in intestinal mucosa and serum [72,100,102,103]. A proposed mechanism suggests that *Lactobacillus* and butyrate synergically enhance bone formation by increasing regulatory T cells in the bone marrow, promoting osteoblast proliferation [104]. Meanwhile, Li *et al*. demonstrated in a murine model that *L. rhamnosus* may prevent bone loss by improving intestinal permeability and reducing microbiota-induced inflammation related to sex hormone deficiency [105].

Other types of intervention have been proposed to alleviate menopause syndrome. In a pilot trial, the use of *Lactobacillus*-containing hygiene products was associated with an improved vaginal ecosystem, indicating better urogenital health [106]. Intervention studies on healthy young women who were given *Saccharomyces cerevisiae* yeast showed increased *Lactobacillus,* especially *L. iners,* and decreased *Prevotella*[107]. Probiotics, particularly strains like *L. reuteri, L. rhamnosus,* and *L. acidophilus,* may help maintain an acidic vaginal pH and alleviate symptoms of VVA and recurrent UTIs [106,108–110]. However, the success of these actions depends on an individual's microbiome composition and may require long-term use to show noticeable effects, especially in older adults and women with less adaptable microbiomes. Moreover, in addition to the vaginal microbiome, many lifestyle factors play significant roles in managing postmenopausal symptoms, and probiotics alone may be insufficient to guarantee a healthy aging trajectory without a holistic approach to health.

Overall, this evidence highlights the need to design customized nutrition strategies to relieve postmenopausal disorders, such as PMO, based on an individual's current diet and microbiome profile and encourage the continued investigation of diet interventions to promote healthy aging in women.

Future perspectives for women's aging research

Our review emphasized biological changes during women's aging from a microbiome perspective and highlighted the potential intervention routes toward healthy aging. We focused on women as the scientific community recognizes the urgent need to improve our knowledge of women's specific mechanisms to provide adequate care to both genders. Historically, biomedical research has been focused on males, often excluding women due to concerns over hormonal fluctuations. This attitude has caused gaps in understanding female health but also resulted in many medical therapies in use today being based on male-oriented studies, potentially offering less effective care for women and higher side effects.

We underlined reported changes in both vaginal and gut microbiomes during aging and their impact on women's health. Since the scientific community has been exploring the role of the human microbiome on a broader scale only in the past decade and mainly focused on the gut microbiome, we expect that understanding of specific bacterial taxa and function, and how they affect health and illness, will consolidate and broaden to include other phenotypes or ecosystem unique to females, like vaginal microbiome. Large-scale studies must include and properly analyze postmenopausal women to improve the understanding of aging-related processes from the microbiome perspective. Ideally, a cohort study approach, which involves collecting samples over extended periods and multi-omics data, should be considered to gather vast amounts of data and allow for an understanding of the full complexity of changes.

Finally, we discussed the yet understudied role of diet in modulating women's health and its potential as a preventive measure to counter the effects of aging in women; moreover, recognizing women's unique challenges as they age is crucial for developing targeted interventions.

Contributions

All authors contributed to the manuscript draft and its revision.

Declaration of interest

The authors declare no conflicts of interest.

Declaration of the use of AI and AI-assisted technologies

We declare that the use of AI (ChatGTP) was limited to correcting the text and making the review more readable. The authors are fully responsible for scientific insights, literature reviews, data analysis and interpretation, and scientific conclusions.

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Figures Legend

Figure 1. The stages of reproductive aging in women and their possible associated symptoms.
A) Visual representation of key events and duration of the MT as established by the World Health Organization Scientific Group on Research in the Menopause [111] (figure modified by Harlow *et al.* [9]). Briefly, the Staging of Reproductive Aging Workshop (STRAW) classification divides a woman's life into seven segments, with segments –2, –1, and 0, including the early MT, the late MT, and the final menstrual period (FMP). Early MT characteristics could include irregular cycles, variable hormone levels, and potential vasomotor symptoms (VMS). Late MT, lasting 1–3 years, might show amenorrhea (absence of the monthly menses), high levels of follicle-stimulating hormone (FSH), varied levels of estradiol (E2), and possibly VMS. Menopause is diagnosed 12 months following the last menstrual cycle. One-year post-menopause, as well as MT phases, constitute perimenopause. Early post-menopause presents high/variable FSH and low E2; late post-menopause is characterized by high FSH, low E2, and genitourinary symptoms (GSM). **B**) Health conditions that may occur during peri- and post-menopause status. Symptoms of postmenopausal syndrome are marked with a *.
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Figure 2. Characteristics of human vaginal Community State Types (CSTs).
CST types, sub-types, and major microbial properties of the dominant bacterial species according to the classification proposed by the VALENCIA study [22]. Four CSTs (I, II, III, and IV) and the corresponding six sub-types are *Lactobacillus*-dominated, while CST IV, dominated by a mixed anaerobic community, has seven sub-CSTs. The A and B subtypes of CST I and CST III differentiate subtypes where the corresponding bacterial species is either fully dominant or highly prevalent, respectively. The symbols "O" in green, yellow, and red represent beneficial, neutral, and potentially harmful bacteria. Created in BioRender. Ferrando, M.L. (2024) https://BioRender.com/ e99t679

Figure 3. Proposed vaginal microbiome changes during MT stages.
Premenopausal women have high estrogen levels that support a thick, glycogen-rich epithelium, with a microbiome community most often dominated by *Lactobacillus* (e.g., *L. crispatus*). During perimenopause and the fluctuation in

estrogen production, *Lactobacillus*-dominated communities become dynamic *(e.g.,* moving toward a higher abundance of *L. iners*), shifting to a mixed anaerobes community after menopause. Postmenopausal estrogen deficiency leads to lower glycogen, higher vaginal pH, and an increase in parabasal cells, potentially causing signs of moderate VVA. *Lactobacillus* levels drop significantly [28–30], while pathobionts bacteria like *Prevotella*, *Streptococcus*, *Gardnerella*, and *Atopobium* increase. Of note, *Lactobacillus spp* remains present – albeit with low abundance - in 30-50% of the vaginal microbiota [23,26,27,29,30] (**Supplementary Table 1A**), maintaining their protective role against severe vaginal inflammation and infections. Created in BioRender. Ferrando, M.L. (2024) https://BioRender.com/z68w440

Figure 4. *Proposed changes in gut microbial taxa associated with aging in postmenopausal women*
A) Gut-vaginal microbiome axis. In healthy women of reproductive age, the ovaries primarily synthesize active estrogens. These estrogens are converted into inactive forms in the liver and excreted into bile. Bacteria producing β-glucuronidases (estrobolome) can deconjugate and reactivate estrogens in the gut. While most reactivated estrogens are excreted, others re-enter systemic circulation through enterohepatic recirculation, potentially targeting tissues like the vagina. Estrogen stimulates vaginal cells to produce glycogen, supporting ana healthy Lactobacillus-dominated vaginal microbiome. **B**) Aging during menopause is linked to decreased gut bacterial diversity, with women's microbiota more similar to males. Key taxa shifts include reductions in SCFA-producers, estrobolome microbes, and beneficial bacteria (e.g., *A. muciniphila*, *Bifidobacterium*, *Lactobacillus*) critical for intestinal integrity (upregulation of TJ via TLR2 receptor), immune support, and bacterial metabolite production. Additionally, an altered Firmicutes-Bacteroidetes ratio, driven by increased Bacteroides, and a rise in pathobionts associated with CVD risk and gut dysbiosis have been observed. **C**) Functional mechanisms of gut taxa involved in the onset of age-related diseases in postmenopausal women. Graphic summary of the key metabolites or effectors produced by the three taxa and the effect each of these microbiome-derived entities has in either negatively or positively regulating various aging-linked diseases and disorders. The reduction in *Bifidobacterium* and *Lactobacillus* lowers acetate production and supports SCFA-producers.
SCFAs interact with G-protein-coupled receptors (GPCRs), influencing a wide range of cell types, including columnar epithelial cells, innate immune cells, and endothelial cells, thereby modulating inflammation, insulin resistance, and blood pressure. A decline in estrobolome diversity (gmGUS-producers) can affect estrogen recycling and contribute to postmenopausal disorders. Reduced folate and cholesterol-α-glucosyltransferase (CGT) producers lead to homocysteine and cholesterol accumulation, raising CVD risk. Enrichment of proteobacteria is linked to elevated blood pressure via inflammatory mediators (IL-8 and TNF-α). Additionally, increased pathobionts (e.g., *Enterobacteriaceae* and *Clostridium*) disrupt gut homeostasis, promoting inflammation (pro-inflammatory interleukins) and dysbiosis, contributing to postmenopausal disorders. Created in BioRender. Ferrando, M.L. (2024) https://BioRender.com/z68w440
SCFAs: Short Chain Fatty acids; gmGUS: gut microbes Glucuronidases; IL-8 and IL-18: pro-inflammatory Interleukins; IFN: Interferon; TJ: cellular Tight Junctions; TLR: Toll-Like Receptors; GPR41/43: G-Protein-Coupled receptors (GPCRs); FFARs: Free Fatty Acid Receptors, and ORs: Olfactory Receptors, families of GPCRs expressed in blood vessels and in vascular smooth muscle cells in a variety of organs; LPS: Lipopolysaccharide; pDC and mDC: premature and mature Dendritic Cells, respectively.
PMO: postmenopausal osteoporosis; VSM: vasomotor symptoms; GSM: genitourinary symptoms of menopause; CVD: cardiovascular diseases.

Figure 5: Potential role of diet on microbiome health during menopause.
A graphic representation shows how diet intervention strategies could promote microbiome changes that prevent the aging-associated decline in postmenopausal women.
Probiotics are different strains of *Lactobacillus*, such as *L. reuteri*, *L. rhamnosus*, *L. plantarum*, and *L. acidophilus.*
Polyphenols include flavonoids such as phytoestrogens (e.g., isoflavonoids) with estrogenic properties, including daidzein (found in soybeans, mung beans, green beans, Red Clover, Thai herb Pueraria Mirifica, and Japanese kudzu) and genistein (found in soybeans, lupin, fava beans, kudzu, and Red Clover), as well as anthocyanins (found in black currant, pomegranate, prunes, berries, grapes, acai, and eggplants) with antioxidant and anti-inflammatory activities. Additionally, polyphenols encompass non-flavonoid compounds such as phenolic acids (found in berries, black and green tea, coffee, whole grains, tomatoes, and apples) and other polyphenols like resveratrol (found in grapes, berries, and red wine), which are known for their strong anti-inflammatory and antioxidant properties.
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TMAO, trimethylamine; IL-1β Interleukin-1 beta pro-inflammatory cytokine; PMO: postmenopausal osteoporosis; VSM: vasomotor symptoms; GSM: genitourinary symptoms of menopause; CMD: cardiometabolic diseases.

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Figure 1

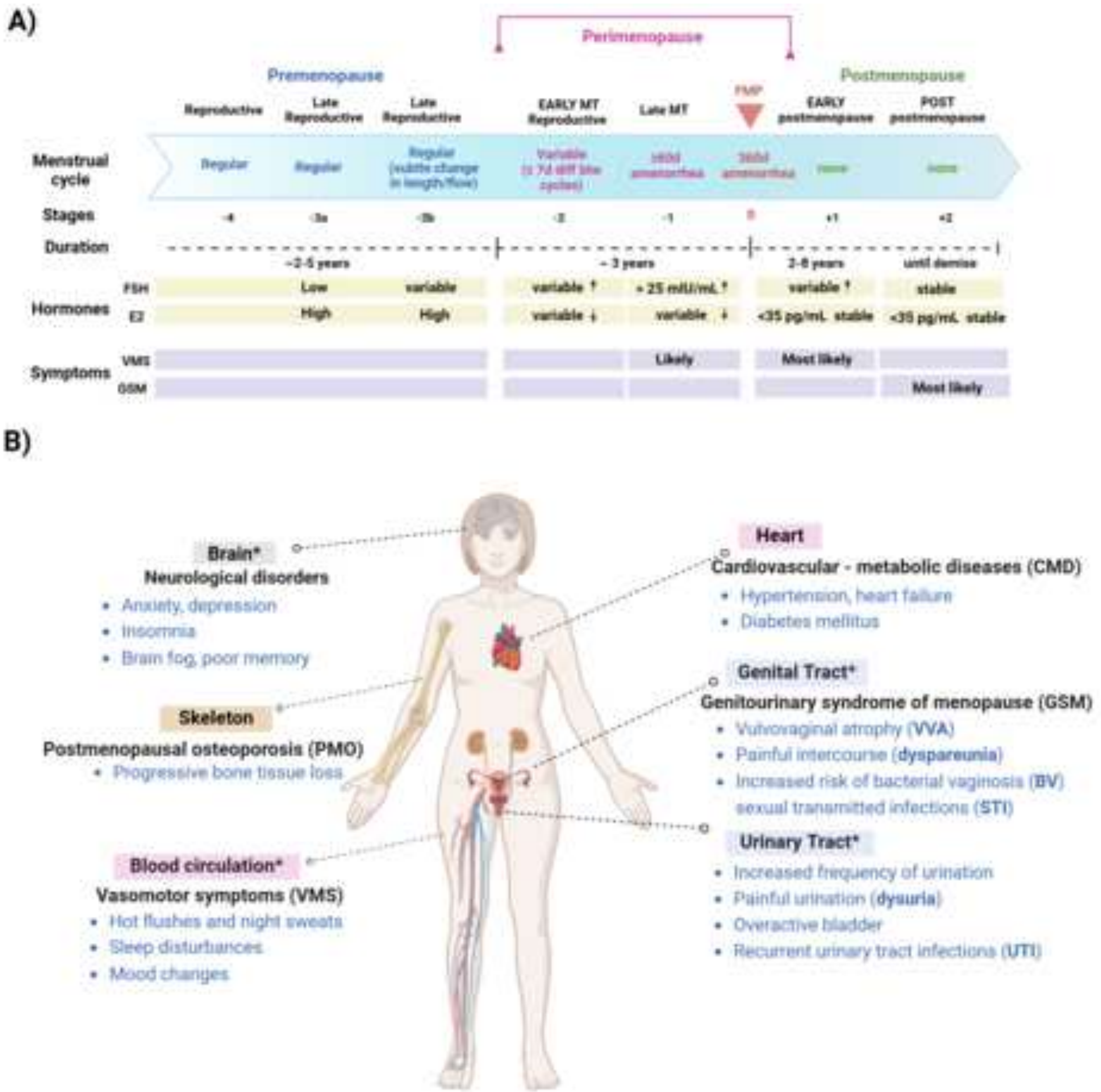
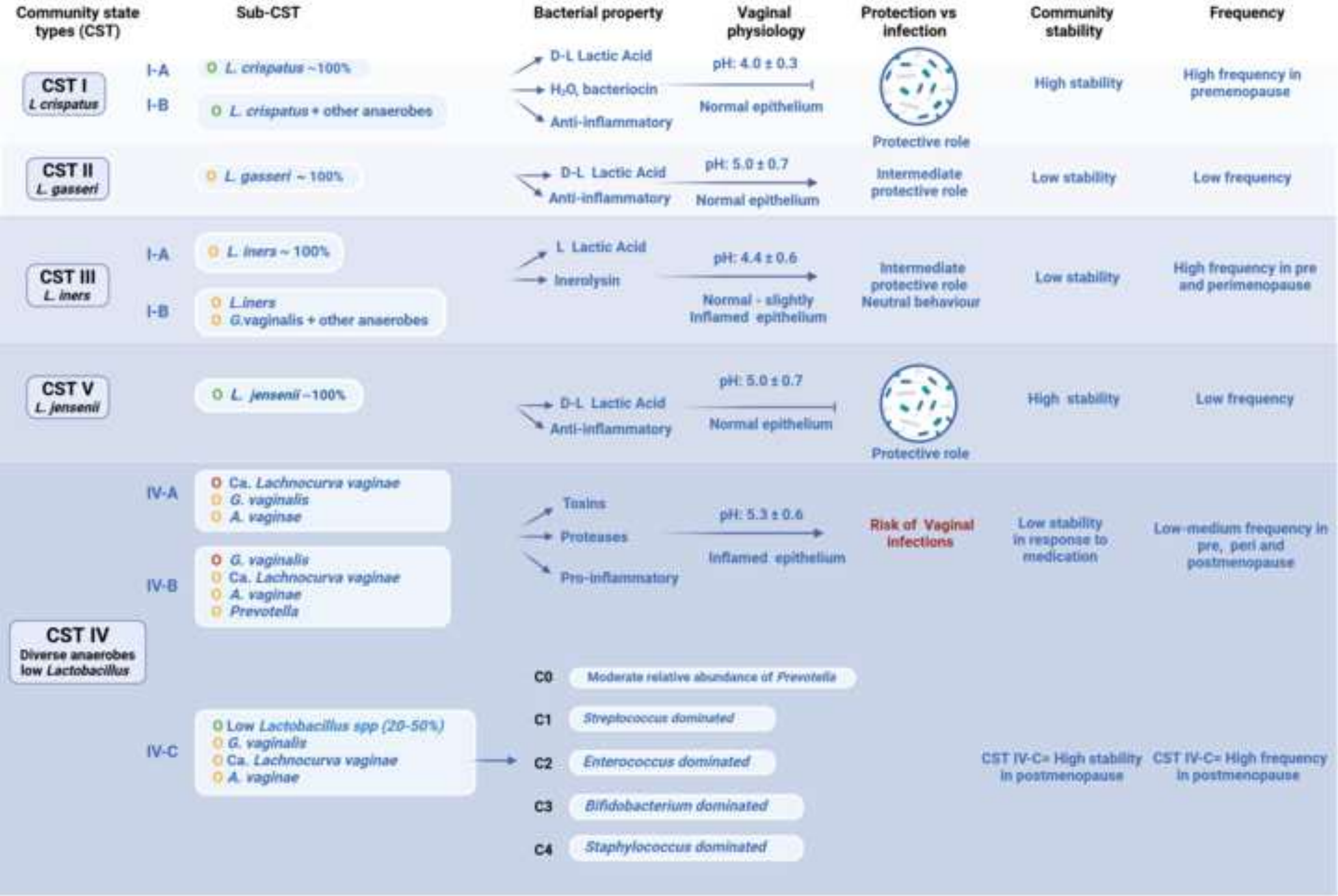
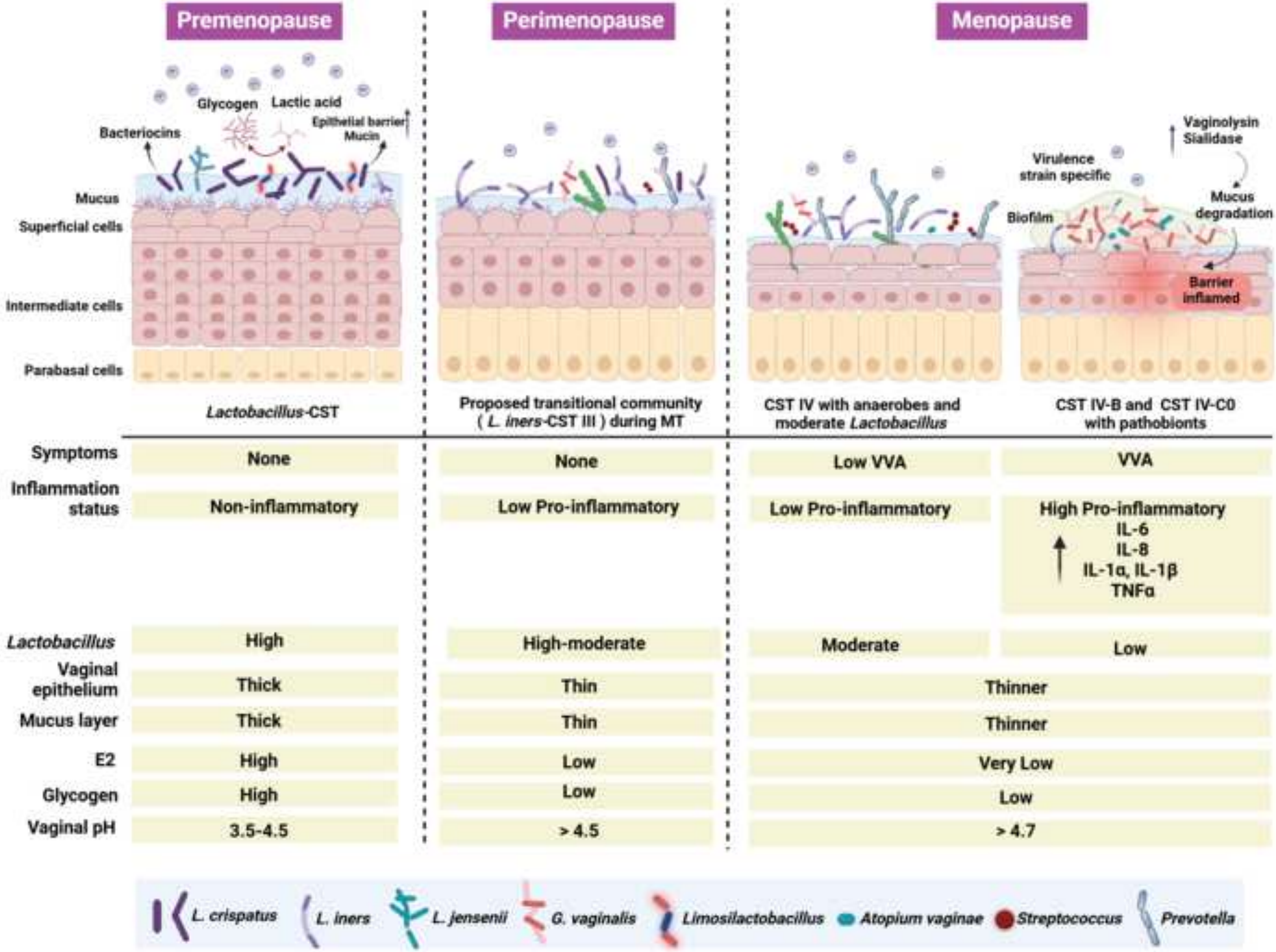

A)
Perimenopause
Premenopause
Postmenopause
Reproductive
Late Reproductive
Late Reproductive
EARLY MT Reproductive
Late MT
FMP
EARLY postmenopause
POST postmenopause
Menstrual cycle
Regular
Regular
Regular (subtle change in length/flow)
Variable (≥ 7d diff btw cycles)
≥60d amenorrhea
365d amenorrhea
none
none
Stages
-4
-3a
-3b
-2
-1
0
+1
+2
Duration
~2-5 years
~ 3 years
2-8 years
until demise
Hormones
FSH
Low
variable
variable ↑
> 25 mIU/mL ↑
variable ↑
stable
E2
High
High
variable ↓
variable ↓
<35 pg/mL stable
<35 pg/mL stable
Symptoms
VMS
Likely
Most likely
GSM
Most likely
B)
Brain*
Neurological disorders
Anxiety, depression
Insomnia
Brain fog, poor memory
Skeleton
Postmenopausal osteoporosis (PMO)
Progressive bone tissue loss
Blood circulation*
Vasomotor symptoms (VMS)
Hot flushes and night sweats
Sleep disturbances
Mood changes
Heart
Cardiovascular - metabolic diseases (CMD)
Hypertension, heart failure
Diabetes mellitus
Genital Tract*
Genitourinary syndrome of menopause (GSM)
Vulvovaginal atrophy (VVA)
Painful intercourse (dyspareunia)
Increased risk of bacterial vaginosis (BV) sexual transmitted infections (STI)
Urinary Tract*
Increased frequency of urination
Painful urination (dysuria)
Overactive bladder
Recurrent urinary tract infections (UTI)

Figure 2



Community state types (CST)	Sub-CST		Bacterial property	Vaginal physiology	Protection vs infection	Community stability	Frequency
CST I *L crispatus*	I-A	○ *L. crispatus* ~100%	D-L Lactic Acid H_2O_2 bacteriocin Anti-inflammatory	pH: 4.0 ± 0.3 Normal epithelium	Protective role	High stability	High frequency in premenopause
	I-B	○ *L. crispatus* + other anaerobes					
CST II *L. gasseri*		○ *L. gasseri* ~ 100%	D-L Lactic Acid Anti-inflammatory	pH: 5.0 ± 0.7 Normal epithelium	Intermediate protective role	Low stability	Low frequency
CST III *L. iners*	I-A	○ *L. iners* ~ 100%	L Lactic Acid Inerolysin	pH: 4.4 ± 0.6 Normal - slightly Inflamed epithelium	Intermediate protective role Neutral behaviour	Low stability	High frequency in pre and perimenopause
	I-B	○ *L.iners* ○ *G.vaginalis* + other anaerobes					
CST V *L. jensenii*		○ *L. jensenii* ~100%	D-L Lactic Acid Anti-inflammatory	pH: 5.0 ± 0.7 Normal epithelium	Protective role	High stability	Low frequency
CST IV Diverse anaerobes low *Lactobacillus*	IV-A	○ Ca. *Lachnocurva vaginae* ○ *G. vaginalis* ○ *A. vaginae*	Toxins Proteases Pro-inflammatory	pH: 5.3 ± 0.6 Inflamed epithelium	Risk of Vaginal infections	Low stability in response to medication	Low-medium frequency in pre, peri and postmenopause
	IV-B	○ *G. vaginalis* ○ Ca. *Lachnocurva vaginae* ○ *A. vaginae* ○ *Prevotella*					
	IV-C	○ Low *Lactobacillus spp (20-50%)* ○ *G. vaginalis* ○ Ca. *Lachnocurva vaginae* ○ *A. vaginae*	C0 Moderate relative abundance of *Prevotella* C1 *Streptococcus dominated* C2 *Enterococcus dominated* C3 *Bifidobacterium dominated* C4 *Staphylococcus dominated*			CST IV-C= High stability in postmenopause	CST IV-C= High frequency in postmenopause

Figure 3



	Premenopause	Perimenopause	Menopause	
	Lactobacillus-CST	Proposed transitional community (*L. iners*-CST III) during MT	CST IV with anaerobes and moderate *Lactobacillus*	CST IV-B and CST IV-C0 with pathobionts
Symptoms	None	None	Low VVA	VVA
Inflammation status	Non-inflammatory	Low Pro-inflammatory	Low Pro-inflammatory	High Pro-inflammatory ↑ IL-6 IL-8 IL-1α, IL-1β TNFα
Lactobacillus	High	High-moderate	Moderate	Low
Vaginal epithelium	Thick	Thin	Thinner	
Mucus layer	Thick	Thin	Thinner	
E2	High	Low	Very Low	
Glycogen	High	Low	Low	
Vaginal pH	3.5-4.5	> 4.5	> 4.7	

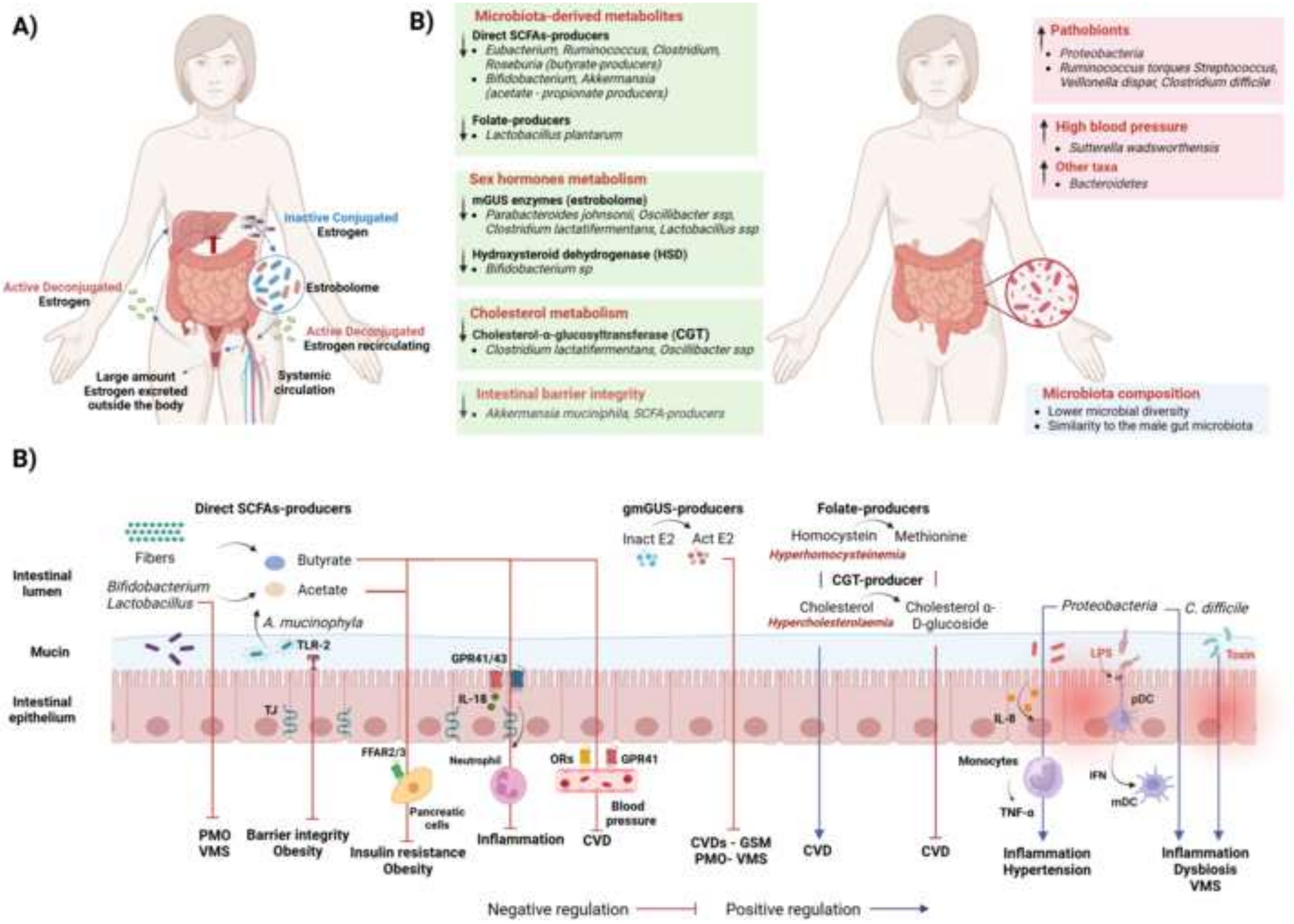
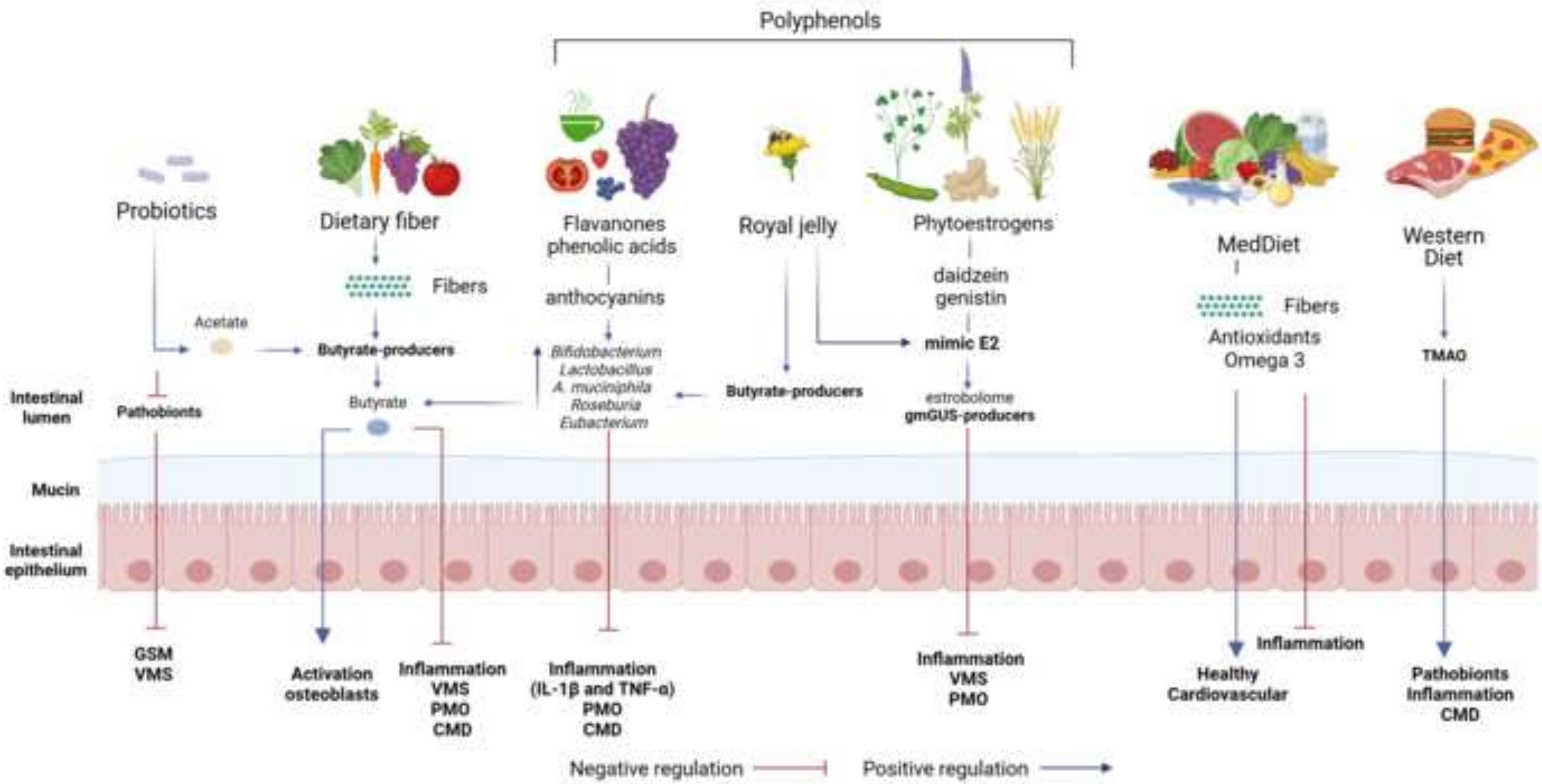
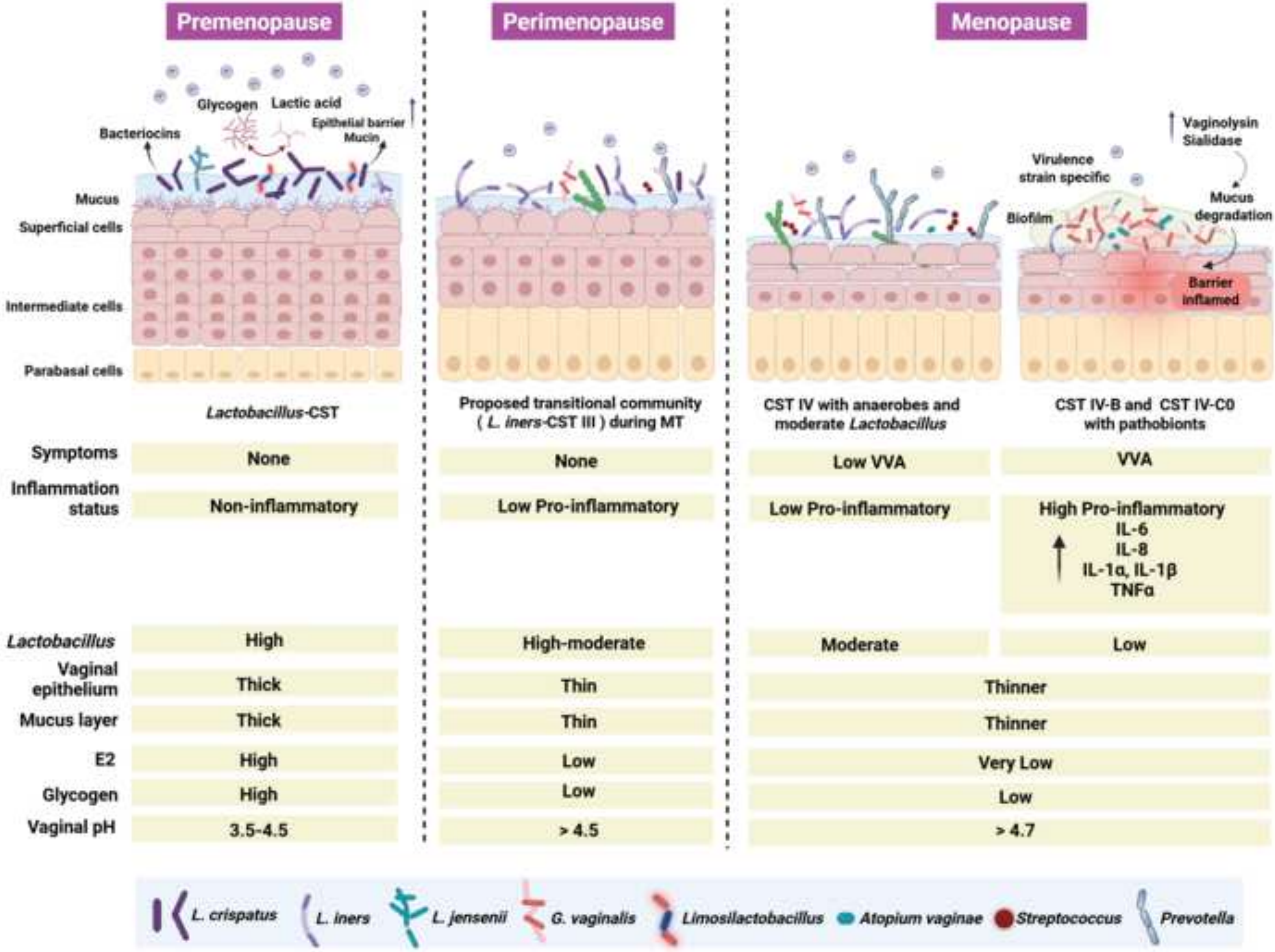
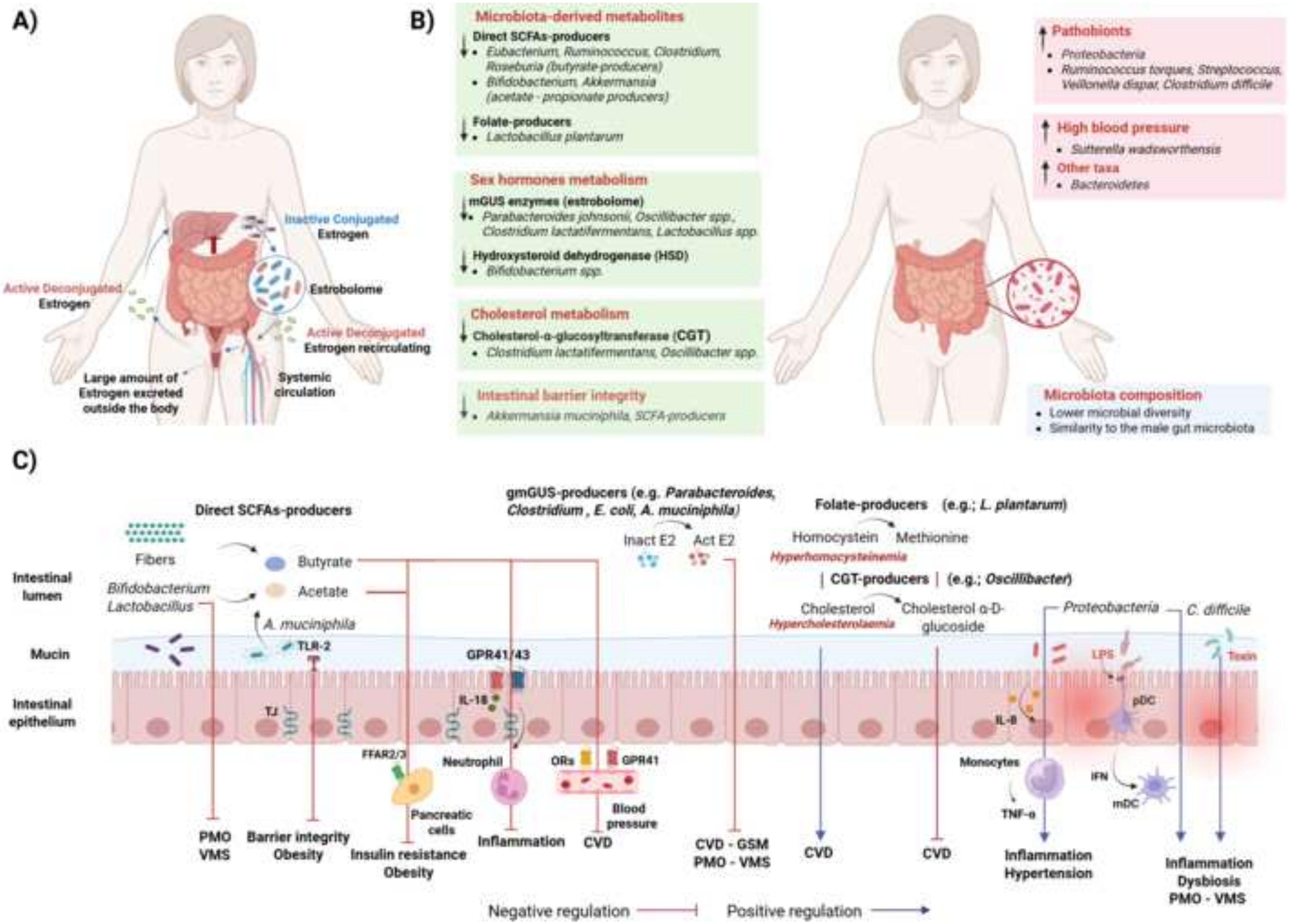

