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THE EFFECT OF EXERCISE AND NUTRITION ON THE GUT MICROBIOME OF PERI- AND POSTMENOPAUSAL WOMEN

by

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Author’s Contribution Statement

I certify that I am the author of this thesis and that any assistance I have had in its preparation is fully acknowledged and referenced in the following text. I have also cited any sources from which I have used data, ideas or words, whether they are quoted exactly or paraphrased. I also certify that this thesis was prepared by me personally specifically for the requirements of obtaining a Master's degree in Lifestyle Medicine by the University of Thessaly. I declare that I will respect co-authorship rights in any linked scientific publication and/or presentation stemming from this thesis, in accordance to my signed affirmation, and in respect of the principles of ethics in research. Likewise, I declare I will recognize any funding sources and will acknowledge my affiliation to the University of Thessaly during the preparation of this work in any future presentation.

Pamela Somers

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Abstract

Introduction: The gut microbiome is important for maintaining human health and contributes to the healthy aging of menopausal women. However, the decline in estrogen during the menopausal transition significantly affects the gut microbiome. These shifts in gut microbiome are associated with menopausal symptoms such as systemic inflammation, metabolic disorders and cardiovascular disease. Both dietary habits and exercise have been shown to have a beneficial impact on the gut microbiota.

Aims: The aim of this study was to evaluate the effect of exercise and nutrition on the gut microbiome of peri-menopausal and menopausal women.

Methodology: This literature study was conducted using the PubMed Advanced Builder, focusing on publications from 2015 to 2025. Studies included specific diets (e.g. ketogenic diet, mediterranean diet, low carbohydrate diet, high fiber diet) and exercise (e.g. physical activity, aerobic training, strength training, resistance training).

Results: The Mediterranean (MED)-diet has the most beneficial effects on the gut microbiome. This can be combined with the Dietary approaches to Stop Hypertension (DASH)- diet to prevent leaky gut. Physical activity plays a role in increasing gut microbial diversity and function. High intensity interval training + resistance training (HIIT + RT) have the greatest effect on obesity related parameters in postmenopausal women. However, individual responses to nutrition and exercise vary based on the baseline microbiota.

Discussion: Individuals who respond well to diets or interventions “responders” typically have a more diverse microbiome, characterized by higher alpha-diversity. These findings reinforce the increasing need for personalized nutrition/exercise approaches as some individuals respond favorably to a lifestyle intervention whereas other not. This thesis

presents the guidelines to set-up a personalized female health plan to optimize the gut microbiome in peri- and postmenopausal women. Ideally, this personalized health plan would encourage peri-menopausal women to implement lifestyle changes before any significant shifts in the gut microbiome occur.

Keywords: peri-menopause, menopause, gut microbiome, exercise, nutrition.

Abstract

Inleiding: Het darmmicrobioom is belangrijk voor het behoud van de gezondheid van de mens en draagt bij aan het gezond ouder worden van vrouwen in de menopauze. De afname van oestrogeen tijdens de overgang naar de menopauze heeft echter een significante invloed op het darmmicrobioom. Deze verschuivingen in het darmmicrobioom worden in verband gebracht met symptomen van de menopauze, zoals systemische ontstekingen, stofwisselingsstoornissen en hart- en vaatziekten. Van zowel voedingsgewoonten als lichaamsbeweging is aangetoond dat ze een gunstige invloed hebben op de darmmicrobiota.

Doel: Het doel van deze studie was om het effect van lichaamsbeweging en voeding op het darmmicrobioom van perimenopauzale en menopauzale vrouwen te evalueren.

Methodologie: Deze literatuurstudie werd uitgevoerd met behulp van de PubMed Advanced Builder, gericht op publicaties van 2015 tot 2025. Studies omvatten specifieke diëten (bv. ketogeen dieet, mediterraan dieet, koolhydraatarm dieet, vezelrijk dieet) en lichaamsbeweging (bv. lichaamsbeweging, aerobe training, krachttraining, weerstandstraining).

Resultaten: Het Mediterraan (MED)-dieet heeft de meest gunstige effecten op het darmmicrobioom. Dit kan worden gecombineerd met het Dietary approaches to Stop Hypertension (DASH)-dieet om een lekkende darm te voorkomen. Lichaamsbeweging speelt een rol bij het vergroten van de diversiteit en functie van het darmmicrobioom. Intervaltraining met hoge intensiteit + weerstandstraining (HIIT + RT) hebben het grootste effect op obesitasgerelateerde parameters bij postmenopauzale vrouwen. Individuele reacties op voeding en lichaamsbeweging variëren echter op basis van de baseline microbiota.

Discussie: Personen die goed reageren op diëten of interventies “responders” hebben doorgaans een meer divers microbioom, gekenmerkt door een hogere alfa-diversiteit. Deze bevindingen versterken de toenemende behoefte aan een gepersonaliseerd voeding-/bewegingsplan, aangezien sommige individuen gunstig reageren op een leefstijlinterventie en anderen niet. Deze thesis presenteert de richtlijnen voor het opstellen van een gepersonaliseerd gezondheidsplan voor vrouwen om het darmmicrobioom te optimaliseren bij peri- en postmenopauzale vrouwen. Idealiter zou dit persoonlijke gezondheidsplan peri-

menopauzale vrouwen aanmoedigen om veranderingen in hun leefstijl door te voeren alvorens er significante verschuivingen in het darmmicrobioom optreden.

Kernwoorden: peri-menopauze, menopauze, darmmicrobioom, lichaamsbeweging, voeding.

Περίληψη

Εισαγωγή: Το μικροβίωμα του εντέρου είναι σημαντικό για τη διατήρηση της ανθρώπινης υγείας και συμβάλλει στην υγιή γήρανση των γυναικών στην εμμηνόπαυση. Ωστόσο, η μείωση των οιστρογόνων κατά τη μετάβαση στην εμμηνόπαυση επηρεάζει σημαντικά το μικροβίωμα του εντέρου. Αυτές οι μετατοπίσεις στο μικροβίωμα του εντέρου σχετίζονται με συμπτώματα της εμμηνόπαυσης, όπως η συστηματική φλεγμονή, οι μεταβολικές διαταραχές και οι καρδιαγγειακές παθήσεις. Έχει αποδειχθεί ότι τόσο οι διατροφικές συνήθειες όσο και η άσκηση έχουν ευεργετικό αντίκτυπο στο μικροβιόκοσμο του εντέρου.

Σκοπός: Σκοπός της παρούσας μελέτης ήταν να αξιολογηθεί η επίδραση της άσκησης και της διατροφής στο μικροβίωμα του εντέρου των περι-εμμηνοπαυσιακών και εμμηνοπαυσιακών γυναικών.

Μεθοδολογία: Η μεσογειακή δίαιτα (MED) έχει τα πιο ευεργετικά αποτελέσματα στο μικροβίωμα του εντέρου. Αυτή μπορεί να συνδυαστεί με τη δίαιτα Dietary approaches to Stop Hypertension (DASH)- dietary approaches to Stop Hypertension (DASH)- diet για την πρόληψη της διαρροής του εντέρου. Η σωματική δραστηριότητα παίζει ρόλο στην αύξηση της ποικιλομορφίας και της λειτουργίας των μικροβίων του εντέρου. Η διαλειμματική προπόνηση υψηλής έντασης + προπόνηση αντίστασης (HIIT + RT) έχουν τη μεγαλύτερη επίδραση στις παραμέτρους που σχετίζονται με την παχυσαρκία σε μετεμμηνοπαυσιακές γυναίκες. Ωστόσο, οι ατομικές αποκρίσεις στη διατροφή και την άσκηση ποικίλλουν ανάλογα με το βασικό μικροβιόκοσμο.

Αποτελέσματα: Η δίαιτα MED έχει τις πιο ευεργετικές επιδράσεις στο μικροβίωμα του εντέρου. Αυτή μπορεί να συνδυαστεί με τη δίαιτα DASH για την πρόληψη της διαρροής του εντέρου. Η σωματική δραστηριότητα παίζει ρόλο στην αύξηση της ποικιλομορφίας και της λειτουργίας των μικροβίων του εντέρου. Η διαλειμματική προπόνηση υψηλής έντασης + προπόνηση αντίστασης (HIT + RT) έχουν τη μεγαλύτερη επίδραση στις παραμέτρους που σχετίζονται με την παχυσαρκία σε μετεμμηνοπαυσιακές γυναίκες. Ωστόσο, οι ατομικές αντιδράσεις στη διατροφή και την άσκηση ποικίλλουν ανάλογα με το βασικό μικροβιόκοσμο.

Συζήτηση: Τα άτομα που ανταποκρίνονται καλά στις δίαιτες ή στις παρεμβάσεις «ανταποκριτές» έχουν συνήθως ένα πιο ποικιλόμορφο μικροβίωμα, το οποίο

χαρακτηρίζεται από υψηλότερη α-ποικιλότητα. Τα ευρήματα αυτά ενισχύουν την αυξανόμενη ανάγκη για εξατομικευμένες προσεγγίσεις διατροφής/άσκησης, καθώς ορισμένα άτομα ανταποκρίνονται ευνοϊκά σε μια παρέμβαση στον τρόπο ζωής, ενώ άλλα όχι. Η παρούσα διατριβή παρουσιάζει τις κατευθυντήριες γραμμές για τη δημιουργία ενός εξατομικευμένου γυναικείου σχεδίου υγείας για τη βελτιστοποίηση του εντερικού μικροβιώματος σε γυναίκες που βρίσκονται σε περι- και μετεμμηνοπαυσιακή περίοδο. Ιδανικά, αυτό το εξατομικευμένο σχέδιο υγείας θα ενθάρρυνε τις περιεμμηνοπαυσιακές γυναίκες να εφαρμόσουν αλλαγές στον τρόπο ζωής πριν από την εμφάνιση σημαντικών αλλαγών στο μικροβίωμα του εντέρου.

Λέξεις -κλειδιά: περιεμμηνόπαυση, εμμηνόπαυση, εντερικό μικροβίωμα, άσκηση, διατροφή.

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List of abbreviations

ALT: alanine aminotransferase

AOX: anti-oxidant

AST: aspartate aminotransferase

BMD: bone mineral density

BMI: body mass index

BMR: basal metabolic rate

CDSA: comprehensive digestive stool analysis

CH: carbohydrate

CRP: c-reactive protein

CVD: cardiovascular disease

DASH: dietary approaches to stop hypertension

FBDG: food based dietary guidelines

FFM: fat-free mass

FSH: follicle-stimulating hormone

GGT: gamma-glutamyl transferase

GI: gastro-intestinal

HDL: high density lipoprotein

HIIT: high intensity interval training

HOMA-IR: homeostatic model assessment of insulin resistance

H_2S: hydrogen sulfide producing bacteria

IBD: inflammatory bowel disease

IBS: irritable bowel syndrome

IFAB: intestinal fatty acid binding protein

IR: insulin resistance

LBP: lipopolysaccharide binding protein

LDL : low-density lipoprotein cholesterol

LDL: low density lipoprotein

LH: luteinizing hormone

LPB: lipopolysaccharide-binding protein

LPS: lipopolysaccharides

MED: mediterranean

MET: metabolic equivalent of task

MVPA: moderate to vigourous physical activity

NAFLD: non-alcoholic fatty liver disease

PA: physical activity

PFHP: personalized female health plan

RT: resistance training

sCD14: soluble CD14

SCFA: short chain fatty acids

SIgA: secretory IgA

SIBO: small intestinal bacterial overgrowth

SMM: skeletal muscle mass

SRB: sulphate reducing bacteria

T3: thyroxine

T4: triiodothyronine

T2DM: type 2 diabetes mellitus

TMAO: trimethylamine N-oxide

TSH: thyroid stimulating hormone

TRP: tryptophan metabolites

VAT: visceral adipose tissue

1. Introduction

As life expectancy increases, the number of menopausal women worldwide is increasing, with an estimated 1.2 billion worldwide by 2030 [1]. Menopause is defined as the point in time when a women has not had a menstrual period for 12 consecutive months, marking the end of her reproductive years. Most women experience menopause between 45 and 54 years of age [2].

Peri-menopause, the period leading up to and one year after menopause, is characterized by the fluctuation and decline of key hormones estrogen and progesterone, which can cause menopausal symptoms in women. The most common symptoms are menstrual irregularities, including amenorrhoea, vasomotor symptoms ("hot flashes" and "night sweats"), genito-urinary symptoms, as well as mental fatigue, "brain fog", joint pain and insomnia can negatively impacts women's wellbeing and quality of life [3]. Hormonal changes - responsible for both physical and psychological effects - begin even earlier, during menopausal transition, the period associated with declining fertility in women over 40 years old [4].

The most obvious change in menopausal women is the decline in ovarian function, resulting in lower estrogen and progesterone levels. After menopause, peripheral tissues (mainly adipose tissue) become the primary producers of estrogens, derived from adrenal androgen precursors [5]. Due to the decrease in estrogen levels, the risk and incidence of several chronic diseases increases. These include cardiovascular disease (CVD), insulin resistance (IR), type 2 diabetes (T2DM), osteoporosis, and hormone-sensitive tumors, especially breast cancer [4,6]. Peri-menopause and menopause are often associated with dysbiosis and gastrointestinal tract (GI) complaints. Moreover, studies have indicated that estrogen levels can affect gut microbiota [7-8].

The microbiota refers to all the commensal microorganisms that inhabit the body, including bacteria, archaea, viruses, and unicellular eukaryotes. The largest microbial community resides in the human gut where the total number of bacterial cells ranges from 10^{13} to 10^{14}. The collection of genes within this microbiota is known as the "gut microbiome".

Four major microbial phyla represent over 90% of the gut microbiota: **Firmicutes**, **Bacteroidota**, **Proteobacteria**, and **Actinobacteriota**. The **Firmicutes** phylum includes genera such as *Lactobacillus*, *Bacillus*, *Clostridium*, *Enterococcus*, and *Ruminococcus*. The

Bacteroidota phylum (synonym Bacteroidetes) is represented by genera such as *Bacteroides* and *Prevotella*. The **Actinobacteriota** phylum, with its main genus *Bifidobacterium*, and the **Proteobacteria** phylum are less abundant [9-10]. When there is an imbalance of either of these phyla of bacteria, dysbiosis can result.

An individual's gut microbiome composition undergoes dynamic changes throughout their lifetime. The most significant shifts occur early in life, particularly during weaning, when microbiome diversity increases markedly as the diet transitions from breastmilk to solid food [11-12]. A second major shift of the gut microbiome may take place during puberty and adolescence, with the emergence of sex differences in gut microbiome composition, strongly implicating sex hormones in its development [13].

In adulthood, gut microbiome diversity tends to increase with age, reflecting age-related changes in immunity, mucosal function, diet, metabolism, and disease burden. This microbiome diversity appears to plateau around the age of 40 [14], while the overall composition of the gut microbiota becomes increasingly individualized with advancing age [15]. Notably, the gut microbiome of extremely long-lived individuals (i.e. nonagenarans and centenarians) differs from that of younger adults [12]. Features like high microbial uniqueness and low *Bacteroides* dominance have been related to healthy aging and longevity.

Sex-based differences in the gut microbiota persist into adulthood. Women generally exhibit higher gut microbiome richness and lower *Prevotella* abundance compared to men [16-17]. Interestingly, women display higher microbiome diversity than men in early adulthood, a difference that diminishes with age, suggesting that microbiome aging is influenced by menopause [14]. Menopause itself is associated with reduced microbiome diversity and a compositional shift toward greater similarity with the male gut microbiome.

While the sex hormone milieu appears to impact the gut microbiome, the gut microbiota are also involved in regulating free circulating hormone levels, suggesting a bi-directional relationship. The microbiome can affect serum estrogen levels, as certain gut microbes - collectively known as the estrobolome - produce β-glucuronidase, a bacterial enzyme that deconjugates estrogens and phytoestrogens. This process converts them into their active forms, which can be reabsorbed in the intestine and enter the bloodstream. Dysbiosis, an imbalance in the gut microbiota, can reduce the activity of the estrobolome, thereby contributing to decreased levels of circulating active estradiol metabolites [18]. This

underscores the potential role of gut microbiota composition in modulating the onset and progression of menopause-related diseases (i.e. osteoporosis, breast cancer, and cardiometabolic diseases) [19].

