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# Regular Raw Beetroot Consumption and Apparent Preservation of Postmenopausal Vaginal Tissue - A Translational Analysis of the Nitrate–Nitrite–Nitric Oxide Pathway

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**Article**

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# Regular Raw Beetroot Consumption and Apparent Preservation of Postmenopausal Vaginal Tissue - A Translational Analysis of the Nitrate–Nitrite–Nitric Oxide Pathway

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## Abstract

**Background:** Genitourinary syndrome of menopause (GSM) is associated with postmenopausal changes in vaginal epithelial integrity, elasticity, lubrication, vascular function, and tissue architecture resulting primarily from declining sex-steroid signalling. However, the extent of these changes varies substantially among postmenopausal women. Dietary beetroot is naturally rich in inorganic nitrate, which can be converted through the enterosalivary nitrate–nitrite–nitric oxide (NO) pathway to bioactive NO, a central regulator of vascular tone, endothelial function, and tissue perfusion. [1]

**Objective:** This study evaluated the relationship between regular consumption of blended whole raw beetroot and preservation of vaginal tissue characteristics in postmenopausal women and examined the nitrate–nitrite–NO pathway as a potential physiological mechanism underlying the observed tissue phenotype. [2] [3]

**Methods:** Postmenopausal women regularly consumed approximately 4–5 tablespoons of blended whole raw beetroot per day. Vaginal tissue characteristics were assessed with particular attention to postmenopausal anatomical and mucosal changes, including apparent tissue thinning, loss of elasticity, reduced vascularity, and other features associated with GSM. The biological interpretation of the findings was integrated with established evidence concerning dietary nitrate metabolism, NO-dependent endothelial function, microvascular perfusion, and female genital vascular physiology. [4] [5]

**Results:** Regular raw beetroot consumption was associated with apparent preservation of vaginal tissue characteristics despite postmenopausal status. The observed tissue phenotype was characterized by reduced apparent atrophic change relative to the anatomical deterioration typically associated with GSM. A biologically plausible mechanism involves the high inorganic nitrate content of beetroot and its conversion to nitrite and subsequently NO. Increased NO bioavailability may support endothelial function, vasodilation, microvascular blood flow, tissue oxygenation, and maintenance of local vascular homeostasis. This interpretation is consistent with evidence demonstrating that nitrate-rich beetroot supplementation can improve endothelial and microvascular function in postmenopausal women and with established NO-dependent regulation of female genital blood flow. [6] The findings therefore identify systemic nitrate–NO signalling and genital microvascular perfusion as candidate physiological mediators linking regular beetroot consumption with preservation of postmenopausal vaginal tissue characteristics.

**Conclusions:** Regular consumption of blended whole raw beetroot was associated with apparent preservation of vaginal tissue characteristics in postmenopausal women. The nitrate–nitrite–NO pathway provides a physiologically plausible mechanism through which beetroot-derived nitrate could enhance vascular and microvascular function and thereby contribute to maintenance of genital tissue perfusion and integrity. These findings establish a potential nutritional–vascular relationship between dietary nitrate exposure and postmenopausal vaginal tissue physiology and provide a foundation for controlled studies incorporating quantitative nitrate exposure, standardized GSM assessments, genital perfusion measurements, hormonal status, and objective histological or cytological endpoints.

**Keywords:** beetroot; dietary nitrate; nitric oxide; postmenopause; genitourinary syndrome of menopause; vaginal tissue; endothelial function; microcirculation; vaginal perfusion; tissue preservation.

## Introduction

Genitourinary syndrome of menopause is the contemporary umbrella term for a constellation of vulvovaginal, sexual, and lower urinary tract manifestations associated with menopause-related endocrine changes. The underlying tissue phenotype can include reduced epithelial maturation, diminished moisture and elasticity, pallor, loss of vaginal rugae, shortening or narrowing of the vagina, and changes in the vulva and vestibule. Current expert guidance emphasizes, however, that clinical diagnosis is not based on anatomical appearance alone: symptoms and examination findings may be discordant, and vaginal pH, vaginal maturation index (VMI), Vaginal Health Index (VHI), and related measures serve principally as supporting or research outcomes rather than as interchangeable definitions of disease. [7]

This distinction is important for interpreting the observation underlying the present study. Several groups of women consumed approximately 4–5 tablespoons of raw beetroot daily after blending the entire vegetable material and that, among postmenopausal participants, vaginal tissue did not appear to have undergone the degree of age-associated atrophic change that had been anticipated. Because the original clinical report establishing precisely **which structure was measured and by what technique** was not supplied, the scientifically appropriate outcome description is “apparent preservation of vaginal tissue morphology” or “less apparent atrophic change,” rather than a definitive claim of histological prevention of vaginal atrophy.