A)
Inactive Conjugated
Estrogen
Active Deconjugated
Estrogen
Estrobolome
Active Deconjugated
Estrogen recirculating
Large amount
Estrogen excreted
outside the body
Systemic
circulation
B)
Microbiota-derived metabolites
Direct SCFAs-producers
Eubacterium, Ruminococcus, Clostridium, Roseburia (butyrate producers)
Bifidobacterium, Akkermanaia (acetate - propionate producers)
Folate-producers
Lactobacillus plantarum
Sex hormones metabolism
mGUS enzymes (estrobolome)
Parabacteroides johnsonii, Oscillibacter ssp, Clostridium lactatifermentans, Lactobacillus ssp
Hydroxysteroid dehydrogenase (HSD)
Bifidobacterium sp
Cholesterol metabolism
Cholesterol-α-glucosyltransferase (CGT)
Clostridium lactatifermentans, Oscillibacter ssp
Intestinal barrier integrity
Akkermanaia muciniphila, SCFA-producers
Pathobionts
Proteobacteria
Ruminococcus torques Streptococcus, Veillonella dispar, Clostridium difficile
High blood pressure
Sutterella wadsworthensis
Other taxa
Bacteroidetes
Microbiota composition
Lower microbial diversity
Similarity to the male gut microbiota
B)
Intestinal lumen
Mucin
Intestinal epithelium
Direct SCFAs-producers
Fibers
Bifidobacterium
Lactobacillus
Butyrate
Acetate
A. mucinophyla
TLR-2
TJ
GPR41/43
IL-18
FFAR2/3
Pancreatic cells
Neutrophil
ORs
GPR41
Blood pressure
PMO
VMS
Barrier integrity
Obesity
Insulin resistance
Obesity
Inflammation
CVD
gmGUS-producers
Inact E2
Act E2
CVDs - GSM
PMO- VMS
Folate-producers
Homocystein
Methionine
Hyperhomocysteinemia
CGT-producer
Cholesterol
Hypercholesterolaemia
Cholesterol α-D-glucoside
CVD
CVD
Proteobacteria
C. difficile
LPS
Toxin
IL-8
pDC
Monocytes
TNF-α
IFN
mDC
Inflammation
Hypertension
Inflammation
Dysbiosis
VMS
Negative regulation
Positive regulation

Figure 5


Polyphenols
Probiotics
Dietary fiber
Flavanones
phenolic acids
Royal jelly
Phytoestrogens
MedDiet
Western
Diet
Fibers
Acetate
Butyrate-producers
anthocyanins
daidzein
genistin
mimic E2
Fibers
Antioxidants
Omega 3
TMAO
Intestinal
lumen
Pathobionts
Butyrate
Bifidobacterium
Lactobacillus
A. muciniphila
Roseburia
Eubacterium
Butyrate-producers
estrobolome
gmGUS-producers
Mucin
Intestinal
epithelium
GSM
VMS
Activation
osteoblasts
Inflammation
VMS
PMO
CMD
Inflammation
(IL-1β and TNF-α)
PMO
CMD
Inflammation
VMS
PMO
Healthy
Cardiovascular
Inflammation
Pathobionts
Inflammation
CMD
Negative regulation
Positive regulation



	Premenopause	Perimenopause	Menopause	
	Lactobacillus-CST	Proposed transitional community (*L. iners*-CST III) during MT	CST IV with anaerobes and moderate *Lactobacillus*	CST IV-B and CST IV-C0 with pathobionts
Symptoms	None	None	Low VVA	VVA
Inflammation status	Non-inflammatory	Low Pro-inflammatory	Low Pro-inflammatory	High Pro-inflammatory ↑ IL-6 IL-8 IL-1α, IL-1β TNFα
Lactobacillus	High	High-moderate	Moderate	Low
Vaginal epithelium	Thick	Thin	Thinner	
Mucus layer	Thick	Thin	Thinner	
E2	High	Low	Very Low	
Glycogen	High	Low	Low	
Vaginal pH	3.5-4.5	> 4.5	> 4.7	


A)
Inactive Conjugated
Estrogen
Estrobolome
Active Deconjugated
Estrogen
Active Deconjugated
Estrogen recirculating
Large amount of
Estrogen excreted
outside the body
Systemic
circulation
B)
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Direct SCFAs-producers
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Bifidobacterium spp.
Cholesterol metabolism
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Clostridium lactatifermentans, Oscillibacter spp.
Intestinal barrier integrity
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Similarity to the male gut microbiota
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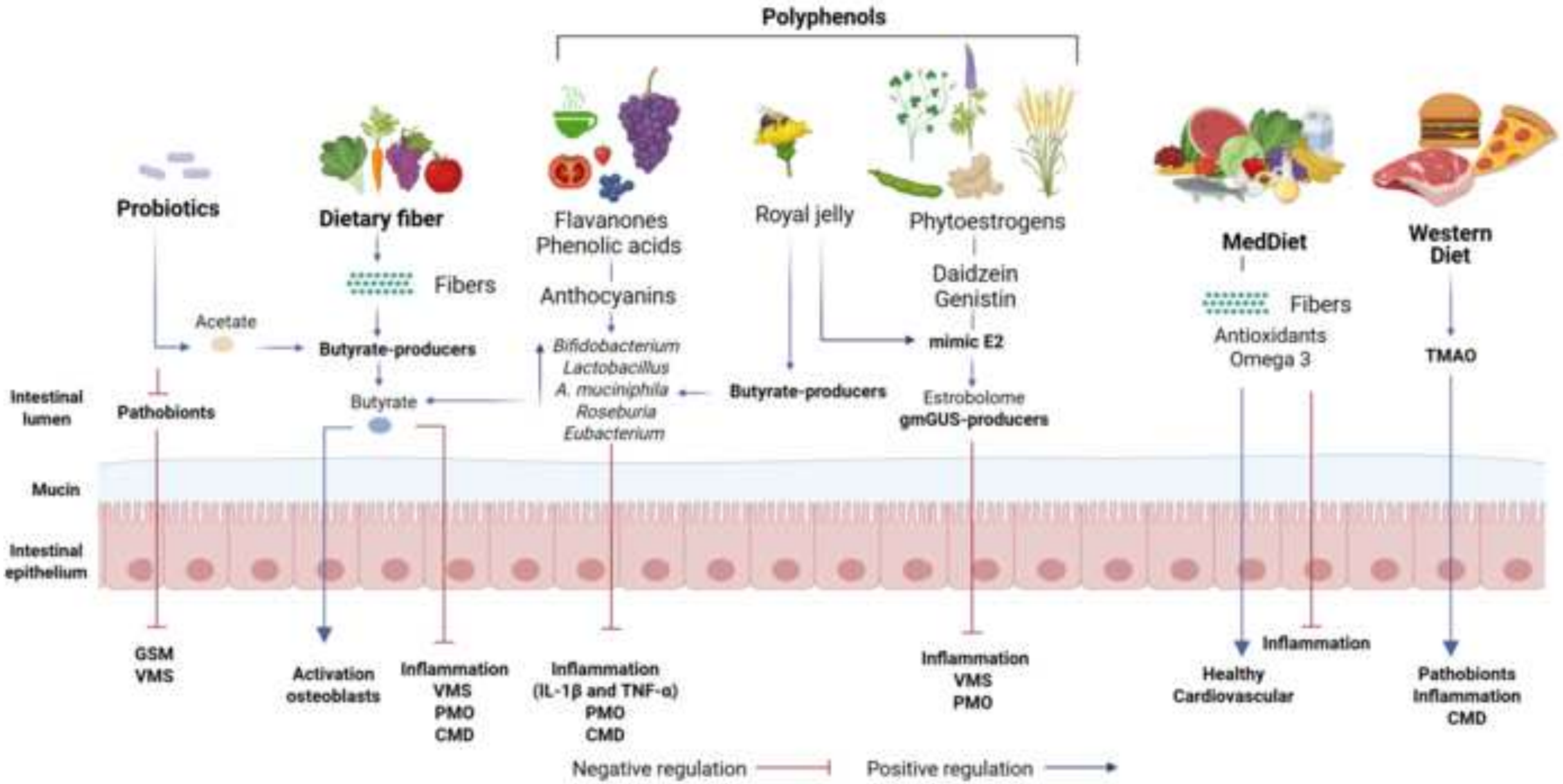
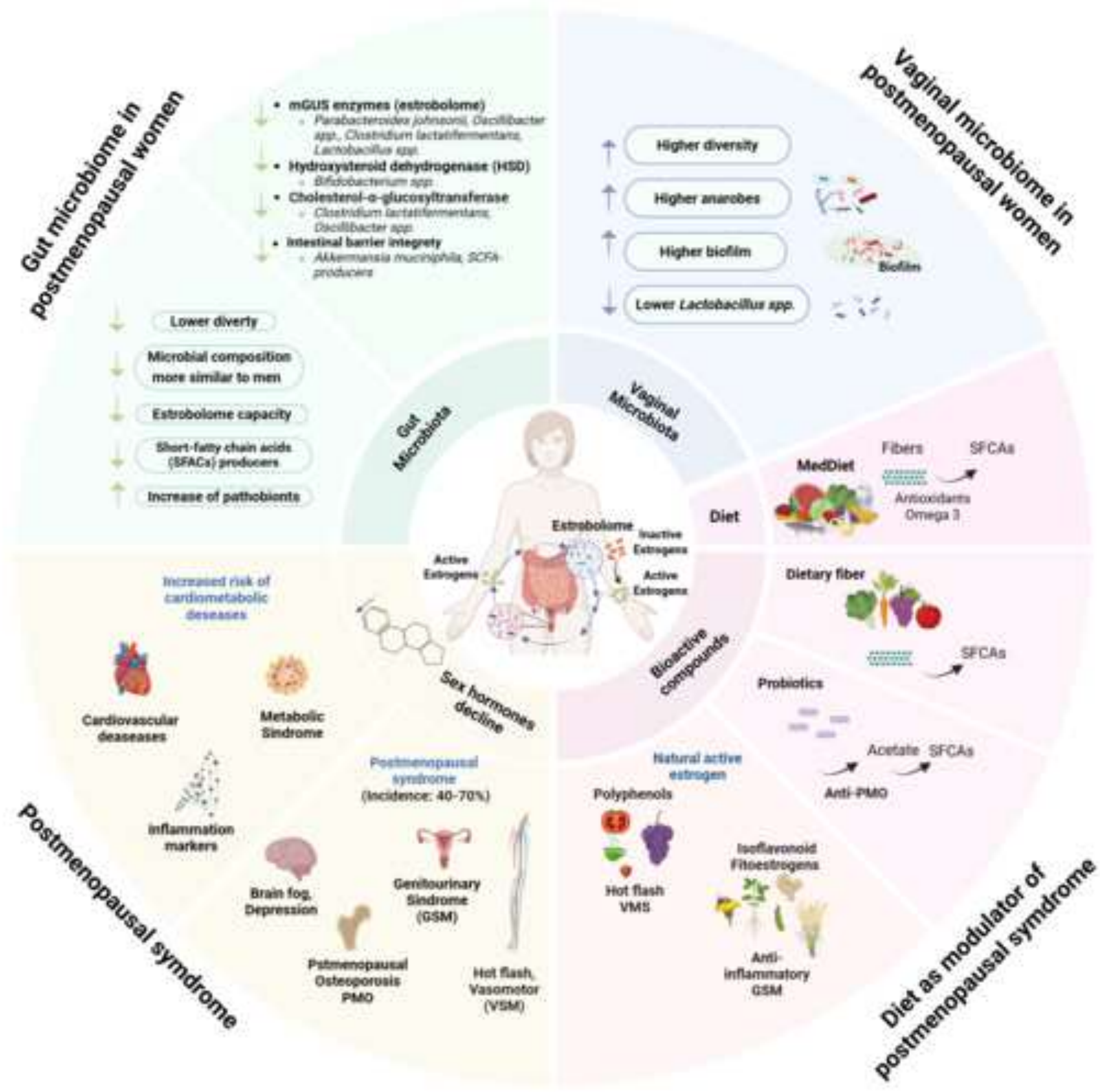
Intestinal lumen
Mucin
Intestinal epithelium
Direct SCFAs-producers
Fibers
Bifidobacterium
Lactobacillus
Butyrate
Acetate
A. muciniphila
TLR-2
TJ
GPR41/43
IL-18
FFAR2/3
Neutrophil
ORs
GPR41
Pancreatic cells
Blood pressure
PMO
VMS
Barrier integrity
Obesity
Insulin resistance
Obesity
Inflammation
CVD
gmGUS-producers (e.g. Parabacteroides, Clostridium, E. coli, A. muciniphila)
Inact E2
Act E2
CVD - GSM
PMO - VMS
Folate-producers (e.g.; L. plantarum)
Homocystein
Methionine
Hyperhomocysteinemia
CGT-producers (e.g.; Oscillibacter)
Cholesterol
Hypercholesterolaemia
Cholesterol α-D-glucoside
CVD
CVD
Proteobacteria
C. difficile
LPS
Toxin
IL-8
pDC
Monocytes
TNF-α
IFN
mDC
Inflammation
Hypertension
Inflammation
Dysbiosis
PMO - VMS
Negative regulation
Positive regulation

Figure_5


Polyphenols
Probiotics
Dietary fiber
Flavanones
Phenolic acids
Royal jelly
Phytoestrogens
MedDiet
Western Diet
Fibers
Anthocyanins
Daidzein
Genistin
Fibers
Antioxidants
Omega 3
Acetate
Butyrate-producers
Bifidobacterium
Lactobacillus
A. muciniphila
Roseburia
Eubacterium
mimic E2
TMAO
Intestinal lumen
Pathobionts
Butyrate
Butyrate-producers
Estrobolome
gmGUS-producers
Mucin
Intestinal epithelium
GSM
VMS
Activation osteoblasts
Inflammation
VMS
PMO
CMD
Inflammation
(IL-1β and TNF-α)
PMO
CMD
Inflammation
VMS
PMO
Inflammation
Healthy
Cardiovascular
Pathobionts
Inflammation
CMD
Negative regulation
Positive regulation

Graphical Abstract



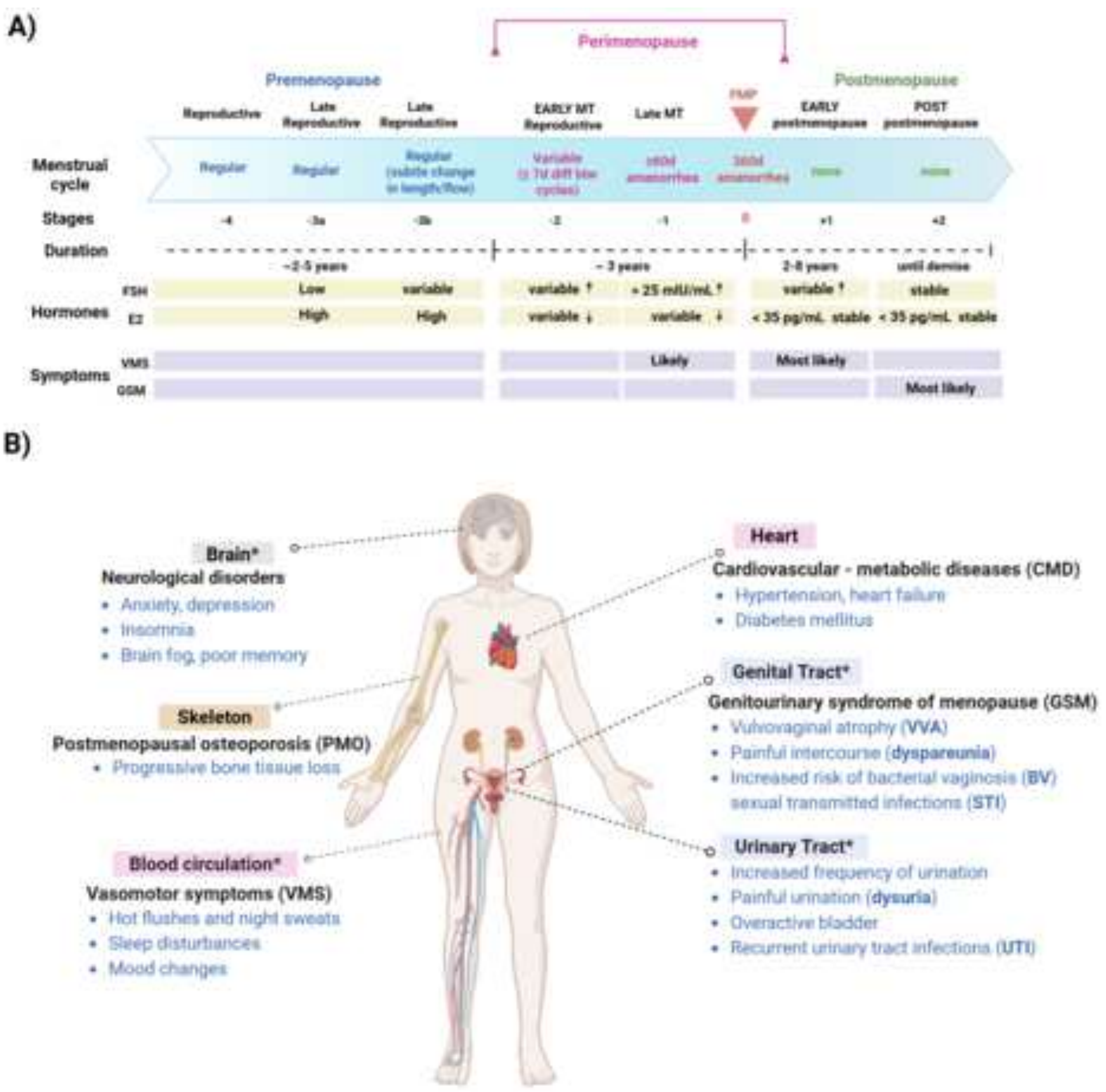
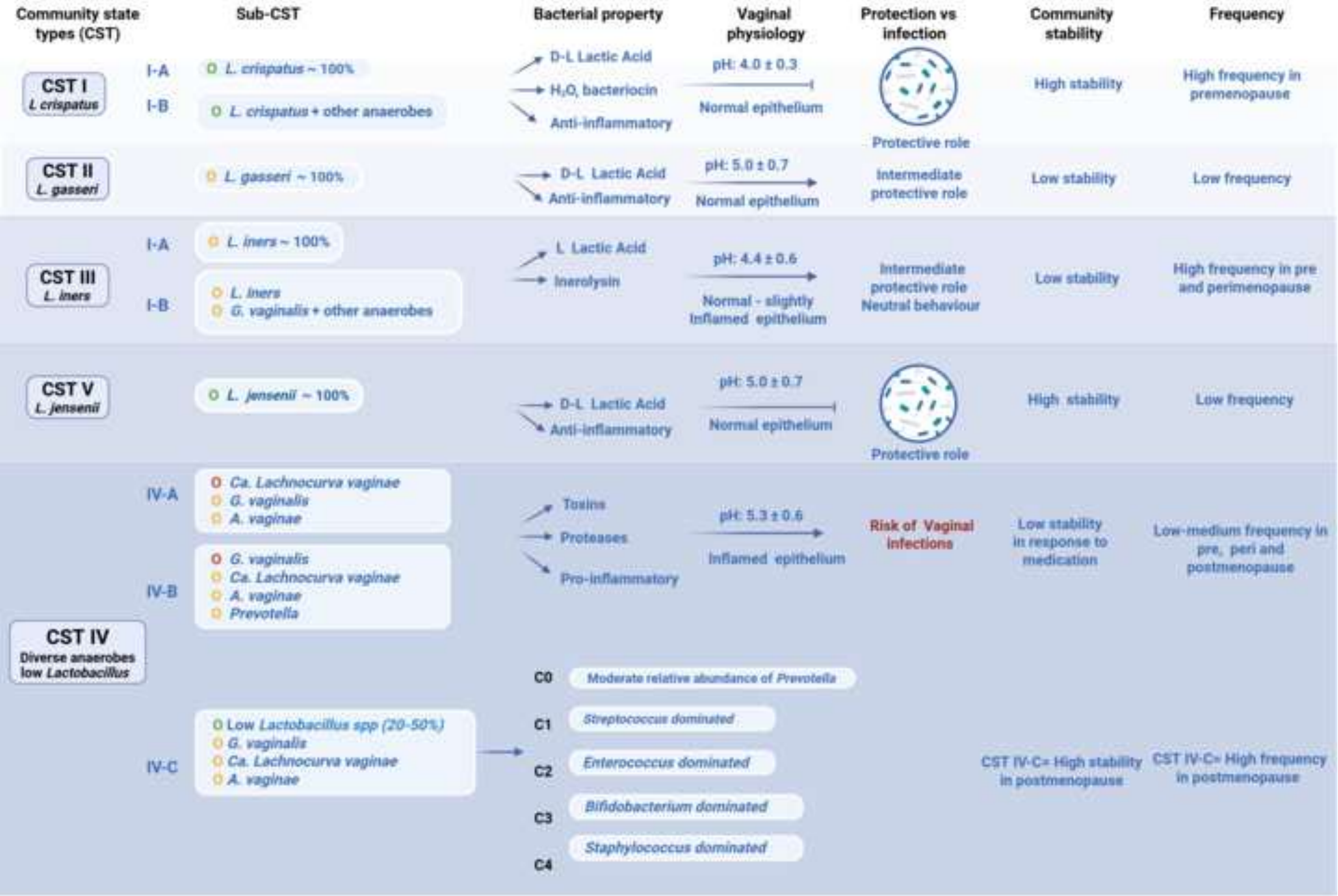

A)
Perimenopause
Premenopause
Postmenopause
Reproductive
Late Reproductive
Late Reproductive
EARLY MT Reproductive
Late MT
FMP
EARLY postmenopause
POST postmenopause
Menstrual cycle
Regular
Regular
Regular (subtle change in length/flow)
Variable (≥ 7d diff btw cycles)
≥60d amenorrhea
360d amenorrhea
none
none
Stages
-4
-3a
-3b
-2
-1
0
+1
+2
Duration
~2-5 years
~ 3 years
2-8 years
until demise
Hormones
FSH
Low
variable
variable ↑
> 25 mIU/mL ↑
variable ↑
stable
E2
High
High
variable ↓
variable ↓
< 35 pg/mL stable
< 35 pg/mL stable
Symptoms
VMS
Likely
Most likely
GSM
Most likely
B)
Brain*
Neurological disorders
Anxiety, depression
Insomnia
Brain fog, poor memory
Skeleton
Postmenopausal osteoporosis (PMO)
Progressive bone tissue loss
Blood circulation*
Vasomotor symptoms (VMS)
Hot flushes and night sweats
Sleep disturbances
Mood changes
Heart
Cardiovascular - metabolic diseases (CMD)
Hypertension, heart failure
Diabetes mellitus
Genital Tract*
Genitourinary syndrome of menopause (GSM)
Vulvovaginal atrophy (VVA)
Painful intercourse (dyspareunia)
Increased risk of bacterial vaginosis (BV) sexual transmitted infections (STI)
Urinary Tract*
Increased frequency of urination
Painful urination (dysuria)
Overactive bladder
Recurrent urinary tract infections (UTI)

Figure_2



Community state types (CST)	Sub-CST		Bacterial property	Vaginal physiology	Protection vs infection	Community stability	Frequency
CST I *L crispatus*	I-A I-B	○ *L. crispatus* ~ 100% ○ *L. crispatus* + other anaerobes	D-L Lactic Acid H_2O, bacteriocin Anti-inflammatory	pH: 4.0 ± 0.3 Normal epithelium	Protective role	High stability	High frequency in premenopause
CST II *L. gasseri*		○ *L. gasseri* ~ 100%	D-L Lactic Acid Anti-inflammatory	pH: 5.0 ± 0.7 Normal epithelium	Intermediate protective role	Low stability	Low frequency
CST III *L. iners*	I-A I-B	○ *L. iners* ~ 100% ○ *L. iners* ○ *G. vaginalis* + other anaerobes	L Lactic Acid Inerolysin	pH: 4.4 ± 0.6 Normal - slightly Inflamed epithelium	Intermediate protective role Neutral behaviour	Low stability	High frequency in pre and perimenopause
CST V *L. jensenii*		○ *L. jensenii* ~ 100%	D-L Lactic Acid Anti-inflammatory	pH: 5.0 ± 0.7 Normal epithelium	Protective role	High stability	Low frequency
CST IV Diverse anaerobes low *Lactobacillus*	IV-A IV-B	○ *Ca. Lachnocurva vaginae* ○ *G. vaginalis* ○ *A. vaginae* ○ *G. vaginalis* ○ *Ca. Lachnocurva vaginae* ○ *A. vaginae* ○ *Prevotella*	Toxins Proteases Pro-inflammatory	pH: 5.3 ± 0.6 Inflamed epithelium	Risk of Vaginal infections	Low stability in response to medication	Low-medium frequency in pre, peri and postmenopause
	IV-C	○ Low *Lactobacillus spp (20-50%)* ○ *G. vaginalis* ○ *Ca. Lachnocurva vaginae* ○ *A. vaginae*	C0 Moderate relative abundance of *Prevotella* C1 *Streptococcus dominated* C2 *Enterococcus dominated* C3 *Bifidobacterium dominated* C4 *Staphylococcus dominated*			CST IV-C= High stability in postmenopause	CST IV-C= High frequency in postmenopause

Declaration of interests

☐The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Serena Sanna reports financial support was provided by Research Institute of Genetics and Biomedical National Research Council. Serena Sanna reports a relationship with Research Institute of Genetics and Biomedical National Research Council that includes: employment and funding grants. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

***Title**:*

Aging in Women – the Microbiome Perspective

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Summary:

~~Represents a hallmark of women's aging, menopause~~Menopause is a hallmark of women's aging and is frequently portrayed as a medical issue. It also encompasses social and biological aspects often neglected and not well-understood, leaving women with insufficient support and attention. With the decline in estrogen levels, starting years before menopause is fully established, women experience various physical symptoms, and the risk of many age-related diseases increases sharply soon after these hormonal changes occur. Notably, these hormonal shifts also~~-~~ significantly impact the vaginal and gut microbiomes, contributing to dysbiosis and influencing the onset and progression of several diseases. Here, we examined the complex and dynamic relationship among aging, menopause, and microbiome changes with a particular focus on the vaginal and gut ecosystems. Emerging research highlights diet as a potential modulator for maintaining microbiome health during menopause. A deeper understanding of microbiome changes across life stages suggests the potential for microbiome-targeted strategies to support well-aging in women.

Panel

Estrogens: Estradiol, also known as 17β-estradiol or estrogen (**E2**), is the most common and essential form of circulating estrogens. In premenopausal women, **E2** reaches its highest levels before ovulation (110–410 pg/mL), and approximately 19–150 pg/mL during the follicular and luteal phases, whereas in postmenopausal women, it is < 35 pg/mL. Two other less abundant forms include estrone (**E1**) and estriol (**E3**); the latter is derived from E1 through the 16α-hydroxylation process. Their roles become more critical during pregnancy when the placenta produces them in large quantities.