The gut microbiota perform numerous functions that contribute to health homeostasis or disease development. These include the metabolism of dietary components (e.g. fiber, amino acids) as well as endogenous compounds (e.g. bile acids, hormones). Additionally, they are involved in the biosynthesis of lipopolysaccharides (LPS), a structural component of gram-negative bacterial cell walls that can trigger inflammation [20]. Metabolites and signaling molecules produced by the gut microbiota can influence systems far beyond the GI tract, including the central nervous system via the “gut microbiota-brain axis”. Studies in humans have identified associations between gut microbiome composition and a wide range of conditions and diseases, including obesity, inflammatory bowel disease, colorectal cancer, depression, diabetes, and CVD [21]. Reduced microbial diversity of gut microbiome – often indicative of dysbiosis – is linked to Western lifestyle and various disease states [22].

Short chain fatty acids (SCFA) are the metabolic end products of bacterial fermentation, which have an effect on host health. [23] Short chain fatty acids like propionate, acetate, and butyrate affect carbohydrate fermentation and play a role in the regulation of intestinal motility, as well as, anti-inflammatory function with prevention of leaky gut. Indole degradation of the amino acid tryptophan increases epithelial-cell tight junction resistance and reduces inflammatory markers. The gut microbiome also plays a role in different vitamin synthesis like Vitamin K, B12, biotin, folate which are co-factors for various metabolic pathways [23].

The gut microbiota influence human health not only through their metabolic activities but also via microbial translocation, a process in which dysfunction of the gut epithelial barrier permits microbes and microbial products to pass from the intestinal lumen into systemic circulation [23]. In the healthy GI tract, multiple defense layers, including the mucus layer, epithelial tight junctions, and resident immune cells such as macrophages, prevent such translocation. However, under conditions such as immunodeficiency, pathogenic infection, or intestinal inflammation, the structural integrity of the gut barrier can become compromised. This disruption facilitates microbial translocation, which triggers systemic immune activation and inflammation and has been implicated in the pathogenesis of numerous diseases [24]. Experimental evidence suggests that estradiol and progesterone

play key roles in maintaining intestinal barrier integrity and limiting microbial translocation [25]. A recent study showed that plasma intestinal fatty acid binding protein (IFAB: a marker of gut epithelial cell function), lipopolysaccharide binding protein (LBP: a marker of microbial translocation), and soluble CD14 (sCD14: a marker of immune activation related to microbial translocation) increased significantly within women from pre- to post-menopause, with lower plasma estradiol associated with higher IFAB and sCD14 [26].

The gut microbiome plays a vital role in various aspects of health. While the composition of the adult gut microbiome is generally considered relatively stable, it can be influenced by aging and age-related physiological changes such peri-menopause and menopause [27]. Several studies [28] have reported that estrogen deficiency and postmenopausal state are associated with changes in the gut microbiome composition. Specifically, postmenopausal individuals tend to exhibit a lower abundance of phylum Firmicutes and the genus Roseburia, along with a higher abundance of the phylum Bacteroidota and the genus Tolumonas, compared to their premenopausal counterparts.

Dysbiosis of the gut microbiome has been linked to various diseases resulting from the presence of harmful bacteria and their byproducts. Dysbiosis can be influenced by genetic factors, lifestyle habbits, diet including ultra-processed foods and food additives, as well as medications. Dysbiosis has been associated with many systemic diseases and can not be diagnosed through standard blood tests or investigations. Microbiota derived metabolites can be analyzed and can be useful in the management of dysbiosis [23].

Comprehensive digestive stool analysis (CDSA) include the analysis different microbiota such as lactobacilli, bifidobacteria, E. Coli, Proteus, Salmonella, Shigella, Vibrio, yeast and microbiome analysis including sequencing technologies, dysbiosis index, metagenomics, metatranscriptomics as well as assessment of microbial metabolites like SCFA. It also includes hydrogen breath test, which detects the presence of gases produced by bacteria in the small intestine and excessive gases indicate imbalance of bacteria [23]

Microbiome analysis using metrics of markers of dybiosis include alpha-diversity and beta-diversity as well as distribution of predominant phyla. The three alpha-diversity indices (Shannon index, Simpson’s Index, Chao-1 Index) and beta-diversity metrics like Bray-Curtis distance will be evaluated. Alpha diversity, which indicates the relative abundance of microbial species in a biological sample, where as beta diversity and gamma diversity

measures species diversity over time. [29] Dysbiosis indexes have to be interpreted in the contecxt of clinical findings [30].

Dysbiosis results in increased inflammation, elevated levels of zonulin, destruction of intestinal tight junctions, and intestinal permeability, which allow LPS to leak into systemic circulation. LPS is a powerful endotoxin that causes chronic inflammation throughout the body. Chronic inflammation is associated with chronic diseases and the acceleration of biological aging [31].

Gut microbiota can function like an endocrine organ with bioactive metabolites like SCFA, trimethylamine N-oxide (TMAO), tryptophan metabolites (TRP) which can circulate in the human blood and be delivered to different target tissues. TMAO, p-cresyl sulfate and indoxyl sulfate have pro-inflammatory effects and may contribute to chronic inflammatory diseases. To treat chronic diseases, a strategy using gut microbiota derived metabolites may be helpful.

The menopausal transition (peri-menopause through postmenopause) significantly affects the gut microbiome, with implications for both GI and systemic health. As estrogen levens drop, the composition of the gut microbiota shifts, often leading to dysbiosis. This menopausal microbiome may promote a pro-inflammatory state, contributing to systemic low-grade inflammation (inflammaging), which is linked to metabolic syndrome, T2DM, IR, osteoporosis and CVD. It is stated that these changes can be reduced by lifestyle changes.

Dietary habits are one of the primary factors influencing the gut microbiome [32]. Additionally, exercise has been shown to have a beneficial impact on the gut microbiota. The aim of this thesis is to evaluate the effects of both exercise and nutrition on the gut microbiome of peri- and postmenopausal women. Based on the findings, the goal is to set up a personalized lifestyle program to optimize the gut microbiome for this demographic. Ideally, the program would encourage peri-menopausal women to implement lifestyle changes before any significant shifts in the gut microbiome occur.

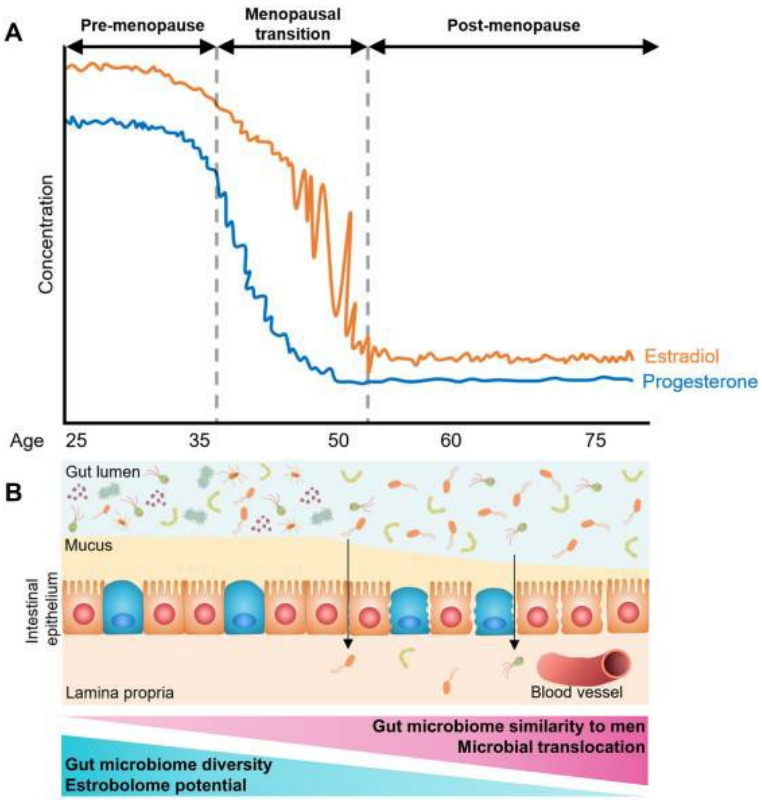


Figure 1: Summary of putative changes in the human gut microbiome related to menopause. (**A**) Trajectory of estradiol and progesterone concentration during a woman's adulthood, showing declines during the menopausal transition and low levels post-menopause. (**B**) Diagram of putative gut microbiome and gut epithelium changes during menopause. With declining estradiol and progesterone, diversity of the gut microbiome and estrobolome potential is reduced, and microbiome composition becomes more similar to men. Additionally, declines in estradiol and progesterone may lead to permeability of the gut barrier, allowing microbial translocation to occur [31].

2. Aims – Significance

Previous studies have suggested that estrogen levels may influence the composition of the intestinal microbiota, indicating that changes in the gut flora could be linked to the onset of menopause. These shifts in the gut microbiome may also be associated with menopausal symptoms such as osteoporosis, metabolic abnormalities, depression, Alzheimers's disease, and cardiovascular disease [6,20].

Dietary habits are one of the primary factors influencing the gut microbiome [32]. Additionally, exercise has been shown to have a beneficial impact on the gut microbiota. The aim of this thesis is to evaluate the effects of both exercise and nutrition on the gut microbiome of peri- and postmenopausal women. Based on the findings, the goal is to set up a personalized lifestyle program to optimize the gut microbiome for this demographic. Ideally, the program would encourage peri-menopausal women to implement lifestyle changes before any significant shifts in the gut microbiome occur.

The added value of this thesis would be to encourage peri-menopausal women in their 40s to undergo a lifestyle assessment, beginning with an evaluation of their gut microbiome. Based on these results, a personal lifestyle plan can then be developed. An example of such a personalized female health plan (PFHP) will be presented in this thesis. The goal is to present this strategy alongside gynaecologists and general practitioners to encourage women to adopt preventive measures for their health and well-being.

3. Literature Review

During peri-menopause and menopause, declining levels of sex hormones – particularly estrogen – lead to significant changes in female metabolism. One major consequence is a decrease in basal metabolic rate (BMR), which can drop as much as 250-300 kcal per day. If lifestyle factors such as diet and physical activity remain unchanged, this metabolic shift may result in an average annual weight gain of approximately 2kg [33]. This weight gain typically manifests as an increase in abdominal (visceral) fat mass [34]. Currently, there is a reduction in fat-free mass (FFM) and skeletal muscle mass (SMM), contributing to the development of sarcopenia. When both excess fat accumulation and loss of muscle mass/function occur together, the condition is referred to as sarcopenic obesity, a pathological body composition state with serious health implications.

Moreover, this excess visceral fat storage leads to the increased secretion of pro-inflammatory signaling molecules and induces a local and low-grade systemic inflammation [35]. This low grade inflammation is due to increased LPS levels which is termed ‘metabolic endotoxemia’ [36].

Increased visceral adipose tissue (VAT) in postmenopausal women is associated with a change in the gut microbiome composition, namely an elevated Proteobacteria abundance, lower Firmicutes/Bacteroidetes ratio and higher immunogenic metabolic endotoxemia markers [36].

The nutrition of the population of developed countries is still far from food-based dietary guidelines (FBDG) [37]. Based on WHO’s data from 2016, 40% of women are overweight and another 15% are obese [38]. Unhealthy Western nutrition is characterized by high fat (38% of energy) and salt intake (9.2g/day in women in the age group of 50-59 years) [39], low vegetable and fruit consumption [40], and low calcium (730+-277mg/day) intake [41].

A diet rich in plant proteins, such as the Mediterranean diet, leads to signifcant increase in anti-inflammatory butyrate-producing bacteria, bacterial diversity and a reduction in pro-inflammatory bacteria [42]. Therefore, this thesis will review the existing literature on the effects of nutrition and exercise on the gut microbiome of peri- and postmenopausal women to identify the specific nutrient/dietary pattern and/or exercise

intervention that can promote the growth of beneficial gut microbiota populations leading to the production of bioactive metabolites.

3.1 Effect of nutrition on the gut microbiome during peri- and postmenopause.

The impact of nutrition on the gut microbiota during menopause is categorized into different sections based on specific menopause-related symptoms and/or diseases.

3.1.1 Dietary strategies for prevention of CVD during menopause.

As a women transitions into menopause, changes in hormonal concentrations and body composition dramatically increase overall cardiometabolic risk. Dietary intervention is one of the most effective lifestyle modifications for reducing CVD risk. However, evidence supporting popular diets like the Dietary Approaches to Stop Hypertension (DASH) and the Mediterranean diet (MED) in postmenopausal women remains limited. Both diets improve CVD risk biomarkers, including total cholesterol and low-density lipoprotein cholesterol (LDL), reduce oxidative stress and inflammation, and enhance endothelial function. Moreover, higher intake of certain individual foods and nutrients (i.e., low fat skimmed milk) has been associated with later menopause onset [43], improvements in menopause symptoms (i.e., fiber) [44], and better CVD risk factors (i.e., blueberries) [45].

Hypertension is the leading CVD risk factor and is estimated to affect approximately 75% of postmenopausal women. A specific dietary intervention – characterized by low sodium consumption (60-70mmol/day) and a low-acid, fruit- and vegetable rich diet – has been shown to reduce blood pressure in postmenopausal women. This underscores the importance of tailored dietary strategies for disease prevention in this population.

The DASH diet is a well-established dietary pattern known to reduce blood pressure across diverse populations [46]. Although the literature is limited regarding the beneficial effects of a DASH diet in postmenopausal women, a systematic review and meta-analysis suggest that the DASH diet approach may be both acceptable and feasible for women transitioning through menopause, offering potential for CVD prevention [47].

The menopause transition is often accompanied by changes in dietary patterns, notably an increase in saturated fat and dietary cholesterol intake [48]. Compared to high fat diets, low fat diets have been shown to improve blood lipid profiles in overweight postmenopausal women within 3 to 8 weeks [49].

Postmenopausal women have an increased risk of endothelial dysfunction, partly due to heightened oxidative stress and elevated levels of proinflammatory cytokines, which can contribute to the development of atherosclerosis [50-51]. The mediterranean diet (MED) diet - rich in nuts, olive oil, fruits - has been shown to reduce oxidative stress and improve endothelial function [52]. In a cross-sectional study involving 176 menopausal women, higher adherence to the MED diet was associated with reduced visceral fat. Additionally, this dietary pattern also improves blood lipid profiles and blood pressure in postmenopausal women with metabolic syndrome [49,53]. Moreover, consumption of isoflavone-rich soy foods can increase levels of gut microbiota-derived estrogen metabolites, which in turn may help reduce markers of systemic inflammation.

3.1.2 Dietary intervention for postmenopausal hot flashes.

Postmenopausal vasomotor symptoms not only cause discomfort and a reduced quality of life, but are also asscociated with an increased risk of CVD and diabetes [54]. Nutrition is a key modifying factor and may play a role in alleviating these symptoms. The Women's Health Initiative demonstrated that low-fat diet rich in whole grains, fruits and vegetables, increased the likelihood of being free from hot flashes at one year by 14% - after adjusting for changes in body weight - and by 23% among women who lost at least 10% of body weight [55].

In a previously published 12-week randomized clinical trial, a low-fat, plant-based diet that included daily consumption of soybeans (86g per day) reduced the frequency of moderate-to-severe postmenopausal hot flashes by 88%, and eliminated all moderate-to-severe hot flashed in 50% of the participants [56].

The beneficial effects of a plant-based diet on vasomotor symptoms in menopausal women may be partly mediated by changes in gut microbiome composition – specifically through the promotion of gut bacteria capable of converting daidzein to equol, an estrogen receptor-beta agonist. Individuals following a plant-based diet are more likely to produce equol compared to omnivores.