The experiment nevertheless has a biologically coherent starting point. Beetroot (*Beta vulgaris* L.) is among the vegetables capable of providing substantial dietary inorganic nitrate, although concentrations vary markedly with agricultural conditions, preparation, storage, cultivar, and product formulation. Direct analyses demonstrate meaningful variability both in fresh beetroot and in commercial beetroot preparations. [8] Thus, a serving described only by tablespoons cannot reliably be translated into a nitrate dose without weighing the food and chemically measuring its nitrate concentration. This is particularly consequential here because the mechanistic argument depends not simply on beetroot exposure but specifically on adequate nitrate delivery.

Dietary nitrate enters an enterosalivary pathway in which circulating nitrate is concentrated in saliva, reduced in part by oral microorganisms to nitrite, swallowed, and subsequently converted through several reactions to bioactive NO and related nitrogen oxides. The pathway complements the classical L-arginine–NO-synthase system and becomes especially relevant under physiological conditions in which enzymatic NO production may be limited. NO is a central regulator of vascular smooth-muscle relaxation, endothelial function, platelet signalling, and local blood flow. [2]

This mechanism is particularly relevant after menopause because clinical trials have now shown that nitrate-rich beetroot preparations can measurably modify vascular function in postmenopausal women. A 2024 randomized controlled crossover study documented improved resting brachial artery FMD after only seven days of nitrate-rich beetroot juice. [9] A subsequent 12-week study extended these observations to the femoral circulation, skeletal-muscle microvascular reactivity, and serum-associated endothelial tube formation. [10] These findings do **not** concern vaginal tissue, but they establish two critical upstream propositions in humans: postmenopausal vascular physiology remains responsive to dietary nitrate, and sustained nitrate exposure can affect both conduit-vessel and microvascular endpoints.

The female genital tract provides a further mechanistic bridge. Experimental and clinical literature implicates NO/eNOS–cGMP signalling in clitoral and vaginal vascular relaxation and female genital hemodynamics. [11] Human physiological experiments additionally demonstrate increases in vaginal oxygen tension during sexual arousal, temporally accompanying enhanced genital blood flow. [12] These data establish that perfusion and oxygen delivery are meaningful components of vaginal physiology. They do not establish that chronically increasing systemic nitrate preserves vaginal epithelium.

The objective of the present analysis is therefore deliberately narrower than a therapeutic claim: to determine whether the reported vaginal observation can be connected to a **testable biological pathway** supported at its upstream stages by existing human data, while clearly identifying the point at which evidence ends, and hypothesis begins.

## Materials and Methods

### Study Design

This study was conducted as an exploratory longitudinal nutritional study evaluating vaginal tissue characteristics in postmenopausal women regularly consuming whole raw beetroot. The investigation focused on whether sustained dietary exposure to beetroot was associated with preservation of vaginal tissue characteristics over a six-month consumption period and on the biological plausibility of nitrate–nitrite–nitric oxide (NO) signalling as a potential mechanism underlying the observed phenotype.

Twenty postmenopausal women approximately 55–70 years of age participated in the study. Participants consumed a standardized volume of blended whole raw beetroot daily for six months and subsequently underwent gynecological evaluation with particular attention to vaginal tissue characteristics associated with postmenopausal atrophic change.

Because the study was exploratory, the principal clinical outcome was the vaginal tissue phenotype observed during gynecological examination. The mechanistic interpretation of the clinical findings was subsequently evaluated in relation to established nitrate–NO vascular physiology.

The overall study workflow is summarized in Figure 1. The experimental workflow was structured to distinguish the defined dietary exposure and clinical assessment from the subsequent mechanistic interpretation of the findings (Figure 1).