Microbiota: the microbial biomass community residing in the host's organs, including bacteria, archaea, fungi, and viruses.

Microbiome: the whole collection of microbiota genomes and their products.

Microbial diversity: the variety and abundance of taxa in the microbiota.

16S rRNA amplicon sequencing: the targeted sequencing of 16S ribosomal RNA (16S rRNA).

Shotgun sequencing: the untargeted sequencing of all microbial genomes (metagenomes).

Dysbiosis: alteration in the microbiome's composition and/or function that can lead to loss of its supportive role in host health.

Pathobionts: normally harmless microorganisms that can become pathogenic under certain conditions, often associated with dysbiosis or immune dysfunction. In the vaginal microbiota, pathobionts include *Prevotella*, *Streptococcus*, *Gardnerella*, and *Atopobium.* In the intestinal microbiota, they include *Bilophila*, *Desulfovibrio*, *Eggerthella*, all *Enterobacteriaceae*, *Campylobacter*, *Fusobacterium*, *Streptococcus*, *Bacteroides fragilis*, *Actinomyces*, *Corynebacterium*, *Staphylococcus*, *Parvimonas*, *Porphyromonas*, *Ruminococcus torques*, and *Clostridium* species such as *C. difficile*.

Short-chain fatty acids (**SCFAs**): fatty acids with 1 to 6 carbon atoms produced through the saccharolytic fermentation of undigested dietary fibers. The main SCFAs—acetic acid (acetate), propionic acid (propionate), and butyric acid (butyrate)—are typically present in a ratio of 60:20:20. *Clostridiales* members are key SCFAs-producers with the families *Ruminococcaceae* (e.g., *Faecalibacterium*, *Ruminococcus*, *Oscillibacter*), *Lachnospiraceae* (i.e., *Roseburia*, *Anaerostipes*, *Blautia*, *Coprococcus, Butyrivibrium, Dorea*), and *Eubacteriaceae* being particularly efficient butyrate-producers. Other bacteria, such as *Bifidobacterium*, can produce non-butyrate metabolites (i.e., acetate, succinate, and lactate) that are utilized by butyrogenic-producing bacteria.

Trimethylamine N-oxide (**TMAO**): a class of amine oxide produced by gut microbes through the metabolism of dietary nutrients, primarily choline, carnitine, and betaine, and its levels increase with the consumption of animal protein.

Estrobolome: a collection of bacterial genes involved in the reactivation of sex hormones (e.g., estrogens), encoding glycoside hydrolase 2 (GH2) family enzymes, which includes gut microbial β-glucuronidases (gmGUS), β-galactosidases, and β-mannosidases. GUS genes are encoded by Bacteroidetes (52%, e.g., *Bacteroides, Prevotella, Alistipes, etc.*), Firmicutes (43%, e.g., *Faecalibacterium, Lactobacillus*, *Streptococcus, Eubacterium, Ruminococcus, Roseburia, Romboutsia, some Clostridium, and Coprococcus*), Actinobacteria (1.6% e.g., *Bifidobacterium*), Verrucomicrobia (1.5% e.g., *Akkermansia*), and Proteobacteria (1.3%, e.g., *Escherichia*).

Abbreviations used for age-related diseases in women:
Genitourinary Syndrome of menopause (GSM)
Vulvovaginal atrophy (VVA)
Vasomotor symptoms (VMS)
Urinary tract infections (UTI)
Postmenopausal osteoporosis (PMO)
Cardiovascular diseases (CVD)
Metabolic diseases (Met)
Type 2 diabetes mellitus (T2DM)

Introduction

Aging is a natural process that leads to profound physiological changes in the human body. Over the past century, global life expectancy has increased significantly, and this trend is projected to continue. Accordingly, the elderly population (over 65) is expected to rise from 761 million in 2021 to 1.6 billion in 2050 (United Nations Department of Economic and Social Affairs, 2023), posing a worldwide challenge to governments and economies. Women generally live longer than men, partly due to the protective effects of female sex hormones, though the precise reasons for this longevity advantage remain unclear (Hägg & Jylhävä, 2021). Sex hormone physiological fluctuations and ~~large~~ extensive modifications, especially those occurring throughout menopause - a major hallmark of aging in women - significantly impact women's health. These hormonal alterations are linked to diverse age-related diseases, such as cardiovascular diseases, metabolic disorders, and osteoporosis (Davis et al., 2015; Harlow, Gass, Hall, Lobo, Maki, Rebar, Sherman, Sluss, de Villiers, et al., 2012; Muka et al., 2016). In contrast, men undergo a progressive fall in testosterone levels, affecting muscle strength and cognitive functions. However, these changes are generally less abrupt than those observed in women. Aging is also associated with changes in the **microbiome,** the microbial communities that are essential for metabolic, immune, and neurological functions. While changes in the microbiome during aging are partially attributable to increased use of medications in late adulthood, it is also known that significant alterations in the composition and diversity of the microbiome (**dysbiosis**) contribute to the onset and progression of age-related conditions (Ghosh et al., 2022). Remarkably, in women, the **estrogen** decline during menopause further influences the gut microbiota, as well as the vaginal microbiome (Muhleisen & Herbst-Kralovetz, 2016; Peters, Santoro, et al., 2022). This is likely to lead to differential mechanisms of the microbiota-host health relationship in men compared to women, which are largely unknown.

This review examines the dynamic relationship between vaginal-gut microbiomes and age-related conditions in women and explores potential avenues to promote women's well-aging through dietary microbial modulation. Understanding these sex-specific mechanisms, including those related to the microbiome, is crucial to fostering personalized healthy aging.

Search strategy and selection criteria

A search of the scientific literature was conducted by using PubMed and Scopus and looking for the following search terms ((vaginal microbiome OR vaginal microbiota) AND (ageing OR aging OR menopause OR postmenopausal women)) (results **269**); ((gut AND (microbiome OR microbiota) AND women AND (ageing OR menopause OR postmenopausal women)) (results **418**); for the dietary intervention on postmenopausal women a different combination of terms was used: diet OR (*Lactobacillus* OR probiotic) OR (phytoestrogens OR soybean OR isoflavone OR flavonoid) AND (microbiome OR microbiota) AND (menopause OR postmenopausal OR older) AND women (results **649**); (diet OR *lactobacillus* OR flavonoid) AND (menopause OR postmenopausal OR postmenopausal) AND women AND trials AND (hypertension OR osteoporosis OR vasomotor) (results **484**), food elements were added to the search including red clover, prunes, blueberry, and royal jelly. More specific search combinations for sex hormone changes in menopause were then used, including the terms mentioned above and additional items; for example, (estrogen) OR (sex hormones) AND (menopause OR perimenopausal OR postmenopausal women) AND (microbiota) was used to find articles related to considerations for sex hormone variation in aging. We examined the studies and included those relevant to this review's topic, as outlined in the following decision flow chart. **Inclusion criteria:** studies with original data, studies conducted in (premenopausal) OR perimenopausal AND postmenopausal women in healthy status; observational studies including prospective, retrospective, longitudinal, cross-sectional study designs, clinical trials, population cohort studies; a measure of microbiota composition by high-throughput sequencing (16S rRNA or shotgun sequencing). **Exclusion criteria**: studies conducted in women only of reproductive age (18-44 years); commentaries, book chapters, letters, editorials, conference proceedings, case reports, conference, abstracts, or non-peer-reviewed articles, publication in a different language than English; studies conducted in gynecologic cancer patients, with infection, chronic diseases; studies conducted in a cohort lower than **30** postmenopausal women. Molecular methods excluded i) quantitative polymerase chain reaction (qPCR), ii) microbiota measured by non-molecular methods (such as culture and microscopy), no relevant outcomes reported, or when there is a lack of quantifiable outcomes (e.g., not defined abundance of microbiota).

Pertinent original articles and reviews (only used for the introduction paragraph) were peer-reviewed and indexed in PubMed and Scopus. We limited the publication dates from 2010 to 2024 and conducted literature searches between May 2024 and July 2024. **Supplementary Table 1** provides a comprehensive list of publications screened, methods used, and a summary of reported results.

The menopausal transition period.

Menopause represents a key milestone in a woman's aging, marking the transition to a new phase of life. Despite its profound impact on health, it remains an often-overlooked aspect of women's well-being. Biologically, it is defined by the permanent cessation of ovarian follicular activity due to the depletion of ovarian follicular reserve. As the number of functional follicles declines, the production of gonadal steroids decreases, ultimately leading to the end of monthly menstruation and of reproductive capacity (Harlow, Gass, Hall, Lobo, Maki, Rebar, Sherman, Sluss, de Villiers, et al., 2012). During this phase, the number of granulosa cells within the ovarian follicles declines (*ovarian aging)*, leading to reduced production of estradiol (and inhibin B) and consequently lower circulating estrogen levels (Harlow, Gass, Hall, Lobo, Maki, Rebar, Sherman, Sluss, de Villiers, et al., 2012). Without the inhibitory effect of estrogen on gonadotropins, the pituitary gland increases the production of follicle-stimulating hormone (FSH ≥ 25 mIU/mL), resulting in the cessation of the menstrual cycle (**Figure 1_A**).

Menopause is not a single event but rather a gradual biological process, often referred to as the menopausal transition (MT). MT occurs in three stages: perimenopause, menopause, and post-menopause. According to the Staging of Reproductive Aging Workshop (STRAW) (**Figure 1_A**), each stage is characterized by variable duration and symptoms (Harlow, Gass, Hall, Lobo, Maki, Rebar, Sherman, Sluss, de Villiers, et al., 2012). Perimenopause encompasses one year preceding the final menstrual period (FMP) and the subsequent year following menopause. During this period, estrogen and progesterone levels fluctuate (**Figure 1_A**), and women may experience some symptoms of the 'postmenopausal syndrome', although ovulatory menstruation can still occur (Harlow, Gass, Hall, Lobo, Maki, Rebar, Sherman, Sluss, de Villiers, et al., 2012). Vasomotor symptoms (VMS), such as hot flashes, followed by abundant menstruation (dysmenorrhea), are often among the earliest signs of perimenopause (Thurston & Joffe, 2011). Menopause is recognized when menstruation stops for 12 consecutive months, typically between the ages of 45 and 55. Post-menopause~~,~~ is characterized by a further decrease in sex hormones, with the adipose tissue becoming the principal producer of estrogens (Harlow, Gass, Hall, Lobo, Maki, Rebar, Sherman, Sluss, de Villiers, et al., 2012). During post-menopause, a woman's body goes through further changes that might influence many organs, leading to a range of symptoms known as postmenopausal syndrome (**Figure 1_B**). Approximately 45–77% of women suffer from the genitourinary syndrome of menopause (GSM) (Cox et al., 2023), which includes urinary, genital, and sexual symptoms. Reduced glycogen in vaginal cells raises vaginal pH and promotes bacterial growth, increasing infection risk (Song et al., 2022). While VMS usually improves with time, GSM might deteriorate without adequate therapy. Sex hormonal variations can also impact the nervous system, creating neurological disorders such as brain fog, anxiety, and sleep disturbances (Freeman et al., 2006). Moreover, the drop in estrogen can accelerate bone density loss and increase the risk of postmenopausal osteoporosis (PMO).

In addition to postmenopausal syndrome, the hypoestrogenic state following menopause can also exacerbate chronic diseases impacting vital systems and accelerate the progression of risk of cardiovascular and metabolic diseases (CMD). Aging in women is, in fact, associated with a significant increase in blood pressure, body mass index (BMI), obesity, and body fat distribution, as well as an increase in insulin resistance (Ryczkowska et al., 2022). Notably, epidemiological studies show that women tend to develop heart diseases later in life compared to men, mainly after menopause (Muka et al., 2016; Paduszyńska et al., 2020). This delay is attributed to the cardioprotective effects of estrogen before menopause, which include enhanced angiogenesis, improved vasodilation, and reduced oxidative stress and fibrosis (Ryczkowska et al., 2022). Similarly, the decline of estrogen can influence insulin resistance and glucose metabolism, increasing the risk of metabolic disorders (e.g., type 2 diabetes mellitus, T2DM) (Davis et al., 2015).

Furthermore, emerging evidence suggests that substantial alterations can occur in intestinal and vaginal microbiomes, altering bacterial populations and increasing the abundance of pathobionts, which can lead to dysbiosis linked to inflammatory diseases such as GSM and PMO.

The Vaginal Microbiota

Vaginal microbiota in premenopausal women

Longitudinal studies have observed that the vaginal microbiome community is overall stable in the short-term period (months), with fluctuations occurring only in a small fraction of the female population (Gajer et al., 2012). Factors such as age, ethnicity, sex hormones, and personal habits influence its composition, and specific events, like vaginal infections (Krog et al., 2022; Lebeer et al., 2023), can introduce substantial changes. Estrogens are also key players in shaping the microbiome by promoting glycogen accumulation in vaginal cells and providing a carbohydrate source for *Lactobacillus,* the dominant bacterial phylum in women's vaginal microbiome during reproductive age (Lebeer et al., 2023). These bacteria maintain vaginal health by creating an acidic environment (pH 3.5–4.5) that inhibits the growth of pathogens and by producing anti-inflammatory compounds that strengthen the vaginal epithelium (Borges et al., 2014). The four main vaginal *Lactobacillus* species are *L. crispatus*, *L. gasseri*, *L. iners*, and *L. jensenii* (**Figure 2**). *L. iners* has been observed in both healthy women and women with vaginal infections, which is why the possibility of *L. iners* strains with potential pathogenic roles is under debate (Petrova et al., 2017). Similarly, vaginal anaerobes, such as *Gardnerella*, *Prevotella*, *Atopobium*, and *Streptococcus*, have pathogenic potential and can destabilize the vaginal microenvironment. To represent and distinguish the different vaginal microbiome types, the community state types (CSTs) classification based on taxa abundance (Ravel et al., 2011) was proposed, with four types representing microbiota dominated by *Lactobacillus spp.* (CST I: *L. crispatus*, CST II: *L. gasseri*, CST III: *L. iners*, CST V: *L. jensenii*) and one, CST IV, which lacks *Lactobacilli* and has high microbial diversity. CST subtypes have further refined this classification (**Figure 2**) (France et al., 2020). While the use of CSTs to define microbiota composition is common, there is a broader call for more dynamic, higher-resolution approaches to understanding microbial communities and modeling their interconnected nature and mutual coexistence within the vaginal microenvironment (Lebeer et al., 2023).

Vaginal microbiome during and after menopausal transition

Most research on vaginal microbiota focused on women of reproductive age, leaving a critical knowledge gap in our understanding of changes that occur throughout and after MT. However, significant changes are expected to exist and are likely to correlate with menopause-related health conditions. One of the pioneering studies tracked the vaginal microbiota over a two-month period in postmenopausal women with and without vulvovaginal atrophy (VVA), observing that asymptomatic women had low bacterial diversity, dominated by *Lactobacillus* species. In contrast, symptomatic women exhibited higher diversity with anaerobes such as *Prevotella timonensis*, *Porphyromonas*, *Peptoniphilus*, and *Bacillus* (Hummelen et al., 2011). Moreover, *Lactobacillus spp.* presence was inversely correlated with dryness. Other studies have also observed that a low percentage of postmenopausal women (10-30%) still maintain *Lactobacillus*-dominated communities, while in the majority of postmenopausal women (approx. 60-70%), the vaginal microbiome is more likely to be characterized by anaerobic communities and increased microbial diversity (rather than having one dominant species) and elevated vaginal pH (Mitchell et al., 2017; Yoshikata, Yamaguchi, Mase, Tatsuzuki, et al., 2022). Larger cohort studies have further confirmed that postmenopausal women often exhibit CST IV, with a moderate prevalence of subtypes (CST IV-C0 and CST IV-B), which were also associated with VVA and GSM biomarkers, though the underlying causal genera were not consistent (Shardell et al., 2021; Shen et al., 2016; Waetjen et al., 2023). Intriguingly, the transition from a vaginal microbiome dominated by *Lactobacillus spp.* (CST I, II, III, V) to CST IV appears to be a gradual process triggered by estrogen decrease beginning in perimenopause, with an abundance of *Lactobacillus spp.* progressively declining from premenopausal to postmenopausal stages (Brotman et al., 2014). Interestingly, Byrne *et al.* found that postmenopausal women with mixed microbiomes lacking *Lactobacillus* exhibited higher levels of proinflammatory cytokines (Byrne et al., 2024)~~, than those in~~compared to premenopausal women with mixed communities dominated by *Prevotella* (Anahtar et al., 2015). Another robust evidence of *Lactobacillus*'s crucial role in maintaining vaginal health, both in pre- and post-menopausal women, comes from the Isala cohort, a comprehensive study of the vaginal microbiome in over 3,000 women of various ages residing in Belgium (Lebeer et al., 2023). In this study, key bacterial modules were correlated with measured host factors using network-based statistical approaches. High estrogen levels and a healthy vaginal microbiome were associated with the *Lactobacillus* module. In contrast, menopausal status was correlated to the *Prevotella* module, confirming on a large scale the shift toward a reduction in *Lactobacillus spp.* after menopause.

Based on the literature results of these studies, we propose a model of expected vaginal microbiota changes during MT stages and the underlying biological mechanisms, as depicted in **Figure 3**. It would, therefore, be ideal to design prevention and treatment strategies for restoring *Lactobacillus* levels to a minimum beneficial level after menopause.

Considering the impact of sex hormone decline on the vaginal microbiome and its consequences for women, it is crucial to prevent and control clinical conditions via estrogen intake. Hormone replacement therapy (HRT) restores estrogen levels (30–60 pg/mL) and is available in various forms, including oral tablets, vaginal creams, rings, and transdermal patches (Stuenkel et al., 2015). Earlier research has shown that postmenopausal women using vaginal estradiol or oral menopausal hormone therapy are more likely to exhibit *Lactobacillus*-dominated vaginal microbiota and decreased microbial diversity (Heinemann & Reid, 2005; Mitchell et al., 2021; Shen et al., 2016; Srinivasan et al., 2022). While HRT effectively alleviates symptoms of VVA in postmenopausal women, it is not recommended for women with a history of breast cancer and cardiovascular or liver diseases (Stuenkel et al., 2015). Additionally, estrogen therapy may cause additional side effects such as vaginal bleeding, breast pain, and perineal pain (Stuenkel et al., 2015). Taken together, HRT is not always a suitable treatment for GSM symptoms, and other solutions should be sought.

The gut microbiome

The rapid expansion of metagenomics to large-scale population-based and disease cohort-based studies has consistently highlighted the association between gut microbiome dysbiosis and many diseases (Gacesa et al., 2022; Imhann et al., 2019). Nevertheless, while a clear distinction can be seen between healthy individuals and those diagnosed with diseases, a "universal" healthy microbiome cannot be defined, as the microbial composition significantly varies between individuals and populations (Kurilshikov et al., 2021). The gut microbiota is primarily composed of phyla including Bacteroidetes (e.g., *Bacteroides*, *Prevotella, Alistipes*), Firmicutes (e.g., *Clostridium*, *Lactobacillus*, *Bacillus*, *Enterococcus*, *Ruminococcus, Faecalibacterium, Roseburia, Blautia*), Actinobacteria (e.g., *Bifidobacterium*), Proteobacteria (e.g., *Escherichia*, *Shigella, Helicobacter, Sutterella*), Fusobacteria (*Leptotrichia*, *Fusobacterium*), and Verrucomicrobia (e.g., *Akkermansia*)(Arumugam et al., 2011). In adults, over 90% of the gut microbiota consists of Bacteroidetes and Firmicutes, reflecting their foundational roles in gut ecology (Arumugam et al., 2011). Unlike the vaginal ecosystem, where a microbiome dominated by one or a few species is preferred, high diversity in the gut microbiome is essential to guarantee resilience to environmental stressors and has been associated with improved human health (Arumugam et al., 2011). Not only is the microbial composition crucial, but also the metabolic activity of the different microbes is essential in maintaining human health. Gut microbes metabolize both dietary and endogenous compounds, producing secondary metabolites that serve as energy sources for themselves and signaling molecules for human metabolism and immune function. Short-chain fatty acids (**SCFAs**) and trimethylamine-N-oxide (**TMAO**) are diet-derived metabolites that significantly impact host health. SCFAs are produced by specific Firmicutes taxa during the breakdown of dietary fiber and act by binding to G-protein-coupled receptors (GPCRs) located on various immune and organ cells (Mann et al., 2024) (**Figure 4_C**). They are essential for maintaining intestinal barrier integrity and immunological homeostasis, and play a significant role in regulating blood pressure and controlling insulin resistance (Mann et al., 2024). In contrast, TMAO, a metabolite produced from dietary phosphatidylcholine and L-carnitine (abundant in meat and processed foods), contributes to atherosclerosis by interfering with cholesterol clearance from the liver, increasing plaque formation, and heart disease risk (Z. Wang et al., 2011).

The gut microbiota ecosystem is dynamic, evolving throughout the human lifespan in response to changes in diet, environment, ~~life style~~lifestyle, and ~~healthy~~ health conditions. Transitions of the gut microbiome during life do not occur equally in both sexes (Zhang et al., 2021). However, only a few studies have investigated the impact of aging on the gut microbiome, particularly in relation to women's health and menopause. In women, we expect that the shifts in sex hormones further complicate the impact of aging. To our knowledge, no current studies have simultaneously interrogated the gut and vaginal microbiomes in population cohorts; however, emerging research supports the existence of a gut-vaginal axis. This complex and reciprocal interaction could be influenced by shifts in sex hormone levels and may have a potential impact on overall health (**Figure 4_A**). Estrogens produced in the ovaries are conjugated in the liver and enter the gut, where bacteria from the

estrobolome can deconjugate estrogen into an active form (Ervin et al., 2019; Hu et al., 2023; Pollet et al., 2017). Dysbiosis and inflammation can alter the gut microbial β-glucuronidases (gmGUS) activity, resulting in a consistent increase or decrease in free active estrogens in the bloodstream, which affects hormonal balance, metabolic processes, and the risk of developing estrogen-related conditions. Similarly, fluctuations in estrogen levels during ~~postmenopause~~post menopause can lead to alterations in gut and vaginal microbial communities, contributing to age-related diseases (**Figure 4_A**).

Gut microbiome diversity in postmenopausal women

Understanding the differences in gut microbiota diversity between premenopausal and postmenopausal women is complex, as confounding factors—such as age-related diseases—can further influence microbiota composition. In this review, we selected studies involving cohorts of healthy older women who may be at increased risk for aging-related diseases. Several of the selected large-scale studies focused on differences in microbial composition across sex differences and age groups, enabling the identification of gut biodiversity in relation to age and sex (de la Cuesta-Zuluaga et al., 2019; Peters, Lin, et al., 2022; Santos-Marcos et al., 2018; Zhang et al., 2021). Generally, younger women exhibit greater microbial diversity than men, but this difference diminishes with age, as middle-aged women display a gut microbiota more similar to men (de la Cuesta-Zuluaga et al., 2019; Peters, Lin, et al., 2022; Santos-Marcos et al., 2018; Zhang et al., 2021) (**Figure 4_B**). In contrast, differences in microbiota composition between younger and older men appear less pronounced (de la Cuesta-Zuluaga et al., 2019). Lifestyle factors—such as alcohol consumption, smoking, drug use, and diet—also contribute to age- and sex-dependent declines in microbiome diversity. However, sex hormones, and in particular the ~~decline~~ decrease in estrogen, remain a crucial determinant of gut microbiome features in women (de la Cuesta-Zuluaga et al., 2019). Notably, two studies reported that the dissimilarity between women and men during the premenopausal period is not significant when restricting the analysis to obese participants, likely due to lower estrogen levels in obesity, which makes the gut microbiome similar across sexes (Mayneris-Perxachs et al., 2020; Zhang et al., 2021).

Bacterial taxa associated with age-related diseases in women

Large cohort studies have identified significant changes in bacterial diversity and microbial taxa abundance in patients with cardiometabolic diseases (Fromentin et al., 2022; Talmor-Barkan et al., 2022; Q. Yan et al., 2017). However, only a minority of these have examined men and women separately. These investigations reported a reduced microbiome potential for the biosynthesis of SCFAs and higher levels of pathobionts belonging to the phylum Proteobacteria, which are associated with systemic inflammation, high adiposity, obesity, and hypertension (**Figure 4_B-C**).

Similar results were found in a recent study investigating postmenopausal women with CVD risk (Gaber et al., 2024). The depletion of SCFA-producing bacteria has been reported to be associated with an increased risk of CVD in postmenopausal women. For instance, in different studies, postmenopausal women had a lower abundance of SFCA-producers (e.g., *Ruminococcus, Eubacterium, Roseburia, Clostridium*) (Y. Liu et al., 2022; Mayneris-Perxachs et al., 2020; Santos-Marcos et al., 2018; Zhao et al., 2019) associated with high-density lipoprotein (HDL) cholesterol levels, lower waist circumference, and an increased risk of metabolic syndrome (Peters, Lin, et al., 2022). Other SCFA-producers, specifically *Parabacteroides* and *Oscillibacter* (**Figure 4_B)**, were found to be depleted in postmenopausal women and associated with lower HDL levels in feces and plasma (Peters, Lin, et al., 2022). Notably, a recent study revealed that *Oscillibacter* encodes cholesterol-α-glucosyltransferase (CgT), which metabolizes cholesterol into secondary metabolites, regulating serum cholesterol levels and thus remarking its importance for cardiovascular health (**Figure 4_C)** (C. Li et al., 2024).

The depletion of SCFA metabolites and SCFA-producers has also been associated with a higher incidence of metabolic diseases, such as T2DM, already in the general population (Sanna et al., 2019). *Akkermansia muciniphila*, which supports butyrate metabolism and regulates insulin resistance (**Figure 4_C)**, was more abundant in premenopausal than postmenopausal women (Sakamuri et al., 2023; Sanna et al., 2019; Wallimann et al., 2021). Intriguingly, this bacterium has been linked to changes in progesterone metabolism (Peters, Lin, et al., 2022).

Additionally, other bacterial metabolic pathways were downregulated in postmenopausal individuals, such as the folate pathway (Mayneris-Perxachs et al., 2020; Santos-Marcos et al., 2018), due to a significant reduction in *Lactobacillus plantarum*, a key folate producer. Folate participates in the methionine cycle, facilitating the conversion of homocysteine to methionine, and low levels can lead to increased homocysteine accumulation in the serum (hyperhomocysteinemia) (**Figure 4_C)**, which is linked to CVD due to its harmful effects on the cardiovascular endothelium (Ganguly & Alam, 2015).

Sex hormone changes affect gut microbiota composition in post-menopause.

Notably, the menopause transition and menopausal symptoms are often accompanied by gastrointestinal complaints, likely attributable to sex hormone fluctuations. Clinical studies ($n < 100$) have found that estrogen levels positively correlate with the abundance of beneficial bacteria, such as *Clostridiales*, especially *Ruminococcaceae*, and negatively with Bacteroides, regardless of age or BMI (Fuhrman et al., 2014). A reduction in estrogen, which decreases the estrobolome's capacity, may lower estrogen reabsorption and lead to gut dysbiosis. Some gmGUS-encoding microbes (mainly species belonging to the *Clostridiales* order) were reduced in obese postmenopausal women (Mayneris-Perxachs et al., 2020; Meng et al., 2021; Peters, Lin, et al., 2022), especially those with CDM risk (Y. Liu et al., 2022). Interestingly, Peters *et al*. examined the menopause-microbiome relationship, finding a decrease in gmGUS enzymes (e.g., β-glucuronidase and aryl-sulfatase orthologs) involved in activating sex hormones (Peters, Lin, et al., 2022). In this study, the low abundance of specific bacterial taxa in postmenopausal women strongly correlated with depleted gmGUS enzymes carried by various beneficial bacteria (e.g., *Parabacteroides johnsonii*, *Clostridium lactatifermentans, Escherichia coli, A. muciniphila*) (Peters, Lin, et al., 2022) (**Figure 4B-C**). However, some bacterial species encoding gmGUS enzymes were also reported to increase in postmenopausal women. Since the discovery of gmGUS is relatively recent, more research is needed to characterize the properties of gmGUS producers.

Bacterial taxa associated with menopause-related disorders

Several studies reported that gut microbiota might have a role in postmenopausal osteoporosis (PMO), as the depletion of SCFAs-producers can affect bone health by altering calcium absorption and metabolism (Ji et al., 2024; Rettedal et al., 2021; L. Yan et al., 2024). PMO severity has been negatively correlated with the abundance of various *Lactobacillus* and *Bifidobacterium* species, suggesting a possible protective role in bone absorption. These two beneficial species were already observed to decline during perimenopause (Chen et al., 2024). Recent studies found that potential pathogens, such as *Klebsiella*, *Streptococcus parasanguinis*, and *Clostridium perfringens*, were negatively associated with bone mineral density (BMD), suggesting their role in bone loss (He et al., 2020; Ji et al., 2024; Q. Wang et al., 2021). Conversely, members of the *Lachnospiraceae* family showed a positive correlation with higher BMD, suggesting their potential role in supporting bone health (He et al., 2020; Ji et al., 2024). A similar association of taxa was found in a study investigating VMS symptoms (Pavlovska et al., 2022) (**Figure 4_B-C**).

These observations suggest that menopause, through physiological and hormonal changes, significantly impacts the gut microbiota, which plays a multifaceted role in women's health.

Diet as a potential modulator of microbiome toward healthy aging in women

Effects of Dietary Intervention on Aging in Women

Emerging evidence supports the effect of diet on healthy aging and longevity in the general population, and the gut microbiome may mediate this effect (Brandhorst et al., 2024). While using diet to modulate the gut microbiome is appealing, targeted modulatory interventions are premature until evidence from multiple studies supports the causal or curative effect of specific bacteria and their functions in age-related conditions. Furthermore, such intervention studies need to be personalized, which means that the baseline microbiome composition must be considered to predict the efficiency and likelihood of persistent microbiome changes as required. The field of personalized nutrition and the microbiome is still in its infancy; however, we foresee the potential impact of using diet to prevent or treat various age-related conditions in both men and women.

For example, in postmenopausal women, increased intake of ‘anti-aging nutrients’ may mitigate the side effects of estrogen decline (**Figure 5**). Likewise, diet regimes that favor the growth of SCFAs-producing bacteria, whose abundance declines with age, might be necessary for a protective effect on the cardiovascular and skeletal systems. In a murine osteotomy model, propionate and butyrate supplementation enhanced bone density and prevented bone loss by inhibiting osteoclast formation and bone reabsorption (Wallimann et al., 2021). Studies conducted on fiber-rich diets such as the Mediterranean diet (MedDiet) showed that a high abundance of SCFAs promotes a healthier cardiovascular system and reduces chronic inflammation (Ghosh et al., 2020). Conversely, a diet high in animal fats, sweets, refined oils, and processed foods exacerbates inflammation, thereby increasing the accumulation of TMAO. In a study on postmenopausal women with and without PMO, those following a diet rich in grains, vegetables, and fruits and low in red meat exhibited normal bone mass and a lower abundance of gut methanogen bacteria responsible for decreasing SCFAs (Brahe et al., 2015; Chen et al., 2024).

Effects of Single Bioactive Compounds Interventions on Aging in Women.

Polyphenols

Polyphenols are another significant food component with anti-inflammatory and antioxidant properties that help mitigate age-related inflammation. This broad class of compounds also includes phytoestrogens (i.e., isoflavones) and anthocyanins, making them a popular dietary supplement for promoting healthy aging (**Figure 5**).

i) Phytoestrogens

Various natural compounds, including phytoestrogens such as daidzein and genistein, mimic human estrogen and are found in soybeans, legumes, mung beans, green beans, and certain plants (e.g., red clover). Soybeans are especially rich in phytoestrogens (~ 150 mg/100 g of cooked soybeans). The recommended intake of isoflavones is ~ 40-50 mg/d, whereas, in European countries, the consumption of phytoestrogens is, on average, ~ 0.76 mg/d (I.-S. Kim, 2022). GmGUS enzymes metabolize these molecules, and once activated, they can bind to the same estrogen receptors (ERs) as endogenous estrogens, thereby regulating downstream signaling pathways (Hu et al., 2023). The advantage of introducing isoflavonoids into the diet lies in their minimal side effects compared to HRT, as they exhibit a strong binding affinity to ERβ and a weaker affinity to ERα. While estrogen is a potent agonist, binding effectively to both receptors, the active metabolite of isoflavones selectively targets the ERβ receptor. This receptor selectivity provides health benefits and lowers cancer risk, as ERβ is primarily expressed in non-gonadal tissues. Moreover, secondary metabolic products of phytoestrogens degradation, produced by gut microbe metabolism, act as potent metabolites with various beneficial effects, including anti-androgenic and antioxidant activities. For instance, *Eggerthella*, *Adlercreutzia*, *Asaccharobacter*, *Slackia*, and *Lactococcus spp.* produce equol, a potent isoflavandiol estrogen metabolized from soy daidzein (Iino et al., 2019). Clinical trial studies have shown that this metabolite reduces the risk of CVD in postmenopausal women (Acharjee et al., 2015; Z.-M. Liu et al., 2014). However, only 30–50% of the population is able to convert daidzein into equol, depending on the composition of their intestinal microbiota. Other derivatives of daidzein are important for women's health. For example, obesity in postmenopausal women was associated with a loss of ability to metabolize the soy isoflavone daidzein to O-desmethylangolensin (Miller et al., 2017). Furthermore, phytoestrogens have been shown to promote the growth of butyrate-producing bacteria (such as *Faecalibacterium* and members of the *Clostridium* clusters), which contribute to intestinal health through their anti-inflammatory and antioxidant properties (Iino et al., 2019). Studies on postmenopausal women indeed suggested that the treatment with phytoestrogens may help prevent many adverse postmenopausal conditions, including osteoporosis and vasomotor symptoms (Arcoraci et al., 2017; Barnard et al., 2023; Lambert, Thorup, et al., 2017). Recent clinical studies have also demonstrated the effectiveness of bioactive isoflavones from red clover in treating osteoporosis with minimal side effects (Lambert, Thybo, et al., 2017). The use of phytoestrogens in the treatment of osteoporosis is well-documented, as they reduce the activity of tumor necrosis factors (RANKL and TNF-α), which limit bone reabsorption, and enhance growth factors (RUNX2 and TGF-β), thereby promoting bone formation. However, and importantly, there is not yet a consensus on the quantity, origin, and type of phytoestrogens to employ for therapy (Patisaul & Jefferson, 2010). For instance, isoflavones have bone benefits regardless of the source (e.g., extracted from red clover or soybeans or consumed in soy foods). In contrast, synthetic ipriflavone did not show a positive effect on bone density. (Alexandersen et al., 2001), a discrepancy that may be explained by differences in the absorption and bioavailability of compounds, which should be assessed on a case-by-case basis (Tanida et al., 2016). Indeed, some intervention studies administering isoflavonoids did not have a positive effect on BMD (Tai et al., 2012). Interestingly, phytoestrogens and probiotics can work synergistically, enhancing their beneficial effects against menopausal comorbidities such as osteoporosis and vasomotor symptoms (Lambert, Thorup, et al., 2017; Lambert, Thybo, et al., 2017).