The study of Kahleova et al. [57] evaluated the effects of a low-fat plant-based diet (including daily consumption of soybeans 86g/day) on gut microbiome composition, with a particular focus on equol-producing gut bacteria and their associations with postmenopausal vasomotor symptoms.

The dietary intervention led to 95% reduction in total hot flashes and a 96% decrease in moderate-to-severe hot flashes. The relative abundance of *Porphyomonas* and *Prevotella corporis* decreased on the intervention diet, and this decrease was associated with the reduction in severe daytime hot flashes. This is a novel finding that has not been previously described [57]. *Prevotella corporis* has been identified in the gut of people with rheumatoid arthritis and appears to have pro-inflammatory properties. It may also be isolated during dental procedures such as root canals, and plays a signifcant role in the development of dental cavities [58].

The decrease in *Clostridium asparagiforme* may be a positive ouctome, as this bacterium produces TMAO, a proatherogenic metabolite, linked to CVD and diabetes [59]. The association with hot flahes is a novel finding. The reduced abundance of *Clostridium asparagiforme* may partially explain the beneficial effects of a plant-based diet on cardiovascular health and offer a potential link between hot flashes and the incidence of CVD [60].

F*usincatenibacter* is typically associated with gut health and has been shown to induce anti-inflammatory cytokines while reducing C-reactive protein (CRP) [61]. Traumatic acid, an anti-oxidant (AOX) found in bean plants and related to injury respsonse, is also positively correlated with the abundance of *Fusincatenibacter* [61-62]. Furthermore, *Fusincatenibacter* can be modulated by prebiotics, particularly supplementation with guar gom, which has been demonstrated to improve menopausal symptoms [63]. Fiber-fermenting bacteria, such as these in the *Fusincatenibacter* genus, are capable of producing SCFA, which have anti-inflammatory effects and may a role in preventing degenerative neurological disease [64].

Fusincatenibacter saccharivorans is also increased on a plant-based diet and is an anaerobic sugar fermenter [65]. This increase may be associated with enhanced breakdown of plant polysaccharides. The higher levels of *F. saccharivoran*s may indicate a reduction in intestinal inflammation, which could have contributed to the reduction in hot flashes observed in this study.

The *Holdemanella* genus increased in abundance on a low fat, plant based diet, which is consistent with existing studies that show increased abundance with a high-fiber diet [66]. Fibrous foods are typically associated with greater satiety, which may contribute to weight loss.

3.1.3 Prunes to maintain hip bone mineral density in postmenopausal women.

Menopause is associated with a rapid decline in BMD and deterioration in bone quality, which compromises bone strength and increases the risk of osteoporosis and fragility fractures in postmenopausal women. Calcium and Vit D supplementation are considered standard nutritional therapies for managing fracture risk [67]. However, there is growing evindence in leveraging specific foods and bioactive food components to help mitigate bone loss and reduce fracture risk. Foods rich in phenolic compounds, such as prunes, blueberries, and soybeans may offer protective effects against postmenopausal bone loss [68]. While the exact mechanisms underlying the osteoprotective effects of phenolic-rich foods and supplements are not fully understood, they are thought to be partly attributed to the ability of host and/or microbial phenolic metabolites to alter endogenous AOX capacity, to exert anti-inflammatory effects, or provide prebiotic-like modulation of the gut microbiome [69-70].

The post-hoc analysis of Simpson et al. [71] aimed to explore the relationship between the gut microbiome, immune responses and bone-protective effects of prunes in postmenopausal women. Women who consumed 50-100g prunes daily were divided into two groups - responders (n=20) and non-responders (n=32) - based on percent change in total hip BMD.

After 12 months of prune consumption, responders exhibited significantly lower levels of anti-inflammatory markers IL-β1 and TNF-α. Sequence analysis revealed that the gut microbiome of responders and non-responders differed in both alpha and beta diversity metrics, before and after prune treament. Notably, responders had higher abundance of bacterial families *Oscillospiraceae* and *Lachnospiraecea* ($p<0.05$). These findings suggest that postmenopausal women with low baseline BMD can benefit from prune supplementation, particularly if they harbor specific gut microbial profiles [71].

The gut microbiome is increasingly recognized as key modulator of the effects of diet on BMD. Observational studies have demonstrated that low BMD is associated with alterations in the gut microbiome composition. The gut microbiome may influence bone health through several mechanisms, including nutrient absorption, metabolite bioavailability, and immune system regulation [72-73].

The primary distinction in the gut microbiome between responders and non-responders was the presence of phylogenetically diverse taxa, a difference that was maintained throughout the 12-month prune intervention. Responders exhibited higher alpha-diversity, which may reflect greater functional diversity and a heightened capacity to metabolize prune components into beneficial metabolites. This concept is supported by models such as equol production from soy, which depends on the presence of specific bacterial species with the metabolic capacity to convert daidzein into equol [74]. In this study *Oscillospiraceae* - detected in responders - was negatively correlated with pro-inflammatory markers IL-β1 and TNF-α. Additionally, *Moryella*, a member of the *Lachnospiraceae* family, emerged as one of the key taxa mediating the health benefits of prune consumption. Members of *Lachnospiraceae* are known producers of SCFAs, which pkay a role in reducing inflamaton and supporting bone health [75]. In particular, *Lachnospiraceae* was most abundant in the group consuming 50g of prunes daily – the same group that demonstrated the greatest bone-protective effects.

This study suggests that, when combined with Vitamin D and calcium supplementation, prunes serve as a promising whole-food nutritional intervention for maintaining BMD in postmenopausal women. In the context of precision nutrition, baseline characteristics indicate that individuals with lower BMD, higher fecal microbiota alpha diversity, and the presence of specific bacterial taxa such as *Oscillospiraeceae* are more likely to benefit incorporating prunes into their diet for bone health [71].

Responders exhibited a more diverse gut microbiome, including the presence of Oscillospiraceae, which exert anti-inflammatory effects by attenuating IL-β1 and TNF-α secretion from peripheral blood mononuclear cells. This reduction in systemic inflammation may help support BMD [76].

3.1.4 Optimizing the estrobolome.

The aggregate of bacterial genes capable of deconjugating and recycling estrogens is referred to as the “estrobolome”. This microbial function may contribute to the retention of estrogen in the body, alleviating hormonal symptoms associated with menopause [77].

Enzymes associated with the estrobolome are expressed across a diverse range of microbes and multiple bacterial phyla, particularly within *Firmicutes* and *Bacteroidetes*, which commonly express β-glucuronidase enzymes [78].

In a large study (n=2300), alpha-diversity analysis revealed that premenopausal women tended to have higher gut microbial diversity compared to postmenopausal women ($p=0.06$) and a significantly higher diversity to both young and older men ($p=0.03$). Postmenopausal women did not significantly differ from men in terms of alpha-diversity. Beta-diversity analysis showed significant difference in gut microbiome composition between premenopausal and postmenopausal women. These findings suggest that menopause is associated with a shift in the gut microbiome towards a composition that is more similar to that of men [79].

B-glucuronidase abundance is significantly higher in premenopausal compared to postmenopausal women, suggesting that the estrobolome potential –specifically, the deconjugation activity toward sex steroid hormones – may be diminished after menopause, likely due to reduced estrogen availability [80].

In a recent study involving 200 postmenopausal women, higher serum estrogen levels were associated with greater gut microbiome alpha-diversity. Beta-diversity analysis revealed that serum levels of oestradiol and oestrone were significantly associated with overall microbiota composition [81]. Estrogen-related bacterial species such as *Alistipes* show a strong correlation with β-glucuronidase abundance. These findings suggest that in postmenopausal women, higher serum oestrogen levels are linked to greater gut microbial diversity [81]. However, further research is needed to clarify the health implications, potential effects on menopause symptoms, and the role of diet and lifestyle on modulating this relationship.

3.1.5 Menopause is associated with postprandial metabolism, metabolic health and lifestyle: ZOE predict study.

Postmenopausal status is associated with unfavoubale changes in body composition, fasting and postprandrial blood profiles, dietary patterns, sleep quality and gut microbiome composition. This study identified a mediating effect of diet and specific gut baterial species on visceral fat accumulation, glycemia and systemic inflammation. These findings suggest that the adverse metabolic changes observed during menopause may be mitigated through modulation of the gut microbiome and dietary interventions [82].

The gut microbiota plays a key role in metabolizing estrogen-like compounds found in plant foods, such as phytoestrogen. These include lignans - present in a variety of plant-based foods - and isoflavonoids, which are abundant in soy products [83]. In postmenopausal women, the administration of isoflavone-rich soy foods have been shown to elevate levels of gut microbial-derived estrogen-like metabolites [84]. This dietary intervention is also associated with shifts in gut microbiota composition, including increased abundance of *Bifidobacterium* and decreased levels of *Clostridiaceae*, the latter of which are implicated in inflammatory conditions [83].

This study identified shifts in species abundance following menopause, including increases in pro-inflammatory and obesogenic bacteria. In this cohort, postmenopausal females reported higher intake of dietary sugars and poorer sleep quality – both of which are associated with exaggerated postprandial glycemic responses and elevated risk for T2DM and CVD [82]. Additionally, a decline in physical activity and an increase in sedentary behavior observed in postmenopausal women have been linked to the direct effects of declining estrogen levels [85].

3.2 The effect of exercise on the gut microbiome.

3.2.1 Equol-producing bacteria influence the effects of exercise training.

Reduced arterial compliance in the cardiovascular system (i.e. carotid artery and aorta) has been identified as an independent risk factor for CVD [86]. In women, arterial compliance declines significantly following menopause [87]. Consequently, identifying lifestyle factors that can help prevent vascular stiffening in postmenopausal women is of critical importance.

As previously described, isoflavones are the most bioactive compounds in soy foods. They possess estrogen-like effects and are classified as phenolic compounds that may complement the action of estrogen after menopause [88]. Epidemiological studies have reported an inverse relation between dietary intake of soy isoflavone and the risk of coronary heart disease [89-90]. Moreover, soy isoflavones have been shown to improve arterial compliance [91]. However, findings from intervention trials remain inconclusive [92-93].

One possible explanation is interpersonal variation in the composition of intestinal microbiota. Equol, a metabolite produced by the gut microbiome from soy isoflavone daidzein, may be a key determinant of anti-atherosclerotic effects of soy isoflavones. Notably, equol exhibits stronger estrogen-like activity and AOX properties than other isoflavones [94]. The conversion of daidzein to equol occurs both in the small intestine and the colon. Importantly, only individuals with equol-producing bacteria appear to benefit from soy or isoflavone intake [95].

Regular aerobic exercise is one of the most effective strategies for improving vascular function in postmenopausal women. However, estrogen presence and/or estrogen status appear to play a key modulatory role in how vascular function adapts to exercise training in this population [96].

In the study by Hayashi et al. [95], the interactive effects of isoflavone intake, aerobic exercise, and equol-producing status on central arterial compliance in post-menopausal women was investigated. A total of 43 postmenopausal women were assigned to one of the two intervention groups: (1) aerobic exercise combined isoflavone supplementation (n=27), or (2) isoflavone supplementation alone (n=16). The exercise intervention consisted of an 8-week aerobic training involving walking or jogging on a treadmill or cycling on an ergometer for 25-30 minutes per day. Isoflavone supplemention was provided via a soy protein bar containing approximately 20mg of soy isoflavones. Equol-producing status (equol producers or non producer) was assessed before and after the intervention.

The primary finding was that isoflavone dietary intervention alone did not influence central arterial compliance, regardless of equol- producing status. However, when isoflavone intake was combined with aerobic exercise, carotid artery compliance significantly increased in equol producers but not in the non producers. This is the first study to suggest that equol-producing status is an important factor modulating vascular adaptation to aerobic exercise training [95].

3.2.2 Effect of training on gut microbiota in overweight or obese postmenopausal women.

Menopause is associated with an increased risk of obesity and abdominal fat mass (FM), along with a reduction in intestinal microbial diversity. The prevalence of obesity is significantly higher in postmenopausal compared to premenopausal women [96].

Diet is the primary factor for shaping the gut microbiota [97], but growing evidence suggests that regular physical activity (PA) is also a significant modulator of gut microbial community structure and diversity [98-100]. Higher levels of PA have been associated with greater fecal bacterial diversity and an increased abundance of certain bacterial phyla, as well as elevated levels of SCFAs in the faeces of healthy adults [101]. In contrast, aging – and particularly menopause – has been linked to a decline in intestinal microbial diversity, a change that may contribute to the higher prevalence of obesity in this population [102].

The study of Dupuit et al. [103] investigated the effects of a combined high-intensity interval training and resistance training (HIIT + RT) program on body composition and gut

microbiota composition in postmenopausal women with overweight or obesity. Over 12 weeks, participants in the training group showed significant improvements in physical fitness (e.g., increased maximal oxygen consumption and peak power output), reductions in total abdominal and visceral FM, and gains in segmental muscle mass compared to sedentary controls.

While the HIIT+RT protocol did not alter alpha-diversity or overall microbial taxonomy, it significantly influenced beta-diversity, indicating changes in the gut microbiota composition. Similarly, Zhong et al [104] reported that a 2-month program, 4-day-per-week combined aerobic and RT program modified beta-diversity in postmenopausal women aged 60-75. Also Rashti et al., [105] confirmed that compared to moderate intensity continuous training + RT, HIIT + RT has a greater effect on obesity related parameters in postmenopausal women.

Importantly, individual responses to exercise appeared to vary based on the baseline microbiota profiles, highlighting the existence of “nonresponders” - individuals who do not exhibit expected physiological adaptations to training.

Recent findings suggest that baseline microbiota composition can predict the effectiveness of HIIT + RT interventions, reinforcing the potential role of the gut microbiome in mediating exercise-induced changes in body composition.

3.2.3 Correlation of daily physical activity and gut microbiome in perimenopause.

Physical activity refers to any bodily movement that results in energy expenditure above the basic metabolic rate. The intensity of physical activity (PA) qualitatively – categorized as low, moderate, or high – is commonly assessed using metrics such as moderate-to-vigorous physical activity (MVPA), metabolic equivalents (METs), and associated metabolic indicators, including BMI, body fat percentage, and energy expenditure [106-107]. However, studies investigating the relationship between daily PA levels in menopausal women and changes in the gut microbiome composition, along with related metabolites, remain limited.

Zheng et al. [108] investigated the correlation between daily physical activity levels and gut microbiota in perimenopausal women compared to younger women. The study found that peri-menopausal women exhibited significantly lower levels of daily PA, including reduced

caloric expenditure (Kcals), METs and MVPA. Notably, the composition of the gut microbiota differed markedly between the two groups. At the phylum levels, the relative abundance of *Acidobacteria*, *Cholorflexi*, *Nitrospirae,* and *Gemmatimonadetes* were significantly lower in perimenopausal women. At the genus level, *Collinsella*, *Ruminococcus gnavus*, *Rothia*, *Haemophilus*, *Sphingomonas*, *Lactobacillales*, and *Lactococcus* were also reduced. In contrast, the genera *Phascolarctobacteium, Paraprevotella*, *Acinetobacter*, *Flavonifractor,* and *Intestimonas* were significantly increased in perimenopausal group.

Acidobacteria, *Cholorflexi*, and *Nitropirae* (all nitrite-oxidizing bacteria) [109] showed a significant positive correlation with METs values, as well as a moderate positive association with moderate-to-high intensity PA and average daily energy expenditure. This suggests that these non-dominant phyla may be involved not only in carbohydrate (CH) and nitrogen metabolism, but also as effector phyla modulated by daily PA.

Lactobacillales and Lactococcus are considered beneficial bacteria and are commonly used in the production of probiotics and fermentated dairy products. Interestingly, *Collinsella*, *Rothia Lactobacillales*, and *Lactococcus* were significantly reduced in perimenopausal women, suggesting that age-related alterations in gut microbiota composition may be linked to an increased risk of abnormal glucolipid metabolism, CVD and osteoporosis.

Spinghomonas was also significantly downregulated in perimenopausal women. This genus showed a significant positive correlation with daily PA related indicators such as energy utilization, moderate to vigoruous PA, and METs, indicating that reduced PA may contribute to its decreased abundance.