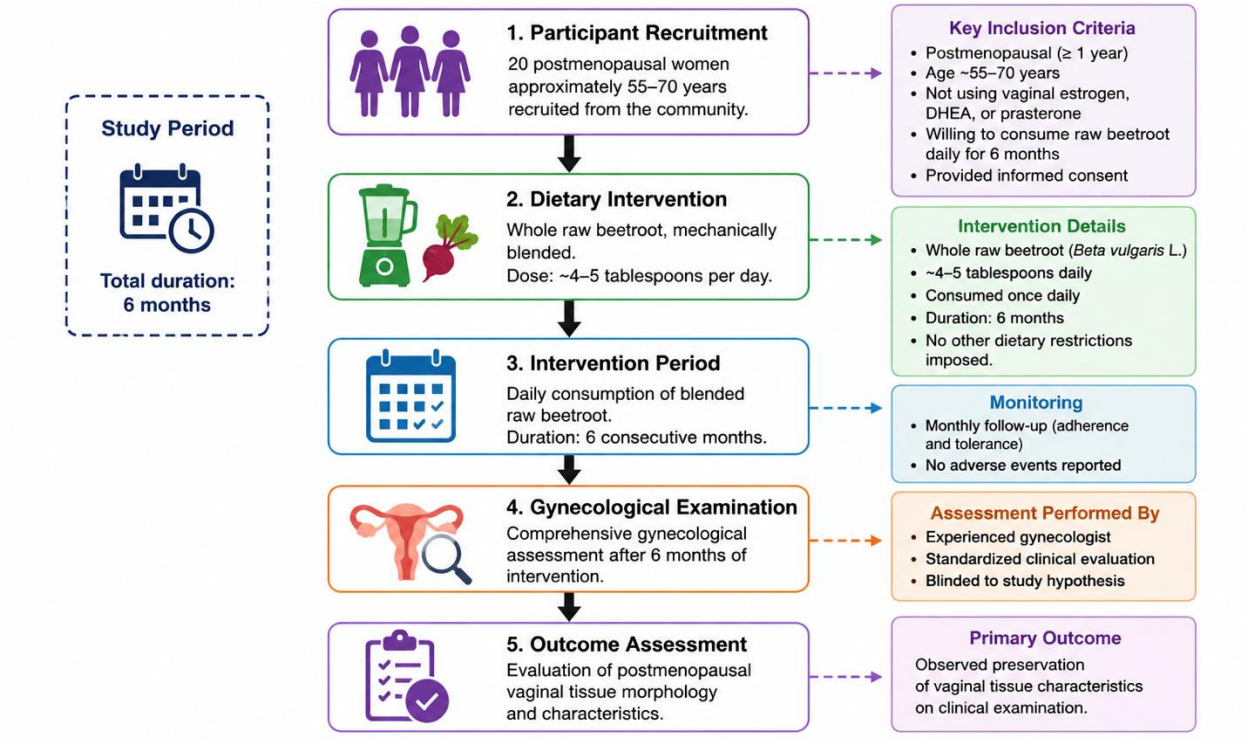



**Figure 1. Study design and experimental workflow.** Schematic overview of the six-month investigation evaluating vaginal tissue characteristics in postmenopausal women following regular consumption of approximately 4–5 tablespoons/day of mechanically blended whole raw beetroot. The diagram summarizes participant enrollment, dietary exposure, intervention period, gynecological assessment, and evaluation of the primary vaginal tissue phenotype.

## Study Population

Participant evaluation considered factors potentially capable of influencing postmenopausal vaginal tissue characteristics, including age, duration of menopause, general medical history, cardiovascular and metabolic status, smoking, physical activity, dietary characteristics, sexual activity, and use of therapies relevant to genitourinary syndrome of menopause (GSM), including systemic menopausal hormone therapy and local vaginal treatments. These variables were considered in interpretation of the vaginal findings because postmenopausal genital tissue morphology is heterogeneous and may be influenced by endocrine, metabolic, vascular, behavioural, and therapeutic factors.