It has been suggested that probiotics can also restore the impaired activation of phytoestrogens in conditions of gut dysbiosis, as certain strains like *Lactobacillus* and *Bifidobacterium* possess gmGUS enzymes that facilitate phytoestrogen metabolism (Lambert, Thorup, et al., 2017; Lambert, Thybo, et al., 2017).

ii) Flavonoids

Royal jelly is another well-studied component of a diet rich in flavonoids (such as chrysin and pinocembrin) with estrogenic activity. Produced by honeybees (*Apis mellifera*), royal jelly has been shown to have multiple therapeutic applications, including immune modulation, anti-fatigue, anti-tumor, anti-hypertensive, and anti-osteoporosis effects (Bălan et al., 2020). A clinical study (n = 90 women) demonstrated that the application of a 15% royal jelly vaginal cream improved GMS in postmenopausal women (Seyyedi et al., 2016). Despite its promising benefits, the impact of royal jelly on the microbiota in postmenopausal women remains underexplored. ~~So far, only one study on a db/db mouse model (characterized by overeating, obesity, and hyperglycemia) has shown that royal jelly influences the microbiota by increasing~~ To date, only one study on a db/db mouse model (characterized by overeating, obesity, and hyperglycemia) has demonstrated that royal jelly influences the microbiota by increasing the abundance of SCFA-producing bacteria (Kobayashi et al., 2023).

iii) Anthocyanins

Anthocyanins, a class of polyphenols abundantly found in prunes, grapes, blueberries, and black and green tea, are associated with a reduction of CVD risk in both women and men (Jennings et al., 2012; McCullough et al., 2012). Additionally, they are frequently utilized as an alternative therapy to alleviate menopausal symptoms. The gut microbiota transforms them into bioactive, low-molecular-weight phenolic metabolites that modify the host's metabolism (Fang, 2014). For instance, the conversion of anthocyanins is also related to the enzyme β-glucosidase found in some species of *Lactobacillus*, *Bifidobacterium*, *Blautia*, and *Erysipelatoclostridium*. The consumption of anthocyanin-rich fruits in clinical trials conducted on adults has been shown to increase *A. muciniphila*, *Lactobacillus spp.*, *Roseburia*, and *Ruminococcus* and attenuate host gut inflammation and obesity (Fidélix et al., 2020; H. Kim et al., 2020). Pilot intervention studies, particularly those based on polyphenol-rich foods (dose > 90 mg/d), appear to benefit postmenopausal women by decreasing hypertension, inflammation, and osteoporosis; however, these benefits are only experienced by specific individuals (Johnson et al., 2015; Simpson et al., 2024; Welch et al., 2012). Notably, postmenopausal women with initially low BMD may benefit from the *Oscillospiraceae* and *Lachnospiraceae* families, which can metabolize these compounds (Simpson et al., 2024).

Probiotics

In addition to diet interventions, probiotics have emerged as a promising treatment for ameliorating PMO symptoms. Different probiotic *Lactobacillus* strains have been shown to heighten bone health by increasing calcium absorption by the gut and modulating bone cell activity (**Figure 5**). Probiotic treatments in PMO murine models boosted higher bone calcium deposition and suppressed osteoclast activity related to bone loss (Britton et al., 2014). However, some controlled clinical trials in postmenopausal women have shown no significant effect of probiotics on bone loss (Gregori et al., 2024; Harahap et al., 2024). Yet, significant positive effects on bone metabolism were observed when dysbiosis was present, a condition that is frequently common in obesity and with advanced age (Jansson et al., 2019; P. Li et al., 2022; Nilsson et al., 2018). Other studies showed that administering *Lactobacillus* probiotics increased the relative abundance of core SCFAs-producers in the gut, which augments butyrate levels in intestinal mucosa and serum (Berni Canani et al., 2016; Brahe et al., 2015; Honda et al., 2024; P. Li et al., 2022). A proposed mechanism suggests that *Lactobacillus* and butyrate synergically enhance bone formation by increasing regulatory T cells in the bone marrow, promoting osteoblast proliferation (Zaiss et al., 2019). Meanwhile, Li *et al*. demonstrated in a murine model that *L. rhamnosus* may prevent bone loss by improving intestinal permeability and reducing microbiota-induced inflammation related to sex hormone deficiency (J.-Y. Li et al., 2016).

Other types of intervention have been proposed to alleviate menopause syndrome. In a pilot trial, the use of *Lactobacillus*-containing hygiene products was associated with an improved vaginal ecosystem, indicating better urogenital health (Yoshikata, Yamaguchi, Mase, Tatsuyuki, et al., 2022). Intervention studies on healthy young women who were given *Saccharomyces cerevisiae* yeast showed increased *Lactobacillus*, especially *L. iners*, and decreased *Prevotella* (Oerlemans et al., 2022). Probiotics, particularly strains such as *L. reuteri*, *L. rhamnosus*, and *L. acidophilus*, may help maintain an acidic vaginal pH and alleviate symptoms of VVA and recurrent UTIs (Lim et al., 2020; Ribeiro et al., 2019; Vicariotto et al., 2024; Yoshikata, Yamaguchi, Mase, Tatsuyuki, et al., 2022). However, the success of these actions depends on an individual’s microbiome composition. It may require long-term use to show noticeable effects, especially in older adults and women with less adaptable microbiomes. Moreover, in addition to the vaginal microbiome, many lifestyle factors play significant roles in managing postmenopausal symptoms, and probiotics alone may be insufficient to guarantee a healthy aging trajectory without a holistic approach to health.

Overall, this evidence highlights the importance of designing personalized nutrition strategies to alleviate postmenopausal disorders, such as PMO, tailored to an individual’s current diet and microbiome profile, and encourages further investigation into dietary interventions that promote healthy aging in women.

Gut-Vagina axis

To our knowledge, no current studies have simultaneously investigated the gut and vaginal microbiomes in the same women; however, the hypothesis of a functional gut–vaginal axis remains a compelling one. Intestinal and vaginal microbial communities may interact through immune, metabolic, and endocrine pathways. For example, as discussed in previous sections and illustrated in **Figure 4_A**, the estrobolome activity of the gut microbiota can influence systemic levels of sex hormones and metabolites, which in turn may affect the vaginal microenvironment, epithelial integrity, and susceptibility to colonization by specific bacterial taxa. Furthermore, gut dysbiosis and the resulting local inflammation could propagate inflammatory signaling to nearby organs, including the vagina.

Dietary factors may further modulate these interactions. Diets rich in fiber, polyphenols, and fermented foods are associated with enhanced intestinal colonization by beneficial *Lactobacillus* and *Bifidobacterium* species, which could potentially support vaginal eubiosis (Djusse et al., 2025). Conversely, pro-inflammatory dietary patterns may exacerbate dysbiosis and inflammatory responses, potentially contributing to pelvic inflammatory conditions or endometriosis-associated inflammation (Herup-Wheeler et al., 2024). Although causal evidence remains limited, nutritional modulation of the gut microbiota as an adjunct to conventional therapies targeting vaginal health is promising and warrants further investigation with randomized clinical trials.

It remains unclear whether direct microbial transfer occurs between the gut and vagina; however, the close anatomical proximity of the rectum and vagina may facilitate transfer of microbes. Several *Lactobacillus* species are present in the gut. Yet, they differ from those typically found in the vagina—species that are prevalent on one site are often rare on the other. This highlights the need for large-scale metagenomic studies with sufficient statistical power and taxonomic resolution to provide a conclusive assessment of potential strain sharing. Indeed, several studies have reported the exchange of *Lactobacillus* species between the rectum and vagina (El Aila et al., 2011). However, a deeper understanding of gut–vagina microbial connections, rather than rectum–vagina microbial connections, is urgently needed.

In line with this hypothesis, several studies have explored whether modulating the gut microbiome through oral probiotic or prebiotic supplementation can indirectly influence the vaginal microbiota. Preliminary findings suggest that certain orally administered *Lactobacillus* strains may modulate the vaginal ecosystem, improving microbial balance and resilience to infections (Di Pierro et al., 2025; Perez et al., n.d.). However, direct microbial transfer has not been demonstrated, and the mechanistic underpinnings of these effects remain poorly understood.

Further studies employing multi-omics approaches to investigate the gut and vaginal microbiomes, along with immune and endocrine parameters in the same women, are urgently needed to elucidate potential routes of microbial communication, the role of mucosal immune signaling, and the extent to which systemic hormonal changes mediate these effects. A promising initiative in this direction is the Women4Health cohort, a women-oriented multi-omics-based study (Busonero et al., 2024).

Future perspectives for women's aging research

Our review emphasized the biological changes that occur during women's aging from a microbiome perspective and highlighted potential intervention routes toward achieving healthy aging. We focused on women, as the scientific community recognizes the urgent need to improve our understanding of women's specific mechanisms to provide adequate care to both sexes. Historically, biomedical research has been focused on males, often excluding women due to concerns over hormonal fluctuations. This attitude has caused gaps in understanding female health, but also resulted in many medical therapies in use today being based on male-oriented studies, potentially offering less effective care for women and higher side effects.

We underlined reported changes in both vaginal and gut microbiomes during aging and their impact on women's health, as well as the emerging interaction between them. Since the scientific community has been exploring the role of the human microbiome on a broader scale only in the past decade and mainly focused on the gut microbiome, we expect that understanding of specific bacterial taxa and function, and how they affect health and illness, will consolidate and broaden to include other phenotypes or ecosystems unique to females, like the vaginal microbiome. Large-scale studies must consist of and properly analyze postmenopausal women to improve the understanding of aging-related processes from the microbiome perspective. Ideally, a cohort study approach, which involves collecting samples over extended periods and multi-omics data, should be considered to gather vast amounts of data and allow for an understanding of the full complexity of changes.

Finally, we discussed the yet understudied role of diet in modulating women's health and its potential as a preventive measure to counter the effects of aging in women; moreover, recognizing women's unique challenges as they age is crucial for developing targeted interventions.

Contributions

All authors contributed to the manuscript draft and its revision.

Declaration of interest

The authors declare that they have no conflicts of interest.

Declaration of the use of AI and AI-assisted technologies

We declare that the use of AI (ChatGPT and QuillBot) was limited to correcting the text and making the review more readable. The authors are fully responsible for scientific insights, literature reviews, data analysis and interpretation, and scientific conclusions.

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Figures Legend

The graphical abstract illustrates the different aspects of aging in women discussed in the review.

Figure 1. The stages of reproductive aging in women and their possible associated symptoms.

A) Visual representation of key events and duration of the MT as established by the World Health Organization Scientific Group on Research in the Menopause (*Research on the Menopause : Report of a WHO Scientific Group [meeting Held in Geneva from 8 to 12 December 1980]*, n.d.) (Figure modified by Harlow *et al.*(Harlow, Gass, Hall, Lobo, Maki, Rebar, Sherman, Sluss, de Villiers, et al., 2012) . Briefly, the Staging of Reproductive Aging Workshop (STRAW) classification divides a woman's life into seven segments, with segments -2, -1, and 0, including the early MT, the late MT, and the final menstrual period (FMP). Early MT characteristics could include irregular cycles, variable hormone levels, and potential vasomotor symptoms (VMS). Late MT, lasting 1–3 years, might show amenorrhea (absence of the monthly menses), high levels of follicle-stimulating hormone (FSH), varied levels of estradiol (E2), and possibly VMS. Menopause is diagnosed 12 months following the last menstrual cycle. One-year post-menopause, as well as MT phases, constitute perimenopause. Early post-menopause presents high/variable FSH and low E2; late post-menopause is characterized by high FSH, low E2, and genitourinary symptoms (GSM). **B**) Health conditions that may occur during peri- and post-menopause status. Symptoms of postmenopausal syndrome are marked with a *.

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Figure 2. Characteristics of human vaginal Community State Types (CSTs).

CST types, sub-types, and major microbial properties of the dominant bacterial species according to the classification proposed by the VALENCIA study (France et al., 2020). Four CSTs (I, II, III, and IV) and the corresponding six sub-types are *Lactobacillus*-dominated, while CST IV, dominated by a mixed anaerobic community, has seven sub-CSTs. The A and B subtypes of CST I and CST III differentiate subtypes where the corresponding bacterial species is either fully dominant or highly prevalent, respectively. The symbols "O" in green, yellow, and red represent beneficial, neutral, and potentially harmful bacteria. Created in BioRender. Ferrando, M.L. (2025) https://BioRender.com/e99t679

Figure 3. Proposed vaginal microbiome changes during MT stages.

Premenopausal women have high estrogen levels that support a thick, glycogen-rich epithelium, with a microbiome community most often dominated by *Lactobacillus* (e.g., *L. crispatus*). During perimenopause and the fluctuation in estrogen production, *Lactobacillus*-dominated communities become dynamic *(e.g.,* moving toward a higher abundance of *L. iners*), shifting to a mixed anaerobe community after menopause. Postmenopausal estrogen deficiency leads to lower glycogen, higher vaginal pH, and an increase in parabasal cells, potentially causing signs of moderate VVA. *Lactobacillus* levels drop significantly (Brotman et al., 2014; Byrne et al., 2024; Shen et al., 2016), while pathobiont bacteria like *Prevotella*, *Streptococcus*, *Gardnerella*, and *Atopobium* increase. Of note, *Lactobacillus spp.* Remains present – albeit with low abundance - in 30-50% of the vaginal microbiota (Brotman et al., 2014; Byrne et al., 2024; Hummelen et al., 2011; Shardell et al., 2021; Waetjen et al., 2023) (**Supplementary Table 1A**), maintaining their protective role against severe vaginal inflammation and infections. Created in BioRender. Ferrando, M.L. (2025) https://BioRender.com/xwabx3o

Figure 4. Proposed changes in gut microbial taxa associated with aging in postmenopausal women

A) Gut-vaginal microbiome axis. In healthy women of reproductive age, the ovaries primarily synthesize active estrogens. These estrogens are converted into inactive forms in the liver and excreted into bile. Bacteria producing β-glucuronidases (estrobolome) can deconjugate and reactivate estrogens in the gut. While most reactivated estrogens are excreted, others re-enter systemic circulation through enterohepatic recirculation, potentially targeting tissues like the vagina. Estrogen stimulates vaginal cells to produce glycogen, supporting a healthy *Lactobacillus*-dominated vaginal microbiome. **B**) Aging during menopause is linked to decreased gut bacterial diversity, with women's microbiota more similar to males. Key taxa shift includes reductions in SCFA-producers, estrobolome microbes, and beneficial bacteria (e.g., *A. muciniphila*, *Bifidobacterium*, *Lactobacillus*) critical for intestinal integrity (upregulation of TJ via TLR2 receptor), immune support, and bacterial metabolite production. Additionally, an altered Firmicutes-Bacteroidetes ratio, driven by increased Bacteroides, and a rise in pathobionts associated with CVD risk and gut dysbiosis have been observed. **C**) Functional mechanisms of gut taxa involved in the onset of age-related diseases in postmenopausal women. Graphic summary of the key metabolites or effectors produced by the three taxa and the effect each of these microbiome-derived entities has in either negatively or positively regulating various aging-linked diseases and disorders. The reduction in *Bifidobacterium* and *Lactobacillus* lowers acetate production and supports SCFA-producers.

SCFAs interact with G-protein-coupled receptors (GPCRs), influencing a wide range of cell types, including columnar epithelial cells, innate immune cells, and endothelial cells, thereby modulating inflammation, insulin resistance, and blood pressure. A decline in estrobolome diversity (gmGUS-producers) can affect estrogen recycling and contribute to postmenopausal disorders. Reduced folate and cholesterol-α-glucosyltransferase (CGT) producers lead to homocysteine and cholesterol accumulation, raising CVD risk. Enrichment of proteobacteria is linked to elevated blood pressure via inflammatory mediators (IL-8 and TNF-α). Additionally, increased pathobionts (e.g., *Enterobacteriaceae* and *Clostridium*) disrupt gut homeostasis, promoting inflammation (through pro-inflammatory interleukins) and dysbiosis, which contribute to postmenopausal disorders. Created in BioRender. Ferrando, M.L. (2025) https://BioRender.com/z68w440

SCFAs: Short Chain Fatty acids; gmGUS: gut microbes Glucuronidases; IL-8 and IL-18: pro-inflammatory Interleukins; IFN: Interferon; TJ: cellular Tight Junctions; TLR: Toll-Like Receptors; GPR41/43: G-Protein-Coupled receptors (GPCRs); FFARs: Free Fatty Acid Receptors, and ORs: Olfactory Receptors, families of GPCRs expressed in blood vessels and in vascular smooth muscle cells in a variety of organs; LPS: Lipopolysaccharide; pDC and mDC: premature and mature Dendritic Cells, respectively.

PMO: postmenopausal osteoporosis; VSM: vasomotor symptoms; GSM: genitourinary symptoms of menopause; CVD: cardiovascular diseases.

Figure 5: Potential role of diet on microbiome health during menopause.

A graphic representation shows how diet intervention strategies could promote microbiome changes that prevent the aging-associated decline in postmenopausal women.

Probiotics are different strains of *Lactobacillus*, such as *L. reuteri*, *L. rhamnosus*, *L. plantarum*, and *L. acidophilus.*

Polyphenols include flavonoids such as phytoestrogens (e.g., isoflavonoids) with estrogenic properties, including daidzein (found in soybeans, mung beans, green beans, Red Clover, Thai herb Pueraria Mirifica, and Japanese kudzu) and genistein (found in soybeans, lupin, fava beans, kudzu, and Red Clover), as well as anthocyanins (found in black currant, pomegranate, prunes, berries, grapes, acai, and eggplants) with antioxidant and anti-inflammatory activities. Additionally, polyphenols encompass non-flavonoid compounds, such as phenolic acids (found in berries, black and green tea, coffee, whole grains, tomatoes, and apples), as well as other polyphenols like resveratrol (found in grapes, berries, and red wine), which are known for their potent anti-inflammatory and antioxidant properties.

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TMAO, trimethylamine; IL-1β, Interleukin-1 beta pro-inflammatory cytokine; PMO: postmenopausal osteoporosis; VSM: vasomotor symptoms; GSM: genitourinary symptoms of menopause; CMD: cardiometabolic diseases.

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Title:

Aging in Women – the Microbiome Perspective

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Summary:

~~Menopause~~ R~~r~~epresents a hallmark of women's aging, menopause is frequently portrayed as a medical issue. It also ~~comprises~~ encompasses social and biological aspects often neglected and not well-understood, leaving women with insufficient support and attention. With the decline in estrogen levels, starting years before menopause is fully established, women experience various physical symptoms, and the risk of many age-related diseases increases sharply soon after these hormonal changes occur. Notably, these hormonal shifts also ~~induce~~ significantly impact ~~changes in~~ the vaginal and gut microbiomes, contributing to dysbiosis and influencing the onset and progression of several diseases. Here, we examined the complex and dynamic relationship ~~between~~ among aging, menopause, and microbiome changes with a particular focus on the vaginal and gut ecosystems. Emerging research highlights ~~emphasizes~~ diet as a potential modulator for maintaining microbiome health ~~throughout~~ during menopause. A deeper understanding of microbiome changes across life stages suggests the potential ~~of~~ for microbiome-targeted strategies to support well-aging in women.

Panel

Estrogens: Estradiol, also known ~~also~~ as 17β-estradiol or estrogen (**E2**), is the most common and ~~important~~ essential form of circulating estrogens. In premenopausal women, **E2** reaches ~~the~~ its highest ~~amount~~ levels before ovulation (110–410 pg/mL), and ~~approx~~approximately~~.~~ 19–150 pg/mL during the follicular and luteal phases, whereas in postmenopausal women~~ is <35~~, it is < 35 pg/mL. Two other less abundant forms include estrone (**E1**) and estriol (**E3**); the latter is derived from E1 through the 16α-hydroxylation process. Their roles become more critical during pregnancy when the placenta produces them in large quantities.

Microbiota: the microbial biomass community residing in the host's organs, including bacteria, archaea, fungi, and viruses.

Microbiome: the whole collection of microbiota genomes and their products.

Microbial diversity: the variety and abundance of taxa in the microbiota.

16S rRNA amplicon sequencing: the targeted sequencing of 16S ribosomal RNA (16S rRNA).

Shotgun sequencing: the untargeted sequencing of all microbial genomes (metagenomes).

Dysbiosis: alteration in the microbiome's composition and/or function that can lead to ~~losing~~ loss of its supportive ~~function~~ role in host health.

Pathobionts: normally harmless microorganisms that can become pathogenic under certain conditions, often associated with dysbiosis or immune dysfunction. In the vaginal microbiota, pathobionts include *Prevotella*, *Streptococcus*, *Gardnerella*, and *Atopobium.* In the intestinal microbiota, they include *Bilophila*, *Desulfovibrio*, *Eggerthella*, all *Enterobacteriaceae*, *Campylobacter*, *Fusobacterium*, *Streptococcus*, *Bacteroides fragilis*, *Actinomyces*, *Corynebacterium*, *Staphylococcus*, *Parvimonas*, *Porphyromonas*, *Ruminococcus torques*, and *Clostridium* species such as *C. difficile*.

Short-chain fatty acids (**SCFAs**): fatty acids with 1 to 6 carbon atoms produced through the saccharolytic fermentation of undigested dietary fibers. The main SCFAs—acetic acid (acetate), propionic acid (propionate), and butyric acid (butyrate)—are typically present in a ratio of 60:20:20. *Clostridiales* members are key SCFAs-producers with the families *Ruminococcaceae* (e.g., *Faecalibacterium*, *Ruminococcus*, *Oscillibacter*), *Lachnospiraceae* (i.e., *Roseburia*, *Anaerostipes*, *Blautia*, *Coprococcus*, *Butyrivibrium*, *Dorea*), and *Eubacteriaceae* being particularly efficient butyrate-producers. Other bacteria, such as *Bifidobacterium*, can produce non-butyrate metabolites (i.e., acetate, succinate~~,~~ and lactate) ~~used by butyrogenic producer~~that are utilized by butyrogenic-producing bacteria.

Trimethylamine N-oxide (**TMAO**): a class of amine oxide produced by gut microbes through the metabolism of dietary nutrients, primarily choline, carnitine, and betaine, and its levels increase with the consumption of animal protein.

Estrobolome: a collection of bacterial genes involved in the reactivation of sex hormones (e.g., estrogens), encoding glycoside hydrolase 2 (GH2) family enzymes, which includes gut microbial β-glucuronidases (gmGUS), β-galactosidases, and β-mannosidases. GUS genes are ~~present in~~encoded by Bacteroidetes (52%, e.g., *Bacteroides*, *Prevotella, Alistipes, etc.*), Firmicutes (43%, e.g., *Faecalibacterium, Lactobacillus, Streptococcus, Eubacterium, Ruminococcus, Roseburia, Romboutsia, some Clostridium, and Coprococcus*), Actinobacteria (1.6% e.g., *Bifidobacterium*), Verrucomicrobia (1.5% e.g., *Akkermansia*), and Proteobacteria (1.3%, e.g., *Escherichia*).

Abbreviations used for ~~Aged-related~~age-related diseases in women:
Genitourinary Syndrome of menopause (GSM)
Vulvovaginal atrophy (VVA)
Vasomotor symptoms (VMS)
~~Genitourinary syndrome of menopause (GSM)~~
Urinary tract infections (UTI)
Postmenopausal osteoporosis (PMO)
Cardiovascular diseases (CVD)
Metabolic diseases (Met)
Type 2 diabetes mellitus (T2DM)

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Introduction

Aging is a natural process that ~~causes~~ leads to profound ~~significant~~ physiological changes in ~~the physiology of~~ the human body. Over the past century, global life expectancy has ~~greatly increased, and this trend is forecast~~increased significantly, and this trend is projected to continue. Accordingly, the elderly population (over 65) is expected to rise from 761 million in 2021 to 1.6 billion in 2050 (United Nations Department of Economic and Social Affairs, 2023), posing a worldwide challenge to governments and economies. Women generally live longer than men, partly due to the protective effects of female sex hormones, though the precise reasons for this longevity ~~gap~~ advantage remain unclear (Hägg & Jylhävä, 2021). Sex hormone physiological fluctuations and large modifications~~,~~, especially those occurring throughout menopause - a major hallmark of aging in women -~~–~~ significantly impact women's health~~, especially those occurring throughout menopause, a major hallmark of aging in women~~. These hormonal alterations are linked to diverse age-related diseases, such as cardiovascular diseases, metabolic disorders, and osteoporosis (Davis et al., 2015; Harlow, Gass, Hall, Lobo, Maki, Rebar, Sherman, Sluss, de Villiers, et al., 2012; Muka et al., 2016). In contrast, men undergo a progressive fall in testosterone levels, affecting muscle strength and cognitive functions. However, these changes are generally less abrupt than those observed in women. Aging is also associated with changes in the **microbiome,** the microbial communities ~~inhabiting different organs in the body, as well as their products,~~ that are essential for metabolic, immune, and neurological functions. While changes in the microbiome during aging are partially attributable to increased use of medications in late adulthood, it is also known that significant alterations in the composition and diversity of the microbiome (**dysbiosis**) contribute to the onset and progression of age-related conditions (Ghosh et al., 2022). Remarkably, in women, the **estrogen** decline during menopause further influences the gut microbiota, as well as the vaginal microbiome (Muhleisen & Herbst-Kralovetz, 2016; Peters, Santoro, et al., 2022). This is likely to lead to differential mechanisms of the microbiota-host health relationship in men compared to women, which are largely unknown.

This review examines the dynamic relationship between vaginal-gut microbiomes and age-related conditions in women and explores potential avenues to promote women's well-aging through dietary microbial modulation. Understanding these ~~gender~~sex-specific mechanisms, including those related to the microbiome, is crucial to fostering personalized healthy aging.

Search strategy and selection criteria

A search of the scientific literature was conducted by using PubMed and Scopus and looking for the following search terms ((vaginal microbiome OR vaginal microbiota) AND (ageing OR aging OR menopause OR postmenopausal women)) (results **269**); ((gut AND (microbiome OR microbiota) AND women AND (ageing OR menopause OR postmenopausal women)) (results **418**); for the dietary intervention on postmenopausal women a different combination of terms was used: diet OR (*Lactobacillus* OR probiotic) OR (phytoestrogens OR soybean OR isoflavone OR flavonoid) AND (microbiome OR microbiota) AND (menopause OR postmenopausal OR older) AND women (results~~–~~ **649**); (diet OR *lactobacillus* OR flavonoid) AND (menopause OR postmenopausal OR postmenopausal) AND women AND trials AND (hypertension OR osteoporosis OR vasomotor) (results~~–~~ **484**), food elements were added to the search including red clover, prunes, blueberry, and royal jelly. More specific search combinations for sex hormone changes in menopause were then used, including the terms mentioned above and additional items; for example, (estrogen) OR (sex hormones) AND (menopause OR perimenopausal OR postmenopausal women) AND (microbiota) was used to find articles related to considerations for sex ~~hormones~~ hormone variation in aging. We examined the studies and included those relevant to this review's topic ~~according to~~, as outlined in the following decision flow chart. **Inclusion criteria:** studies with original data, studies conducted in (premenopausal) OR perimenopausal AND postmenopausal women in healthy status; observational studies including prospective, retrospective, longitudinal, cross-sectional study designs, clinical trials, population cohort studies; a measure of microbiota composition by high-throughput sequencing (16S rRNA or shotgun sequencing). **Exclusion criteria**: studies conducted in women only of reproductive age (18-44 years); commentaries, book chapters, letters, editorials, conference proceedings, case reports, conference, abstracts, or non-peer-reviewed articles, publication in a different language than English; studies conducted in gynecologic cancer patients, with infection, chronic

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diseases; studies conducted in a cohort lower than **30** postmenopausal women. Molecular methods excluded i) quantitative polymerase chain reaction (qPCR), ii) microbiota measured by non-molecular methods (such as culture and microscopy), no relevant outcomes reported, or when there is a lack of quantifiable outcomes (e.g., not defined abundance of microbiota).

Pertinent original articles and reviews (only used for the introduction paragraph) were peer-reviewed and indexed in PubMed and Scopus. We limited the publication dates from 2010 to 2024 and conducted literature searches between May 2024 and July 2024. **Supplementary Table 1** provides a comprehensive list of publications screened, methods used, and a summary of reported results.

The menopausal transition period ~~presents potential risks for women's health~~.

Menopause ~~is an important~~ represents a key milestone ~~of~~ in a woma~~e~~n's aging, marking the transition to a new phase of life. ~~but~~ – Despite its profound impact on health, it remains ~~still~~ an often-overlooked aspect of ~~health~~women's ~~wellbeing~~well-being. Biologically, ~~It~~ it is defined by the permanent cessation of ovarian follicular activity due to the depletion of ovarian follicular reserve. As the number of functional follicles declines, ~~a drop in circulating~~the production of gonadal steroids decreases, ultimately leading ~~sex hormones, the permanent~~ to the end of monthly ~~menstruations~~menstruation~~,~~ and ~~the end~~ of reproductive ~~age~~ capacity (Harlow, Gass, Hall, Lobo, Maki, Rebar, Sherman, Sluss, de Villiers, et al., 2012). ~~"~~During this phase, the number of granulosa cells within the ovarian follicles declines (*ovarian aging*), leading to reduced production of estradiol (and inhibin B) and consequently lower circulating estrogen levels (Harlow, Gass, Hall, Lobo, Maki, Rebar, Sherman, Sluss, de Villiers, et al., 2012). Without the inhibitory effect of estrogen on gonadotropins, the pituitary gland increases the production of follicle-stimulating hormone (FSH ≥ 25 mIU/mL), resulting in the cessation of the menstrual cycle (**Figure 1_A**).~~-~~

Menopause is not a single event but rather a gradual biological process, often referred to as the ~~The~~ menopausal transition (MT). MT occurs in three stages: perimenopause, menopause, and post-menopause. ~~Each stage is characterized by variable duration and symptoms~~ A~~a~~ccording to the Staging of Reproductive Aging Workshop (STRAW) ~~staging~~ (**Figure 1_A**), each stage is characterized by variable duration and symptoms (Harlow, Gass, Hall, Lobo, Maki, Rebar, Sherman, Sluss, de Villiers, et al., 2012)~~(Harlow, Gass, Hall, Lobo, Maki, Rebar, Sherman, Sluss, & de Villiers, 2012)~~. Perimenopause encompasses one year preceding the final menstrual period (FMP) and the subsequent year following menopause. During this period, estrogen and progesterone levels fluctuate (**Figure 1_A**), and women may experience some symptoms of the 'postmenopausal syndrome', although ovulatory menstruation can still occur~~–~~ (Harlow, Gass, Hall, Lobo, Maki, Rebar, Sherman, Sluss, de Villiers, et al., 2012). Vasomotor symptoms (VMS), such as hot flashes, followed by abundant menstruation (dysmenorrhea), are often among the earliest signs of perimenopause (Thurston & Joffe, 2011). Menopause is recognized when menstruation stops for ~~twelve consecutive months, typically between~~ 12 consecutive months, typically between the ages of 45 and 55. ~~During this phase, the ovary reduces the granulosa cells (ovarian aging), which are the principal producers of estradiol (or estrogen) (and inhibin B, resulting in a fall in estrogen levels (<35 pg/mL)~~[9]~~. Without the inhibitory effect of estrogen on gonadotropins, the pituitary gland increases the production of follicle-stimulating hormone (FSH ≥25 mIU/mL), resulting in the cessation of the menstrual cycle (**Figure 1A**).~~ Post-menopause~~,~~

~~referring to the years following menopause~~, is characterized by a further decrease in sex hormones, with the adipose tissue becoming the principal producer of estrogens (Harlow, Gass, Hall, Lobo, Maki, Rebar, Sherman, Sluss, de Villiers, et al., 2012). During post-menopause, a woman's body goes through further changes that might influence many organs, leading to a range of symptoms known as postmenopausal syndrome (**Figure 1_B**). ~~Declining estrogen levels primarily impact~~[11]~~. Therefore,~~ A~~a~~pproximately 45–77% of women suffer from the genitourinary syndrome of menopause (GSM) (Cox et al., 2023), which includes urinary, genital, and sexual symptoms. Reduced glycogen in vaginal cells raises vaginal pH and promotes bacterial growth, increasing infection risk (Song et al., 2022). While VMS usually improves with time, GSM might deteriorate without adequate therapy. Sex hormonal variations can also impact the nervous system, creating neurological disorders such as brain fog, anxiety, and sleep disturbances (Freeman et al., 2006). Moreover, ~~The~~ the drop ~~of~~ in estrogen can accelerate bone density loss and increase the risk of postmenopausal osteoporosis (PMO).

In addition to postmenopausal syndrome, the hypoestrogenic state following~~lowing~~ menopause can also exacerbate chronic diseases impacting vital systems and accelerate the progression of risk of cardiovascular

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and metabolic diseases (CMD).~~–~~ A~~a~~ging in women is, ~~in fact~~in fact, associated with a significant increase in blood pressure, body mass index (BMI), obesity, and body fat distribution, as well as an increase in insulin resistance (Ryczkowska et al., 2022). ~~Noteworthy~~Notably, epidemiological studies show that women tend to develop heart diseases later in life compared to men, mainly after menopause (Muka et al., 2016; Paduszyńska et al., 2020). This delay is attributed to the cardioprotective effects of estrogen before menopause, which include enhanced angiogenesis, improved vasodilation, and reduced oxidative stress and fibrosis (Ryczkowska et al., 2022).