The abundance of *Phascolarctobacterium* and *Acinetobacter* was elevated in perimenopausal women compared to younger women, and showed a negative correlation with METs, suggesting a potential association with aging, weight gain, and altered host metabolism.

Other genera, includingas *Paraprevotella*, *Flavonifractor*, and I*ntestimonas* were enriched in the perimenopausal women. However, the precise function of *Intestimonas* remains unclear.

4. Methodology

4.1. Literature Searching Plan

This study was undertaken using the PubMed advanced search builder, with a publication period of 2015 to 2025. Studies involving obese adult subjects were included. This review did not include subjects with genetic disorders, autoimmune diseases or cancer.

To review the effect of nutrition and exercise on the gut microbiome of peri- and postmenopausal women, a search was done based on several keywords. The following combinations of keywords were used:

- Nutrition OR mediterranean diet OR ketogenic diet OR low carbohydrate diet OR high fiber diet.

- Exercise OR physical activity OR resistance training OR strength training OR aerobic training OR sport.

- Gut microbiome OR gut microbiota OR intestinal flora OR gut diversity OR gut dysbiosis.

- Menopause OR menopausal women OR peri-menopause OR peri-menopausal women OR perimenopause OR perimenopausal women OR menopausal transition.

The initial search combining all 4 keywords and synonyms - nutrition, exercise, gut microbiome and menopause - yielded no results. This indicates that no studies have simultaneously investigated the combined effects of nutrition and exercise on the gut microbiome in menopausal women.

To broaden the scope, the search was divided into two separate queries focusing on either nutrition or exercise. The search examining the effect of exercise alone on the gut microbiome in menopausal women retrieved 8 articles. The search investigating the nutritional effects on the gut microbiome in this population yielded 9 articles.

In total, 15 relevant articles were identified based on these search strategies.

4.2. Articles Inclusion criteria

Research question: Studies that investigate the effect of nutrition and exercise on the gut microbiome of peri- and postmenopausal women.

Population: Studies that include adult women from 40 – 75 years old. No animal studies.

Intervention: Studies that include specific diets (e.g. ketogenic diet, mediterranean diet, low carbohydrate diet, high fiber diet) and exercise (e.g. physical activity, aerobic training, strength training, resistance training).

Outcome measures: Studies that characterize fecal microbiota through DNA sequencing and alpha- and beta- diversity metrics.

Study design: Both observational as experimental studies are included.

4.3. Articles Exclusion criteria

Irrelevant studies: Three studies were excluded from the literature review on nutrition. One was based on the probiotic ingestion (supplementation) with specific bacteria and its effect on estrogen and testosteron levels, rather than examining the gut microbiome after intake of nutrition. The other study investigated the effect of genetic polymorphisms on precision nutrition medicine in perimenopausal women. The third study was a study performed in rats.

Five articles were excluded from the literature search on exercise. Two studies evaluated the effect on cancer patients and two studies were performed on rats. Another study evaluated the effect of dietary fiber on mental health/depression in menopause.

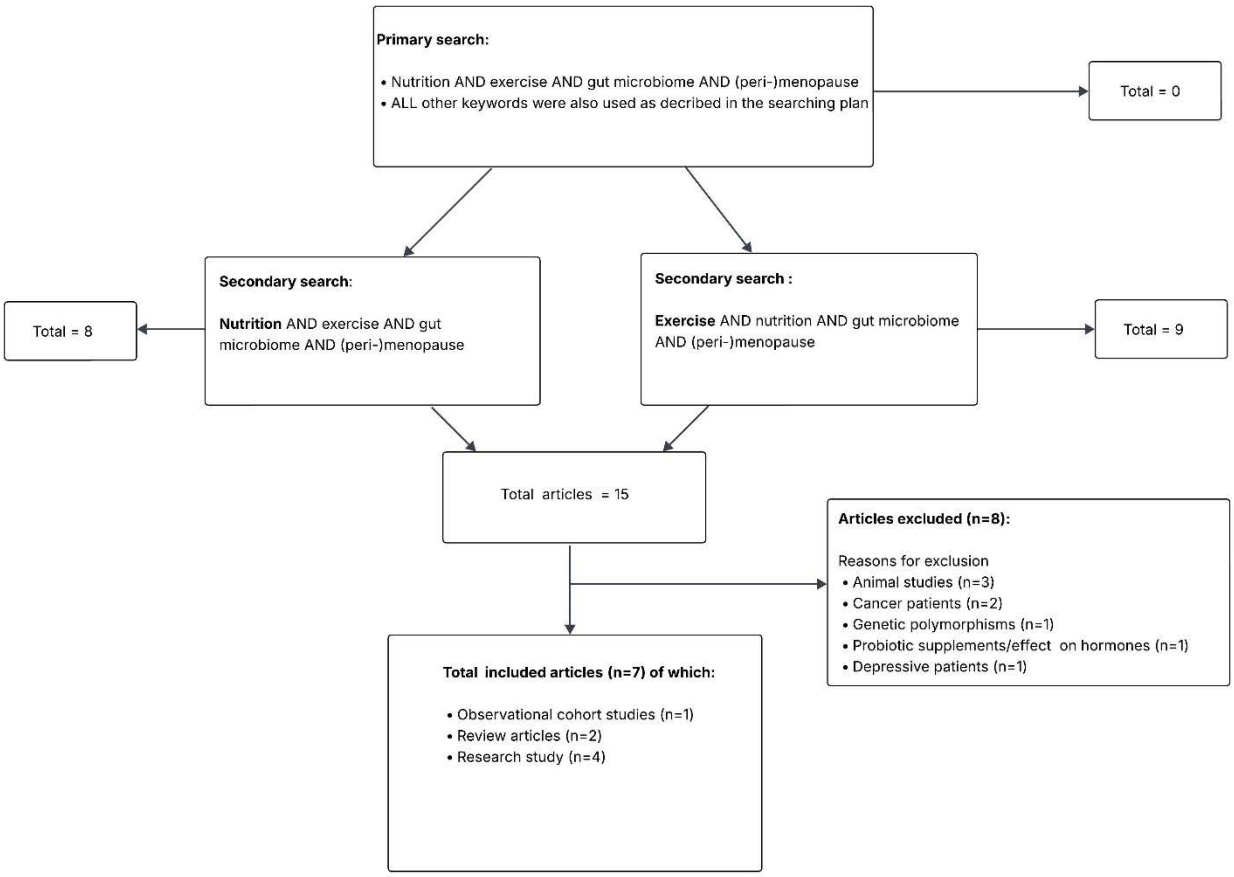
Methodological flaws: Culture-based methods for evaluating the composition of the gut microbiome are excluded. These can not be used to identify bacterial groups in clinical situations.

Biases: Women using hormone replacement therapy (HRT) were excluded from the study. HRT can influence the gut microbiota composition by affecting hormone levels, immune function and metabolism.

Duplicate studies: No duplicate studies were identified in this search.

4.4. Flow Chart

Of the 15 articles, the search identified 7 articles that we included in this review: 1 observational study, 2 review articles and 4 research articles. This process is summarized in the flowchart below.



Flowchart : Process of inclusion/exclusion of studies on exercise and nutrition on the gut microbiome of peri- and postmenopausal women.

5. Results

5.1. Gut microbiota composition: Peri-menopause vs Menopause.

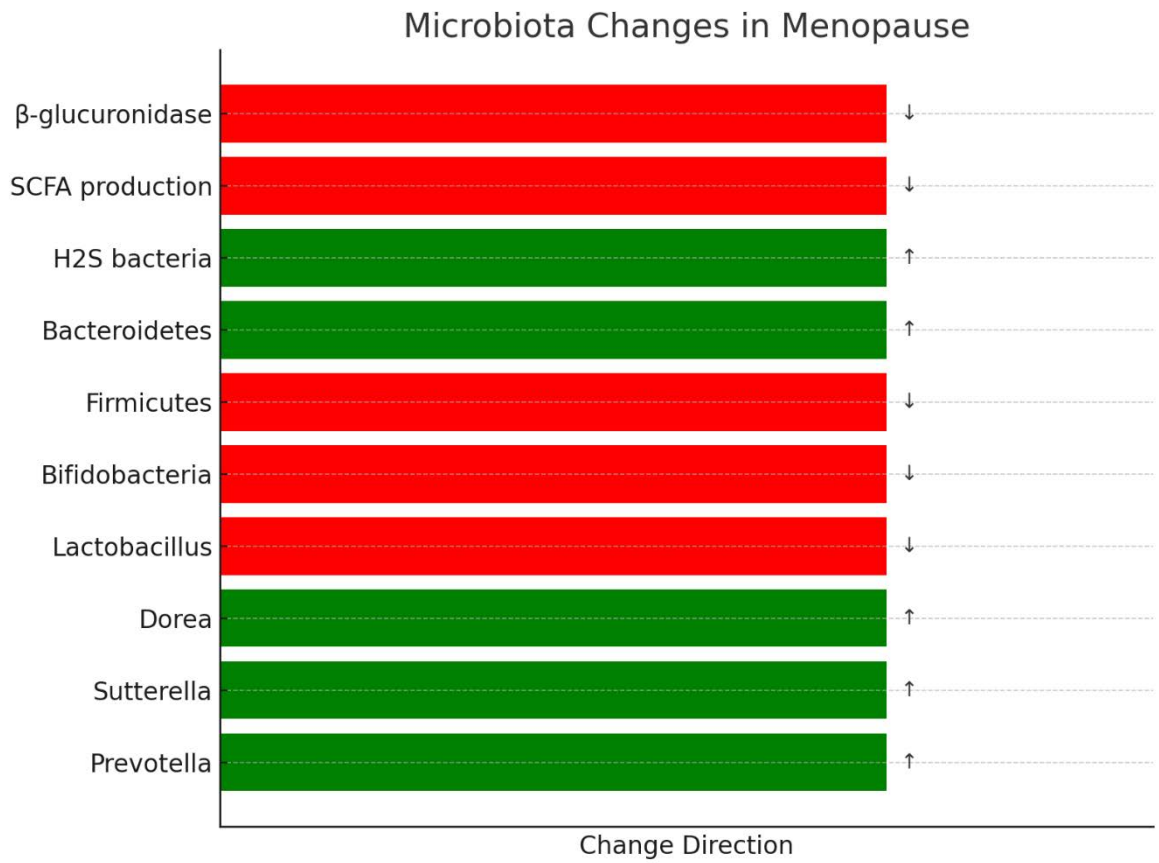
Before discussing the impact of nutrition and exercise on the gut microbiota, it is crucial to first understand the baseline difference in gut microbial diversity between peri-menopausal and postmenopausal women. The table below summarizes reported changes in key taxa when comparing peri- and menopausal (postmenopausal) women in human studies.

Taxon	Peri-menopause (menopausal transition)	Menopause (postmenopause)	References
Firmicutes	Relatively high abundance (as in pre-menopause)	Significantly decreased abundance	[110]
Bacteroidetes	Relatively low abundance (as in pre-menopause)	Significantly increased abundance	[110]
Roseburia (Firmicutes genus)	Abundant in perimenopause	Depleted by menopause	[110]
Prevotella (Bacteroidetes genus)	Lower abundance	Enriched in menopause	[31]
Sutterella (Proteobacteria genus)	Lower abundance	Enriched in menopause	[31]
Akkermansia (Verrucomicrobia genus)	Detectable before menopause	Reduced abundance in menopause	[31]
Escherichia/Shigella (Proteobacteria genus)	Present in perimenopause	Lower abundance in menopause	[31]
Veillonella (Firmicutes genus)	Present in perimenopause	Decreased in menopause	[31]
Parabacteroides (Bacteroidetes genus)	Present in perimenopause	Decreased in menopause	[111]
Odoribacter (Bacteroidetes genus)	Relatively low abundance	Elevated in menopause	[111]
Bilophila (Proteobacteria genus)	Relatively low abundance	Elevated in menopause	[111]

Table 1: These human studies collectively indicate that the gut community shifts in menopause, often with a decrease in *Firmicutes* and certain mucin-degraders (*Akkermansia***)** and an increase in certain *Bacteroidetes* and *Proteobacteria* genera. Also the butyrate producing *Roseburia* is decreased in menopause.

5.2 Microbiota and functional shifts related to menopause.

Based on the literature reviewed in the initial search, the following represent the primary alterations in gut microbiota and function observed during menopause.



Graph 1 presents alterations in gut microbiota composition and microbial metabolic activity associated with menopause (green is increased, red is decreased).

Taxonomic shifts include an increased relative abundance of *Prevotella*, *Sutterella*, *Dorea*, and hydrogen sulfide (H_2S)-producing bacteria, alongside a reduction in beneficial genera such as *Lactobacillus* and *Bifidobacteria*. Broader phylum-level changes include decreased *Firmicutes* and increased *Bacteroidetes*. Functionally, menopause is associated with reduced short-chain fatty acid (SCFA) production and lower β-glucuronidase activity. These microbial and metabolic shifts may contribute to the systemic inflammation, metabolic alterations, and hormonal dysregulation observed during the menopausal transition [27, 54, 79].

5.3 Diet-induced microbiota changes

The most important findings of the literature search are summarized in Table 2 and Table 3.

Microbiota	Change	Observed effect	Diet	Duration
Porphyromonas	↑	Less daytime hot flashes	Low fat diet + 86g soya	12 weeks
Prevotella corporis	↑	Less daytime hot flashes	Low fat diet + 86g soya	12 weeks
Clostridium asparagiforme	↑	Less total severe hot flashed and night hot flashes	Low fat diet + 86g soya	12 weeks
Fusicatenibacter saccharivorans	↑	Less total severe hot flashes	Low fat diet + 86g soya	12 weeks
Holdemanella	↑	Less total severe hot flashes	Low fat diet + 86g soya	12 weeks
Oscillospiraceae	↑	Improved BMD, lower IL-β1 and TNF-α	50-100 g prunes/day	12 months
Lachnospiraecea	↑	Improved BMD, lower IL-β1 and TNF-α	50-100 g prunes/day	12 months

Table 2: Summary of the effects of prunes [71] and a low fat diet, soy-based diet [57] on various bacterial strains.

Diet	Health effects
MED Diet	• Improves lipid blood profiles (↑ HDL, ↓ LDL, ↓ triglycerides) • ↓ Visceral adiose tissue (VAT) • ↓ Blood pressure (BP) • ↓ Oxidative stress
DASH Diet	• Blood pressure (BP) • Cardiovascular disease (CVD) prevention
Low-fat Diet	• Improves lipid blood profiles (↑ HDL, ↓ LDL, ↓ triglycerides) • ↓ Overweight/obesity
Isoflavone-rich soy food	• ↑ Gut microbiota derived estrogen metabolites • ↓ Systemic inflammation

Table 3: Summary of the dietary interventions and health outcomes [43].

5.4 Exercise-induced microbiota changes.

The most important findings of the literature study are summarized in Table 4 and Table 5.

Intervention Group	Equol-Producing Status	Change in Arterial Compliance	Key Finding
Isoflavone supplementation only	Equol producers	No significant change	Isoflavones alone had no effect on compliance regardless of equol status
Isoflavone supplementation only	Non-producers	No significant change	
Isoflavones + aerobic exercise	Equol producers	**Significant increase** in carotid artery compliance	Vascular adaptation occurred only in equol producers with combined intervention
Isoflavones + aerobic exercise	Non-producers	No significant change	No vascular benefit despite combined intervention

Table 4 summarizes the findings from the study of Hayashi et al. [95]. The most important finding is that isoflavone intake alone did not improve arterial compliance regardless of equol-producing status. When combined with exercise a significant increase in artery compliance was observed exclusively in equol producers.