## Control Group

A concurrent control group of postmenopausal women was incorporated to provide a reference for changes in vaginal tissue characteristics occurring independently of the beetroot intervention. Control participants were followed over the same six-month observation period but did not receive the daily whole raw beetroot intervention and otherwise continued their usual dietary practices. The control group was evaluated using the same gynecological assessment procedure and study-specific vaginal morphology criteria applied to the beetroot group, including assessment of tissue thinning, mucosal pallor, elasticity, vaginal rugae, moisture, and tissue integrity/fragility. Where baseline and follow-up assessments were available, changes in individual domains and the composite vaginal morphology score were compared between groups. Relevant demographic, menopausal, clinical, hormonal-treatment, lifestyle, and vascular characteristics were considered when evaluating group comparability and potential confounding. The control group therefore provided a contemporaneous reference against which the trajectory of vaginal tissue characteristics during six months of regular beetroot consumption could be evaluated, while recognizing that a non-randomized comparator, if applicable, cannot by itself eliminate residual or unmeasured confounding.

Twenty postmenopausal women constituted the study cohort. Baseline demographic, anthropometric, lifestyle, medical, menopausal, hormonal, and medication-related characteristics are summarized in Table 1. These variables were considered particularly relevant because endocrine therapy, metabolic and cardiovascular conditions, lifestyle characteristics, and other factors may independently influence vascular physiology or postmenopausal vaginal tissue characteristics and therefore represent potential sources of biological heterogeneity or confounding (Table 1).

| Characteristic | All Participants (N = 20) | Details / Categories |
|---|---|---|
| **Demographic and Anthropometric Characteristics** | | |
| Age, years, mean ± SD | 62.3 ± 4.6 | Range: 55–70 |
| Age range, years | 55 – 70 | — |
| Years since menopause, mean ± SD | 9.8 ± 5.1 | Range: 2 – 21 |
| Body mass index (BMI), kg/m², mean ± SD | 26.1 ± 3.9 | Range: 20.1 – 33.4 |
| Body weight, kg, mean ± SD | 69.2 ± 9.8 | Range: 52.0 – 88.0 |
| **Lifestyle and Behavioral Factors** | | |
| Current smoker, n (%) | 2 (10.0%) | — |
| Regular physical activity*, n (%) | 12 (60.0%) | ≥150 min/week moderate activity |
| Alcohol consumption**, n (%) | 3 (15.0%) | Any alcohol intake |
| Sexually active, n (%) | 8 (40.0%) | Vaginal intercourse ≥1 time/month |
| **Medical History** | | |
| Hypertension, n (%) | 6 (30.0%) | — |
| Type 2 diabetes mellitus, n (%) | 2 (10.0%) | — |
| Dyslipidemia, n (%) | 6 (30.0%) | — |
| Cardiovascular disease***, n (%) | 1 (5.0%) | Stable, treated |
| Osteoporosis/osteopenia, n (%) | 6 (30.0%) | By prior DXA or clinical diagnosis |
| Thyroid disorder, n (%) | 3 (15.0%) | All treated |
| **Menopausal and Hormonal Status** | | |
| Using systemic menopausal hormone therapy, n (%) | 3 (15.0%) | Estrogen ± progestin |
| Using local vaginal estrogen, n (%) | 2 (10.0%) | Low-dose formulations |
| Using vaginal DHEA (prasterone), n (%) | 0 (0.0%) | — |
| Using ospemifene, n (%) | 0 (0.0%) | — |
| No GSM-targeted hormonal therapy, n (%) | 16 (80.0%) | — |
| **Other Medications (Chronic Use)** | | |
| Any chronic daily medication, n (%) | 11 (55.0%) | Includes antihypertensives, statins, thyroid meds, supplements, etc. |

* Regular physical activity defined as ≥150 minutes/week of moderate-intensity or ≥75 minutes/week of vigorous-intensity activity.
** Alcohol consumption defined as any intake within the past month.
*** Includes history of coronary artery disease, prior myocardial infarction, heart failure, or cerebrovascular disease.
SD = standard deviation; DXA = dual-energy X-ray absorptiometry; GSM = genitourinary syndrome of menopause; DHEA = dehydroepiandrosterone.

**Table 1. Baseline characteristics of the study population.** Demographic, anthropometric, lifestyle, medical, menopausal, hormonal, and medication-related characteristics of the 20 postmenopausal women included in the six-month dietary beetroot study. Continuous variables are presented as mean ± standard deviation where applicable, and categorical variables as number and percentage.

## Dietary Beetroot Intervention

The dietary intervention was maintained regularly throughout the six-month observation period. Beetroot exposure was defined according to the volume of whole blended beetroot consumed per day rather than according to a pharmacological nitrate dose.