Similarly, the decline of estrogen can influence insulin resistance and glucose metabolism, increasing the risk of ~~metabolism~~ metabolic disorders (e.g., type 2 diabetes mellitus, T2DM) (Davis et al., 2015).

Furthermore, emerging evidence suggests that substantial alterations can occur in intestinal and vaginal microbiomes, altering bacterial populations and ~~boosting the abundance of pathobionts, ensuing~~increasing the abundance of pathobionts, which can lead to dysbiosis linked to inflammatory diseases such as GSM and PMO.

The Vaginal Microbiota

Vaginal microbiota in premenopausal women

Longitudinal studies have observed that the vaginal microbiome community is overall stable in the~~a~~ short-term period (months), with fluctuations occurring only in a small fraction of the female population (Gajer et al., 2012).~~-~~ Factors ~~like age, ethnicity, sex hormones, and personal habits influence its composition, and some specific event may introduce substantial changes like vaginal infections (Krog et al., 2022; Lebeer et al., 2023), may~~such as age, ethnicity, sex hormones, and personal habits influence its composition, and specific events, like vaginal infections (Krog et al., 2022; Lebeer et al., 2023), can introduce substantial changes. Estrogens are also key players in shaping the microbiome by promoting glycogen accumulation in vaginal cells and providing a carbohydrate source for *Lactobacillus,* the~~ main~~ dominant ~~bacteria~~ bacterial phylum ~~present~~ in women's vaginal microbiome during reproductive age (Lebeer et al., 2023). These bacteria maintain vaginal health by creating an acidic environment (pH 3.5–4.5) that inhibits the growth of pathogens and ~~produce~~ by producing anti-inflammatory compounds that strengthen the vaginal epithelium (Borges et al., 2014). The four main vaginal *Lactobacillus* species are *L. crispatus*, *L. gasseri*, *L. iners*, and *L. jensenii* (**Figure 2**). *L. iners* has been observed in both healthy women ~~as well as in~~and women with vaginal infections, ~~reason~~which is why ~~and~~ the possibility of *L. iners* strains with potential pathogenic roles is under debate (Petrova et al., 2017). Similarly, vaginal anaerobes, such as *Gardnerella*, *Prevotella*, *Atopobium*, and *Streptococcus*, have pathogenic potential and can destabilize the vaginal microenvironment. To represent and distinguish the different vaginal microbiome types, the community state types (CSTs) classification based on taxa abundance (Ravel et al., 2011) was proposed, with four ~~classes~~ types representing microbiota dominated by *Lactobacillus spp.* (CST I: *L. crispatus*, CST II: *L. gasseri*, CST III: *L. iners*, CST V: *L. jensenii*) and one, CST IV, which lacks *Lactobacilli* and has high microbial diversity. CST subtypes have further refined this classification (**Figure 2**) (France et al., 2020). While the use of CSTs to define microbiota composition is common, there is a broader call for more dynamic, higher-resolution approaches to understanding microbial communities and modeling their interconnected nature and mutual coexistence within the vaginal microenvironment (Lebeer et al., 2023).

Vaginal microbiome during and after menopausal transition

Most research on vaginal microbiota focused on women of reproductive age, leaving a critical knowledge gap in our understanding of changes that occur throughout and after MT. However, ~~major~~ significant changes are expected to exist and are likely to correlate with menopause-related health conditions. One of the ~~pioneer studies tracked over two months the vaginal microbiota~~ pioneering studiesy~~ies~~ tracked the vaginal microbiota over a two-month period in postmenopausal women with and without vulvovaginal atrophy (VVA), observing that asymptomatic women had low bacterial diversity, dominated by *Lactobacillus* species~~asymptomatic in postmenopausal women with and without vulvovaginal atrophy (VVA), observing that women without symptoms had low bacterial diversity dominated by *Lactobacilli*~~. In contrast, symptomatic women ~~had~~ exhibited higher diversity with anaerobes ~~like~~ such as *Prevotella timonensis*, *Porphyromonas*, *Peptoniphilus*, and *Bacillus* (Hummelen et al., 2011). Moreover, *Lactobacillus spp.* presence was inversely correlated with dryness. Other studies have also observed that a low percentage of postmenopausal women (10-30%) still maintain *Lactobacillus*-dominated communities, while in the majority of postmenopausal women (approx. 60-

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70%), the vaginal microbiome is more likely to be characterized by anaerobic communities and increased microbial diversity (rather than having one dominant species) and elevated vaginal pH (Mitchell et al., 2017; Yoshikata, Yamaguchi, Mase, Tatsuzuki, et al., 2022). Larger cohort studies have further confirmed that postmenopausal women often exhibit CST IV, with a moderate prevalence of subtypes (CST IV-C0 and CST IV-B), which were also associated with ~~the risk of~~ VVA and GSM biomarkers, though the underlying causal genera were not consistent (Shardell et al., 2021; Shen et al., 2016; Waetjen et al., 2023). Intriguingly, the transition from a vaginal microbiome dominated by *Lactobacillus spp*. (CST I, II, III, V) to CST IV appears to be a gradual process triggered by estrogen decrease beginning in perimenopause, with an abundance of *Lactobacillus spp*. progressively declining from premenopausal to postmenopausal stages (Brotman et al., 2014). Interestingly, Byrne *et al.* found that postmenopausal women with mixed microbiomes lacking *Lactobacillus* exhibited higher levels of proinflammatory cytokines (Byrne et al., 2024), ~~like~~ ~~similar~~than those in premenopausal women with mixed communities dominated by *Prevotella* (Anahtar et al., 2015). Another robust evidence of *Lactobacillus*'s crucial role in maintaining vaginal health, ~~also~~ ~~including~~ both in pre- and~~in~~ post-menopausal women, comes from the Isala cohort~~study~~, a comprehensive study of the vaginal microbiome ~~of~~ in over 3,000 women of ~~different~~ various ages ~~living~~ residing in Belgium (Lebeer et al., 2023). In this study, key bacterial modules were ~~linked~~ correlated with measured host factors using network-based statistical approaches~~ and linked with measured host factors~~. High estrogen levels and a healthy vaginal microbiome were associated with the *Lactobacillus* module~~, while menopausal~~. In contrast, menopausal status was ~~linked to the *Prevotella* module, confirming at~~correlated to the *Prevotella* module, confirming on a large scale the shift toward a reduction in *Lactobacillus spp*. after menopause.

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Based on ~~literature results of these studies, we propose a model of expected vaginal microbiota changes during MT stages and the underlying biological mechanisms that we~~the literature results of these studies, we propose a model of expected vaginal microbiota changes during MT stages and the underlying biological mechanisms, as depicted in **Figure 3**. It would, therefore, be ideal to design prevention and treatment strategies for restoring *Lactobacillus* levels to a minimum beneficial level after menopause.

Considering the impact of sex hormone decline ~~in~~ on the vaginal microbiome and its consequences for women, ~~preventing and controlling~~it is crucial to prevent and control clinical conditions via estrogen intake. Hormone replacement therapy (HRT) restores estrogen levels (30–60 pg/mL) and is available in various forms, including oral tablets, vaginal creams, rings, and transdermal patches (Stuenkel et al., 2015). Earlier research has shown that postmenopausal women using vaginal estradiol or oral menopausal hormone therapy are more likely to exhibit *Lactobacillus*-dominated vaginal microbiota and decreased microbial diversity (Heinemann & Reid, 2005; Mitchell et al., 2021; Shen et al., 2016; Srinivasan et al., 2022). While HRT effectively alleviates symptoms of VVA in postmenopausal women, it is not recommended for women with a history of breast cancer and cardiovascular ~~and~~ or liver diseases (Stuenkel et al., 2015). Additionally, estrogen therapy may cause additional side effects such as vaginal bleeding, breast pain, and perineal pain (Stuenkel et al., 2015). Taken together, HRT is not always a suitable treatment for GSM symptoms, and other solutions should be sought.

The gut microbiome ~~*and its connection to aging and women's health*~~

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The rapid expansion of metagenomics ~~studies~~ to large-scale population-based ~~studies~~ and disease ~~cohorts-based~~cohort-based studies has consistently highlighted the association between gut microbiome dysbiosis and many diseases (Gacesa et al., 2022; Imhann et al., 2019). Nevertheless, while a clear distinction can be seen between healthy individuals and those diagnosed with diseases, a "universal" healthy microbiome cannot be defined, ~~since~~ as the microbial composition significantly varies ~~greatly~~ ~~significantly~~ between individuals and populations (Kurilshikov et al., 2021). The gut microbiota is primarily composed of phyla including Bacteroidetes (e.g., *Bacteroides*, *Prevotella, Alistipes*), Firmicutes (e.g., *Clostridium*, *Lactobacillus*, *Bacillus*, *Enterococcus*, *Ruminococcus, Faecalibacterium, Roseburia, Blautia*), Actinobacteria (e.g., *Bifidobacterium*), Proteobacteria (e.g., *Escherichia*, *Shigella, Helicobacter, Sutterella*), Fusobacteria (*Leptotrichia*, *Fusobacterium*), and Verrucomicrobia (e.g., *Akkermansia*)(Arumugam et al., 2011). In adults, over 90% of the gut microbiota ~~comprises~~ consists of Bacteroidetes and Firmicutes, reflecting their foundational roles in gut ecology (Arumugam et al., 2011). Unlike the vaginal ecosystem, where a microbiome dominated by one or a few species is preferred, high diversity in the gut microbiome is essential to guarantee resilience to environmental stressors and has been associated with ~~better~~ improved human health (Arumugam et al., 2011). Not only ~~microbial composition~~ is the microbial composition crucial, but also the metabolic activity of the different microbes is essential in maintaining human health. Gut microbes metabolize both dietary and endogenous compounds, producing secondary metabolites that serve as energy sources for themselves and signaling molecules for human metabolism and immune function. Short-chain fatty acids (**SCFAs**) and trimethylamine-N-oxide (**TMAO**) are diet-derived ~~metabolites~~metabolites that significantly impact~~-~~ ~~important for their impact on~~ host health. SCFAs are produced by specific Firmicutes taxa during the breakdown of dietary fiber and act by binding to G-protein-coupled receptors (GPCRs) located on various immune and organ cells (Mann et al., 2024) **(Figure 4_C)**. They are essential for maintaining intestinal barrier integrity and immunological homeostasis, and play a significant role in regulating blood pressure and controlling insulin resistance (Mann et al., 2024). In contrast, TMAO, a metabolite produced from dietary phosphatidylcholine and L-carnitine (abundant in meat and processed foods), contributes to atherosclerosis by interfering with cholesterol clearance from the liver, increasing plaque formation, and heart disease risk (Z. Wang et al., 2011). The gut microbiota ecosystem is dynamic, evolving throughout the human lifespan~~, shaped by~~ in response to ~~dietary, environmental, and physiological changes~~changes in diet, environment, life style and healthy conditions ~~physiology~~. Transitions of the gut microbiome during life do not occur equally in both ~~genders~~ sexes (Zhang et al., 2021). However, only a few studies have investigated the impact of aging on the gut microbiome, ~~especially regarding~~particularly in relation to women's health and menopause. In women, we expect that the ~~occurring shifts in sex hormones~~ shifts in sex hormones further complicate the impact of aging. ~~While, to our knowledge, no current studies have interrogated simultaneously gut and vaginal microbiomes in population cohorts~~To our knowledge, no current studies have simultaneously interrogated the gut and vaginal microbiomes in population cohorts; however, emerging research supports the existence of a gut-vaginal axis. This complex and reciprocal interaction could be ~~affected by the shifts in sex hormone levels and potentially impacts~~influenced by shifts in sex hormone levels and may have a potential impact on overall health (**Figure 4_A**). Estrogens produced in the ovaries are conjugated in the liver and enter the gut, where bacteria from the **estrobolome** can deconjugate estrogen into an active form (Ervin et al., 2019; Hu et al., 2023; Pollet et al., 2017). Dysbiosis and inflammation ~~could alter gmGUS activity, with a consistent increase or decrease of free active estrogens in the bloodstream affecting~~can alter the gut microbial β-glucuronidases (gmGUS) activity, resulting in a consistent increase or decrease in free active estrogens in the bloodstream, which affects hormonal balance, metabolic processes, and the risk of developing estrogen-related conditions. Similarly, fluctuations in estrogen levels during postmenopause can lead to alterations in gut and vaginal microbial communities, contributing to age-related diseases **(Figure 4_A)**.

~~*Changes in gut microbiome composition and function in postmenopausal women*~~

Gut microbiome diversity in postmenopausal women

Understanding the differences in gut microbiota diversity between premenopausal and postmenopausal women is complex, as ~~;~~ confounding factors—such as age-related diseases—can further influence microbiota composition. In this review, we selected studies involving cohorts ~~comprising~~ of healthy ~~elderlyolder women who, while generally~~ older women~~healthy, may be~~ who may be~~-~~ at ~~an~~ increased risk for aging-related diseases. Several of~~Some~~ the selected large-scale studies focused on differences in microbial composition across ~~gender~~ sex differences and age groups, enabling the identification of gut biodiversity in relation to age and sex (de la Cuesta-Zuluaga et al., 2019; Peters, Lin, et al., 2022; Santos-Marcos et al., 2018; Zhang et al., 2021). Generally, younger women exhibit greater microbial diversity than men, but this difference diminishes with age, as middle-aged women display a gut microbiota more similar to men (de la Cuesta-Zuluaga et al., 2019; Peters, Lin, et al., 2022; Santos-Marcos et al., 2018; Zhang et al., 2021) (**Figure 4_B**). In contrast, differences in microbiota composition between younger and older men appear less pronounced (de la Cuesta-Zuluaga et al., 2019). Lifestyle factors—such as alcohol consumption, smoking, drug use, and diet—also contribute to age- and ~~gender~~sex-dependent declines in microbiome diversity. However, sex hormones, ~~especially~~ and in particular the decline in estrogen, ~~decline,~~ remains a crucial determinant of gut microbiome ~~specificities~~ features in women (de la Cuesta-Zuluaga et al., 2019). Notably, two studies reported that the dissimilarity between women and men during the premenopausal period is not significant when restricting the analysis to obese participants, likely due to lower estrogen levels in obesity, which makes the gut microbiome similar across~~-~~ sexes ~~difference genders~~ (Mayneris-Perxachs et al., 2020; Zhang et al., 2021).

Bacterial taxa associated with age-related diseases in women

~~Studies involving large~~ Large ~~cohorts~~ cohort studies have identified significant changes in bacterial diversity and microbial taxa abundance in patients with cardiometabolic diseases (Fromentin et al., 2022; Talmor-Barkan et al., 2022; Q. Yan et al., 2017).~~,~~ ~~albeit~~ However, only a minority of these have examined men and

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women separately. These ~~studies~~ investigations reported ~~reduced microbiome potential for the biosynthesis of SCFAs and higher levels of pathobionts belonging to Proteobacteria linked to systemic inflammation, high adiposity, obesity, and hypertension (**Figure 4 B-C**~~a reduced microbiome potential for the biosynthesis of SCFAs and higher levels of pathobionts belonging to the phylum Proteobacteria, which are associated with systemic inflammation, high adiposity, obesity, and hypertension (**Figure 4 B-C**).

Similar results were found in a recent study investigating postmenopausal women with CVD risk (Gaber et al., 2024). The depletion of SCFA-producing bacteria ~~was reported to be associated with CVD risk~~ has been reported to be associated with an increased risk of CVD in postmenopausal women. For instance, in different studies, postmenopausal women had a lower abundance of SFCA-producers (e.g., *Ruminococcus, Eubacterium, Roseburia, Clostridium*) (Y. Liu et al., 2022; Mayneris-Perxachs et al., 2020; Santos-Marcos et al., 2018; Zhao et al., 2019) associated with high-density lipoprotein (HDL) cholesterol levels, lower waist circumference, and ~~a~~ an ~~reduced~~ increased risk of metabolic syndrome (Peters, Lin, et al., 2022). Other SCFA-producers, specifically *Parabacteroides* and *Oscillibacter* **(Figure 4 B)**, were found to be depleted in postmenopausal women and associated with lower HDL levels in feces and plasma (Peters, Lin, et al., 2022). Notably, a recent study revealed that *Oscillibacter* encodes cholesterol-α-glucosyltransferase (CgT), which metabolizes cholesterol into secondary metabolites, regulating serum cholesterol levels and thus remarking its importance for cardiovascular health **(Figure 4 C)** (C. Li et al., 2024).

The depletion of SCFA metabolites and SCFA-producers has also been associated with a higher incidence of metabolic diseases, such as T2DM, already in the general population (Sanna et al., 2019). *Akkermansia~~.~~ muciniphila*, which supports butyrate metabolism and regulates insulin resistance **(Figure 4 C)**, was more abundant in premenopausal than postmenopausal women (Sakamuri et al., 2023; Sanna et al., 2019; Wallimann et al., 2021). Intriguingly, this bacterium has been linked to changes in progesterone metabolism (Peters, Lin, et al., 2022).

Additionally, other bacterial metabolic pathways were downregulated in postmenopausal individuals, such as the folate pathway (Mayneris-Perxachs et al., 2020; Santos-Marcos et al., 2018)~~-~~, due to a significant reduction in *Lactobacillus plantarum*, a key folate producer. Folate participates in the methionine cycle, facilitating the conversion of homocysteine to methionine, and low levels can lead to increased homocysteine accumulation in the serum (hyperhomocysteinemia) **(Figure 4 C)**, which is linked to CVD due to its harmful effects on the cardiovascular endothelium (Ganguly & Alam, 2015) ~~**(Figure 4 B-C)**~~.

Sex hormone changes affect gut microbiota composition in post-menopause.

~~Noteworthy~~Notably, the menopause transition and menopausal symptoms are often accompanied by gastrointestinal complaints, likely attributable to sex hormone fluctuations. Clinical studies ($n < 100$) have found that estrogen levels positively correlate with the~~an~~ a~~a~~bundance of beneficial bacteria, such as *Clostridiales*, especially *Ruminococcaceae*, and negatively with Bacteroides, regardless of age or BMI (Fuhrman et al., 2014). A reduction in estrogen, which decreases the estrobolome's capacity, may lower estrogen reabsorption ~~and also~~and lead to gut dysbiosis. Some gmGUS-encoding microbes (mainly species belonging to the *Clostridiales* order) were ~~decreased~~ reduced in obese postmenopausal women (Mayneris-Perxachs et al., 2020; Meng et al., 2021; Peters, Lin, et al., 2022), especially those with CDM risk (Y. Liu et al., 2022). ~~Notably~~Interestingly, Peters *et al*. examined the menopause-microbiome relationship, finding a decrease in gmGUS enzymes (e.g., β-glucuronidase and aryl-sulfatase orthologs) involved in activating sex hormones (Peters, Lin, et al., 2022). In this study, the low abundance of specific bacterial taxa in postmenopausal women strongly correlated with depleted gmGUS enzymes carried by various beneficial bacteria (e.g., *Parabacteroides johnsonii*, *Clostridium lactatifermentans, Escherichia coli,* ~~*Akkermansia*~~ *A. muciniphila*) (Peters, Lin, et al., 2022) (**Figure 4**~~-~~**B-C**). However, some bacterial species encoding gmGUS enzymes were also reported to increase in postmenopausal women. Since the discovery of gmGUS is relatively recent, more research is needed to characterize the properties of gmGUS producers.

Bacterial taxa associated with menopause-related disorders

Several studies reported that gut microbiota might have a role in postmenopausal osteoporosis (PMO), as the depletion of SCFAs-producers can affect bone health by altering calcium absorption and metabolism (Ji et al., 2024; Rettedal et al., 2021; L. Yan et al., 2024). PMO severity~~symptoms~~ ~~have~~ has been negatively correlated with the abundance of various ~~with different species of~~ *Lactobacillus* and *Bifidobacterium* species, ~~indicating~~ suggesting a possible protective role ~~on~~ in bone absorption. These two beneficial species were already observed to decline during perimenopause (Chen et al., 2024). Recent studies found that potential pathogens, such as *Klebsiella*, *Streptococcus parasanguinis,* and *Clostridium perfringens*, were negatively associated with bone mineral density (BMD), suggesting their role in bone loss (He et al., 2020; Ji et al., 2024; Q. Wang et al., 2021). ~~On the contrary~~Conversely, members of the *Lachnospiraceae* family showed a positive correlation with

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higher BMD, suggesting their potential role in supporting bone health (He et al., 2020; Ji et al., 2024). A similar association of taxa was found in a study investigating VMS symptoms (Pavlovska et al., 2022) (**Figure ~~5~~ 4 B-C**).

These observations suggest that menopause, through physiological and hormonal changes, significantly impacts the gut microbiota, which plays a multifaceted role in women's health.

Diet as a potential modulator of microbiome toward healthy aging in women

Effects of Dietary Intervention on Aging in Women

Emerging evidence supports the effect of diet on healthy aging and longevity in the general population, and the gut microbiome may mediate this effect (Brandhorst et al., 2024). While using diet to modulate the gut microbiome is appealing, targeted modulatory interventions are premature until evidence from multiple studies supports the causal or curative effect of specific bacteria and ~~function~~ their functions in age-related conditions. Furthermore, such intervention studies ~~will~~ ~~have to be personalized, which means that the baseline microbiome composition will have to be considered to predict the efficiency and likelihood of persistent microbiome change~~need to be personalized, which means that the baseline microbiome composition must be considered to predict the efficiency and likelihood of persistent microbiome changes as ~~needed~~required. The field of personalized nutrition ~~on the microbiome is yet in the infancy stage, but we foresee the potential impact of using the diet for preventing or treating different~~and the microbiome is still in its infancy; however, we foresee the potential impact of using diet to prevent or treat various age-related conditions in both men and women.

For example, in postmenopausal women, increased intake of 'anti-aging nutrients' may~~could~~ mitigate~~improve~~ the side effects of estrogen decline (**Figure 5**). Likewise, diet regimes that favor the growth~~production~~ of SCFAs-producing bacteria, whose abundance declines with age, might be ~~important~~ necessary for a protective effect on the cardiovascular and skeletal~~on~~ systems. In a murine osteotomy model, propionate and butyrate supplementation enhanced bone density and prevented bone loss by inhibiting osteoclast formation and bone reabsorption (Wallimann et al., 2021). Studies conducted on fiber-rich diets ~~like~~ such as the Mediterranean diet (MedDiet) showed that a high abundance of SCFAs promotes a healthier cardiovascular system and reduces chronic inflammation (Ghosh et al., 2020). Conversely, a diet ~~with a high amount of animal fats, sweets, refined oils, and processed food exacerbates inflammation,~~ high in animal fats, sweets, refined oils, and processed foods exacerbates inflammation, thereby increasing the accumulation of TMAO. In a study on postmenopausal women with and without PMO, those following a diet rich in grains, vegetables, and fruits and low in red meat exhibited normal bone mass and a lower abundance of gut methanogen bacteria responsible for decreasing SCFAs (Brahe et al., 2015; Chen et al., 2024).

Effects of Single Bioactive Compounds Interventions on Aging in Women.

Polyphenols

Polyphenols are another ~~important~~significant food component with anti-inflammatory and antioxidant properties that help mitigate age-related inflammation. This broad class of compounds also includes phytoestrogens (i.e., isoflavones)~~,~~ and anthocyanins, making them a popular dietary supplement for promoting healthy aging **(Figure 5)**.

i) Phytoestrogens

Various natural compounds ~~of phytoestrogens, particularly daidzein and genistein, mimic human estrogen and are present in soybeans, legumes, mung beans, green beans, and~~ , including phytoestrogens such as daidzein and genistein, mimic human estrogen and are found in soybeans, legumes, mung beans, green beans, and certain plants (e.g., red clover). Soybeans are especially rich in phytoestrogens (~ 150 mg/100 g of cooked soybeans). The recommended intake of isoflavones is ~ 40-50 mg/d, whereas, in European countries, the consumption of phytoestrogens is, on average, ~ 0.76 mg/d (I.-S. Kim, 2022). GmGUS enzymes metabolize these molecules, and once activated, they can bind to the same estrogen receptors (ERs) as endogenous estrogens, thereby regulating downstream signaling pathways (Hu et al., 2023). The advantage of introducing isoflavonoids ~~with diet lies in their minimal side effects compared to HRT, as they show~~into the diet lies in their minimal side effects compared to HRT, as they exhibit a strong binding affinity to ERβ and a weaker affinity to ERα. While estrogen is a ~~strong~~ potent agonist, binding effectively to both receptors, the active metabolite of isoflavones selectively targets ~~ERβ~~the ERβ receptor. This receptor selectivity provides health benefits and lowers cancer risk, as ERβ is primarily expressed in non-gonadal tissues. Moreover, secondary metabolic products of phytoestrogens degradation, produced by gut microbe metabolism, act as potent

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metabolites with various beneficial effects, including anti-androgenic and antioxidant activities. For instance, *Eggerthella*, *Adlercreutzia*, *Asaccharobacter*, *Slackia*, and *Lactococcus spp*. produce equol, a potent isoflavandiol estrogen metaboliz~~t~~ed ~~of~~ from soy daidzein (Iino et al., 2019)~~.~~. Clinical trial studies have shown~~ed~~ that this metabolite ~~had been shown to reduce~~ reduces the risk of CVD in postmenopausal women (Acharjee et al., 2015; Z.-M. Liu et al., 2014). However, only 30–50% of the population is able to convert daidzein into equol, depending on ~~intestinal microbiota composition~~the composition of their intestinal microbiota. Other derivatives of daidzein are important for women's health. For example, obesity in postmenopausal women was associated with a loss of ability to metabolize the soy isoflavone daidzein to O-desmethylangolensin (Miller et al., 2017). Furthermore, phytoestrogens have been shown to promote the growth of ~~are believed to induce an increase in~~ butyrate-producing bacteria (such as *Faecalibacterium* and members of the *Clostridium* clusters), which contribute to intestinal health through their anti-inflammatory and ~~showing potential~~ antioxidant properties (Iino et al., 2019). Studies on postmenopausal women, indeed suggested that the treatment with phytoestrogens may help prevent many adverse postmenopausal conditions, including osteoporosis and vasomotor symptoms (Arcoraci et al., 2017; Barnard et al., 2023; Lambert, Thorup, et al., 2017). Recent clinical studies have also demonstrated the effectiveness of~~-~~ bioactive isoflavones from red clover in treating osteoporosis with ~~few side effects [81]~~ minimal side effects (Lambert, Thybo, et al., 2017). The use of phytoestrogens in the treatment of osteoporosis is well-documented, as they reduce ~~tumor necrosis factors (RANKL and TNF-α) activity, which limit bone reabsorption, and enhance growth factors (es RUNX2 and TGF-β),~~ the activity of tumor necrosis factors (RANKL and TNF-α), which limit bone reabsorption, and enhance growth factors (RUNX2 and TGF-β), thereby promoting bone formation. However, and importantly, there is ~~debate~~ not yet a consensus on the quantity, origin, and type of phytoestrogens to employ for therapy (Patisaul & Jefferson, 2010). For instance, isoflavones have bone benefits regardless of the source (e.g., extracted from red clover or soybeans or consumed in soy foods)~~, while the synthetic ipriflavone did not show a positive effect on bone density [83,84]~~. In contrast, synthetic ipriflavone did not show a positive effect on bone density. (Alexandersen et al., 2001)~~[83,84]. This~~ a discrepancy~~n effect~~ that may be explained by differences in the absorption and bioavailability of ~~the various~~ compounds, which should be assessed on a case-by-case basis (Tanida et al., 2016). Indeed, some intervention studies administering isoflavonoids did not have a positive effect on BMD (Tai et al., 2012). Interestingly, phytoestrogens and probiotics can work synergistically, enhancing their beneficial effects against menopausal comorbidities such as osteoporosis and vasomotor symptoms (Lambert, Thorup, et al., 2017; Lambert, Thybo, et al., 2017).

It has been suggested that probiotics can also restore the impaired activation of phytoestrogens in conditions of gut dysbiosis, as certain strains like *Lactobacillus* and *Bifidobacterium* possess gmGUS enzymes that facilitate phytoestrogen metabolism (Lambert, Thorup, et al., 2017; Lambert, Thybo, et al., 2017).

ii) Flavonoids

Royal jelly is another well-studied component of a diet rich in flavonoids (such as chrysin and pinocembrin) with estrogenic activity. Produced by honeybees (*Apis mellifera*), royal jelly has been shown to have multiple therapeutic applications, including immune modulation, anti-fatigue, anti-tumor, anti-hypertensive, and anti-osteoporosis effects (Bălan et al., 2020). A clinical study (n = 90 women) demonstrated that the application of a 15% royal jelly vaginal cream improved GMS in postmenopausal women (Seyyedi et al., 2016). Despite its promising benefits, the impact of royal jelly on the microbiota in postmenopausal women remains underexplored. So far, only one study on a db/db mouse model (characterized by overeating, obesity, and hyperglycemia) has shown that royal jelly influences the microbiota by increasing SCFA-producing bacteria (Kobayashi et al., 2023).

iii) Anthocyanins

Anthocyanins, a class of polyphenols abundantly found in prunes, grapes, blueberries, and black and green tea, are associated with a reduction of CVD risk in both women and men (Jennings et al., 2012; McCullough et al., 2012). Additionally, they are frequently utilized as an alternative therapy to alleviate menopausal symptoms. The gut microbiota transforms them into bioactive, low-molecular-weight phenolic metabolites that modify the host's metabolism (Fang, 2014). For instance, the conversion of anthocyanins is also related to the enzyme β-glucosidase found in some species of *Lactobacillus, Bifidobacterium, Blautia,* and *Erysipelatoclostridium*. The consumption of anthocyanin-rich fruits in clinical trials conducted on adults has been shown to increase *A. muciniphila, Lactobacillus spp., Roseburia,* and *Ruminococcus* and attenuate host gut inflammation and obesity (Fidélix et al., 2020; H. Kim et al., 2020). Pilot intervention studies, particularly those based on polyphenol-rich ~~food (dose > 90 mg/d), appeared to affect postmenopausal women, decreasing hypertension, inflammation, and osteoporosis, but~~foods (dose > 90 mg/d), appear to benefit postmenopausal women by decreasing hypertension, inflammation, and osteoporosis; however, ~~only specific individuals experience these~~

~~benefits~~these benefits are only experienced by specific individuals (Johnson et al., 2015; Simpson et al., 2024; Welch et al., 2012). ~~Importantly~~Notably, postmenopausal women with initially low BMD may benefit from the ~~, postmenopausal women with initial low BMD could benefit from~~ *Oscillospiraceae* and *Lachnospiraceae* families, which can metabolize these compounds (Simpson et al., 2024).

Probiotics

In addition to diet interventions, probiotics have emerged as a promising treatment for ameliorating PMO symptoms. Different probiotic *Lactobacillus* strains have been shown to heighten bone health by increasing calcium absorption by the gut and modulating bone cell activity (**Figure 5**). Probiotic treatments in PMO murine models boosted higher bone calcium deposition and suppressed osteoclast activity related to bone loss (Britton et al., 2014). However, some controlled clinical trials in postmenopausal women have shown no significant effect of probiotics ~~use~~ on bone loss (Gregori et al., 2024; Harahap et al., 2024). Yet, significant positive effects on bone metabolism were observed when dysbiosis was present, a condition that is frequently common in obesity and with advanced age (Jansson et al., 2019; P. Li et al., 2022; Nilsson et al., 2018). Other studies showed that administering *Lactobacillus* probiotics increased the relative abundance of core SCFAs-producers in the gut, which augments butyrate levels in intestinal mucosa and serum (Berni Canani et al., 2016; Brahe et al., 2015; Honda et al., 2024; P. Li et al., 2022). A proposed mechanism suggests that *Lactobacillus* and butyrate synergically enhance bone formation by increasing regulatory T cells in the bone marrow, promoting osteoblast proliferation (Zaiss et al., 2019). Meanwhile, Li *et al*. demonstrated in a murine model that *L. rhamnosus* may prevent bone loss by improving intestinal permeability and reducing microbiota-induced inflammation related to sex hormone deficiency (J.-Y. Li et al., 2016).

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Other types of intervention have been proposed to alleviate menopause syndrome. In a pilot trial, the use of *Lactobacillus*-containing hygiene products was associated with an improved vaginal ecosystem, indicating better urogenital health (Yoshikata, Yamaguchi, Mase, Tatsuyuki, et al., 2022). Intervention studies on healthy young women who were given *Saccharomyces cerevisiae* yeast showed increased *Lactobacillus*, especially *L. iners*, and decreased *Prevotella* (Oerlemans et al., 2022). Probiotics, particularly strains such as *L. reuteri*, *L. rhamnosus*, and *L. acidophilus*, may help maintain an acidic vaginal pH and alleviate symptoms of VVA and recurrent UTIs (Lim et al., 2020; Ribeiro et al., 2019; Vicariotto et al., 2024; Yoshikata, Yamaguchi, Mase, Tatsuyuki, et al., 2022). However, the success of these actions depends on an individual's microbiome composition. It may require long-term use to show noticeable effects, especially in older adults and women with less adaptable microbiomes. Moreover, in addition to the vaginal microbiome, many lifestyle factors play significant roles in managing postmenopausal symptoms, and probiotics alone may be insufficient to guarantee a healthy aging trajectory without a holistic approach to health.

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Overall, this evidence highlights the importance of designing personalized nutrition strategies to alleviate postmenopausal disorders, such as PMO, tailored to an individual's current diet and microbiome profile, and encourages further investigation into dietary interventions that promote healthy aging in women.

Gut-Vagina axis

To our knowledge, no current studies have simultaneously investigated the gut and vaginal microbiomes in the same women; however, the hypothesis of a functional gut–vaginal axis remains a compelling one. Intestinal and vaginal microbial communities may interact through immune, metabolic, and endocrine pathways. For example, as discussed in previous sections and illustrated in **Figure 4** **A**, the estrobolome activity of the gut microbiota can influence systemic levels of sex hormones and metabolites, which in turn may affect the vaginal microenvironment, epithelial integrity, and susceptibility to colonization by specific bacterial taxa. Furthermore, gut dysbiosis and the resulting local inflammation could propagate inflammatory signaling to nearby organs, including the vagina.