Intervention	Health changes	References
Physical Activity (PA)	• ↑ Microbial diversity • ↑ Beneficial phyla • ↑ SCFAs in feces	[98–101]
HIIT + RT	• ↓ Total and visceral fat mass • ↑ Muscle mass • Altered beta-diversity • No change in alpha-diversity	[103]
Combined aerobic + RT	• Changed beta-diversity	[104]
HIIT + RT vs. Moderate PA	• Greater effect on obesity-related parameters	[105]
METs & PA	• **Positive correlation**: Acidobacteria, Chloroflexi, Nitrospirae, Sphingomonas • **Negative correlation**: Phascolarctobacterium, Acinetobacter	[108]

Table 5 outlines the effects of PA on gut microbiota and body composition in postmenopausal women. Exercise, particularly HITT combined with RT is associated with improved body composition and alterations in gut microbial beta-diversity. While alpha-diversity remains unchanged, increased microbial diversity, beneficial phyla, and SCFA levels are observed with higher PA. HIIT + RT appears more effective than moderate-intensity exercise in reducing obesity-related parameters.

Moreover, higher levels of PA (METSs) significantly increase the abundance of nitrite-oxidizing bacteria in the gut. The more physically active the individual (as measured by METs), the more prevalent these beneficial, nitrite oxidizing bacteria tend to be in the gut.

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6. Discussion

Menopause remodels the gut microbiota. These alterations are mostly driven by hormonal changes rather than aging per se. These combined changes substantially raise CVD risk, hot flashes, osteoporosis, and metabolic changes. Conventional heart-healthy diets (like DASH and MED) can improve many risk factors in older women. Other dietary interventions such as “healthy diet” (defined as >25 servings of fruit and vegetables, whole grains, high-fiber food, and fish), plant-based (MED-vegetarian), energy reduction, low-fat, and low-fat + energy intake reduction, have also beneficial effects on CVD risk factors in healthy postmenopausal women [54].

However, these are all “one-size-fits-all" approaches that may not fully address menopause-specific metabolic and microbiome changes. Menopausal women are a clinical population that would greatly benefit from precision nutrition since the gut microbiome clearly contributes to the heterogeneity among these women. Tailoring these diets (for example by adding soy food or extra fiber) may further enhance benefits. In theory, raising gut-derived estrogens and SCFAs could lower pro-inflammatory markers (such as CRP). Early evidence suggests that aligning diet with a women’s metabolic profile does attenuate menopause associated risks [112]. Though large clinical trials are still needed

This thesis highlights the potential for gut microbiome composition to influence the effectiveness of dietary interventions targeting general health in menopausal women. Prunes, which are rich in phenolics, have been shown to exert beneficial effects on bone mineral density (BMD). However, these effects are not uniform across individuals. Only a subset of participants - classified as responders - experienced significant improvements in inflammatory markers. A distinguishing feature of these responders was their gut microbial composition. They exhibited greater alpha and beta diversity, as well as higher abundances of bacterial families *Oscillospiraceae* and *Lachnospiraecea* [71]. These taxa haven been previously associated with anti-inflammatory properties and enhanced SCFA production, which may contribute to bone health.

Hayashi et al. [95] reported similar findings in which isoflavone treatment combined with aerobic exercise had significant effect on carotid artery compliance only in responders or equol producers. These findings suggest that equol-producing status may be a critical determinant of vascular responsiveness to lifestyle interventions in postmenopausal women.

Only about 20-35% of adults in Western countries, whereas 50-60% of Asian adults have the gut microbiota capable of converting the soy isoflavone daidzein to equol. Asian diets are richer in foods such as meat, fish, soy, and vegetables [113]. A study by Selin et al. [114] reported that equol-producing status may be modulated by microflora diversity and dietary habbits. It is common for the abundance of gut bacteria to increase when dietary intake of their substrate is increased. The primary susbstrate for equol production is soy-rich food and high-fiber plant foods.

It is important to note that individuals who respond well to a diet typically have a more diverse gut microbiome, characterized by higher alpha-diversity. This greater diversity often reflects a more “functional” microbial community, with an enhanced ability to metabolize components into beneficial metabolites. The observed differential response underscores the importance of considering host-microbiome interactions when evaluating the efficacy of nutritional interventions. This again supports the growing evidence for precision nutrition, suggesting that tailoring dietary recommendations to an individual's microbiome profile may enhance outcomes.

An effective enhancement to standard therapy for BMD in postmenopausal women could involve adding 50-100 grams of prunes per day alongside vitamin D and calcium supplements. This may be a promising whole-food nutritional supplement to maintain BMD in PM women [71].

Research indicates that a significant proportion of women seek medical support during menopausal transition. These findings highlight a critical window during which healthcare providers can effectively engage women to address modifiable factors including diet and lifestyle [115]. Moreover, based on these findings, it is imperative to promote a diverse and healthy dietary pattern even before menopausal transition occurs, before the onset of bothersome symptoms, to optimize the gut microbiome composition. Several studies have demonstrated that a 12- week intervention period is sufficient to induce measurabale adaptations in the gut microbiome [57].

During peri-menopause, women often become less physically active and consume more sugar and fat contributing to an increase in VAT. This rise is VAT is associated with an imbalance in the gut microbiome and elevated levels of LBP, LPS and hsCRP. These changes promote systemic inflammation. Reduced PA also correlates with lower estrogen levels, further impacting metabolic and vascular health. Optimizing the estrobolome - the

collection of gut bacteria involved in estrogen metabolism - is therefore essential. Equol, a non-steroidal estrogen-like compound, plays a key role in this process. It selectively binds to estrogen receptor beta, modulating hormonal activity in the body. In addition to its estrogenic effects, equol also increases nitric oxide production and promotes endothelium-independent vasodilatation. These vascular effects are comparable to the benefits seen with regular aerobic exercise, highlighting equol's potential in supporting cardiovascular and hormonal health during menopausal transition [116].

Enzymes associated with the estrobolome are expressed across a diverse range of microbes and multiple bacterial phyla, particularly within *Firmicutes* and *Bacteroidetes,* which commonly express β-glucuronidase enzymes [78]. Several dietary factors can balance β-glucuronidase activity such as cruciferous vegetables, fibers, prebiotics and postbiotics.

Specific dietary patters can significantly influence the gut microbiota [43]. Among these, the MED diet – characterized by high consumption of olive oil, fruits, and vegetables – has shown the most beneficial effects. In menopausal women with metabolic syndrome, the MED diet has been associated with reduced VAT, improved blood lipid profiles, and lower BP [43]. To support a healthy gut microbiota, dietary changes should emphasize key nutrients that are central to the MED diet, including fiber, polyphenols, and omega-3 fatty acids [117]. Additionally, a diet rich in minimally processed plant-based foods – such as whole grains, fruits, vegetables, nuts, and seeds – and low in refined grains, red or processed meats, and saturated fats is linked to both improved gut microbiota composition and better cardiometabolic health. This is especially important during menopause [118]. However, low grain intake is not only a concern for postmenopausal women aged 50 to 64, but also for women in the 19-34 and 35-79 age groups [47]. To address this, the MED diet can be combined with the DASH diet, which is rich in fiber and low in saturated fats. Moreover, Lin et al. [119] showed that DASH diet may improve intestinal permeability which may be related to the lowering of BP.

Menopause-related factors such as reduced PA, changes in energy expenditure and metabolic alterations increase the risk of weight gain and obesity. Gut microbial dysbiosis may also contribute to the development of obesity. Obesity has been associated with significant alterations in gut microbiota composition and metabolic function, including reduced alpha- and beta-diversity and an imbalance between beneficial and pathogenic bacteria. These changes can disrupt carbohydrate metabolism, suggesting a bidirectional

relationship between the intestinal microbiota and human metabolic health [97]. Therefore, it is important to motivate premenopausal women to increase their PA. In addition, Yang et al. [120] showed that higher cardiorespiratory fitness levels were associated with a more favorable gut microbiota profile, including higher abundance of beneficial bacteria such as *Bacteroidetes* and lower levels of harmful bacteria such as *Clostridium*. These associations were independent of age or dietary intake, suggesting that physical fitness itself may play a role in shaping gut microbiota composition.

Several studies indicated that compared to moderate intensity continuous training + RT, HIIT + RT has a greater effect on obesity related parameters in postmenopausal women [103-105]. Most importantly individual responses to exercise appeared to vary based on the baseline microbiota profiles, highlighting the existence of “nonresponders” - individuals who do not exhibit expected physiological adaptations to training.

Additionally, recent studies highlight that machine learning, particularly random forest algorithms, can predict individual responses to exercise based on baseline gut microbiota composition. Dupuit et al. [103] used a random forest model to integrate microbial features and accurately predict changes in body composition – such as fat mass reduction and muscle mass gain – after a 12-week HITT combined with RT. This demonstrates the gut microbiota’s potential as a biomarker for exercise responsiveness. By stratifying these individuals into likely responders and nonresponders these algorithms enable personalized exercise prescriptions. Such findings reinforce the growing evidence linking the gut microbiome to exercise-induced adaptations and suggest microbiome-informed strategies could enhance health outcomes in postmenopausal women with overweight or obesity.

Zheng et al [108] showed that when METs increase the abundance of *Acidobacteria*, *Choloflexi*, *Nitrospirae* and *Sphingomonas* increase. In other words, higher levels of physical activity are associated with greater relative abundance of these nitrite-oxidizing bacterial phyla in the gut. Their increased presence in physically active individuals suggests that PA may enhance gut microbial diversity and function, including nitrogen-related metabolic pathways. Moreover, specific phyla could act as indicators or mediators of a healthier, more metabolically active gut environment influenced by regular physical activity.

The results of this thesis indicate that precision nutrition and precision exercise could be critical tools in optimizing health, managing symptoms, and preventing chronic disease in

menopausal women, due to the complex biological and metabolic shifts that occur during menopausal transition. Precision strategies based on gut microbiome profiling, blood biomarkers, metabolomics, body composition and lifestyle are more effective than generalized advice. Moreover, future research may leverage machine learning to personalize diet, such as the DASH or MED diet, by tailoring them to individual profiles based on data like microbiome composition or blood biomarkers. In addition, probiotic supplementation shows promise for supporting gut health, bone density, metabolic function, and also vaginal health. However, this is beyond the scope of this study because of its complexity. Commonly used strains are Lactobacillus and Bifidobacterium [121].

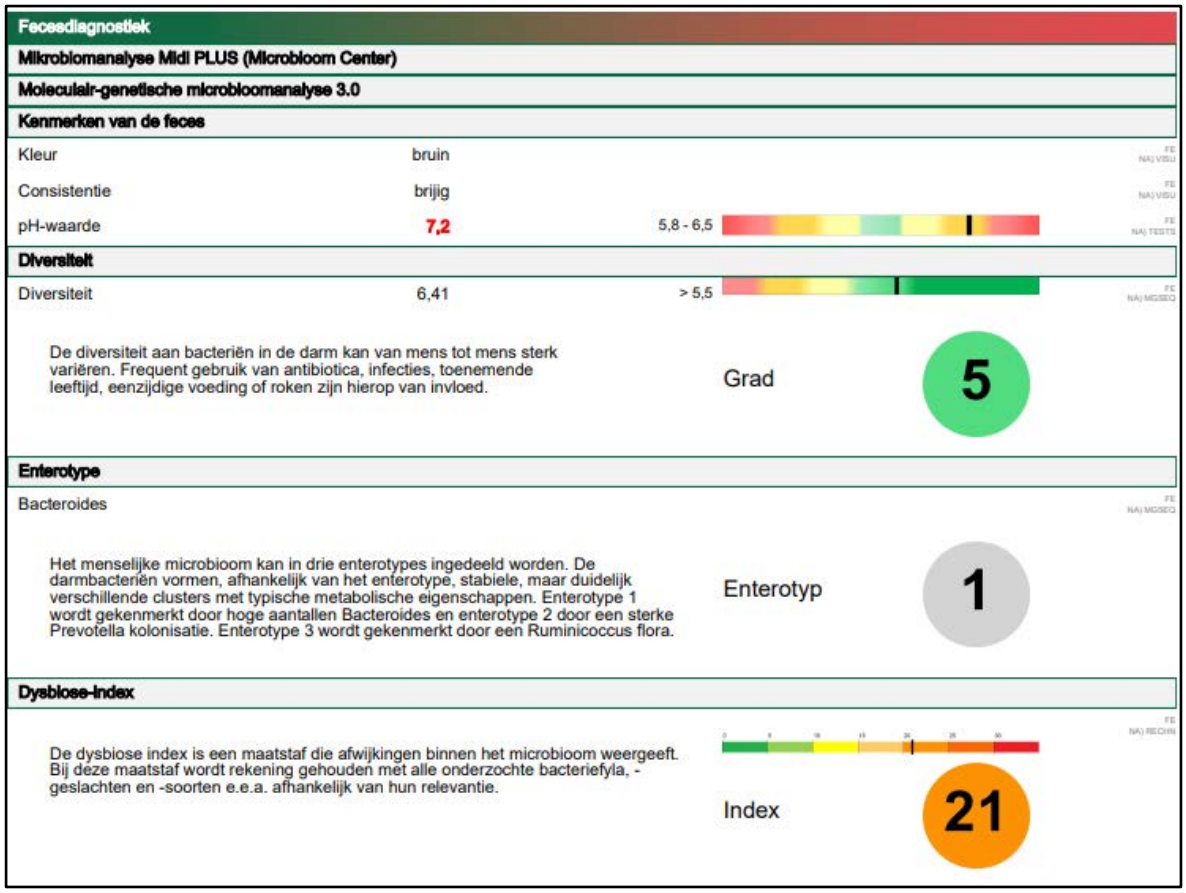
The next chapter is first step towards a personalized precision health plan based on the findings of this literature review combined with my personal experience in this research field.

Towards Precision Health in Women: The Personalized Female Health Plan (PFHP).

The goal of this thesis is to set-up guidelines for a Personalized Female Health Plan for peri- and postmenopausal women. This is a comprehensive, individualized health strategy tailored to the unique physiological, hormonal, microbiological, and lifestyle related needs of peri- and postmenopausal women. The PHPF should integrate data from clinical biomarkers, microbiome profiling, hormonal assessments, nutritional status and also psychosocial factors to promote optimal health during menopausal transition.

💉 STEP 1: Comprehensive Gut Microbiome Profiling

Below an illustrative example of a comprehensive microbiome analysis assessing all relevant parameters necessary for evaluating intestinal microbial diversity (Dutch-language report based on Biovis Diagnostics Lab, Germany). The most important findings in this report are being discussed.



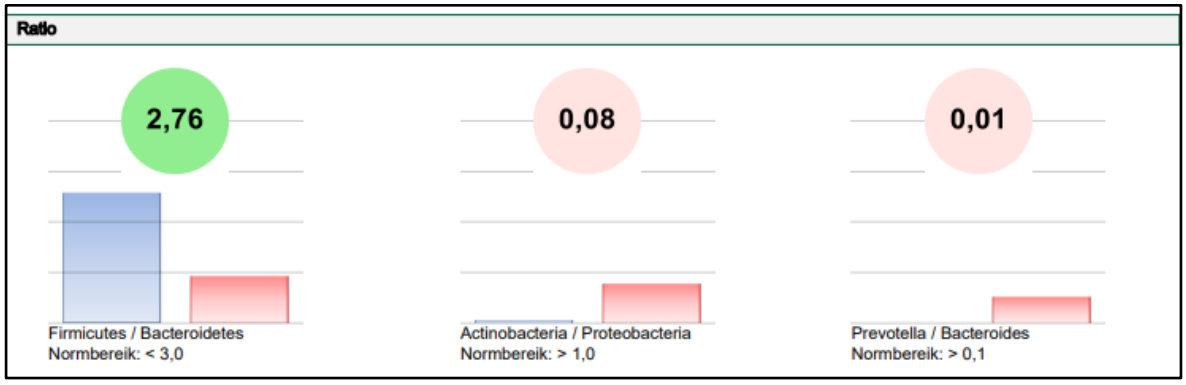
✅ Macroscopic properties of the feces, including coloration, form, and acidity (pH), are evaluated. Fecal pH is a critical indicator of intestinal health, reflecting the balance of

microbial fermentation processes and the overal composition of the gut microbiota. Deviations from the normal pH range may signal dysbiosis, malabsorption, or inflammation. A slightly acidic pH (5.8-6.5) is ideal for butyrate-producing bacteria such as *Faecalibacterium* and *Roseburia*, as well as for lactate production. It also inhibits the growth of a pathogenic bacteria such as Clostridium difficile, E. Coli and proteobacteria. Meat, alcohol and soda drinks increase fecal pH. Vegetables decrease pH.