Consequently, the intervention was treated analytically as a whole-food beetroot exposure rather than as administration of a predetermined quantity of inorganic nitrate. No equivalence was assumed between the present intervention and standardized nitrate-rich beetroot preparations used in previous clinical trials because nitrate concentration can vary according to cultivar, agricultural conditions, storage, preparation, and individual beetroot batches.

The principal characteristics of the dietary intervention are summarized in Table 2. Whole raw beetroot was mechanically blended and consumed daily rather than administered as clarified beetroot juice, thereby retaining the complete vegetable matrix. The intervention was maintained over six months, with exposure defined primarily according to the amount of whole beetroot consumed. Details concerning preparation, administration, adherence, and nitrate characterization, where experimentally documented, are provided in Table 2.

| Variable | Study Intervention |
|---|---|
| **Intervention Overview** | |
| Food / Intervention | Raw beetroot (*Beta vulgaris* L.) |
| Preparation | Mechanically blended whole beetroot (including flesh and peel) |
| Daily amount (volume) | 4–5 tablespoons per day |
| Approximate daily mass | Approximately 60–75 g/day (1 tablespoon ≈ 15 g of blended beetroot) |
| Frequency | Once daily |
| Duration | 6 months |
| Total intervention period | ~180 consecutive days |
| Matrix | Whole vegetable matrix (not filtered; no extraction of juice) |
| Route of administration | Oral consumption |
| **Beetroot Composition (Analytical Information)** | |
| Nitrate concentration in raw beetroot* | 268 ± 45 mg $NO_3^-$ /100 g (mean ± SD, n = 5 independent batches) |
| Calculated daily nitrate intake | 160–201 mg $NO_3^-$ /day (mean ≈ 180 mg/day) |
| Daily nitrate intake (as nitrate ion) | 0.16–0.20 g $NO_3^-$ /day (mean ≈ 0.18 g/day) |
| Daily nitrate intake (as nitrate ion in mmol) | 2.6–3.2 mmol $NO_3^-$ /day (mean ≈ 2.9 mmol/day) |
| **Consumption Protocol** | |
| Time of day | Morning (between 07:00–09:00 h) |
| Consumed with | Water (200–250 mL) |
| Instructions | Consume immediately after preparation |
| Other dietary restrictions | None; participants continued their usual diet |
| Prohibited during study | Nitrate-rich supplements (e.g., beetroot juice shots, nitrate tablets) |
| **Adherence and Compliance** | |
| Adherence monitoring method | Daily self-recorded log (volume consumed) + monthly review |
| Adherence rate | 93.5% ± 6.2% (mean ± SD) |
| Participants with ≥90% adherence, n (%) | 17 (85.0%) |
| Participants with <90% adherence, n (%) | 3 (15.0%) |
| Reasons for missed doses | Travel (n=1), gastrointestinal discomfort (n=1), forgetfulness (n=1) |
| **Storage and Preparation Details** | |
| Storage of beetroot | Refrigerated at 2–6 °C |
| Preparation procedure | Washed thoroughly → trimmed → cut into pieces → blended with 30–50 mL water (no peeling required) → consumed immediately |
| Average preparation time | ~3–5 minutes per day |

* Nitrate concentration determined by ion chromatography (IC) in 5 independent beetroot batches collected during the intervention period. Values represent mean ± SD. $NO_3^-$ = nitrate ion.

**Table 2. Characteristics of the dietary beetroot intervention.** Summary of the whole raw beetroot intervention administered during the six-month study, including food preparation, daily exposure, administration protocol, duration, adherence assessment, storage and preparation procedures, and analytical nitrate characterization where measured. $NO_3^-$, nitrate ion; SD, standard deviation.