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Dietary factors may further modulate these interactions. Diets rich in fiber, polyphenols, and fermented foods are associated with enhanced intestinal colonization by beneficial *Lactobacillus* and *Bifidobacterium* species, which could potentially support vaginal eubiosis (Djusse et al., 2025). Conversely, pro-inflammatory dietary patterns may exacerbate dysbiosis and inflammatory responses, potentially contributing to pelvic inflammatory conditions or endometriosis-associated inflammation (Herup-Wheeler et al., 2024). Although causal evidence remains limited, nutritional modulation of the gut microbiota as an adjunct to conventional therapies targeting vaginal health is promising and warrants further investigation with randomized clinical trials.

It remains unclear whether direct microbial transfer occurs between the gut and vagina; however, the close anatomical proximity of the rectum and vagina may facilitate ~~the~~ transfer of microbes. Several *Lactobacillus* species are present in the gut. Yet, they differ from those typically found in the vagina—species that are prevalent on one site~~ and~~ are often rare on the other. This highlights the need for large-scale metagenomic studies with sufficient statistical power and taxonomic resolution to provide a conclusive assessment of potential strain sharing. Indeed, several studies have reported the exchange of *Lactobacillus* species between the rectum and vagina (El Aila et al., 2011). However, a deeper understanding of gut–vagina microbial connections, rather than rectum–vagina microbial connections, is urgently needed.

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In line with this hypothesis, several studies have explored whether modulating the gut microbiome through oral probiotic or prebiotic supplementation can indirectly influence the vaginal microbiota. Preliminary findings suggest that certain orally administered *Lactobacillus* strains may modulate the vaginal ecosystem, improving microbial balance and resilience to infections (Di Pierro et al., 2025; Perez et al., n.d.). However, direct microbial transfer has not been demonstrated, and the mechanistic underpinnings of these effects remain poorly understood.

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Further studies employing multi-omics approaches to investigate the gut and vaginal microbiomes, along with immune and endocrine parameters in the same women, are urgently needed to elucidate potential routes of microbial communication, the role of mucosal immune signaling, and the extent to which systemic hormonal changes mediate these effects. A promising initiative in this direction is the Women4Health cohort, a women-oriented multi-omics-based study (Busonero et al., 2024).

Future perspectives for women's aging research

Our review emphasized the biological changes that occur during women's aging from a microbiome perspective and highlighted potential intervention routes toward achieving healthy aging. We focused on women, as the scientific community recognizes the urgent need to improve our understanding of women's specific mechanisms to provide adequate care to both sexes. Historically, biomedical research has been focused on males, often excluding women due to concerns over hormonal fluctuations. This attitude has caused gaps in understanding female ~~health, but~~health,~~,~~ but also resulted in many medical therapies in use today being based on male-oriented studies, potentially offering less effective care for women and higher side effects.

We underlined reported changes in both vaginal and gut microbiomes during aging and their impact on women's health, as well as the emerging interaction between them. Since the scientific community has been exploring the role of the human microbiome on a broader scale only in the past decade and mainly focused on the gut microbiome, we expect that understanding of specific bacterial taxa and function, and how they affect health and illness, will consolidate and broaden to include other phenotypes or ecosystems unique to females, like the vaginal microbiome. Large-scale studies must consist of and properly analyze postmenopausal women to improve the understanding of aging-related processes from the microbiome perspective. Ideally, a cohort study approach, which involves collecting samples over extended periods and multi-omics data, should be considered to gather vast amounts of data and allow for an understanding of the full complexity of changes.

Finally, we discussed the yet understudied role of diet in modulating women's health and its potential as a preventive measure to counter the effects of aging in women; moreover, recognizing women's unique challenges as they age is crucial for developing targeted interventions.

Contributions

All authors contributed to the manuscript draft and its revision.

Declaration of interest

The authors declare that they have no conflicts of interest.

Declaration of the use of AI and AI-assisted technologies

We declare that the use of AI (ChatGPT and QuillBot) was limited to correcting the text and making the review more readable. The authors are fully responsible for scientific insights, literature reviews, data analysis and interpretation, and scientific conclusions.

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Resilience Plan, Investment PE8 – Project Age-It: "Ageing Well in an Ageing Society, " [DM 1557 11.10.2022] . The views and opinions expressed are only those of the authors and do not necessarily reflect those of the European Union or the European Commission. Neither the European Union nor the European Commission can be held responsible for them.
We thank Simona Incollu for the critical reading of the manuscript.

Figures Legend

The graphical abstract illustrates the different aspects of aging in women discussed in the review.

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Figure 1. The stages of reproductive aging in women and their possible associated symptoms.
A) Visual representation of key events and duration of the MT as established by the World Health Organization Scientific Group on Research in the Menopause (*Research on the Menopause : Report of a WHO Scientific Group [meeting Held in Geneva from 8 to 12 December 1980]*, n.d.) (Figure modified by Harlow *et al.*(Harlow, Gass, Hall, Lobo, Maki, Rebar, Sherman, Sluss, de Villiers, et al., 2012) ~~(Harlow, Gass, Hall, Lobo, Maki, Rebar, Sherman, Sluss, & de Villiers, 2012))~~. Briefly, the Staging of Reproductive Aging Workshop (STRAW) classification divides a woman's life into seven segments, with segments -2, -1, and 0, including the early MT, the late MT, and the final menstrual period (FMP). Early MT characteristics could include irregular cycles, variable hormone levels, and potential vasomotor symptoms (VMS). Late MT, lasting 1–3 years, might show amenorrhea (absence of the monthly menses), high levels of follicle-stimulating hormone (FSH), varied levels of estradiol (E2), and possibly VMS. Menopause is diagnosed 12 months following the last menstrual cycle. One-year post-menopause, as well as MT phases, constitute perimenopause. Early post-menopause presents high/variable FSH and low E2; late post-menopause is characterized by high FSH, low E2, and genitourinary symptoms (GSM). **B**) Health conditions that may occur during peri- and post-menopause status. Symptoms of postmenopausal syndrome are marked with a *.
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Figure 2. Characteristics of human vaginal Community State Types (CSTs).
CST types, sub-types, and major microbial properties of the dominant bacterial species according to the classification proposed by the VALENCIA study (France et al., 2020). Four CSTs (I, II, III, and IV) and the corresponding six sub-types are *Lactobacillus*-dominated, while CST IV, dominated by a mixed anaerobic community, has seven sub-CSTs. The A and B subtypes of CST I and CST III differentiate subtypes where the corresponding bacterial species is either fully dominant or highly prevalent, respectively. The symbols "O" in green, yellow, and red represent beneficial, neutral, and potentially harmful bacteria. Created in BioRender. Ferrando, M.L. (2025) https://BioRender.com/e99t679~~Created in BioRender. Ferrando, M.L. (2024) https://BioRender.com/ e99t679~~

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Figure 3. Proposed vaginal microbiome changes during MT stages.
Premenopausal women have high estrogen levels that support a thick, glycogen-rich epithelium, with a microbiome community most often dominated by *Lactobacillus* (e.g., *L. crispatus*). During perimenopause and the fluctuation in estrogen production, *Lactobacillus*-dominated communities become dynamic *(e.g.,* moving toward a higher abundance of *L. iners*), shifting to a mixed anaerobe community after menopause. Postmenopausal estrogen deficiency leads to lower glycogen, higher vaginal pH, and an increase in parabasal cells, potentially causing signs of moderate VVA. *Lactobacillus* levels drop significantly (Brotman et al., 2014; Byrne et al., 2024; Shen et al., 2016), while pathobiont bacteria like *Prevotella*, *Streptococcus*, *Gardnerella*, and *Atopobium* increase. Of note, *Lactobacillus spp.* Remains present – albeit with low abundance - in 30-50% of the vaginal microbiota (Brotman et al., 2014; Byrne et al., 2024; Hummelen et al., 2011; Shardell et al., 2021; Waetjen et al., 2023) (**Supplementary Table 1A**), maintaining their protective role against severe vaginal inflammation and infections. Created in BioRender. Ferrando, M.L. (2025) https://BioRender.com/xwabx3o~~Created in BioRender. Ferrando, M.L. (2024) https://BioRender.com/z68w440~~

Figure 4. Proposed changes in gut microbial taxa associated with aging in postmenopausal women
A) Gut-vaginal microbiome axis. In healthy women of reproductive age, the ovaries primarily synthesize active estrogens. These estrogens are converted into inactive forms in the liver and excreted into bile. Bacteria producing β-glucuronidases (estrobolome) can deconjugate and reactivate estrogens in the gut. While most reactivated estrogens are excreted, others re-enter systemic circulation through enterohepatic recirculation, potentially targeting tissues like the vagina. Estrogen stimulates vaginal cells to produce glycogen, supporting a healthy *Lactobacillus*-dominated vaginal microbiome. **B**) Aging during menopause is linked to decreased gut bacterial diversity, with women's microbiota more similar to males. Key taxa shift includes reductions in SCFA-producers, estrobolome microbes, and beneficial bacteria (e.g., *A. muciniphila*, *Bifidobacterium*, *Lactobacillus*) critical for intestinal integrity (upregulation of TJ via TLR2 receptor), immune support, and bacterial metabolite production. Additionally, an altered Firmicutes-Bacteroidetes ratio, driven by

increased Bacteroides, and a rise in pathobionts associated with CVD risk and gut dysbiosis have been observed. **C**) Functional mechanisms of gut taxa involved in the onset of age-related diseases in postmenopausal women. Graphic summary of the key metabolites or effectors produced by the three taxa and the effect each of these microbiome-derived entities has in either negatively or positively regulating various aging-linked diseases and disorders. The reduction in *Bifidobacterium* and *Lactobacillus* lowers acetate production and supports SCFA-producers.

SCFAs interact with G-protein-coupled receptors (GPCRs), influencing a wide range of cell types, including columnar epithelial cells, innate immune cells, and endothelial cells, thereby modulating inflammation, insulin resistance, and blood pressure. A decline in estrobolome diversity (gmGUS-producers) can affect estrogen recycling and contribute to postmenopausal disorders. Reduced folate and cholesterol-α-glucosyltransferase (CGT) producers lead to homocysteine and cholesterol accumulation, raising CVD risk. Enrichment of proteobacteria is linked to elevated blood pressure via inflammatory mediators (IL-8 and TNF-α). Additionally, increased pathobionts (e.g., *Enterobacteriaceae* and *Clostridium*) disrupt gut homeostasis, promoting inflammation (through pro-inflammatory interleukins) and dysbiosis, which contribute to postmenopausal disorders. Created in BioRender. Ferrando, M.L. (2025) https://BioRender.com/z68w440~~Created in BioRender. Ferrando, M.L. (2024)~~

SCFAs: Short Chain Fatty acids; gmGUS: gut microbes Glucuronidases; IL-8 and IL-18: pro-inflammatory Interleukins; IFN: Interferon; TJ: cellular Tight Junctions; TLR: Toll-Like Receptors; GPR41/43: G-Protein-Coupled receptors (GPCRs); FFARs: Free Fatty Acid Receptors, and ORs: Olfactory Receptors, families of GPCRs expressed in blood vessels and in vascular smooth muscle cells in a variety of organs; LPS: Lipopolysaccharide; pDC and mDC: premature and mature Dendritic Cells, respectively.

PMO: postmenopausal osteoporosis; VSM: vasomotor symptoms; GSM: genitourinary symptoms of menopause; CVD: cardiovascular diseases.

Figure 5: Potential role of diet on microbiome health during menopause.

A graphic representation shows how diet intervention strategies could promote microbiome changes that prevent the aging-associated decline in postmenopausal women.

Probiotics are different strains of *Lactobacillus*, such as *L. reuteri*, *L. rhamnosus*, *L. plantarum*, and *L. acidophilus.*

Polyphenols include flavonoids such as phytoestrogens (e.g., isoflavonoids) with estrogenic properties, including daidzein (found in soybeans, mung beans, green beans, Red Clover, Thai herb Pueraria Mirifica, and Japanese kudzu) and genistein (found in soybeans, lupin, fava beans, kudzu, and Red Clover), as well as anthocyanins (found in black currant, pomegranate, prunes, berries, grapes, acai, and eggplants) with antioxidant and anti-inflammatory activities. Additionally, polyphenols encompass non-flavonoid compounds, such as phenolic acids (found in berries, black and green tea, coffee, whole grains, tomatoes, and apples), as well as other polyphenols like resveratrol (found in grapes, berries, and red wine), which are known for their potent anti-inflammatory and antioxidant properties.

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TMAO, trimethylamine; IL-1β, Interleukin-1 beta pro-inflammatory cytokine; PMO: postmenopausal osteoporosis; VSM: vasomotor symptoms; GSM: genitourinary symptoms of menopause; CMD: cardiometabolic diseases.

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Aging in Women – the Microbiome Perspective

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Supplementary Material

Supplementary Table 1A. Metadata and summary of results gathered for articles investigating the vaginal microbiome

PubMed ID	First Author	Title	Journal	Year	Country	Criteria of inclusion/exclusion	Sequencing technology	Study type	Number of enrolled women	Overall age range	Age by category (mean ± SD)			Ethnicity	CSTs classification with dominant species	Proportions of the prominent bacterial communities of CSTs		
											Premenopausal	Perimenopausal	Postmenopausal			premenopausal	perimenopausal	postmenopausal
https://pubmed.ncbi.nlm.nih.gov/22073175/	Hummelen R.	Vaginal microbiome and epithelial gene array in post-menopausal women with moderate to severe dryness	*PlosOne*	2011	Canada	Inclusion criteria: •12 months of amenorrhea Exclusion criteria: •Not used antibiotics •No vaginal infection one month before sampling •No systemic estrogen products 6 months before sampling •No topical estrogen one week before sampling	16S rRNA V6 region	Longitudinal: 2 times sampling over 10 weeks	N total = 32 21 postmenopausal women without VVA 11 postmenopausal women with moderate-severe VVA	42-77 y	NA	NA	59.5±4.5	NA	NA	NA	NA	*L. iners - G. vaginalis* constantly present. Postmenopausal women with **VVA** ↑ *Prevotella timonensis,* ↑ *Porphyromonas uenonis,* ↑ *Peptoniphilus* ↑ *Bacillus* present in
https://pubmed.ncbi.nlm.nih.gov/24080849/	Brotman R.M.	Association between the vaginal microbiota, menopause status, and signs of vulvovaginal atrophy	*Menopause*	2014	USA	Inclusion criteria: • The menopausal stage was determined using the Stages of STRAW Exclusion criteria: •Not currently pregnant intact uterus •No history of organ transplantation •Human immunodeficiency virus infection	16S rRNA V1-V2 region	Cross-sectional prospective cohort study	N total=90 30 premenopausal women 30 perimenopausal women 30 postmenopausal women	35-60 y	40 ± 5.0	40.5± 7.0	50.0± 5.0	White=77.0% African-American=16.1% Asian other race=6.9%	CST I = *L. crispatus*	46.7%	20.7%	25.0%
															CST II= *L. gasseri*	3.3%	20.7%	4.6~~%~~
															CST III= *L. iners*	33.3%	37.9%	17.9~~%~~
															CSTV= *L. jensenii*	0.0%	3.5%	7.1~~%~~
															CST IV-A=*Streptococcus, Prevotella*	0.0%	6.9%	28.6%
															CST IV-B=*Gardnerella, Atopium*	16.7%	10.3%	17.9%
https://pubmed.ncbi.nlm.nih.gov/27103314/	Shen J.	Effects of low dose estrogen therapy on the vaginal microbiomes of women with atrophic vaginitis	*Sci Rep*	2016	China	Inclusion criteria: VVA group: • The menopausal stage was determined using STRAW • Obvious GSMs and VVA • FSH > 40 IU/L • E1 < 20 pg/mL • Endometrial thickness ≤ 5 mm • BMI bwteen 18.0 and 35.0 kg/m2 • No smoking • Trichomoniasis or candidiasis healthy post-menopausal group: Exclusion criteria: • No Antibiotics or all sorts of vaginal medications 6 months before sampling • Systemic or chronic diseases	16S rRNA V1-V3 region	Longitudinal: 0, 2, 4 weeks cohort study	N total =59 29 postmenopausal women without AVV 30 postmenopausal women with AVV	NA	NA	NA	Without= 55.8 ± 3.2 With AVV= 55.6 ± 2.6	NA	Cluster I: *Lactobacillus* communities	NA	NA	Cluster I= >50% 53.2 ± 40.3 % without VVA 11.2 ± 16.4 % with VVA
															Cluster II: *Prevotella* , *Atopobium* , *Streptococcus, Gardnerella*	NA	NA	Cluster II= >10.3% 7.4 ± 13.3 % without VVA 6.0 ± 10.3 % with VVA
															Cluster III: *Gardnerella, Prevotella, Streptococcus, Atopobium, Anaerococcus, Sneathia, Aerococcus, Peptoniphilus, Dialister* and *Finegoldia* . Low *Lactobacillus*	NA	NA	Cluster III= 39.1% 16.7 ± 21.7 % without VVA 41.7 ± 31.0 % with VVA
															Specifically *Atopobium*	NA	NA	2.7 ± 6.9 % without VVA 4.8 ± 5.5 % with VVA
https://pubmed.ncbi.nlm.nih.gov/28640154/	Mitchell C.M.	Vaginal microbiota and genitourinary menopausal symptoms: a cross-sectional and analysis	*Menopause*	2017	USA	Self-reported genitourinary symptoms (≥ 14 hot flashes per week) MsFLASH Eligibility Criteria Applied to All MsFLASH trials	16S rRNA V1–V3 region	Cross-sectional cohort study	N total =88 (all postmenopausal women)	40-62 y	NA	NA	54 ± 4	White=55% African-American=39%	NA	NA	NA	CST I
https://pubmed.ncbi.nlm.nih.gov/30837959/	Gliniewicz K.	Comparison of the Vaginal Microbiomes of Premenopausal and Postmenopausal Women	*Frontiers in microbiology*	2019	USA	Exclusion criteria: • Full hysterectomy, • Irregular menstrual cycle • Skin abnormalities in the vulvar area • TD1DM or TD2DM • Kidney, heart, circulatory disease • Pregnant or breastfeeding • Immunosuppressive or anti- inflammatory therapy	16S rRNA V1–V3 region	Cross-sectional cohort study	N total=45 15 premenopausal women 15 postmenopausal women 15 postmenopausal women under HT	23-67 y	33.0 ± 6.4	NA	60.7 ± 3.6	NA	Cluster A= *L. crispatus* Cluster B= *Gardnerella* Cluster C= *L. iners* Cluster D= *Enterococcus* , *Atopobium* , *Finegoldia* , *Gardnerella* , *Prevotella* , *Streptococcus* Cluster E= *Bifidobacterium* Cluster F= *L. gasseri*	NA	NA	NA
https://pubmed.ncbi.nlm.nih.gov/33903897/	Shardell M.	Association of Vaginal Microbiota With Signs and Symptoms of the Genitourinary Syndrome of Menopause Across Reproductive Stages	*Journals of Gerontology: Biological Sciences*	2021	USA	Inclusion criteria: • The menopausal stage was determined using the Stages of STRAW • Women with intact uterus Exclusion criteria: • Women pregnant or planning to become pregnant during the 2 years of the study •No history of organ transplantation •Human immunodeficiency virus (HIV) infection	16S rRNA V3–V4 region	Mix of cross-sectional and longitudinal cohort study over 2 years	N total= 750 270 premenopausal women 310 perimenopausal women 375 postmenopausal women	35-60 y	40 ± 5.0	40.5 ± 7.0	50.0± 5.0	White=75.5% African-American=17.1% Other=7.5%	CST I = *L. crispatus*	38.6%	33.0%	19.0%
															CST II= *L. gasseri*	10.2%	9.9	8.4%
															CST III= *L. iners*	23.7%	27.0%	18.3%
															CST IV-A= BVAB1, *G vaginalis* , anaerobes	2.1%	2.0%	1.4%
															CST IV-B= *G vaginalis* , anaerobes, low BVAB1	13.5%	11.2%	14.8%
															CST IV-C= diverse anaerobes: *Streptococcus* , *Anaerococcus* , *Peptoniphilus* and *Finegoldia*	5.5%	9.7%	33.5%
															CSTV= *L. jensenii*	6.4%	7.2%	4.6%
https://pubmed.ncbi.nlm.nih.gov/36374244/	Yoshikata R.	Age-Related Changes, Influencing Factors, and Crosstalk Between Vaginal and Gut Microbiota: A Cross-Sectional Comparative Study of Pre- and Postmenopausal Women	*J Womens Health*	2022	Japan	Inclusion criteria: • Menopause established by hormone measurement Exclusion criteria: • Genitourinary symptoms • Irregular menstrual cycle • Unstable ovarian function	16S rRNA V1–V2 region	Cross-sectional cohort study	N total=70 35 premenopausal women 35 postmenopausal women	27-76 y	37.3 ± 6.1	NA	59.9 ± 4.8	Japanese	CST I = *L. crispatus*	NA	28.9%	1.1%
															CST II= *L. gasseri*	NA	5.0%	1.6%
															CST III= *L. iners*	NA	35.0%	4.8%
															CST V= *L. jensenii*	NA	1.0%	1.0%
															Pathogenic bacteria (CST IV)	NA	16.7%	26.8%
															Others		11.1%	63.8%
https://pubmed.ncbi.nlm.nih.gov/37788422/	Waetjen L.E.	Relationships between the vaginal microbiota and genitourinary syndrome of menopause symptoms in postmenopausal women: the Study of Women's Health Across the Nation. Menopause	*Menopause*	2023	USA	The menopausal stage was determined using the Stages of STRAW Inclusion criteria: • Women with intact uterus Exclusion criteria: • Women pregnant or planning to become pregnant during the 2 years of the study •No history of organ transplantation •No Human immunodeficiency virus (HIV) infection	16S rRNA V3-V4 regions	Multicentre, prospective cohort study with a cross-sectional survey	N total =1320 Subset of postmenopausal women with microbiome with complete biomarkers=994	60-72	NA	NA	66.5 ± 6.5	Black, Hispanic, Japanese, Chinese, White	CST I = *L. crispatus*	NA	NA	10.5%
															CST II= *L. gasseri*	NA	NA	5.2%
															CST III= *L. iners*	NA	NA	18.9%
															CSTV= *L. jensenii*	NA	NA	1.7%
															CST IV= diverse anaerobes	NA	NA	63.6%
															subgroup CTS IV:			
															CST IV-A, IV-B= different proportion of *G. vaginalis* , BVAB1	NA	NA	13.8%
															CST IV-C0= moderate R.A. of *Prevotella*	NA	NA	28.9%
															CST IV-C1= dominated by *Streptococcus*	NA	NA	14.1%
															CST IV-C2, IV-C3, IV-C4= other anaerobes	NA	NA	6.8%
https://pubmed.ncbi.nlm.nih.gov/38713891/	Byrne E.H.	Association between vaginal microbiota and vaginal inflammatory immune markers in postmenopausal women	*Menopause*	2024	USA	Inclusion criteria: • The menopausal stage was determined using the Stages of STRAW • Women with intact uterus • at least 1 moderate to severe GSM symptoms Exclusion criteria: • Women pregnant or planning to become pregnant during the 2 years of the study •No history of organ transplantation •No Human immunodeficiency virus infection	16S rRNA V3-V4 regions	Multicentre, prospective cohort study with a cross-sectional survey	N total =119 (all postmenopausal women)	45-70	NA	NA	61± 6.5	NA	*Lactobacillus* dominant	NA	NA	29.5%
															CST I= *L. crispatus*	NA	NA	5.7%
															Lactobacillus no-dominant	NA	NA	70.6%

Abbreviations:
NA= Not Applicable because the data was not reported
BVAB1= *Ca Lachnocurva vaginae*
RA: Relative abundance
STRAW= Reproductive Aging Workshop staging system
HRT= Hormone Replacement Therapy
E1= Estrone
E2= Estradiol
GSM= Genitourinary Syndrome of menopause
VVA= Vulvovaginal atrophy
VMS= Vasomotor symptoms
*VVA and GSM markers= vaginal dryness, vulvovaginal irritation, sexual pain, dysuria and urinary urgency
VMI= Vaginal maturation index
AVV= Vulvar and vaginal atrophy
T2DM=Type 2 diabetes mellitus

Meaningful Results on Vaginal Microbiota
Microbial profile associated with VVA: • Postmenopausal women without VVA had low bacterial diversity dominated by *Lactobacillus spp* , while those with moderate or severe VVA had decreased *Lactobacillus spp* and higher species diversity. • VVA was associated with ↓ of genes involved in maintaining the vaginal barrier function, and ↑ inflammation-related genes
Microbial profile • Premenopausal women: predominantly *Lactobacillus dominat* CSTs, ↑ *L. crispatus* and *L. iners* • Perimenopausal women (transition stage): ↑ *L. iners* and *L. gasseri* ↑ CST IV-A (with *Streptococcus* and *Prevotella*) • Postmenopausal women: Often ↑ CST IV-A, marked by a higher presence of *Streptococcus* and *Prevotella* **Microbial profile associated with VVA:** • ↑ CST IV-A associated with mild to moderate VVA • ↓ *Lactobacillus* spp. associated with increased microbial diversity and higher vaginal pH
Microbial profile • Over 50% of postmenopausal women presented Cluster I (*Lactobacillus* community) *Lactobacillus spp* was present in 83% of women with VVA but with low RA (11.2%) • About 40% of postmenopausal women presented Cluster III (*Gardnerella* community). *Gardnerella spp* was present in 83% of the participants, with high RA with VVA (41.7%) and low RA without VVA (16.7%) Microbial profile under HRT • Estrogen treatment altered the vaginal microbiota, with ↑ *Lactobacillus* (from 11.2% → 71.0%) and ↓ *Gardnerella* (from 41.7% → 9.6%), *Prevotella* and *Atopobium* .
Microbial profile • The majority of postmenopausal women (66%) did not present *Lactobacillus* **Microbial profile associated with sex hormones (E1-E2), glycogen, GMS** • Women with *Lactobacillus* -dominant communities had ↑ unconjugated E1. • No difference in vaginal glycogen levels between women with *Lactobacillus* -dominant and non-*Lactobacillus* -communities • High serum E2 or E1 levels were not associated with either higher vaginal glycogen or detection of individual genera.
Microbial profile under HTR • The vaginal communities of most postmenopausal women receiving HRT (10/15) were dominated by species of lactobacilli and belonged to clusters A (*L.crispatus*), C (*L.iners*), and F (*L. gasseri*) • Postmenopausal women without HT were in cluster D (*Lactobacilli* depleted) • HRT greatly improved vaginal atrophy scores ↓vaginal pH ↓ microbial diversity
Microbial profile: • Premenopausal Women: ↑ *Lactobacillus* species, particularly *L. crispatus* and *L. iners* . • Perimenopausal Women: ↑ *L. iners* ↑ CST IV-C (characterized by anaerobes) and *L. iners* • Postmenopausal Women: low-*Lactobacillus* communities and a significant ↑ of CST IV with **Microbiota associated with VVA**: • Postmenopausal women with CST IV-B (low *Lactobacillus* and high R.A. *G vaginalis*) was associate with VVA
Microbial profile: Postmenopausal women exhibited ↓ levels of *Lactobacillus* and ↑ levels of nonoptimal species, ↑ microbial diversity, and ↑ vaginal pH.
Microbial profile: • 36% of postmenopausal women's microbiomes were dominated by *Lactobacillus* spp. • CST IV was the most frequent (63.6%), with CST IV-C being the most prevalent (49.8%). • Within CST IV-C, 1) CST IV-C0 (28.9%, *Prevotella* -dominant), 2) CST IV-C1 (14%, *Streptococcus* -dominant). • CST IV-C is rare in premenopausal women. **Microbial profile associated with estrogen hormones, VVA* and GSM* biomarkers** • Women with CSTs *Lactobacillus* -dominant (particularly CST I) was associated ↑ E1 and ↑ E2 • Low vaginal pH was associated with CST I, while high vaginal pH was associated with CST IV-C0 • Low VMI was associated with CST IV-C0 and IV-C1
Microbial profile: • Only a minority of postmenopausal women had a microbiome profile dominated by *Lactobacillus spp* • Postmenopausal women with *Lactobacillus spp* dominant profile had: ↓ Low alpha microbial diversity ↓ Concentrations of proinflammatory cytokines (IL-1a, IL-1b, IL-10, IL-8, and IL-6) After menopause *Lactobacilli* have beneficial effects on vaginal immune homeostasis, despite lower prevalence.

Supplementary Table 1B. Metadata and summary of results gathered for articles investigating the gut microbiome