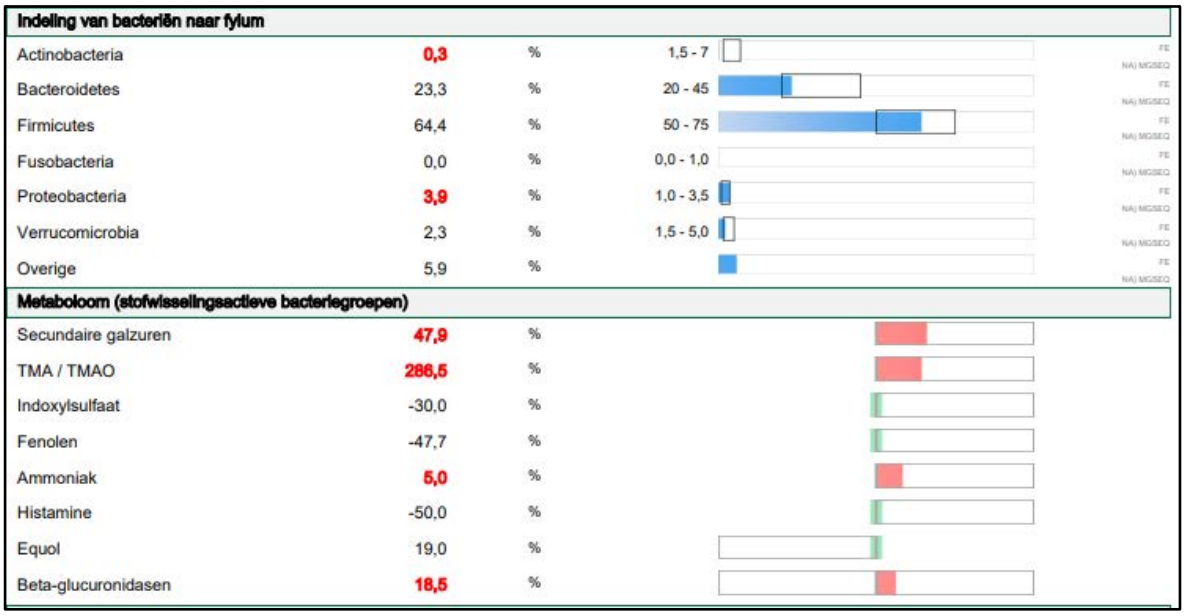
✅ The diversity index (alpha-diversity) is calculated to quantify microbiome diversity. The diversity index of 5 is the absolute minimum.

✅ The enterotype classification of the gut microbiome is determined. The enterotype 1 (*Bacteroides*-dominant) is associated with diets high in protein and animal fats (Western diet). Enterotype 2 is dominated by the genus *Prevotella* and is more common in individuals consuming high-fiber, carbohydrate-rich diets (plant-based).

✅ The dysbiosis index is evaluated. This reflects the degree of microbial imbalance, often indicating a shift away from healthy, diverse microbiome toward a composition associated with disease or dysfunction.



✅ The ratio *Firmicutes/Bacteroidetes* is analysed. This represents the balance between the two dominant phyla of bacteria. *Firmicutes* is involved in absorption, fermentation of dietary fibers and production of SCFAs like butyrate. *Bacteroidetes* is known for breaking down complex carbohydrates and proteins, contributing to nutrient absorption and immune regulation.

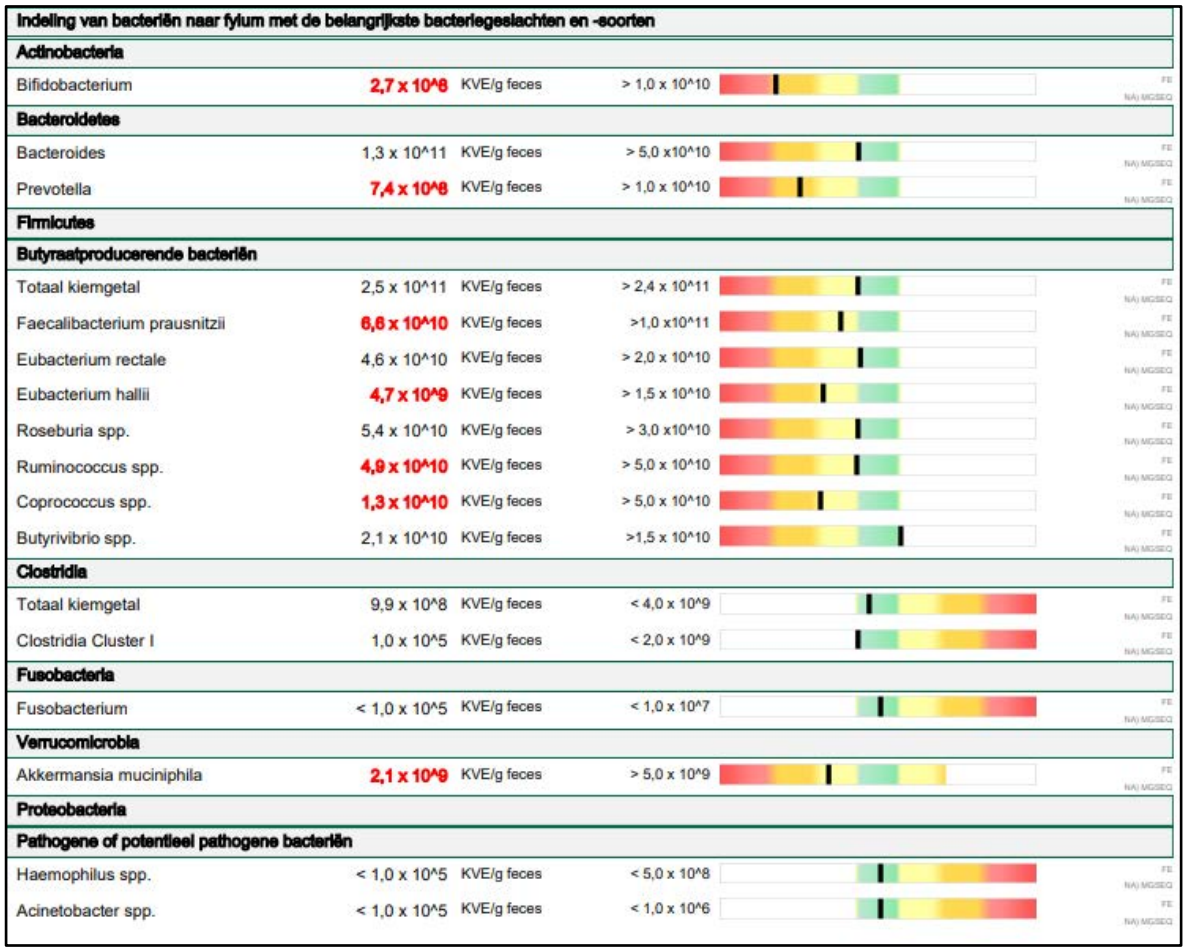


Indeling van bacteriën naar fylum			
Actinobacteria	**0,3**	%	1,5 - 7
Bacteroidetes	23,3	%	20 - 45
Firmicutes	64,4	%	50 - 75
Fusobacteria	0,0	%	0,0 - 1,0
Proteobacteria	**3,9**	%	1,0 - 3,5
Verrucomicrobia	2,3	%	1,5 - 5,0
Overige	5,9	%	
Metaboloom (stofwisselingsactieve bacteriegroepen)			
Secundaire galzuren	**47,9**	%	
TMA / TMAO	**286,5**	%	
Indoxylsulfaat	-30,0	%	
Fenolen	-47,7	%	
Ammoniak	**5,0**	%	
Histamine	-50,0	%	
Equol	19,0	%	
Beta-glucuronidasen	**18,5**	%	

✅ The predominant bacterial phyla within the gut microbiome are analyzed. In parallel, key microbiological metabolites - including TMAO, bile acids, equol and others - are evaluated. Secondary bile salts are elevated most probably due to dysbiosis (increases the conversion of primary to secondary bile salts). This can lead to fatigue. Also TMAO is increased (characteristic of dysbiosis), which is linked to CVD, systemic inflammation and metabolic disorders (IR and T2DM).

The increased level of ammonia is also a sign of dysbiosis. Some hut bacteria such as *Clostridium* overproduce ammonia. This also could be a sign of SIBO (small intestinal bacterial overgrowth). High levels can lead to fatigue and brain fog.

Beta-glucuronidases are overproduced by certain bacteria. These can raise active estrogen levels possibly increasing the risk of estrogen-dominant symptoms (PMS, fibroids, weight gain) or hormone dependent cancers. Elevated levels can also reflect overgrowth of certain bacteria such as *E. coli*, *Clostridia* and *Bacteroides* that produce this enzyme. This can be due to low intake of polyphenols or fiber (which support detox and inhibit beta-glucuronidase).



Indeling van bacteriën naar fylum met de belangrijkste bacteriegeslachten en -soorten			
Actinobacteria			
Bifidobacterium	**2,7 x 10^8**	KVE/g feces	> 1,0 x 10^10
Bacteroidetes			
Bacteroides	1,3 x 10^11	KVE/g feces	> 5,0 x10^10
Prevotella	**7,4 x 10^8**	KVE/g feces	> 1,0 x 10^10
Firmicutes			
Butyraatproducerende bacteriën			
Totaal kiemgetal	2,5 x 10^11	KVE/g feces	> 2,4 x 10^11
Faecalibacterium prausnitzii	**6,6 x 10^10**	KVE/g feces	>1,0 x10^11
Eubacterium rectale	4,6 x 10^10	KVE/g feces	> 2,0 x 10^10
Eubacterium hallii	**4,7 x 10^9**	KVE/g feces	> 1,5 x 10^10
Roseburia spp.	5,4 x 10^10	KVE/g feces	> 3,0 x10^10
Ruminococcus spp.	**4,9 x 10^10**	KVE/g feces	> 5,0 x 10^10
Coprococcus spp.	**1,3 x 10^10**	KVE/g feces	> 5,0 x 10^10
Butyrivibrio spp.	2,1 x 10^10	KVE/g feces	>1,5 x 10^10
Clostridia			
Totaal kiemgetal	9,9 x 10^8	KVE/g feces	< 4,0 x 10^9
Clostridia Cluster I	1,0 x 10^5	KVE/g feces	< 2,0 x 10^9
Fusobacteria			
Fusobacterium	< 1,0 x 10^5	KVE/g feces	< 1,0 x 10^7
Verrucomicrobia			
Akkermansia muciniphila	**2,1 x 10^9**	KVE/g feces	> 5,0 x 10^9
Proteobacteria			
Pathogene of potentieel pathogene bacteriën			
Haemophilus spp.	< 1,0 x 10^5	KVE/g feces	< 5,0 x 10^8
Acinetobacter spp.	< 1,0 x 10^5	KVE/g feces	< 1,0 x 10^6

✅ The predominant phyla are investigated.

For instance, a low abundance of *Prevotalla* suggests insufficient dietary fibre intake. Similarly, reduced levels of *Faecalibacterium prausnitzii* – a key butyrate producing bacterium - are also indicative of a fiber-deficient diet.

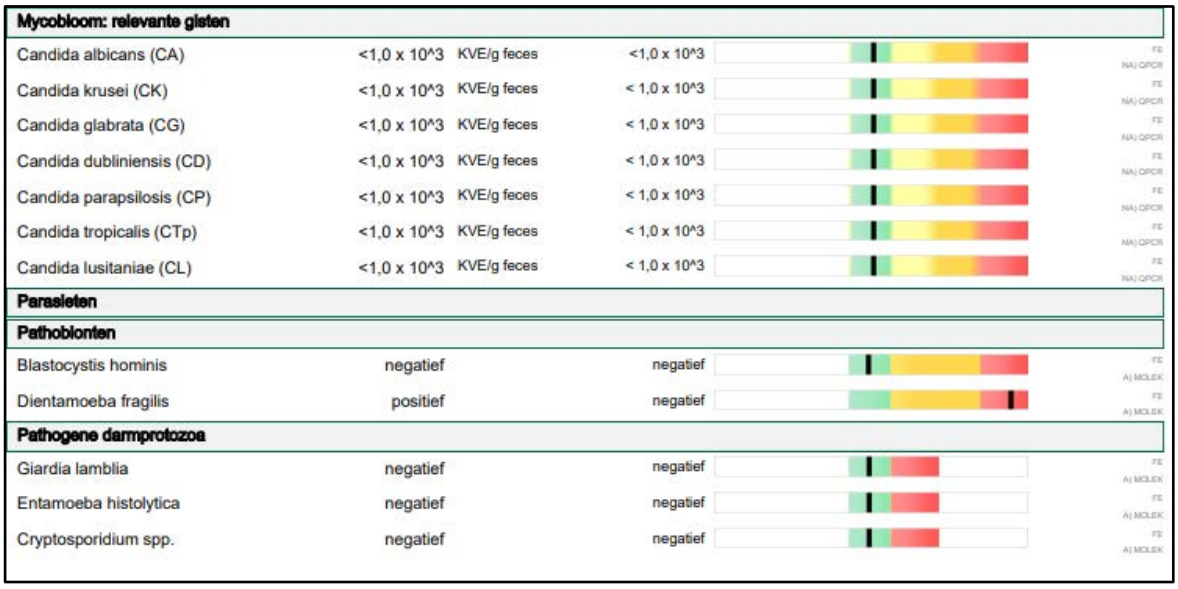
Akkermansia muciniphila plays a key role in maintaining gut health. It reinforces the intestinal lining and helps to prevent “leaky gut”. Thus this bacteria is important in lowering the risk of systemic inflammation. Low levels are linked to obesity, T2DM and leaky gut.

Both *Faecalibacterium prausnitzii* and *Akkermansia muciniphila* thrive in a fiber-rich environment, as dietary fiber is a crucial substrate for their growth and metabolic activity.