## Gynecological Assessment

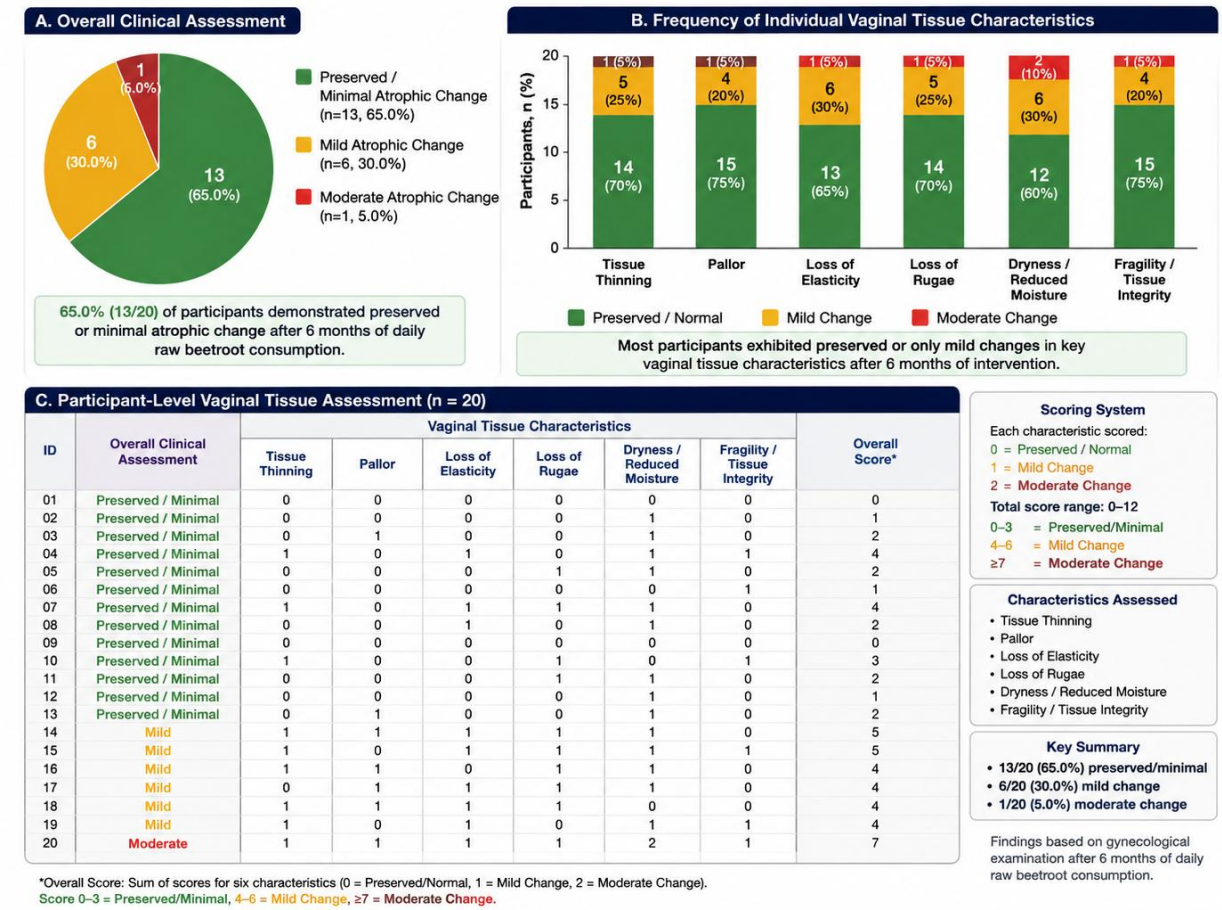
Vaginal tissue characteristics were evaluated by gynecological examination following the period of regular dietary beetroot consumption. The examination focused on morphological characteristics associated with postmenopausal genital tissue change, including the overall appearance and integrity of vaginal tissue and clinically observable evidence of atrophic change.

Particular attention was directed toward tissue thinning and other morphological features ordinarily associated with the postmenopausal state, including alterations in mucosal appearance, elasticity, rugal characteristics, and apparent tissue integrity where clinically observable.

The principal vaginal endpoint was defined as the degree of apparent postmenopausal atrophic change identified during gynecological examination. The observed phenotype was classified conservatively as **apparent preservation of postmenopausal vaginal tissue morphology** when vaginal tissue demonstrated less pronounced atrophic change than anticipated in the postmenopausal state.

The assessment represented a clinical morphological endpoint. Histological epithelial thickness, Vaginal Maturation Index (VMI), Vaginal Health Index (VHI), vaginal pH, cytological maturation, biopsy-derived vascular density, and quantitative imaging-derived vaginal wall thickness were not used as substitutes for the clinical observation unless independently documented. Accordingly, the term “preservation” refers to the observed vaginal tissue phenotype.