PubMed ID	First Author	Title	Journal	Year	Country	Criteria of inclusion/exclusion	Sequencing technology	Study type	Number of enrolled participants	Overall age range	Age (mean +/- SD)		Ethnicity	Microbial diversity	Taxa differential abundance	Differences in microbial function	Meaningful Results on Gun Microbiota in postmenopausal women
											Premenopausal	Postmenopausal			Postmenopausal women vs premenopausal women	Postmenopausal women vs premenopausal women	
https://pubmed.ncbi.nlm.nih.gov/37341612/	Fuhrman J.B.	Associations of the faecal microbiome with urinary estrogen and estrogen metabolites in postmenopausal women	*J Clin Endocrinol Metab* .	2014	USA	Exclusion criteria: • Any medical conditions or use of medicines that impact gut microbiome or hormone levels; • Any history of cancer or intestinal diseases • No systemic estrogen/progesterone use in the past 12 months • No antibiotics in the past 6 months.	16S rRNA pyrosequencing V1-V2 region	Cross-sectional study	Healthy Postmenopausal women=60	55-69 y	NA	NA	NA	α-diversity significantly associated with estrogen	*Firmicutes/Bacteroidetes* ratio *Firmicutes* 65% *Bacteroidetes* 32%	NA	**Correlation with estrogen** *Clostridiales* specifically with *Ruminococcaceae* were positively associated with the estrogen ratio, while *Bacteroides* were negatively associated.
https://pubmed.ncbi.nlm.nih.gov/30244778/	Santos-Marcos J.A.	Influence of gender and menopausal status on gut microbiota	*Maturitas*	2018	Spain	Inclusion criteria postmenopausal • years after the last menstrual cycle: 6.38 ± 1.14; Exclusion criteria: • Population without oncological diseases or disabling diseases • Use of antibiotics in the 3 months prior to sampling	16S rRNA V1-V2 region Illumina MiSeq	Case-control study Cross-sectional	Total participants=76 Premenopausal women=17 Postmenopausal women=20 Young men= 19 Old men =20 Groups matched by BMI and diet	NA	♂ 46.1 ± 0.8 ♀ 46.6 ± 0.6	♂ 55.8 ± 0.6 ♀ 56.2 ± 0.7	NA	No difference in α-and β-diversity between groups Post-menopausal microbiome more similar to age-matched men than pre-menopausal to age-matched men	↑ *Firmicutes* /*Bacteroidetes* ratio **Phylum**: ↑ *Firmicutes* ↓ *Actinobacteria* ↓ *Bacteroidetes* **Genera**: ↑ *Lachnospira* ↑ *Roseburia* (SCFA-producer, gmGUS) ↓ *Prevotella* (SCFA-producer, gmGUS) ↓ *Parabacteroides* (SCFA-producer, sulphate assimilation, gmGUS) ↓ *Biophilia* (sulphate metabolism) ↓ *Ruminococcus* (butyrate-produce)	↓ Pyrimidine pathway ↓ One carbon pool by folate pathways	**Microbial diversity:** Sexual dimorphism in microbiota composition between young women and men Postmenopausal women had microbiota more similar to men No significant difference in bacterial richness and diversity between postmenopausal and premenopausal women **Microbial composition in postmenopausal compared to premenopausal women**: Increased Firmicutes/Bacteroidetes ratio Decreased SCFA-producer genera in postmenopausal women (*Prevotella* , *Parabacteroides* , *Ruminococcus*) **Correlation with estrogen** Gammaproteobacteria positively correlated with E2, while *Prevotellaceae* negatively correlated with E2 Lower inflammatory markers (IL-6 and MCP-1) in the premenopausal women group compared to postmenopausal women and old and young men
https://pubmed.ncbi.nlm.nih.gov/31273779/	Zhao H.	Compositional and functional features of the female premenopausal and postmenopausal gut microbiota	*FEBS*	2019	China	Inclusion criteria • Natural menopause Exclusion criteria • History of chronic serious infection • malignant cancer • No antibiotics in the past 1 months	Single-end metagenomic seq was conducted on the BGISEQ-500 platform	Case-control study Cross-sectional	Total women=48 Premenopausal women=24 Postmenopausal women=24	NA	52.5 ± 5.6	53.9 ± 3.8	NA	α-diversity (Shannon index) ↑ Premenopausal (1.8) ↓ Postmenopausal (1.3) (*p* =5.0e-6)	↑ *Firmicutes* /Bacteroidetes ratio **Phylum:** ↑ *Bacteroidetes* ↑ *Verrucomicrobia* ↓ *Firmicutes* ↓ *Proteobacteria* **Genera** ↑ *Tolumonas* (toluene-producing genus) ↓ *Faecalibacterium* (butyrate-producer, gmGUS) ↓ *Alistipes* (gmGUS, protective effects against CVD, colitis, but marker CRC) ↓ *Eubacterium* (butyrate-producer) ↓ *Roseburia* (butyrate-producer, gmGUS)	↑ Glycolysis, ↑ Lactate consumption, ↑ Cysteine biosynthesis, ↑ Homocysteine degradation ↑ Lysine and arginine degradation ↑ Methionine degradation ↓ Pentose phosphate pathway ↓ Proline degradation process	**Microbial diversity:** Postmenopausal women presented lower richness and diversity than premenopausal women **Microbial composition in post-menopausal compared premenopausal women:** Increased Firmicutes/Bacteroidetes ratio Decreased Firmicutes and Roseburia spp. Decreased SCFAs (*Faecalibacterium* , *Alistipes* , *Eubacterium* , *Roseburia*) Increased Bacteroidetes and the toluene-producing genus Tolumonas.
https://pubmed.ncbi.nlm.nih.gov/31098397/	de la Cuesta-Zuluaga J.	Age- and Sex-Dependent Patterns of Gut Microbial Diversity in Human Adults	*mSystem*	2019	USA / UK Colombia Chinese	Inclusion criteria • BMI between 18.5-30 kg/m Exclusion criteria: • Underweight • Pregnancy • Neurodegenerative diseases, • Current or recent cancer • Gastrointestinal diseases • No history in the past year of inflammatory bowel disease, TD1DM or TD2DM, antibiotic use	16S rRNA V4 region with the Illumina MiSeq platform	Prospective, population-based cohort study (Four large cohort (healthy partecipants)	Total participants (USA) = 3023 Premenopausal women=736 Postmenopausal women=881 Young men= 732 Old men =674 Total participants (UK)= 936 Premenopausal women=195 Postmenopausal women=344 Young men= 173 Old men =224 Total participants (China)= 4963 Premenopausal women=946 Postmenopausal women=1826 Young men= 670 Old men =1521 Total participants (Colombia)= 437 Premenopausal women=143 Postmenopausal women=83 Young men= 133	Young adults (ages 20–45) Middle-aged adults (ages 46–69)	NA	NA	Diverse ethnicities	Premenopausal women had higher α-diversity than young and old men. Reduced α-diversity starts above age 40 years.	NA	NA	**Microbial diversity**: Sexual dimorphism in microbiota composition between young women and men Postmenopausal women had gut microbiota more similar to age-matched men.
https://pubmed.ncbi.nlm.nih.gov/32951609/	Mayneris-Perxachs J.	Gut microbiota steroid sexual dimorphism and its impact on gonadal steroids: influences of obesity and menopausal status	*Microbiome*	2020	Spain	Inclusion criteria: • subjects with obesity (BMI ≥ 30kg/m2) and age- and sex-matched non-obese subjects (18.5 < BMI < 30kg/m2), with an age range of 27.2-66.6 years (n = 131) Exclusion criteria: • TD2DM • Chronic inflammatory systemic diseases • Acute or chronic infections in the previous month • Use of antibiotic, antifungal, antiviral, • No treatment with proton pump inhibitors • Severe disorders of eating behavior or major psychiatric antecedents • Excessive alcohol intake	Whole-genome shotgun seq	• Case-control cross-sectional sudy • Small subgroup (n=81) longitudinal was followed for 1year	Total women=131; after 1-year n=81 Premenopausal women= 44 Postmenopausal women= 45 Middle-age Men = 42	Premenopause women 34.4-48.4 Age-matched man: 39.2-54.3 Postmenopausal women 55.4-59.6	♂ 41.9 [+/-NA] ♀ 46.1 [+/-NA]	♂ 58.6 [+/-NA]	Hispanic	No difference in α-diversity among pre- and post-menopausal women and men, but β-diversity differed among groups	**Bacteroidetes species:** ↑ *Bacteroides sp. CAG:661* , *Bacteroides sp.* ↑ *Mediterranean massilensis* , ↑ *Marseille P3166* , ↑ *Bacteroides thetaiotaomicron CAG 4* 0 ↓ Alistipes ssp. AL-1 , ↓ Alistipes ssp. CAG 29, ↓ Alistipes onderdonkii (small amounts of SCFA, gmGUS) **Firmicutes species:** ↑ *Anaerovibrio spp JC8* ↑ *Veillonella sp. ICM51a,* ↑ *Veillonella dispar,* ↑ *Veillonella sp. DORA_A_3_16_22* ↑ *Dorea sp. CAG:317* ↑ *Lactobacillus rhamnosus (gmGUS)* ↑ *Faecalitalea cylindroides* ↓ *Ruminococcus sp. CAG:379 (butyrate-producer, gmGUS)* ↓*Clostridium neonatal, Clostridium sp. CAG:411 (gmGUS)* ↓ *Firmicutes CAG 534, Firmicutes bacterium CAG:194 (gmGUS)* ↓ *Enterococcus faecalis* ↓ *Eubacterium sp. CAG:603 (butyrate-producer)* ↓ *Blautia sp. CAG:257 (gmGUS)* ↓ *Lactobacillus plantarum (butyrate-producer, gmGUS)* ↓ *Christensenella massiliensis* **Actinobacteria species:** ↓ *Bifidobacterium angulatum (butyrate-producer, gmGUS)* ↓ *Eggerthella sp. HGA1* **Proteobacterium species:** ↑ *Haemophilus, Haemophilus sp. HMSC71H05 Haemophilus sp. HMSC068C11* ↑ *Haemophilus parainfluenza* ↑ *Pasteurellaceae* ↑ *Aggregatibacter actinomycetemcomitans*	↓ Pyrimidine pathway ↓ One carbon pool by folate pathways ↓ bacterial steroid degradation pathway	**Microbial diversity:** No significant difference in bacterial alpha diversity between pre- and post-menopausal women Sexual dimorphism in microbiota between young women and men Postmenopausal women had microbiota more similar to men. **Microbial composition:** Decreased SCFAs producers and gmGUS taxa (*Bifidobacterium* , *Ruminococcus* , *Eubacterium* , *Alipses)* Decreased *Lactobacillus plantarum* , a high folate producer Increase of possible pathobionts (*Haemophilus* and *Aggregatibacter spp*). **Difference Metabolic pathways:** Decrease Pyrimidine pathway Decrease to One carbon pool by folate pathway No metabolic differences between pre- and post-menopausal obese women.
https://pubmed.ncbi.nlm.nih.gov/36374244/	Yoshikata R.	Age-Related Changes, Influencing Factors, and Crosstalk Between Vaginal and Gut Microbiota: A Cross-Sectional Comparative Study of Pre- and Postmenopausal Women	*J Womens Health*	2021	Japan	Inclusion criteria: • Menopause established by hormone measurement Exclusion criteria: • Genitourinary symptoms • Irregular menstrual cycle • Unstable ovarian function	16S rRNA V1–V2 region	Case-control study Cross-sectional	Total women=70 Premenopausal women=35 Postmenopausal women=35	NA	37.3 ± 6.1	59.9 ± 4.8	NA	No difference in in α-and β-diversity between groups	No difference	NA	No difference was observed in the gut microbiota composition between pre and postmenopausal women Positive correlation between Equol production and *Firmicutes* and gut microbial diversity
https://pubmed.ncbi.nlm.nih.gov/37118004/	Zhang X.	Sex- and age-related trajectories of the adult human gut microbiota shared across populations of different ethnicities	*Nature Aging*	2021	China	Exclusion criteria: • Participants with intake of drugs for the treatment of TD2DM, hypertension, or dyslipidemia	Whole-genome shotgun seq	Prospective, population-based cohort study	China place in Pinggu (PG)= 1,741 adults: 892 women - 849 men Plus other 3 validated cohorts from previous large metagenomics study: China= 876 Israel= 875 Dutch=1,133	26-76 y	younger (≤50 years; predominantly premenopausal women) and older (>50 years; predominantly postmenopausal women) groups		Diverse ethnicities	Premenopausal women had higher microbial diversity compared to men. The difference decreased in an age-dependent manner, with the microbiota in postmenopausal women becoming more similar to that of men.	Taxa differentially abundant despite gender during aging in the 3 cohorts ↑ *Streptococcus gordonii* ↑ *Lactobacillus salivarius* ↑ *Veillonella atypica* ↑ *Akkermansia muciniphila* (gmGUS) ↑ *Clostridium perfringens* ↑ *Veillonella atypica* ↑ *Butyrivibrio crossotus* ↑ *Lactobacillus salivarius* ↓ *Bifidobacterium adolescentis* (gmGUS) ↓ *Bifidobacterium bifidum* (gmGUS) ↓ *Bifidobacterium breve* (gmGUS)	↑ *Enterobacteriaceae* -encoding genes ↓ genes encoding butyrate biosynthesis ↓ genes encoding β-glucuronidase	**Microbial diversity:** Sexual dimorphism in microbiota composition. Higher alpha diversity in young women compared to men and a higher abundance of beneficial microbes in premenopausal women (such as *A. muciniphila* and numerous SCFA-producers). **Microbial composition in young adults compared to older adults:** Decrease of beneficial taxa (*Bifidobacterium* e.g.: *B. breve, B. bifidum, B. longum, B. adolescentis,* and numerous SCFA-producers) Increase of microbial clusters comprising oral microbiota and possible pathobionts **Correlation with sex hormones**: No significant associations between gut microbiota (microbial diversity, richness, and species abundances) and estradiol levels in pre or postmenopausal women Significant correlation of testosterone with different taxa (positive association with butyrate-producing members and probiotics)
https://pubmed.ncbi.nlm.nih.gov/35675542/	Peters B.A.	Menopause Is Associated with an Altered Gut Microbiome and Estrobolome, with Implications for Adverse Cardiometabolic Risk in the Hispanic Community Health Study/Study of Latino	*mSystem*	2022	USA	Inclusion criteria for menopause • Menstrual periods stopped permanently Inclusion of men: • Men were matched to pre- or postmenopausal women using case-control and nearest-neighbor matching Exclusion criteria for menopause • No systemic or topical HRT • Taking hormonal birth control • Younger than 35 years old • Older than 55 years • Did not have a period within 90 days prior to the visit • Cancer	Whole-genome shotgun seq	Prospective, population-based cohort study	Total participants=16,415 Premenopausal women=295 Postmenopausal women=1,027 Young men=258 Old men=720	NA	40.1 ± 8.8	61.0 ± 7.2	Hispanic/Latino	Postmenopausal women lower α-diversity than premenopausal women Difference in β-diversity between groups Post-menopausal microbiome was more similar to age-matched men than pre-menopausal to age-matched men	**Firmicutes species:** ↓ *Oscillibacter sp. strain KLE1745 (butyrate-producer)* ↓ *Clostridium lactatifermentans (gmGUS)* ↓ *Veillonella seminalis* **Bacteroidetes species:** ↑ *Bacteroides sp. strain Ga6A1* (gmGUS) ↑ *Prevotella marshi* (gmGUS) ↓ *Parabacteroides johnsonii* (gmGUS) **Proteobacteria species:** ↑ *Sutterella wadsworthensis* ↓ *Escherichia coli-Shigella* spp., (gmGUS) ↓ *Escherichia coli* (gmGUS) **Verrucomicrobia species:** ↓ *Akkermansia muciniphila* (gmGUS)	↑ Alpha-hemolysin/cyclolysin transport system ↑ Type III secretion system ↑ Type IV secretion system, ↑ Enterohemorrhagic *E. coli* /enteropathogenic *E. coli* [EHEC/EPEC] pathogenicity signature ↓ Sulphate transport system ↓ β-glucuronidase ortholog ↓ Aryl-sulfatase ortholog	**Microbial diversity:** Postmenopausal women had lower alpha diversity than pre-menopausal women Postmenopausal women had microbiota more similar to men. **Microbial composition in postmenopausal women compared to premenopausal women**: Decrease of estrobolome taxa (*Clostridium lactatifermentans* , *Akkermansia muciniphila)* **Correlation with Estrogen:** Decrease in estrobolome capacity associated with a decrease of b-glucuronidase ortholog Decrease of aryl-sulfatase ortholog **Association of microbiota and diseases**: *Clostridium lactatifermentans* was associated with higher HDL cholesterol and lower waist circumference *Sutterella wadsworthensis* was associated with higher systolic and diastolic blood pressure
https://pmc.ncbi.nlm.nih.gov/articles/PMC11453046/	Gaber M.	Visceral adiposity in postmenopausal women is associated with a pro-inflammatory gut microbiome and immunogenic metabolic endotoxemia	*Microbiome*	2024	USA	Exclusion criteria: • Fewer than six teeth, a bilateral hip replacement • Bone disease or osteoporosis, • Cancer within the previous 10 years, or treatment for serious illness • History of hypertension • TD1DM or TD2DM • HRT	Whole-genome shotgun seq	Prospective, population-based cohort study	Total participants 50 participants n = 25 low VAT area (45.6 ± 12.5 cm^2) n = 25 high VAT area (177.5 ± 31.3 cm^2)	NA	Mean age=78.8 ± 5.3		NA	No significant differences in α- and β-diversity between the VAT groups	↓ Firmicutes/Bacteroidetes ↑ Proteobacteria **Burkholderiales species** ↑ *Emergencia* ↑ *Klebsiella,* ↑ *Parabacteroides* ↑ *Parasutterella* ↑ *Shigella* ↓ *Anaerostipes* ↓ *Barnesiella* ↓*Desulfovibrio,* ↓*Intestinimonas*	NA	**Association of microbiota and diseases:** Proteobacteria (*Escherichia col* i, *Shigella* spp., and *Klebsiella spp* .) and *Veillonella atypica* were associated with both adiposity and inflammation
https://pubmed.ncbi.nlm.nih.gov/32392181/	He J.	Gut microbiota and metabolite alterations associated with reduced bone mineral density or bone metabolic indexes in postmenopausal osteoporosis	*Aging*	2020	China	Exclusion criteria: • Participants with cancer kidney disease • Metabolic or genetic bone disease, • Digestive system disease • Psychiatric disease • No antibiotics in the past 3 months	16S rRNA V3-V4	Case-control study Cross-sectional (Cases = women with Osteoporosis)	Total postmenopausal women=106 Normal BMD=31 Osteoporosis=42 Osteopenia=33	NA	NA	PMO= 59.7±5.5 Osteopenia=57.4±5.1 Normal BMD=57.3±4.0	NA	Bacterial richness lower in PMO	**Enterobacteriaceae family:** ↑ *Klebsiella* ↑ *Escherichia/Shigella* ↑ *Enterobacter* ↑ *Citrobacter* ↑ *Pseudomonas* **Others***:* ↑ *Succinivibrio* ↑ *Desulfovibrio* **Lachnospiraceae family**: ↓ *Blautia* ↓ *Lachnospiraceae_UCG-001* ↓ *Lachnospiraceae_UCG-004* **Others:** ↓ *Fusicatenibacter* ↓ *Prevotella_7*	NA	**Microbial diversity:** Decreased bacterial richness and diversity in postmenopausal osteoporosis **Microbial composition:** Higher abundance of Bacteroides and core SCFA-producers in healthy postmenopausal women Pathobionts were enriched in postmenopausal women with osteopenia and osteoporosis **Correlation with Estrogen**: significant correlation between bacterial diversity and estrogen
https://pubmed.ncbi.nlm.nih.gov/33778322/	Rettedal E.A.	The Gut Microbiome Is Altered in Postmenopausal Women With Osteoporosis and Osteopenia	*JBMR Plus*	2021	New Zealand	Inclusion criteria: • Menopause from at least 5 years based on no menstruation Exclusion criteria: • Systemic disease • Food intolerances that affect the gut • Smokers • High intake of alcohol (>2 units/day) • No antibiotics in the past 1 months • Any treatment for osteoporosis or osteopenia	Whole-genome shotgun seq	Case-control study Cross-sectional (Cases = women with Osteoporosis)	Total Postmenopausal women= 86 Healthy (H)= 18 Osteoporosis (OP) =26 Osteopenia (OPN) = 42	54-81y	NA	NA	NA	No significant difference in α-diversity. The healthy group presented a microbial composition different from the osteoporosis and osteopenia groups	Order ↓ Clostridiales Genus ↑ *Clostridia* ↑ methanogenic archaea *(Methanobacteriaceae)* Genus: ↓ *Rombousia* (*Peptostreptococcus*) ↓ *Clostridium*	NA	**Microbial diversity:** Microbiota dissimilarity between healthy women and women with osteoporosis and osteopenia **Microbial composition:** Higher abundance of SCFA-producers and methanogenic archaea in healthy postmenopausal women Bacteroides are more abundant in osteoporosis and osteopenia
https://pubmed.ncbi.nlm.nih.gov/36348390/	Liu Y.	The relationship between menopausal syndrome and gut microbes	*BMC Women's Health*	2022	China	Inclusion: female, 40–60 years old Inclusion of women in the postmenopausal syndrome group (P group) • At least one of these autonomic nerve changes (hot flashes, night sweats, insomnia, irritability, and other symptoms) • Menstrual disorders (two consecutive cycle length changes > 7 days in 10 months); • Modified KI score > 15 points Inclusion of women in the health postmenopausal women group (H group): • Modified KI score > 15 points • No hot flashes Exclusion criteria: • Irregular vaginal bleeding; • No HRT within 3 months; • No antibiotics within 2 weeks • No history of severe, cardiac, hepatic, renal, mental, or haematological diseases	16S rRNA V3-V4	Case-control study (Cases = women with postmenopausal syndrome)	Total participants=101 Postmenopausal women with MPS= 77 Postmenopausal women without MPS=24	40-60 y	Healthy group 47.88 ± 3.04	Postmenopausal syndrome group 49.55 ± 3.77	NA	No significant α-and β-diversity differences were found between women with and without postmenopausal syndrome	Enriched in healthy postmenopausal: ↑ *Aggregatibacter segnis* ↑ *Bifidobacterium animalis* (gmGUS) ↑ *Acinetobacter guillouiae* Correlated with MPS postmenopausal women ↑ *Ruminococcus torques* (gmGUS) ↑ *Blautia obeum* (gmGUS) ↑ *Butyricicoccus pullicaecorum* (gmGUS) ↓ *Lactobacillus delbrueckii* (gmGUS)	NA	**Association of microbiota and menopausal symptoms:** The menopausal symptoms were positively correlated with *Ruminococcus torques* , *Blautia obeum* and *Butyricicoccus pullicaecorum* , while inversely correlated with *Lactobacillus delbrueckii* **Association of microbiota with hot flash symptoms** Hot flash symptoms were positively related to some pathobionts such as *Ruminococcus torques* , while negatively related to *Clostridium cocleatum* . **Correlation with Estrogen:** E2 had a positive correlation with *Aggregatibacter segnis* , *Bifidobacterium animalis* , *Acinetobacter guillouiae* and decreased in menopausal syndrome

https://pubmed.ncbi.nlm.nih.gov/38832037/	Ji J.	Gut microbiota alterations in postmenopausal women with osteoporosis and osteopenia from Shanghai, China	*PeerJ.*	2024	China	Inclusion criteria: • Menopause of at least 12 months based on no menstruation • Age > 45 Exclusion criteria: • No use of HRT, antibiotics, or antiosteoporosis medications within 6 months before sampling • disability or inability to care for themselves • Disease history of cancer, metabolic bone disease, digestive system disease, kidney disease, psychiatric disease, hyper or hypothyroidism	16S rRNA V3-V4	Cohort Cross-sectional (Cases = women with Osteoporosis and Osteopenia)	Total postmenopausal women=94 Normal BMD (NC)= 23 Osteoporosis (ON)= 44 Osteopenia (OP)=27	NA	NC=59.7 ± 5.5	ON=59.3 ± 6.5 PO=61.7 ± 6.7	NA	No significant differences in α- and β-diversity	**Phylum** ↑ *Fusobacteriota* ↑ *Synergistota* ↑ *Proteobacteria* **Genera** ↓ *Roseburia* ↓ *Clostridia_UCG.014* ↓ *Agathobacter* ↓ *Dialister*	NA	**Microbial diversity:** Difference in α-diversity and β-diversity in women no-PMO compared to PMO-patients **Association of microbiota and osteoporosis:** Pathobionts were negatively associated with low bone mass SCFA-producers were positively associated with high bone mass
https://pmc.ncbi.nlm.nih.gov/articles/PMC11190063/	Yan L.	Characteristics of the gut microbiota and serum metabolites in postmenopausal women with reduced bone mineral density	*Front Cell Infect Microbiol.*	2024	China	Inclusion criteria: • Menopause • Age > 50 Exclusion criteria: • No use of antibiotics, or antiosteoporosis medications within 3 months before sampling • disability or inability to care for themselves • Disease history of cancer, kidney disease, metabolic disease, digestive system disease, psychiatric illness	16S rRNA V4	Case-control study Cross-sectional (Cases = women with Osteoporosis)	Total postmenopausal women=64 Normal (N) n=38 Osteoporosis (OS) n=25	NA	N=65.2 ± 7.5	OS= 63.7± 6.6	NA	Microbial richness was higher in women with osteoporosis. β-diversity different between the two gropus	**Genera** ↓ *Bacteroides* ↓ *Blautia (SCFA-producer)* ↓ *Fusicatenibacter* ↓ *Ruminococcus (butyrate-producer, gmGUS)* ↓ *Anaerostipes (SCFA-producer)* ↑ *Agathobacter* ↑ *Lactobacillus* **Species** ↓ *Bacteroides stercoris* ↓ *Bacteroides plebeius* ↓ *Bacteroides coprocola*	↓ Tryptophan-indole metabolism pathway ↓ Steroid hormone biosynthesis (testosterone) ↑7-Ketocholesterol	**Microbial diversity:** α-diversity and β-diversity were higher in women with PMO compared to women with normal BMD **Association of microbiota and osteoporosis:** *Bacteroides,* and some SCFAs-producers were lower in PMO women *Agathobacter* and *Lactobacillus* were higher in PMO women Tryptophan-indole metabolism pathway decrease in PMO women
https://pubmed.ncbi.nlm.nih.gov/39060403/ in dubbio	Chen T.	The Characteristics of Gut Microbiota and Its Relation with Diet in Postmenopausal Osteoporosis	*Calcified Tissue International*	2024	China	Inclusion criteria: • Menopause from at least 12 months based on no menstruation • Age > 50 • Diagnosis of osteoporosis Exclusion criteria: • No use of antibiotics, or antiosteoporosis medications within 3 months before sampling • Disability or inability to care for themselves • Disease history of cancer, kidney disease, metabolic disease, digestive disorders, individuals with secondary osteoporosis-related ailments (hyperthyroidism, steroid abuse, Cushing's syndrome, hyperparathyroidism)	Shallow shotgun sequencing	Case-control study Cross-sectional (Cases = women with Osteoporosis)	Total postmenopausal women=109 Normal BMD (Con)= 55 Osteoporosis (Op)= 54	NA	60.9±7.2	63.3±6.2	NA	No significant differences in α- and β-diversity	**Genera** ↑ *Anaerostipes* (SCFA-producer) **Species** ↑ *Methanobrevibacter smithii* ↑ *Bifidobacterium animalis* ↑ *Rhodococcus defuvii* ↑ *Lactobacillus plantarum* ↑ *Carnobacterium mobile* ↓ *Bifidobacterium breve* ↓ *Bacillus luciferensis* ↓ *Acetivibrio cellulolyticus* ↓ *Citrobacter amalonaticus*	NA	**Microbial diversity:** No microbial difference between women with PMO and women with normal BMD **Association of microbiota and osteoporosis:** *Methanobrevibacter smithii* was higher in PMO women (associated to a reduction of butyrate in the gut and bone loss) *Bacillus luciferensis* , *Acetivibrio cellulolyticus* , *Citrobacter amalonaticus* , and *Bifidobacterium breve* were lower in women with PMO

Abbreviations:
NA= Not Applicable because the data was not reported
RA= Relative abundance
STRAW= Reproductive Aging Workshop Staging System
HRT= Hormone Replacement Therapy
PMS= Postmenopausal Syndrome
HRT= Hormone Replacement Therapy
Kupperman index (KI)= Menopause Rating Scale
gmGUS= Current gut microbial β-glucuronidase-encoding species
BMD= Bone Mineral Density
T-score= the T-score measures bone density in DEXA scans. T-scores are classified as normal (> -1 SD), osteopenia (-1 to -2.5 SD), or osteoporosis (< -2.5 SD).
Core SCFA-producers include members of the following genera: *Faecalibacterium, Roseburia, Eubacterium, Dorea, Coprococcus and Blautia* .
VAT= visceral adipose tissue
*VVA and GSM markers= vaginal dryness, vulvovaginal irritation, sexual pain, dysuria and urinary urgency
TD1DM or T2DM= Type 1 or Type 2 diabetes mellitus

Supplementary Table 1

Supplementary Table 1A. Metadata and summary of results gathered for articles investigating the vaginal microbiome

PubMed ID	First Author	Title	Journal	Year	Country	Criteria of inclusion/exclusion	Sequencing technology	Study type	Number of enrolled women	Overall age range	Age by category (mean ± SD)			Ethnicity	CSTs classification with dominant species	Proportions of the prominent bacterial communities of CSTs		
											Premenopausal	Perimenopausal	Postmenopausal			premenopausal	perimenopausal	postmenopausal
https://pubmed.ncbi.nlm.nih.gov/22073175/	Hummelen R.	Vaginal microbiome and epithelial gene array in post-menopausal women with moderate to severe dryness	*PlosOne*	2011	Canada	Inclusion criteria: •12 months of amenorrhea Exclusion criteria: •Not used antibiotics •No vaginal infection one month before sampling •No systemic estrogen products 6 months before sampling •No topical estrogen one week before sampling	16S rRNA V6 region	Longitudinal: 2 times sampling over 10 weeks	N total = 32 21 postmenopausal women without VVA 11 postmenopausal women with moderate-severe VVA	42-77 y	NA	NA	59.5±4.5	NA	NA	NA	NA	*L. iners - G. vaginalis* constantly present. Postmenopausal women with **VVA** ↑ *Prevotella timonensis,* ↑ *Porphyromonas uenonis,* ↑ *Peptoniphilus* ↑ *Bacillus* present in
https://pubmed.ncbi.nlm.nih.gov/24080849/	Brotman R.M.	Association between the vaginal microbiota, menopause status, and signs of vulvovaginal atrophy	*Menopause*	2014	USA	Inclusion criteria: • The menopausal stage was determined using the Stages of STRAW Exclusion criteria: •Not currently pregnant intact uterus •No history of organ transplantation •Human immunodeficiency virus infection	16S rRNA V1-V2 region	Cross-sectional prospective cohort study	N total=90 30 premenopausal women 30 perimenopausal women 30 postmenopausal women	35-60 y	40 ± 5.0	40.5± 7.0	50.0± 5.0	White=77.0% African-American=16.1% Asian other race=6.9%	CST I = *L. crispatus* CST II= *L. gasseri* CST III= *L. iners* CSTV= *L. jensenii* CST IV-A=*Streptococcus, Prevotella* CST IV-B=*Gardnerella, Atopium*	46.7% 3.3% 33.3% 0.0% 0.0% 16.7%	20.7% 20.7% 37.9% 3.5% 6.9% 10.3%	25.0% 4.6% 17.9% 7.1% 28.6% 17.9%
https://pubmed.ncbi.nlm.nih.gov/27103314/	Shen J.	Effects of low dose estrogen therapy on the vaginal microbiomes of women with atrophic vaginitis	*Sci Rep*	2016	China	Inclusion criteria: VVA group: • The menopausal stage was determined using STRAW • Obvious GSMs and VVA • FSH > 40 IU/L • E1 < 20 pg/mL • Endometrial thickness ≤ 5 mm • BMI bwteen 18.0 and 35.0 kg/m2 • No smoking • Trichomoniasis or candidiasis healthy post-menopausal group: Exclusion criteria: • No Antibiotics or all sorts of vaginal medications 6 months before sampling • Systemic or chronic diseases	16S rRNA V1-V3 region	Longitudinal: 0, 2, 4 weeks cohort study	N total =59 29 postmenopausal women without AVV 30 postmenopausal women with AVV	NA	NA	NA	Without= 55.8 ± 3.2 With AVV= 55.6 ± 2.6	NA	Cluster I: *Lactobacillus* communities Cluster II: *Prevotella* , *Atopobium* , *Streptococcus, Gardnerella* Cluster III: *Gardnerella, Prevotella, Streptococcus, Atopobium, Anaerococcus, Sneathia, Aerococcus, Peptoniphilus, Dialister* and *Finegoldia* . Low *Lactobacillus* Specifically *Atopobium*	NA NA NA NA	NA NA NA NA	Cluster I= >50% 53.2 ± 40.3 % without VVA 11.2 ± 16.4 % with VVA Cluster II= >10.3% 7.4 ± 13.3 % without VVA 6.0 ± 10.3 % with VVA Cluster III= 39.1% 16.7 ± 21.7 % without VVA 41.7 ± 31.0 % with VVA 2.7 ± 6.9 % without VVA 4.8 ± 5.5 % with VVA
https://pubmed.ncbi.nlm.nih.gov/28640154/	Mitchell C.M.	Vaginal microbiota and genitourinary menopausal symptoms: a cross-sectional and analysis	*Menopause*	2017	USA	Self-reported genitourinary symptoms (≥ 14 hot flashes per week) MsFLASH Eligibility Criteria Applied to All MsFLASH trials	16S rRNA V1–V3 region	Cross-sectional cohort study	N total =88 (all postmenopausal women)	40-62 y	NA	NA	54 ± 4	White=55% African-American=39%	NA	NA	NA	CST I
https://pubmed.ncbi.nlm.nih.gov/30837959/	Gliniewicz K.	Comparison of the Vaginal Microbiomes of Premenopausal and Postmenopausal Women	*Frontiers in microbiology*	2019	USA	Exclusion criteria: • Full hysterectomy, • Irregular menstrual cycle • Skin abnormalities in the vulvar area • TD1DM or TD2DM • Kidney, heart, circulatory disease • Pregnant or breastfeeding • Immunosuppressive or anti- inflammatory therapy	16S rRNA V1–V3 region	Cross-sectional cohort study	N total=45 15 premenopausal women 15 postmenopausal women 15 postmenopausal women under HT	23-67 y	33.0 ± 6.4	NA	60.7 ± 3.6	NA	Cluster A= *L. crispatus* Cluster B= *Gardnerella* Cluster C= *L. iners* Cluster D= *Enterococcus* , *Atopobium* , *Finegoldia* , *Gardnerella* , *Prevotella* , *Streptococcus* Cluster E= *Bifidobacterium* Cluster F= *L. gasseri*	NA	NA	NA
https://pubmed.ncbi.nlm.nih.gov/33903897/	Shardell M.	Association of Vaginal Microbiota With Signs and Symptoms of the Genitourinary Syndrome of Menopause Across Reproductive Stages	*Journals of Gerontology: Biological Sciences*	2021	USA	Inclusion criteria: • The menopausal stage was determined using the Stages of STRAW • Women with intact uterus Exclusion criteria: • Women pregnant or planning to become pregnant during the 2 years of the study •No history of organ transplantation •Human immunodeficiency virus (HIV) infection	16S rRNA V3–V4 region	Mix of cross-sectional and longitudinal cohort study over 2 years	N total= 750 270 premenopausal women 310 perimenopausal women 375 postmenopausal women	35-60 y	40 ± 5.0	40.5 ± 7.0	50.0± 5.0	White=75.5% African-American=17.1% Other=7.5%	CST I = *L. crispatus* CST II= *L. gasseri* CST III= *L. iners* CST IV-A= BVAB1, *G vaginalis* , anaerobes CST IV-B= *G vaginalis* , anaerobes, low BVAB1 CST IV-C= diverse anaerobes: *Streptococcus* , *Anaerococcus* , *Peptoniphilus* and *Finegoldia* CSTV= *L. jensenii*	38.6% 10.2% 23.7% 2.1% 13.5% 5.5% 6.4%	33.0% 9.9 27.0% 2.0% 11.2% 9.7% 7.2%	19.0% 8.4% 18.3% 1.4% 14.8% 33.5% 4.6%
https://pubmed.ncbi.nlm.nih.gov/36374244/	Yoshikata R.	Age-Related Changes, Influencing Factors, and Crosstalk Between Vaginal and Gut Microbiota: A Cross-Sectional Comparative Study of Pre- and Postmenopausal Women	*J Womens Health*	2022	Japan	Inclusion criteria: • Menopause established by hormone measurement Exclusion criteria: • Genitourinary symptoms • Irregular menstrual cycle • Unstable ovarian function	16S rRNA V1–V2 region	Cross-sectional cohort study	N total=70 35 premenopausal women 35 postmenopausal women	27-76 y	37.3 ± 6.1	NA	59.9 ± 4.8	Japanese	CST I = *L. crispatus* CST II= *L. gasseri* CST III= *L. iners* CST V= *L. jensenii* Pathogenic bacteria (CST IV) Others	NA NA NA NA NA	28.9% 5.0% 35.0% 1.0% 16.7% 11.1%	1.1% 1.6% 4.8% 1.0% 26.8% 63.8%
https://pubmed.ncbi.nlm.nih.gov/37788422/	Waetjen L.E.	Relationships between the vaginal microbiota and genitourinary syndrome of menopause symptoms in postmenopausal women: the Study of Women's Health Across the Nation. Menopause	*Menopause*	2023	USA	The menopausal stage was determined using the Stages of STRAW Inclusion criteria: • Women with intact uterus Exclusion criteria: • Women pregnant or planning to become pregnant during the 2 years of the study •No history of organ transplantation •No Human immunodeficiency virus (HIV) infection	16S rRNA V3-V4 regions	Multicentre, prospective cohort study with a cross-sectional survey	N total =1320 Subset of postmenopausal women with microbiome with complete biomarkers=994	60-72	NA	NA	66.5 ± 6.5	Black, Hispanic, Japanese, Chinese, White	CST I = *L. crispatus* CST II= *L. gasseri* CST III= *L. iners* CSTV= *L. jensenii* CST IV= diverse anaerobes *subgroup CTS IV:* CST IV-A, IV-B= different proportion of *G. vaginalis* , BVAB1 CST IV-C0= moderate R.A. of *Prevotella* CST IV-C1= dominated by *Streptococcus* CST IV-C2, IV-C3, IV-C4= other anaerobes	NA NA NA NA NA NA NA NA NA	NA NA NA NA NA NA NA NA NA	10.5% 5.2% 18.9% 1.7% 63.6% 13.8% 28.9% 14.1% 6.8%
https://pubmed.ncbi.nlm.nih.gov/38713891/	Byrne E.H.	Association between vaginal microbiota and vaginal inflammatory immune markers in postmenopausal women	*Menopause*	2024	USA	Inclusion criteria: • The menopausal stage was determined using the Stages of STRAW • Women with intact uterus • at least 1 moderate to severe GSM symptoms Exclusion criteria: • Women pregnant or planning to become pregnant during the 2 years of the study •No history of organ transplantation •No Human immunodeficiency virus infection	16S rRNA V3-V4 regions	Multicentre, prospective cohort study with a cross-sectional survey	N total =119 (all postmenopausal women)	45-70	NA	NA	61± 6.5	NA	*Lactobacillus* dominant CST I= *L. crispatus* *Lactobacillus* no-dominant	NA NA NA	NA NA NA	29.5% 5.7% 70.6%

Abbreviations:
NA= Not Applicable because the data was not reported
BVAB1= *Ca Lachnocurva vaginae*
RA: Relative abundance
STRAW= Reproductive Aging Workshop staging system
HRT= Hormone Replacement Therapy
E1= Estrone
E2= Estradiol
GSM= Genitourinary Syndrome of menopause
VVA= Vulvovaginal atrophy
VMS= Vasomotor symptoms
*VVA and GSM markers= vaginal dryness, vulvovaginal irritation, sexual pain, dysuria and urinary urgency
VMI= Vaginal maturation index
AVV= Vulvar and vaginal atrophy
T2DM=Type 2 diabetes mellitus

Meaningful Results on Vaginal Microbiota
Microbial profile associated with VVA: • Postmenopausal women without VVA had low bacterial diversity dominated by *Lactobacillus spp*, while those with moderate or severe VVA had decreased *Lactobacillus spp* and higher species diversity. • VVA was associated with ↓ of genes involved in maintaining the vaginal barrier function, and ↑ inflammation-related genes
Microbial profile • Premenopausal women: predominantly *Lactobacillus dominat* CSTs, ↑ *L. crispatus* and *L. iners* • Perimenopausal women (transition stage): ↑ *L. iners* and *L. gasseri* ↑ CST IV-A (with *Streptococcus* and *Prevotella*) • Postmenopausal women: Often ↑ CST IV-A, marked by a higher presence of *Streptococcus* and *Prevotella* **Microbial profile associated with VVA:** • ↑ CST IV-A associated with mild to moderate VVA • ↓ *Lactobacillus* spp. associated with increased microbial diversity and higher vaginal pH
Microbial profile • Over 50% of postmenopausal women presented Cluster I (*Lactobacillus* community) *Lactobacillus spp* was present in 83% of women with VVA but with low RA (11.2%) • About 40% of postmenopausal women presented Cluster III (*Gardnerella* community). *Gardnerella spp* was present in 83% of the participants, with high RA with VVA (41.7%) and low RA without VVA (16.7%) Microbial profile under HRT • Estrogen treatment altered the vaginal microbiota, with ↑ *Lactobacillus* (from 11.2% → 71.0%) and ↓ *Gardnerella* (from 41.7% → 9.6%), *Prevotella* and *Atopobium*.
Microbial profile • The majority of postmenopausal women (66%) did not present *Lactobacillus* **Microbial profile associated with sex hormones (E1-E2), glycogen, GMS** • Women with *Lactobacillus*-dominant communities had ↑ unconjugated E1. • No difference in vaginal glycogen levels between women with *Lactobacillus*-dominant and non-*Lactobacillus*-communities • High serum E2 or E1 levels were not associated with either higher vaginal glycogen or detection of individual genera.
Microbial profile under HTR • The vaginal communities of most postmenopausal women receiving HRT (10/15) were dominated by species of lactobacilli and belonged to clusters A (*L.crispatus*), C (*L.iners*), and F (*L. gasseri*) • Postmenopausal women without HT were in cluster D (*Lactobacilli* depleted) • HRT greatly improved vaginal atrophy scores ↓vaginal pH ↓ microbial diversity
Microbial profile: • Premenopausal Women: ↑ *Lactobacillus* species, particularly *L. crispatus* and *L. iners*. • Perimenopausal Women: ↑ *L. iners* ↑ CST IV-C (characterized by anaerobes) and *L. iners* • Postmenopausal Women: low-*Lactobacillus* communities and a significant ↑ of CST IV with **Microbiota associated with VVA**: • Postmenopausal women with CST IV-B (low *Lactobacillus* and high R.A. *G vaginalis*) was associate with VVA
Microbial profile: Postmenopausal women exhibited ↓ levels of *Lactobacillus* and ↑ levels of nonoptimal species, ↑ microbial diversity, and ↑ vaginal pH.
Microbial profile: • 36% of postmenopausal women's microbiomes were dominated by *Lactobacillus* spp. • CST IV was the most frequent (63.6%), with CST IV-C being the most prevalent (49.8%). • Within CST IV-C, 1) CST IV-C0 (28.9%, *Prevotella*-dominant), 2) CST IV-C1 (14%, *Streptococcus*-dominant). • CST IV-C is rare in premenopausal women. **Microbial profile associated with estrogen hormones, VVA* and GSM* biomarkers** • Women with CSTs *Lactobacillus*-dominant (particularly CST I) was associated ↑ E1 and ↑ E2 • Low vaginal pH was associated with CST I, while high vaginal pH was associated with CST IV-C0 • Low VMI was associated with CST IV-C0 and IV-C1
Microbial profile: • Only a minority of postmenopausal women had a microbiome profile dominated by *Lactobacillus spp* • Postmenopausal women with *Lactobacillus spp* dominant profile had: ↓ Low alpha microbial diversity ↓ Concentrations of proinflammatory cytokines (IL-1a, IL-1b, IL-10, IL-8, and IL-6) After menopause *Lactobacilli* have beneficial effects on vaginal immune homeostasis, despite lower prevalence.