Test	Uitslag	Eenheid	Normbereik	Vorig onderzoek
Proteus spp.	< 1,0 x 10^5	KVE/g feces	< 1,0 x 10^6	
Klebsiella spp.	< 1,0 x 10^5	KVE/g feces	< 1,0 x 10^7	
Enterobacter spp.	< 1,0 x 10^5	KVE/g feces	< 1,0 x 10^6	
Serratia spp.	< 1,0 x 10^5	KVE/g feces	< 1,0 x 10^7	
Hafnia spp.	< 1,0 x 10^5	KVE/g feces	< 1,0 x 10^6	
Morganella spp.	< 1,0 x 10^5	KVE/g feces	< 1,0 x 10^6	
Citrobacter spp.	< 1,0 x 10^5	KVE/g feces	< 5,0 x 10^8	
Pseudomonas spp.	< 1,0 x 10^5	KVE/g feces	< 5,0 x 10^7	
Providencia spp.	< 1,0 x 10^5	KVE/g feces	< 5,0 x 10^7	
H2S-vorming				
Sulfaatreducerende bacteriën (SRB)	**8,0 x 10^9**	KVE/g feces	< 2,5 x 10^9	
Desulfovibrio piger	< 1,0 x 10^5	KVE/g feces	< 1,0 x 10^9	
Desulfomonas pigra	< 1,0 x 10^5	KVE/g feces	< 1,0 x 10^9	
Bilophila wadsworthii	< 1,0 x 10^5	KVE/g feces	< 2,0 x 10^9	
Immunogeniciteit / mucine vorming				
Immunogeen werkende bacteriën				
Escherichia coli	**1,4 x 10^8**	KVE/g feces	10^6 - 10^7	
Enterococcus spp.	**< 1,0 x 10^5**	KVE/g feces	10^6 - 10^7	
Lactobacillus spp.	**8,0 x 10^4**	KVE/g feces	10^5 - 10^7	
Mucine vorming / slijmvliesbarrière				
Akkermansia muciniphila	**2,1 x 10^9**	KVE/g feces	> 5,0 x 10^9	
Faecalibacterium prausnitzii	**6,6 x 10^10**	KVE/g feces	>1,0 x10^11	
Archaea				
Methanogenen				
Methanobrevibacter spp.	< 1,0 x 10^5	KVE/g feces	< 5,0 x 10^8	

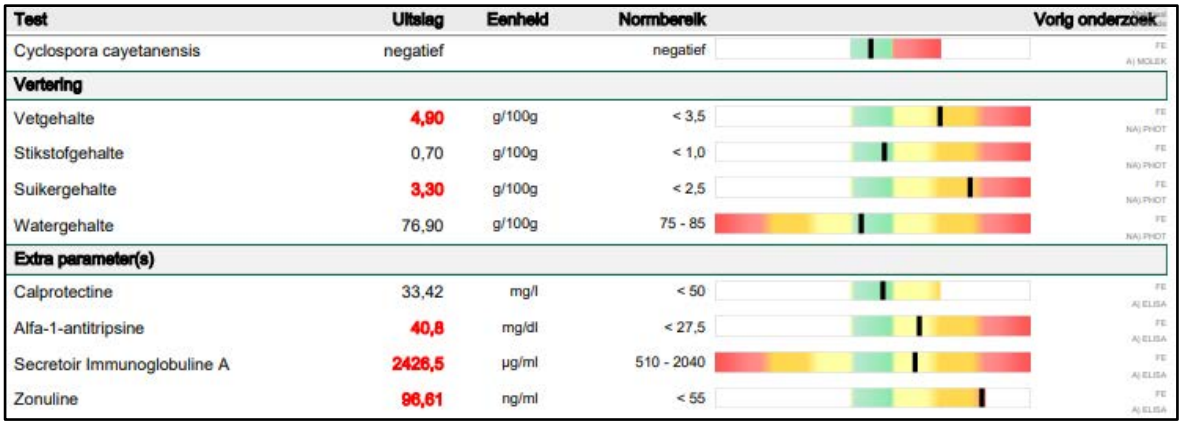
✅ Increased sulphate reducing bacteria (SRB) are associated with chronic low-grade inflammation. Overgrowth of H_2S producers often reflects an imbalance in the gut, especially a fiber-deficient or high-fat, high-protein diet. These bacteria may suppress butyrate producing bacteria such as *Faecalibacterium prausnitzii* and are linked to metabolic syndrome. An increase is mostly associated with an animal-based diet, high in meat, cheese and eggs.

✅ Immunomodulatory bacteria are analyzed. Important are *Lactobacillus* who produce lactic acid which lower gut pH and inhibit pathogens. Moreover, it promotes mucin production and tight junction expression. *Enterococcus* is also involved in lactid acid production and carbohydrate metabolism. *Escherichia coli* is a normal part of the gut microbiota. Low levels are important for stimulating the immune system and aiding in mucosal immune development. A disbalance of *Escherichia Coli* and *Enterococcus* is linked to increased intestinal permeability and chronic low-grade inflammation.



Mycobloom: relevante gisten			
Candida albicans (CA)	<1,0 x 10^3 KVE/g feces	<1,0 x 10^3	
Candida krusei (CK)	<1,0 x 10^3 KVE/g feces	< 1,0 x 10^3	
Candida glabrata (CG)	<1,0 x 10^3 KVE/g feces	< 1,0 x 10^3	
Candida dubliniensis (CD)	<1,0 x 10^3 KVE/g feces	< 1,0 x 10^3	
Candida parapsilosis (CP)	<1,0 x 10^3 KVE/g feces	< 1,0 x 10^3	
Candida tropicalis (CTp)	<1,0 x 10^3 KVE/g feces	< 1,0 x 10^3	
Candida lusitaniae (CL)	<1,0 x 10^3 KVE/g feces	< 1,0 x 10^3	
Parasieten			
Pathobionten			
Blastocystis hominis	negatief	negatief	
Dientamoeba fragilis	positief	negatief	
Pathogene darmprotozoa			
Giardia lamblia	negatief	negatief	
Entamoeba histolytica	negatief	negatief	
Cryptosporidium spp.	negatief	negatief	

✅ The fecal sample is screened for the presence of yeasts, intestinal parasites, and pathogenic protozoa. It is important to check for Candida infections. Lower sugar intake is imperative to reduce its abundance.

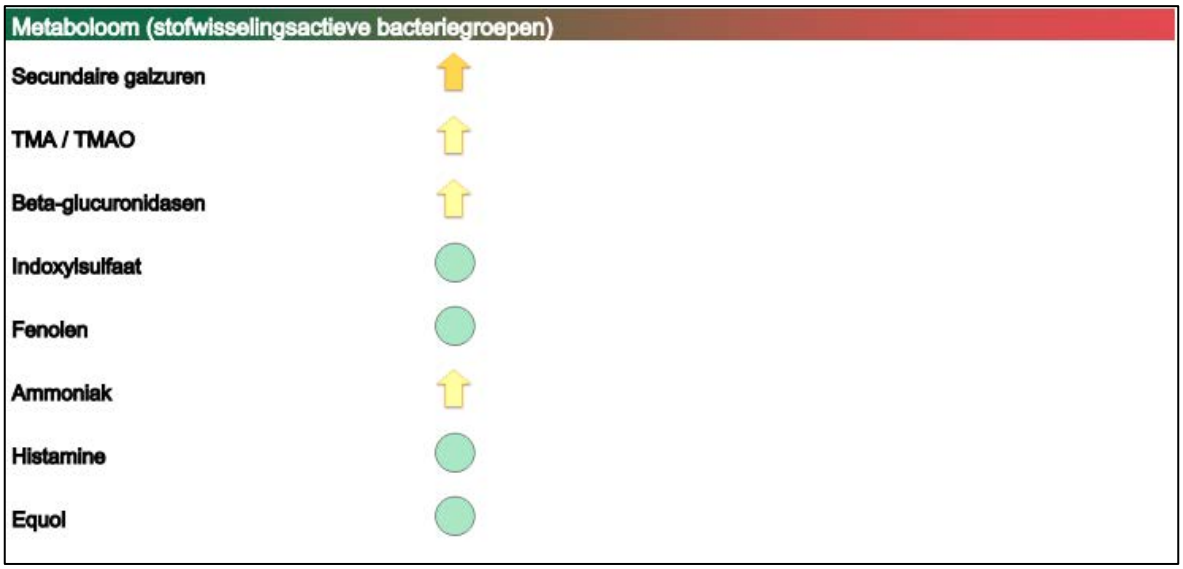


Test	Uitslag	Eenheid	Normbereik	Vorig onderzoek
Cyclospora cayetanensis	negatief		negatief	
Vertering				
Vetgehalte	**4,90**	g/100g	< 3,5	
Stikstofgehalte	0,70	g/100g	< 1,0	
Suikergehalte	**3,30**	g/100g	< 2,5	
Watergehalte	76,90	g/100g	75 - 85	
Extra parameter(s)				
Calprotectine	33,42	mg/l	< 50	
Alfa-1-antitripsine	**40,8**	mg/dl	< 27,5	
Secretoir Immunoglobuline A	**2426,5**	µg/ml	510 - 2040	
Zonuline	**96,61**	ng/ml	< 55	

✅ Digestion parameters are investigated. Fats and carbohydrate digestion appears to be suboptimal. The carbohydrate malabsorption might be due to SIBO or lactose intolerance. These excess undigested carbohydrate feed gas-producing or pathogenic bacteria which leads to dysbiosis. It may promote overgrowth of H_2S bacteria or Candida. Fat malabsoprtion can lead to defficiency in fat-soluble vitamins A, D, E and K. A possible underlying cause is intestinal mucosal damage.

✅ Extra parameters for intestinal permeability are evaluated such as α-1-antitrypsin, zonulin and SecretoryI gA (sIgA). Calprotectin is analyzed to distinguish between inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS).

α-1-Antitrypsin serves as marker for mucosal damage. Elevated levels can be indicative for increased permeability or intestinal inflammation. Zonulin is a protein that opens the tight junctions of the gut cells. It is a good indicator of leaky gut, dysbiosis or celiac disease. Secretory IgA are the main immunoglobulins in the gut mucosa. They serve as a first line defence in the gastrointestinal tract. Increased levels may indicate active inflammation or immune response (e.g. pathogens, food antigens, dysbiosis).



✅ The dysbiosis index is presented as a numerical score that evaluates the degree of imbalance in the gut microbiota. Additionally, a summary of key findings is provided along with therapeutic options.

🔵 STEP 2: Urinary Organic Metabolites (UOMs).

The microbiome test can be supplemented with a UOMs analysis. As the microbiome analysis measures the composition and diversity of bacteria in the gut via a feces sample, the UOM analysis measures metabolic byproducts (organic acids) excreted in urine. It reflects the metabolic activity of the microbiota. It can also indicate fungal overgrowth, which microbiome tests may miss.

In the presence of dysbiosis, the profile of excreted metabolites changes. Elevated levels of certain metabolites serve as diagnostic markers, helping to distinguish between fungal and bacterial imbalances. This information supports a more precise and targeted therapeutic strategy. Additionally, the UOM analysis can be used to monitor recovery by tracking the return of metabolite levels to their baseline, indicating the restoration of a healthy gut flora.

Panel of organic urinary metabolites (test by SYNLAB Belgium).

ORGANISCHE METABOLIETEN URINE - Bacteriën module

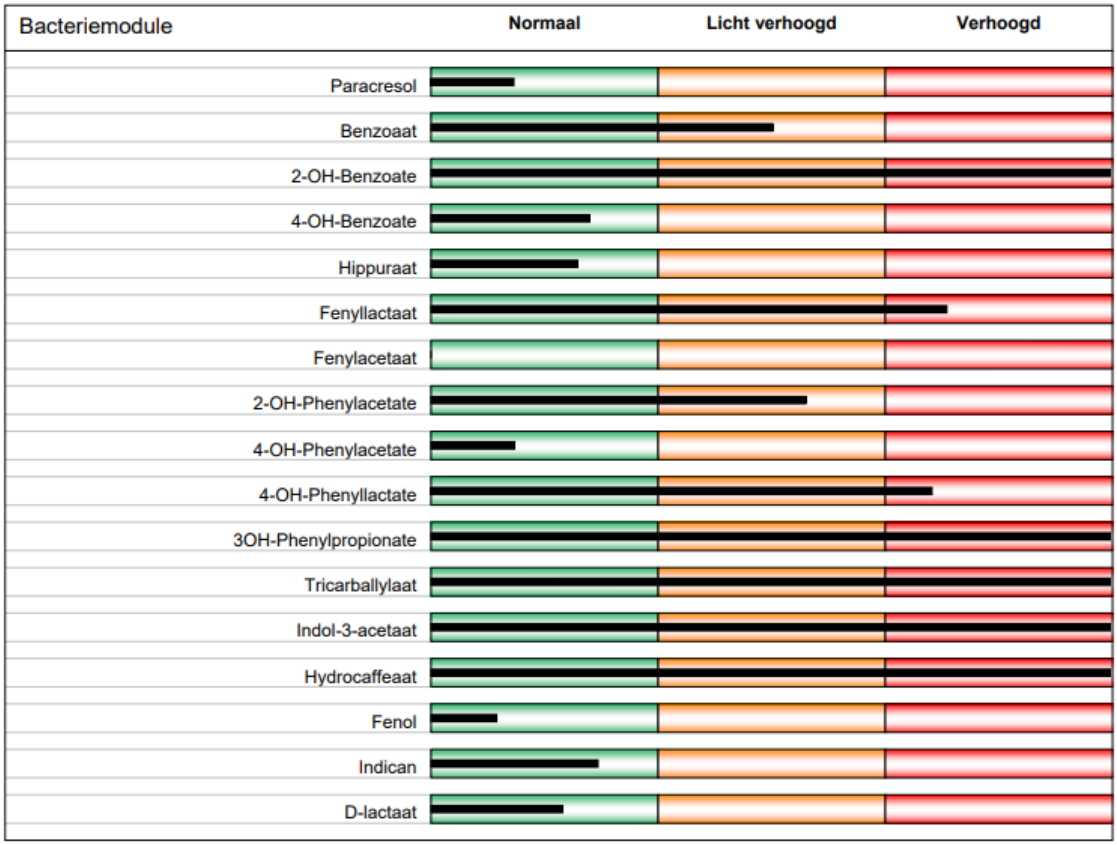
Paracresol	64.41
Benzoate	↑ 7.26
2-OH-Benzoate	↑↑10.33
4-OH-Benzoate	1.10
Hippurate	349
Phenyllactate	↑↑0.28
Phenylacetate	0.00
2-OH-Phenylacetate	↑ 0.64
4-OH-Phenylacetate	5.12
4-OH-Phenyllactate	↑↑1.05
3OH-Phenylpropionate	↑↑0.69
Tricarballylate	↑↑1.45
Indole-3-Acetate	↑↑4.95
Hydrocaffeate	↑↑1.84
Phenol	9.22
Indican	29.82
D-Lactate	3.86

✅ Bacteria module.

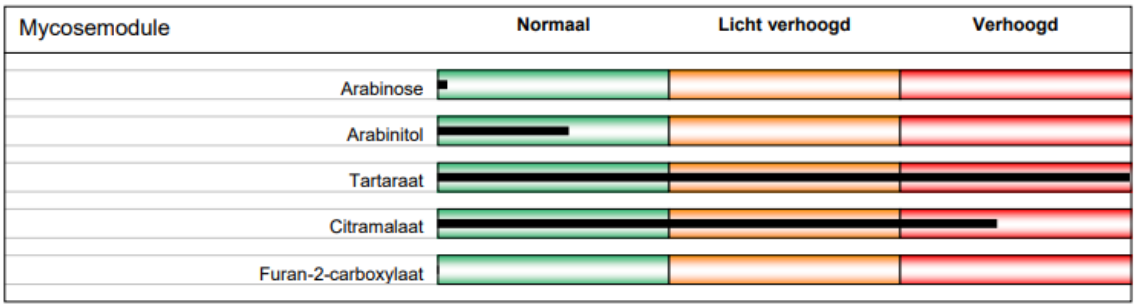
ORGANISCHE METABOLIETEN URINE - Fungi module

Arabinose	0.31
Arabinitol	16.31
Tartarate	↑↑43.49
Citramalate	↑↑2.30
Furan-2-Carboxylate	0.00

✅ Fungi module.



✅ The results of the module indicate significant intestinal bacterial overgrowth. The presence of excessive amounts of one or more bacterial metabolites primarily indicates the growth of putrefactive bacteria of the *Clostridium* type. The consumption of protein-rich foods such as meat, cheese and eggs should be limited. Fiber-rich foods such as vegetables, fruit and whole grains should be increased.



✅ The overall results indicate significant intestinal fungal proliferation. The presence of excessive amounts of arabinose and/or arabitol and/or tartrate and/or citramalate primarily indicates a proliferation of yeast of the *Candida* species. The presence of excessive amounts of furan-2-carboxylate primarily indicates a proliferation of yeast of the *Geotrichum* species. Bread, pasta, foods containing lactose and refined sugar should be eliminated due to the reduced efficiency of the enzyme amylase and the discaccharidases in candidiasis.

🧬 STEP 3: Blood Tests Related to Gut Health.

Microbiome tests should ideally be combined with blood tests to get a fuller picture. Especially LPS or LBP is important because this endotoxin is related to metabolic endotoxemia. Also SCFAs (acetate, propionate and butyrate) which are key metabolites produced by gut bacteria, can be checked.

Panel to analyze the intestinal ecosystem (test by SYNLAB Belgium).

Clinical relevance of SCFAs in blood

SCFA	Function in Blood	Potential Clinical Significance
Acetate	Lipid synthesis, appetite regulation	Elevated in obesity, insulin resistance
Propionate	Gluconeogenesis, satiety signals	Supports glycemic control
Butyrate	Anti-inflammatory, HDAC inhibition	Neuroprotection, immune modulation

🩸 STEP 4: Core Blood Markers for Peri- and Postmenopause.