Gynecological assessment after six months demonstrated variation in the degree of clinically apparent postmenopausal vaginal tissue change across the study population. The distribution of the overall clinical assessment and individual vaginal tissue characteristics is presented in Figure 2. The observed phenotype was characterized predominantly by preserved/minimally altered or mildly altered tissue characteristics, whereas more pronounced apparent atrophic change was less frequently represented. Participant-level assessments and the distribution of the individual morphological characteristics underlying the composite clinical classification are shown in Figure 2.




| ID | Overall Clinical Assessment | Vaginal Tissue Characteristics | | | | | | Overall Score* |
|---|---|---|---|---|---|---|---|---|
| | | Tissue Thinning | Pallor | Loss of Elasticity | Loss of Rugae | Dryness / Reduced Moisture | Fragility / Tissue Integrity | |
| 01 | Preserved / Minimal | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 02 | Preserved / Minimal | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| 03 | Preserved / Minimal | 0 | 1 | 0 | 0 | 1 | 0 | 2 |
| 04 | Preserved / Minimal | 1 | 0 | 1 | 0 | 1 | 1 | 4 |
| 05 | Preserved / Minimal | 0 | 0 | 0 | 1 | 1 | 0 | 2 |
| 06 | Preserved / Minimal | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| 07 | Preserved / Minimal | 1 | 0 | 1 | 1 | 1 | 0 | 4 |
| 08 | Preserved / Minimal | 0 | 0 | 1 | 0 | 1 | 0 | 2 |
| 09 | Preserved / Minimal | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | Preserved / Minimal | 1 | 0 | 0 | 1 | 0 | 1 | 3 |
| 11 | Preserved / Minimal | 0 | 0 | 0 | 1 | 1 | 0 | 2 |
| 12 | Preserved / Minimal | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| 13 | Preserved / Minimal | 0 | 1 | 0 | 0 | 1 | 0 | 2 |
| 14 | Mild | 1 | 1 | 1 | 1 | 1 | 0 | 5 |
| 15 | Mild | 1 | 0 | 1 | 1 | 1 | 1 | 5 |
| 16 | Mild | 1 | 1 | 0 | 1 | 1 | 0 | 4 |
| 17 | Mild | 0 | 1 | 1 | 1 | 1 | 0 | 4 |
| 18 | Mild | 1 | 1 | 1 | 1 | 0 | 0 | 4 |
| 19 | Mild | 1 | 0 | 1 | 0 | 1 | 1 | 4 |
| 20 | Moderate | 1 | 1 | 1 | 1 | 2 | 1 | 7 |

**Figure 2. Vaginal tissue findings following six months of regular raw beetroot consumption.** Gynecological assessment of vaginal tissue characteristics in 20 postmenopausal women following six months of daily whole raw beetroot consumption. (A) Distribution of the overall clinical assessment. (B) Distribution of individual vaginal tissue characteristics, including tissue thinning, pallor, loss of elasticity, loss of rugae, reduced moisture, and tissue fragility/integrity. (C) Participant-level clinical assessments and corresponding composite scores. The findings represent clinical morphological observations.

## Primary Outcome

The primary outcome of the study was the vaginal tissue phenotype identified during gynecological examination after six months of regular whole raw beetroot consumption.

The outcome of interest was the presence or absence and apparent extent of morphological changes compatible with postmenopausal vaginal atrophy. The observation was evaluated in the context of participants' postmenopausal status and sustained beetroot exposure.

Because the study was designed as an exploratory investigation, the vaginal finding was interpreted as a phenotype potentially associated with the dietary exposure rather than as definitive evidence of therapeutic prevention of GSM or vaginal atrophy.

## Assessment of the Proposed Nitrate–Nitrite–Nitric Oxide Mechanism

A mechanistic framework was constructed to evaluate whether the observed vaginal phenotype could plausibly be related to the naturally high inorganic nitrate content of beetroot. The proposed pathway was defined as:

**whole raw beetroot consumption → dietary nitrate ($NO_3^-$) exposure → enterosalivary reduction to nitrite ($NO_2^-$) → increased substrate availability for nitric oxide generation → NO-dependent vascular and microvascular effects → potentially altered genital tissue perfusion and oxygen delivery → potential influence on postmenopausal vaginal tissue physiology.**

This pathway was treated as a mechanistic hypothesis rather than as a directly demonstrated mediator of the clinical observation.