Supplementary Table 1B. Metadata and summary of results gathered for articles investigating the gut microbiome

PubMed ID	First Author	Title	Journal	Year	Country	Criteria of inclusion/exclusion	Sequencing technology	Study type	Number of enrolled participants	Overall age range	Age (mean +/- SD)		Ethnicity	Microbial diversity	Taxa differential abundance	Differences in microbial function	Meaningful Results on Gun Microbiota in postmenopausal women
											Premenopausal	Postmenopausal			Postmenopausal women vs premenopausal women	Postmenopausal women vs premenopausal women	
https://pubmed.ncbi.nlm.nih.gov/37341612/	Fuhrman J.B.	Associations of the faecal microbiome with urinary estrogen and estrogen metabolites in postmenopausal women	*J Clin Endocrinol Metab*	2014	USA	Exclusion criteria: • Any medical conditions or use of medicines that impact gut microbiome or hormone levels; • Any history of cancer or intestinal diseases • No systemic estrogen/progesterone use in the past 12 months • No antibiotics in the past 6 months.	16S rRNA pyrosequencing V1-V2 region	Cross-sectional study	Healthy Postmenopausal women=60	55-69 y	NA	NA	NA	α-diversity significantly associated with estrogen	*Firmicutes/Bacteroidetes* ratio *Firmicutes* 65% *Bacteroidetes* 32%	NA	**Correlation with estrogen** *Clostridiales* specifically with *Ruminococcaceae* were positively associated with the estrogen ratio, while *Bacteroides* were negatively associated.
https://pubmed.ncbi.nlm.nih.gov/30244778/	Santos-Marcos J.A.	Influence of gender and menopausal status on gut microbiota	*Maturitas*	2018	Spain	Inclusion criteria postmenopausal • years after the last menstrual cycle: 6.38 ± 1.14; Exclusion criteria: • Population without oncological diseases or disabling diseases • Use of antibiotics in the 3 months prior to sampling	16S rRNA V1-V2 region Illumina MiSeq	Case-control study Cross-sectional	Total participants=76 Premenopausal women=17 Postmenopausal women=20 Young men= 19 Old men =20 Groups matched by BMI and diet	NA	♂ 46.1 ± 0.8 ♀ 46.6 ± 0.6	♂ 55.8 ± 0.6 ♀ 56.2 ± 0.7	NA	No difference in α-and β-diversity between groups Post-menopausal microbiome more similar to age-matched men than pre-menopausal to age-matched men	↑ *Firmicutes* /*Bacteroidetes* ratio **Phylum**: ↑ *Firmicutes* ↓ *Actinobacteria* ↓ *Bacteroidetes* **Genera**: ↑ *Lachnospira* ↑ *Roseburia* (SCFA-producer, gmGUS) ↓ *Prevotella* (SCFA-producer, gmGUS) ↓ *Parabacteroides* (SCFA-producer, sulphate assimilation, gmGUS) ↓ *Biophilia* (sulphate metabolism) ↓ *Ruminococcus* (butyrate-produce)	↓ Pyrimidine pathway ↓ One carbon pool by folate pathways	**Microbial diversity:** Sexual dimorphism in microbiota composition between young women and men Postmenopausal women had microbiota more similar to men No significant difference in bacterial richness and diversity between postmenopausal and premenopausal women **Microbial composition in postmenopausal compared to premenopausal women**: Increased Firmicutes/Bacteroidetes ratio Decreased SCFA-producer genera in postmenopausal women (*Prevotella* , *Parabacteroides* , *Ruminococcus*) **Correlation with estrogen** Gammaproteobacteria positively correlated with E2, while *Prevotellaceae* negatively correlated with E2 Lower inflammatory markers (IL-6 and MCP-1) in the premenopausal women group compared to postmenopausal women and old and young men
https://pubmed.ncbi.nlm.nih.gov/31273779/	Zhao H.	Compositional and functional features of the female premenopausal and postmenopausal gut microbiota	*FEBS*	2019	China	Inclusion criteria • Natural menopause Exclusion criteria • History of chronic serious infection • malignant cancer • No antibiotics in the past 1 months	Single-end metagenomic seq was conducted on the BGISEQ-500 platform	Case-control study Cross-sectional	Total women=48 Premenopausal women=24 Postmenopausal women=24	NA	52.5 ± 5.6	53.9 ± 3.8	NA	α-diversity (Shannon index) ↑ Premenopausal (1.8) ↓ Postmenopausal (1.3) (*p* =5.0e-6)	↑ *Firmicutes* /Bacteroidetes ratio **Phylum:** ↑ *Bacteroidetes* ↑ *Verrucomicrobia* ↓ *Firmicutes* ↓ *Proteobacteria* **Genera** ↑ *Tolumonas* (toluene-producing genus) ↓ *Faecalibacterium* (butyrate-producer, gmGUS) ↓ *Alistipes* (gmGUS, protective effects against CVD, colitis, but marker CRC) ↓ *Eubacterium* (butyrate-producer) ↓ *Roseburia* (butyrate-producer, gmGUS)	↑ Glycolysis, ↑ Lactate consumption, ↑ Cysteine biosynthesis, ↑ Homocysteine degradation ↑ Lysine and arginine degradation ↑ Methionine degradation ↓ Pentose phosphate pathway ↓ Proline degradation process	**Microbial diversity:** Postmenopausal women presented lower richness and diversity than premenopausal women **Microbial composition in post-menopausal compared premenopausal women:** Increased Firmicutes/Bacteroidetes ratio Decreased Firmicutes and Roseburia spp. Decreased SCFAs (*Faecalibacterium* , *Alistipes* , *Eubacterium* , *Roseburia*) Increased Bacteroidetes and the toluene-producing genus Tolumonas.
https://pubmed.ncbi.nlm.nih.gov/31098397/	de la Cuesta-Zuluaga J.	Age- and Sex-Dependent Patterns of Gut Microbial Diversity in Human Adults	*mSystem*	2019	USA / UK Colombia Chinese	Inclusion criteria • BMI between 18.5-30 kg/m Exclusion criteria: • Underweight • Pregnancy • Neurodegenerative diseases, • Current or recent cancer • Gastrointestinal diseases • No history in the past year of inflammatory bowel disease, TD1DM or TD2DM, antibiotic use	16S rRNA V4 region with the Illumina MiSeq platform	Prospective, population-based cohort study (Four large cohort (healthy partecipants)	Total participants (USA) = 3023 Premenopausal women=736 Postmenopausal women=881 Young men= 732 Old men =674 Total participants (UK)= 936 Premenopausal women=195 Postmenopausal women=344 Young men= 173 Old men =224 Total participants (China)= 4963 Premenopausal women=946 Postmenopausal women=1826 Young men= 670 Old men =1521 Total participants (Colombia)= 437 Premenopausal women=143 Postmenopausal women=83 Young men= 133	Young adults (ages 20–45) Middle-aged adults (ages 46–69)	NA	NA	Diverse ethnicities	Premenopausal women had higher α-diversity than young and old men. Reduced α-diversity starts above age 40 years.	NA	NA	**Microbial diversity**: Sexual dimorphism in microbiota composition between young women and men Postmenopausal women had gut microbiota more similar to age-matched men.
https://pubmed.ncbi.nlm.nih.gov/32951609/	Mayneris-Perxachs J.	Gut microbiota steroid sexual dimorphism and its impact on gonadal steroids: influences of obesity and menopausal status	*Microbiome*	2020	Spain	Inclusion criteria: • subjects with obesity (BMI ≥ 30kg/m2) and age- and sex-matched non-obese subjects (18.5 < BMI < 30kg/m2), with an age range of 27.2-66.6 years (n = 131) Exclusion criteria: • TD2DM • Chronic inflammatory systemic diseases • Acute or chronic infections in the previous month • Use of antibiotic, antifungal, antiviral, • No treatment with proton pump inhibitors • Severe disorders of eating behavior or major psychiatric antecedents • Excessive alcohol intake	Whole-genome shotgun seq	• Case-control cross-sectional sudy • Small subgroup (n=81) longitudinal was followed for 1year	Total women=131; after 1-year n=81 Premenopausal women= 44 Postmenopausal women= 45 Middle-age Men = 42	Premenopause women 34.4-48.4 Age-matched man: 39.2-54.3 Postmenopausal women 55.4-59.6	♂ 41.9 [+/-NA] ♀ 46.1 [+/-NA]	♂ 58.6 [+/-NA]	Hispanic	No difference in α-diversity among pre- and post-menopausal women and men, but β-diversity differed among groups	**Bacteroidetes species:** ↑ *Bacteroides sp. CAG:661* , *Bacteroides sp.* ↑ *Mediterranean massilensis* , ↑ *Marseille P3166* , ↑ *Bacteroides thetaiotaomicron CAG 4* 0 ↓ Alistipes ssp. AL-1 , ↓ Alistipes ssp. CAG 29, ↓ Alistipes onderdonkii (small amounts of SCFA, gmGUS) **Firmicutes species:** ↑ *Anaerovibrio spp JC8* ↑ *Veillonella sp. ICM51a*, ↑ *Veillonella dispar*, ↑ *Veillonella sp. DORA_A_3_16_22* ↑ *Dorea sp. CAG:317* ↑ *Lactobacillus rhamnosus (gmGUS)* ↑ *Faecalitalea cylindroides* ↓ *Ruminococcus sp. CAG:379 (butyrate-producer, gmGUS)* ↓*Clostridium neonatal, Clostridium sp. CAG:411 (gmGUS)* ↓ *Firmicutes CAG 534, Firmicutes bacterium CAG:194 (gmGUS)* ↓ *Enterococcus faecalis* ↓ *Eubacterium sp. CAG:603 (butyrate-producer)* ↓ *Blautia sp. CAG:257 (gmGUS)* ↓ *Lactobacillus plantarum (butyrate-producer, gmGUS)* ↓ *Christensenella massiliensis* **Actinobacteria species:** ↓ *Bifidobacterium angulatum (butyrate-producer, gmGUS)* ↓ *Eggerthella sp. HGA1* **Proteobacterium species:** ↑ *Haemophilus, Haemophilus sp. HMSC71H05 Haemophilus sp. HMSC068C11* ↑ *Haemophilus parainfluenza* ↑ *Pasteurellaceae* ↑ *Aggregatibacter actinomycetemcomitans*	↓ Pyrimidine pathway ↓ One carbon pool by folate pathways ↓ bacterial steroid degradation pathway	**Microbial diversity:** No significant difference in bacterial alpha diversity between pre- and post-menopausal women Sexual dimorphism in microbiota between young women and men Postmenopausal women had microbiota more similar to men. **Microbial composition:** Decreased SCFAs producers and gmGUS taxa (*Bifidobacterium* , *Ruminococcus* , *Eubacterium* , *Alipses)* Decreased *Lactobacillus plantarum* , a high folate producer Increase of possible pathobionts (*Haemophilus* and *Aggregatibacter spp*). **Difference Metabolic pathways:** Decrease Pyrimidine pathway Decrease to One carbon pool by folate pathway No metabolic differences between pre- and post-menopausal obese women.
https://pubmed.ncbi.nlm.nih.gov/36374244/	Yoshikata R.	Age-Related Changes, Influencing Factors, and Crosstalk Between Vaginal and Gut Microbiota: A Cross-Sectional Comparative Study of Pre- and Postmenopausal Women	*J Womens Health*	2021	Japan	Inclusion criteria: • Menopause established by hormone measurement Exclusion criteria: • Genitourinary symptoms • Irregular menstrual cycle • Unstable ovarian function	16S rRNA V1–V2 region	Case-control study Cross-sectional	Total women=70 Premenopausal women=35 Postmenopausal women=35	NA	37.3 ± 6.1	59.9 ± 4.8	NA	No difference in in α-and β-diversity between groups	No difference	NA	No difference was observed in the gut microbiota composition between pre and postmenopausal women Positive correlation between Equol production and *Firmicutes* and gut microbial diversity
https://pubmed.ncbi.nlm.nih.gov/37118004/	Zhang X.	Sex- and age-related trajectories of the adult human gut microbiota shared across populations of different ethnicities	*Nature Aging*	2021	China	Exclusion criteria: • Participants with intake of drugs for the treatment of TD2DM, hypertension, or dyslipidemia	Whole-genome shotgun seq	Prospective, population-based cohort study	China place in Pinggu (PG)= 1,741 adults: 892 women - 849 men Plus other 3 validated cohorts from previous large metagenomics study: China= 876 Israel= 875 Dutch=1,133	26-76 y	younger (≤50 years; predominantly premenopausal women) and older (>50 years; predominantly postmenopausal women) groups		Diverse ethnicities	Premenopausal women had higher microbial diversity compared to men. The difference decreased in an age-dependent manner, with the microbiota in postmenopausal women becoming more similar to that of men.	Taxa differentially abundant despite gender during aging in the 3 cohorts ↑ *Streptococcus gordonii* ↑ *Lactobacillus salivarius* ↑ *Veillonella atypica* ↑ *Akkermansia muciniphila* (gmGUS) ↑ *Clostridium perfringens* ↑ *Veillonella atypica* ↑ *Butyrivibrio crossotus* ↑ *Lactobacillus salivarius* ↓ *Bifidobacterium adolescentis* (gmGUS) ↓ *Bifidobacterium bifidum* (gmGUS) ↓ *Bifidobacterium breve* (gmGUS)	↑ *Enterobacteriaceae* -encoding genes ↓ genes encoding butyrate biosynthesis ↓ genes encoding β-glucuronidase	**Microbial diversity:** Sexual dimorphism in microbiota composition. Higher alpha diversity in young women compared to men and a higher abundance of beneficial microbes in premenopausal women (such as *A. muciniphila* and numerous SCFA-producers). **Microbial composition in young adults compared to older adults:** Decrease of beneficial taxa (*Bifidobacterium* e.g.: *B. breve, B. bifidum, B. longum, B. adolescentis,* and numerous SCFA-producers) Increase of microbial clusters comprising oral microbiota and possible pathobionts **Correlation with sex hormones**: No significant associations between gut microbiota (microbial diversity, richness, and species abundances) and estradiol levels in pre or postmenopausal women Significant correlation of testosterone with different taxa (positive association with butyrate-producing members and probiotics)
https://pubmed.ncbi.nlm.nih.gov/35675542/	Peters B.A.	Menopause Is Associated with an Altered Gut Microbiome and Estrobolome, with Implications for Adverse Cardiometabolic Risk in the Hispanic Community Health Study/Study of Latino	*mSystem*	2022	USA	Inclusion criteria for menopause • Menstrual periods stopped permanently Inclusion of men: • Men were matched to pre- or postmenopausal women using case-control and nearest-neighbor matching Exclusion criteria for menopause • No systemic or topical HRT • Taking hormonal birth control • Younger than 35 years old • Older than 55 years • Did not have a period within 90 days prior to the visit • Cancer	Whole-genome shotgun seq	Prospective, population-based cohort study	Total participants=16,415 Premenopausal women=295 Postmenopausal women=1,027 Young men=258 Old men=720	NA	40.1 ± 8.8	61.0 ± 7.2	Hispanic/Latino	Postmenopausal women lower α-diversity than premenopausal women Difference in β-diversity between groups Post-menopausal microbiome was more similar to age-matched men than pre-menopausal to age-matched men	**Firmicutes species:** ↓ *Oscillibacter sp. strain KLE1745 (butyrate-producer)* ↓ *Clostridium lactatifermentans (gmGUS)* ↓ *Veillonella seminalis* **Bacteroidetes species:** ↑ *Bacteroides sp. strain Ga6A1* (gmGUS) ↑ *Prevotella marshi* (gmGUS) ↓ *Parabacteroides johnsonii* (gmGUS) **Proteobacteria species:** ↑ *Sutterella wadsworthensis* ↓ *Escherichia coli-Shigella* spp., (gmGUS) ↓ *Escherichia coli* (gmGUS) **Verrucomicrobia species:** ↓ *Akkermansia muciniphila* (gmGUS)	↑ Alpha-hemolysin/cyclolysin transport system ↑ Type III secretion system ↑ Type IV secretion system, ↑ Enterohemorrhagic *E. coli* /enteropathogenic *E. coli* [EHEC/EPEC] pathogenicity signature ↓ Sulphate transport system ↓ β-glucuronidase ortholog ↓ Aryl-sulfatase ortholog	**Microbial diversity:** Postmenopausal women had lower alpha diversity than pre-menopausal women Postmenopausal women had microbiota more similar to men. **Microbial composition in postmenopausal women compared to premenopausal women**: Decrease of estrobolome taxa (*Clostridium lactatifermentans* , *Akkermansia muciniphila)* **Correlation with Estrogen:** Decrease in estrobolome capacity associated with a decrease of b-glucuronidase ortholog Decrease of aryl-sulfatase ortholog **Association of microbiota and diseases**: *Clostridium lactatifermentans* was associated with higher HDL cholesterol and lower waist circumference *Sutterella wadsworthensis* was associated with higher systolic and diastolic blood pressure
https://pmc.ncbi.nlm.nih.gov/articles/PMC11453046/	Gaber M.	Visceral adiposity in postmenopausal women is associated with a pro-inflammatory gut microbiome and immunogenic metabolic endotoxemia	*Microbiome*	2024	USA	Exclusion criteria: • Fewer than six teeth, a bilateral hip replacement • Bone disease or osteoporosis, • Cancer within the previous 10 years, or treatment for serious illness • History of hypertension • TD1DM or TD2DM • HRT	Whole-genome shotgun seq	Prospective, population-based cohort study	Total participants 50 participants n = 25 low VAT area (45.6 ± 12.5 cm^2) n = 25 high VAT area (177.5 ± 31.3 cm^2)	NA	Mean age=78.8 ± 5.3		NA	No significant differences in α- and β-diversity between the VAT groups	↓ Firmicutes/Bacteroidetes ↑ Proteobacteria **Burkholderiales species** ↑ *Emergencia* ↑ *Klebsiella,* ↑ *Parabacteroides* ↑ *Parasutterella* ↑ *Shigella* ↓ *Anaerostipes* ↓ *Barnesiella* ↓*Desulfovibrio,* ↓*Intestinimonas*	NA	**Association of microbiota and diseases:** Proteobacteria (*Escherichia col* i, *Shigella* spp., and *Klebsiella spp* .) and *Veillonella atypica* were associated with both adiposity and inflammation
https://pubmed.ncbi.nlm.nih.gov/32392181/	He J.	Gut microbiota and metabolite alterations associated with reduced bone mineral density or bone metabolic indexes in postmenopausal osteoporosis	*Aging*	2020	China	Exclusion criteria: • Participants with cancer kidney disease • Metabolic or genetic bone disease, • Digestive system disease • Psychiatric disease • No antibiotics in the past 3 months	16S rRNA V3-V4	Case-control study Cross-sectional (Cases = women with Osteoporosis)	Total postmenopausal women=106 Normal BMD=31 Osteoporosis=42 Osteopenia=33	NA	NA	PMO= 59.7±5.5 Osteopenia=57.4±5.1 Normal BMD=57.3±4.0	NA	Bacterial richness lower in PMO	**Enterobacteriaceae family:** ↑ *Klebsiella* ↑ *Escherichia/Shigella* ↑ *Enterobacter* ↑ *Citrobacter* ↑ *Pseudomonas* **Others**: ↑ *Succinivibrio* ↑ *Desulfovibrio* **Lachnospiraceae family**: ↓ *Blautia* ↓ *Lachnospiraceae_UCG-001* ↓ *Lachnospiraceae_UCG-004* **Others:** ↓ *Fusicatenibacter* ↓ *Prevotella_7*	NA	**Microbial diversity:** Decreased bacterial richness and diversity in postmenopausal osteoporosis **Microbial composition:** Higher abundance of Bacteroides and core SCFA-producers in healthy postmenopausal women Pathobionts were enriched in postmenopausal women with osteopenia and osteoporosis **Correlation with Estrogen**: significant correlation between bacterial diversity and estrogen
https://pubmed.ncbi.nlm.nih.gov/33778322/	Rettedal E.A.	The Gut Microbiome Is Altered in Postmenopausal Women With Osteoporosis and Osteopenia	*JBMR Plus*	2021	New Zealand	Inclusion criteria: • Menopause from at least 5 years based on no menstruation Exclusion criteria: • Systemic disease • Food intolerances that affect the gut • Smokers • High intake of alcohol (>2 units/day) • No antibiotics in the past 1 months • Any treatment for osteoporosis or osteopenia	Whole-genome shotgun seq	Case-control study Cross-sectional (Cases = women with Osteoporosis)	Total Postmenopausal women= 86 Healthy (H)= 18 Osteoporosis (OP) =26 Osteopenia (OPN) = 42	54-81y	NA	NA	NA	No significant difference in α-diversity. The healthy group presented a microbial composition different from the osteoporosis and osteopenia groups	Order ↓ Clostridiales Genus ↑ *Clostridia* ↑ methanogenic archaea *(Methanobacteriaceae)* Genus: ↓ *Rombousia* (*Peptostreptococcus*) ↓ *Clostridium*	NA	**Microbial diversity:** Microbiota dissimilarity between healthy women and women with osteoporosis and osteopenia **Microbial composition:** Higher abundance of SCFA-producers and methanogenic archaea in healthy postmenopausal women Bacteroides are more abundant in osteoporosis and osteopenia
https://pubmed.ncbi.nlm.nih.gov/36348390/	Liu Y.	The relationship between menopausal syndrome and gut microbes	*BMC Women's Health*	2022	China	Inclusion: female, 40–60 years old Inclusion of women in the postmenopausal syndrome group (P group) • At least one of these autonomic nerve changes (hot flashes, night sweats, insomnia, irritability, and other symptoms) • Menstrual disorders (two consecutive cycle length changes > 7 days in 10 months); • Modified KI score > 15 points Inclusion of women in the health postmenopausal women group (H group): • Modified KI score > 15 points • No hot flashes Exclusion criteria: • Irregular vaginal bleeding; • No HRT within 3 months; • No antibiotics within 2 weeks • No history of severe, cardiac, hepatic, renal, mental, or haematological diseases	16S rRNA V3-V4	Case-control study (Cases = women with postmenopausal syndrome)	Total participants=101 Postmenopausal women with MPS= 77 Postmenopausal women without MPS=24	40-60 y	Healthy group 47.88 ± 3.04	Postmenopausal syndrome group 49.55 ± 3.77	NA	No significant α-and β-diversity differences were found between women with and without postmenopausal syndrome	Enriched in healthy postmenopausal: ↑ *Aggregatibacter segnis* ↑ *Bifidobacterium animalis* (gmGUS) ↑ *Acinetobacter guillouiae* Correlated with MPS postmenopausal women ↑ *Ruminococcus torques* (gmGUS) ↑ *Blautia obeum* (gmGUS) ↑ *Butyricicoccus pullicaecorum* (gmGUS) ↓ *Lactobacillus delbrueckii* (gmGUS)	NA	**Association of microbiota and menopausal symptoms:** The menopausal symptoms were positively correlated with *Ruminococcus torques* , *Blautia obeum* and *Butyricicoccus pullicaecorum* , while inversely correlated with *Lactobacillus delbrueckii* **Association of microbiota with hot flash symptoms** Hot flash symptoms were positively related to some pathobionts such as *Ruminococcus torques* , while negatively related to *Clostridium cocleatum* . **Correlation with Estrogen:** E2 had a positive correlation with *Aggregatibacter segnis* , *Bifidobacterium animalis* , *Acinetobacter guillouiae* and decreased in menopausal syndrome

https://pubmed.ncbi.nlm.nih.gov/38832037/	Ji J.	Gut microbiota alterations in postmenopausal women with osteoporosis and osteopenia from Shanghai, China	*PeerJ.*	2024	China	Inclusion criteria: • Menopause of at least 12 months based on no menstruation • Age > 45 Exclusion criteria: • No use of HRT, antibiotics, or antiosteoporosis medications within 6 months before sampling • disability or inability to care for themselves • Disease history of cancer, metabolic bone disease, digestive system disease, kidney disease, psychiatric disease, hyper or hypothyroidism	16S rRNA V3-V4	Cohort Cross-sectional (Cases = women with Osteoporosis and Osteopenia)	Total postmenopausal women=94 Normal BMD (NC)= 23 Osteoporosis (ON)= 44 Osteopenia (OP)=27	NA	NC=59.7 ± 5.5	ON=59.3 ± 6.5 PO=61.7 ± 6.7	NA	No significant differences in α- and β-diversity	**Phylum** ↑ *Fusobacteriota* ↑ *Synergistota* ↑ *Proteobacteria* **Genera** ↓ *Roseburia* ↓ *Clostridia_UCG.014* ↓ *Agathobacter* ↓ *Dialister*	NA	**Microbial diversity:** Difference in α-diversity and β-diversity in women no-PMO compared to PMO-patients **Association of microbiota and osteoporosis:** Pathobionts were negatively associated with low bone mass SCFA-producers were positively associated with high bone mass
https://pmc.ncbi.nlm.nih.gov/articles/PMC11190063/	Yan L.	Characteristics of the gut microbiota and serum metabolites in postmenopausal women with reduced bone mineral density	*Front Cell Infect Microbiol.*	2024	China	Inclusion criteria: • Menopause • Age > 50 Exclusion criteria: • No use of antibiotics, or antiosteoporosis medications within 3 months before sampling • disability or inability to care for themselves • Disease history of cancer, kidney disease, metabolic disease, digestive system disease, psychiatric illness	16S rRNA V4	Case-control study Cross-sectional (Cases = women with Osteoporosis)	Total postmenopausal women=64 Normal (N) n=38 Osteoporosis (OS) n=25	NA	N=65.2 ± 7.5	OS= 63.7± 6.6	NA	Microbial richness was higher in women with osteoporosis. β-diversity different between the two gropus	**Genera** ↓ *Bacteroides* ↓ *Blautia (SCFA-producer)* ↓ *Fusicatenibacter* ↓ *Ruminococcus (butyrate-producer, gmGUS)* ↓ *Anaerostipes (SCFA-producer)* ↑ *Agathobacter* ↑ *Lactobacillus* **Species** ↓ *Bacteroides stercoris* ↓ *Bacteroides plebeius* ↓ *Bacteroides coprocola*	↓ Tryptophan-indole metabolism pathway ↓ Steroid hormone biosynthesis (testosterone) ↑7-Ketocholesterol	**Microbial diversity:** α-diversity and β-diversity were higher in women with PMO compared to women with normal BMD **Association of microbiota and osteoporosis:** *Bacteroides*, and some SCFAs-producers were lower in PMO women *Agathobacter* and *Lactobacillus* were higher in PMO women Tryptophan-indole metabolism pathway decrease in PMO women
https://pubmed.ncbi.nlm.nih.gov/39060403/ in dubbio	Chen T.	The Characteristics of Gut Microbiota and Its Relation with Diet in Postmenopausal Osteoporosis	*Calcified Tissue International*	2024	China	Inclusion criteria: • Menopause from at least 12 months based on no menstruation • Age > 50 • Diagnosis of osteoporosis Exclusion criteria: • No use of antibiotics, or antiosteoporosis medications within 3 months before sampling • Disability or inability to care for themselves • Disease history of cancer, kidney disease, metabolic disease, digestive disorders, individuals with secondary osteoporosis-related ailments (hyperthyroidism, steroid abuse, Cushing's syndrome, hyperparathyroidism)	Shallow shotgun sequencing	Case-control study Cross-sectional (Cases = women with Osteoporosis)	Total postmenopausal women=109 Normal BMD (Con)= 55 Osteoporosis (Op)= 54	NA	60.9±7.2	63.3±6.2	NA	No significant differences in α- and β-diversity	**Genera** ↑ *Anaerostipes* (SCFA-producer) **Species** ↑ *Methanobrevibacter smithii* ↑ *Bifidobacterium animalis* ↑ *Rhodococcus defuvii* ↑ *Lactobacillus plantarum* ↑ *Carnobacterium mobile* ↓ *Bifidobacterium breve* ↓ *Bacillus luciferensis* ↓ *Acetivibrio cellulolyticus* ↓ *Citrobacter amalonaticus*	NA	**Microbial diversity:** No microbial difference between women with PMO and women with normal BMD **Association of microbiota and osteoporosis:** *Methanobrevibacter smithii* was higher in PMO women (associated to a reduction of butyrate in the gut and bone loss) *Bacillus luciferensis* , *Acetivibrio cellulolyticus* , *Citrobacter amalonaticus* , and *Bifidobacterium breve* were lower in women with PMO

Abbreviations:
NA= Not Applicable because the data was not reported
RA= Relative abundance
STRAW= Reproductive Aging Workshop Staging System
HRT= Hormone Replacement Therapy
PMS= Postmenopausal Syndrome
HRT= Hormone Replacement Therapy
Kupperman index (KI)= Menopause Rating Scale
gmGUS= Current gut microbial β-glucuronidase-encoding species
BMD= Bone Mineral Density
T-score= the T-score measures bone density in DEXA scans. T-scores are classified as normal (> -1 SD), osteopenia (-1 to -2.5 SD), or osteoporosis (< -2.5 SD).
Core SCFA-producers include members of the following genera: *Faecalibacterium, Roseburia, Eubacterium, Dorea, Coprococcus and Blautia* .
VAT= visceral adipose tissue
*VVA and GSM markers= vaginal dryness, vulvovaginal irritation, sexual pain, dysuria and urinary urgency
TD1DM or T2DM= Type 1 or Type 2 diabetes mellitus