1. Fasting Blood Glucose

- Detects impaired fasting glucose or diabetes.
- *Normal*: <100 mg/dL (5.6 mmol/L)
- *Metabolic syndrome*: ≥100 mg/dL (5.6 mmol/L) or on medication.

2. Hemoglobin A1c

- Reflects average glucose over past 2–3 months.
- *Pre-diabetes*: 5.7–6.4%
- *Diabetes*: ≥6.5%

3. Lipid Profile

- Measures cholesterol and triglycerides:
    - **Total Cholesterol**
    - **HDL Cholesterol**:
      Low for women: <50 mg/dL (1.3 mmol/L)
    - **LDL Cholesterol**
    - **Triglycerides**:
      High: ≥150 mg/dL (1.7 mmol/L)

4. Insulin (Fasting)

- Used to calculate HOMA-IR (insulin resistance index).

5. High-Sensitivity C-Reactive Protein (hs-CRP)

- Marker of low-grade inflammation; often elevated in metabolic syndrome.

6. Liver Function Tests (ALT, AST, GGT)

- Assesses for NAFLD (non-alcoholic fatty liver disease), commonly associated with metabolic syndrome.

7. Thyroid Panel (TSH, Free T4, ± T3)

- Hypothyroidism may mimic or worsen metabolic features.

8. Hormonal Panel

- **Estradiol (E2)**
- **FSH / LH**
  Helps confirm menopausal status if not yet fully menopausal.

9. Vitamin D (25-OH D)

- Deficiency is common in menopause and can contribute to metabolic disturbances.

10. Bone health panel

- **Calcium**: total and ionized calcium levels, essential for bone health.
- **Parathyroid hormone (PTH)**: regulates calcium levels.

11. Vitamin panel

- **Vitamin B12**: essential for cognitive health, nervous system.
- **Folate (Vitamin B9)**: important for hearth health, cognitive function.
- **Zinc**: often deficient, important for hormone balance.
- **Omega-3 index**: heart and brain health, anti-inflammatory function.

🧘 STEP 5: Health Check-Up

- BMI: estimates body fat using height and weight. A healthy BMI range is 18.5 to 24.9.
- Body composition: measures the percentage of body fat, visceral fat, muscle mass and hydration levels.
- Blood pressure: assesses the risk of cardiovascular disease.
- Waist-to-hip ratio: evaluates fat distribution to identify risk related to abdominal fat.

Criteria for Metabolic Syndrome Diagnosis (3 or more):

Criteria	Threshold
Waist circumference	> 88 cm (35 inches)
Triglycerides	≥ 150 mg/dL
HDL cholesterol	< 50 mg/dL
Blood pressure	≥ 130/85 mmHg or on treatment
Fasting glucose	≥ 100 mg/dL

📝 STEP 6: Lifestyle review

- Assessment of diet, physical activity, smoking and alcohol use.
- Food, physical activity and sleep questionnaires should be used.
- Evaluate mental health: depression, anxiety, sleep and stress.
- A gut microbiome checklist for peri- and menopausal women is evaluated (**Appendix 2**).

💲 STEP 6: Nutrition and exercise plan

Based on the research findings, the following nutrition and exercise plan is suggested to optimize the gut microbiome in peri- and postmenopausal women:

NUTRITION

- Low-fat
- Soy-rich (isoflavones)
- High-fiber
- Prune supplementation
- Mediterranean-inspired
- DASH-aligned (for heart health)

Appendix 3 shows an example of a menopause friendly diet plan & weekly meal plan.

EXERCISE

🏋 Resistance Training Focus (RT)

3× per week (Mon/Wed/Fri), targeting:

- Legs & Glutes: squats, lunges
- Core: planks, Russian twists
- Upper body: push-ups, rows, shoulder presses
- Aim: 2–3 sets of 8–12 reps per exercise

⚡ High-Intensity Interval Training (HIIT)

2× per week (Mon/Fri):

- 30-sec sprint / 90-sec walk × 6–8 rounds
- Total session: 20–25 minutes
- Use bike, rowing machine, or treadmill

🌿 Aerobic Exercise

2–3× per week:

- Moderate-intensity (50–70% HRmax)
- Examples: Brisk walking, dancing, swimming
- Duration: 30–60 minutes

Appendix 4 shows an example of a weekly exercise plan for peri- and postmenopausal women.

7. Conclusions

This thesis highlights the critical role of diet and physical activity in optimizing gut health, particularly for peri- and postmenopausal women. The MED-diet, especially when complemented with elements of the DASH-diet, demonstrates the most beneficial effects on gut microbiota and intestinal integrity. Physical activity – particularly high-intensity interval training combined with resistance training – further enhances microbial diversity and supports metabolic health. However, individual variability, driven largely by baseline gut microbiota composition, underscores the necessity of personalized interventions. By identifying responders based on microbiome diversity, we can better tailor nutrition and exercise strategies. Ultimately, early implementation of a personalized health plan in the perimenopausal phase offers a proactive strategy to support gut health, reduce disease risk, and enhance overall well-being during the menopausal transition.

8. Strengths & Weaknesses of this work

This thesis explores a critical and under-researched aspect of women's health: the interplay between gut microbiota and physiological changes associated with menopause. Specifically, it investigates how nutrition and exercise during the peri- and postmenopausal stages influence microbial diversity. The findings carry significant implications for the prevention of menopause-related symptoms. By highlighting interindividual variability in gut microbiome responses – including the distinction between responders and non-responders - the study underscores the need for personalized, preventive health strategies during or even before the menopausal transition to support microbiota stability. Moreover, this interindividual variation should be considered as a valuable factor in microbiome research.

A limitation of this work lies in the current research trend, where studies evaluating the gut microbiome's role in predicting treatment response often concentrate on a limited number of pre-identified or enriched bacterial species with specific functions. However, treatment outcomes are likely influenced by the broader microbial community, not just a few selected taxa. This highlights the need for studies that examine the entire microbiome ecosystem to better understand the collective contributions of diverse microbial populations.

Inconsistencies across studies may be attributed to differences in food supplements, varying intervention durations (ranging from 12 weeks to 12 months), and the number of years since menopause (up to 12 years and more). Additionally, the absence of diet monitoring presents a significant limitation. It can not be guaranteed that a participant's food intake remained consistent or that specific dietary components – known to influence gut microbiota – were not introduced. To address these issues, long-term longitudinal studies with multiple sampling points are needed to more accurately assess the sustained effects of nutritional interventions on the gut microbiome.

To support the intraindividual variation and its role in microbiota research in humans a growing body of literature suggest that case-wise/intrapersonal assessment in a functional capacity may be a positive way to understand microbiota changes.

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Appendices

Appendix 1: Search engine algorithm.

History and Search Details

Download Delete

Search	Actions	Details	Query	Results	Time
#14	•••	›	Search: **#1 AND #3 AND #4 AND #11**	0	07:12:32
#13	•••	›	Search: **#2 AND #3 AND #4 AND #11**	0	07:11:00
#12	•••	›	Search: **#2 AND #3 AND #4 AND #8 AND #9**	0	07:10:07
#11	•••	›	Search: **systematic review**	392,444	07:09:12
#10	•••	›	Search: **#1 AND #3 AND #4 AND #8 AND #9**	0	07:08:01
#7	•••	›	Search: **#2 AND #3 AND #4**	8	07:06:57
#6	•••	›	Search: **#1 AND #3 AND #4**	9	07:05:56
#9	•••	›	Search: **meta-analysis**	336,929	07:05:45
#8	•••	›	Search: **(((randomized control trials) OR (randomised control trial)) OR (randomized controlled trial)) OR (randomised controlled trial)**	946,956	07:05:23
#5	•••	›	Search: **#1 AND #2 AND #3 AND #4**	0	06:58:43
#4	•••	›	Search: **((((((menopause[Title/Abstract]) OR (menopausal women[Title/Abstract])) OR (peri-menopause[Title/Abstract])) OR (peri-menopausal women[Title/Abstract])) OR (perimenopause[Title/Abstract])) OR (perimenopausal women[Title/Abstract])) OR (menopausal transition[Title/Abstract])**	46,495	06:57:43
#3	•••	›	Search: **((((gut microbiome[Title/Abstract]) OR (gut microbiota[Title/Abstract])) OR (intestinal flora[Title/Abstract])) OR (gut diversity[Title/Abstract])) OR (gut dysbiosis[Title/Abstract])**	81,614	06:56:54
#2	•••	›	Search: **((((exercise[Title/Abstract]) OR (physical activity[Title/Abstract])) OR (resistance training[Title/Abstract])) OR (strength training[Title/Abstract])) OR (sport[Title/Abstract])**	533,007	06:56:15
#1	•••	›	Search: **((((nutrition[Title/Abstract]) OR (mediterranean diet[Title/Abstract])) OR (ketogenic diet[Title/Abstract])) OR (low carbohydrate diet[Title/Abstract])) OR (high fiber diet[Title/Abstract])**	267,680	06:55:26

Appendix 2: Gut Microbiome Health Checklist for Menopausal Women

Dietary Habits

- ❑ Regular intake of **soy-based foods** (e.g., tofu, tempeh, soy milk) for isoflavones
- ❑ Daily consumption of **prunes** (5–10 pieces) for bone and gut health
- ❑ High intake of **fiber-rich foods** (whole grains, legumes, fruits, vegetables)
- ❑ Preference for **low-fat diet** components
- ❑ Incorporation of **Mediterranean or DASH diet principles** (olive oil, nuts, fish, vegetables)
- ❑ Avoidance or limitation of processed foods, high sugar, and saturated fats

Lifestyle Factors

- ❑ Regular **physical activity or aerobic exercise**
- ❑ Adequate **hydration** (6–8 glasses water daily)
- ❑ Limiting alcohol and caffeine intake

Symptoms and Gut Health Monitoring

- ❑ Tracking frequency and consistency of bowel movements
- ❑ Monitoring for gastrointestinal symptoms (bloating, gas, discomfort)
- ❑ Awareness of changes in hot flashes or menopausal symptoms related to diet

*S*upplement and Medication Review

- ❑ Use of **probiotics or fermented foods** (e.g., yogurt, kefir, sauerkraut)
- ❑ Review of antibiotic use or other medications affecting gut flora

Testing and Assessment

- ❑ Consider **stool microbiota analysis**
- ❑ Blood markers for gut health and **inflammation**
- ❑ UOMs analysis to assess **metabolic activity** of the gut microbiome

Appendix 3: Example of a daily meal plan for menopause.

🗓️ Daily Meal Plan Overview

🥣 Breakfast

- Oatmeal with:
  - 1 tbsp ground flaxseed
  - Soy milk (unsweetened)
  - Berries (rich in antioxidants)
- Green tea or water

✅ *Supports microbiota diversity, provides phytoestrogens, and aids lipid metabolism.*

🥗 Lunch

- Mediterranean-style salad:
  - Mixed greens, chickpeas or lentils, cherry tomatoes, red onion, olives
  - Extra virgin olive oil + lemon dressing
- 1 slice whole grain bread or quinoa on the side
- Optional: 1 boiled egg or tofu cubes

✅ *High fiber, plant protein, and healthy fats improve cholesterol and support microbiome balance.*

🍥 Dinner

- Stir-fry with:
  - Tofu or tempeh (for soy isoflavones)
  - Broccoli, bell peppers, carrots, spinach
  - Ginger, garlic, tamari sauce
- Brown rice or whole grain noodles

✅ *Soy-rich, anti-inflammatory, and supports gut health.*

🍎 Snacks (choose 2–3 per day)

- **Prunes** (5–10 daily) ✅ *Supports bone mineral density*
- Handful of walnuts or almonds
- Soy yogurt with chia seeds
- Edamame (steamed)
- Sliced cucumber or bell peppers with hummus

Hydration

- 6–8 glasses of water daily
- Herbal teas (e.g., peppermint, chamomile)
- Limit caffeine and alcohol

Focus Area	Strategy
Hot Flashes	86g soy/day, low-fat diet (↑ *Porphyromonas*, *Prevotella*, etc.)
Bone Health	Prunes (5–10/day), calcium-rich foods, vitamin D, ↓ inflammation
Cardiovascular Health	MED + DASH elements: ↑ HDL, ↓ LDL, ↓ triglycerides, ↓ BP
Microbiota Diversity	High-fiber, fermented soy, legumes, low saturated fats

Foods to Limit

- Red and processed meats
- Refined carbs and sugars
- Saturated/trans fats
- Excess salt and alcohol

🗓️ Weekly meal plan

Day	Breakfast	Lunch	Dinner	Snacks (2–3/day)
Mon	Oatmeal with soy milk, flaxseed & berries	Mediterranean quinoa salad with chickpeas	Stir-fried tofu & broccoli with brown rice	Prunes (5), soy yogurt, cucumber + hummus
Tue	Soy yogurt parfait (berries, oats, chia)	Lentil soup + whole grain bread + side salad	Grilled tempeh with roasted veg + barley	Prunes (5), walnuts, edamame
Wed	Smoothie (soy milk, banana, spinach, flaxseed)	Tofu wrap with hummus, lettuce, tomato	Vegetable curry with tofu + quinoa	Prunes (5), almonds, carrots + hummus
Thu	Oatmeal with prunes & almonds	Chickpea & avocado salad	Stir-fry soba noodles with tofu & vegetables	Prunes (5), soy yogurt, bell peppers
Fri	Soy smoothie bowl (berries, granola)	Whole grain pasta with lentil tomato sauce	Grilled salmon (or tofu) + steamed greens + sweet potato	Prunes (5), edamame, chia pudding
Sat	Whole grain toast + avocado + boiled egg	Couscous with roasted vegetables & chickpeas	Stuffed bell peppers (quinoa, black beans, corn)	Prunes (5), soy yogurt, apple slices
Sun	Chia pudding (soy milk, berries) + 1 slice whole grain toast	Greek-style salad + lentil patties	Miso soup, brown rice, stir-fried greens + tofu	Prunes (5), walnuts, celery sticks

Appendix 4: Example of an exercise plan for peri- and postmenopausal women.

Day	Type	Details
Mon	**HIIT** + Resistance	20 min HIIT (e.g., intervals on bike or treadmill) + 30 min weight training
Tue	**Active Recovery**	Light walk, yoga, or stretching (30–45 min)
Wed	**Aerobic + Resistance**	30 min brisk walking, elliptical or cycling + 20 min strength work
Thu	**Rest or gentle activity**	Optional walk or stretching
Fri	**HIIT + Resistance**	Repeat Monday or vary with stair sprints and full-body circuit
Sat	**Aerobic/Cardio**	45–60 min moderate hike, swimming, dance, etc.
Sun	**Rest**	Full recovery

Appendix 5: Copyright Statement

Affirmation

The undersigned Pamela Somers postgraduate student of the Postgraduate Program "LIFESTYLE MEDICINE" of the University of Thessaly

I declare responsibly that I accept the following terms concerning:

(a) the copyright of my Master's Thesis entitled "The effect of exercise and nutrition on the gut microbiome of peri- and postmenopausal women."

(b) the management of the research data I will collect in the course of its elaboration:

1. The copyright of the resulting volume of the master's thesis will belong to me. I will follow the instructions for writing, printing and depositing copies of the dissertation in the relevant repositories (in printed and/or electronic form).
2. The management of the dissertation data belongs jointly to me and the Director of Studies/Main Supervisor.
3. Any scientific publication or announcement (posted or oral), or reference derived from the material/data of this work will be made with authors myself, the main supervisor and/or other researchers (such as a member of the three-member advisory committee), depending on their contribution to the research or the writing of the research paper(s).
4. The order of names in scientific publications or scientific announcements will be decided jointly by me and the main supervisor (DOS) of the project before it begins. This decision will be certified in writing between myself and the main supervisor.

Finally, I declare that I am aware of the rules on plagiarism and intellectual property and that I will strictly follow them throughout my studies, I will attend to the educational obligations arising from this MSc, as well as will attend the publication procedures that will arise after the completion of my studies.

06.06.2025

Pamela Somers

Pamela Somers