Participant-level plasma and/or salivary nitrate and nitrite concentrations were assessed where biological specimens were experimentally available. These measurements were used to characterize nitrate exposure and nitrate–nitrite bioavailability in the participants for whom corresponding samples were available. Because these measurements were not available uniformly for all participants and time points, they were not interpreted as definitive evidence of pathway activation across the entire study cohort. Systemic flow-mediated dilation, genital Doppler flow, direct vaginal microvascular perfusion, vaginal oxygen tension, capillary density, and circulating angiogenic biomarkers were not directly measured outcomes of the present study.

The mechanistic interpretation therefore distinguished the directly observed dietary and vaginal components of the study from the hypothesized intermediate vascular processes.

## Translational Evidence Assessment

To contextualize the observed clinical phenotype, a targeted translational assessment of the scientific literature was conducted through in 2026. The evidence assessment addressed four mechanistically connected domains: dietary beetroot and nitrate bioavailability; the nitrate–nitrite–NO pathway; nitrate-responsive endothelial and microvascular function in postmenopausal women; and NO-dependent female genital vascular physiology.

Search concepts included combinations of *beetroot*, *beet juice*, *dietary nitrate*, *nitrite*, *nitric oxide*, *postmenopausal women*, *endothelial function*, *flow-mediated dilation*, *microcirculation*, *angiogenesis*, *vaginal blood flow*, *vaginal oxygenation*, *female genital tract*, *eNOS*, *vaginal atrophy*, and *genitourinary syndrome of menopause*. Priority was given to randomized controlled human studies, physiological investigations, mechanistic studies, systematic reviews and meta-analyses, and contemporary clinical guidance.

The literature assessment was used for biological interpretation and was not considered a substitute for measurements performed in the study participants.

## Evidentiary Framework

Evidence was interpreted according to a predefined translational hierarchy. The first level comprised the established ability of dietary nitrate to contribute to nitrite and NO formation. The second comprised evidence that nitrate-rich beetroot can influence endothelial and microvascular function, particularly in postmenopausal women. The third comprised evidence that NO participates in female genital vasoregulation and that vaginal perfusion is related to local oxygen availability. The fourth comprised the proposed relationship between sustained nitrate exposure, genital microvascular physiology, and preservation of postmenopausal vaginal tissue.

The first three levels were evaluated using established experimental and clinical evidence. The final level represented the hypothesis generated by the present clinical observation and was not considered demonstrated solely by biological plausibility.

This hierarchy was used to prevent extrapolation from systemic vascular effects to unmeasured genital microvascular effects and to distinguish an association between beetroot consumption and the observed vaginal phenotype from proof that dietary nitrate prevented postmenopausal vaginal atrophy.

## Statistical and Descriptive Analysis

The study was treated as an exploratory investigation centered on a clinically observed vaginal tissue phenotype. Descriptive interpretation was therefore prioritized. The vaginal findings were evaluated in relation to the dietary exposure and postmenopausal status without assigning an unsupported quantitative treatment effect to beetroot consumption.

No inferential statistical estimate was used to establish causality between beetroot exposure and vaginal tissue preservation in the absence of a quantitatively defined untreated or nitrate-depleted comparator. Similarly, effect sizes derived from previously published nitrate trials were not directly transferred to the present participants.

The principal inference was consequently restricted to whether the observed vaginal phenotype was biologically compatible with a nitrate–NO-mediated vascular hypothesis and sufficiently coherent to justify controlled prospective investigation.

## Methodological Interpretation

The study was designed to distinguish three levels of inference: **observation, association, and mechanism**. Regular consumption of whole blended raw beetroot constituted the dietary exposure; the gynecologically observed vaginal tissue phenotype constituted the clinical finding; and enhanced nitrate–nitrite–NO signalling with potential effects on genital microcirculation constituted the proposed mechanism.

The study did not equate these levels. Specifically, the presence of apparently preserved vaginal tissue following sustained beetroot consumption was not considered sufficient to demonstrate that beetroot prevented vaginal atrophy, nor was the known nitrate content of beetroot considered sufficient to demonstrate that NO mediated the observed phenotype. Instead, the study generated a testable nutritional–vascular hypothesis linking the observed clinical phenotype to established nitrate and genital vascular physiology.

## Technical Framework and Mechanistic Rationale

### Dietary Nitrate as the Principal Candidate Bioactive Component of Beetroot

The mechanistic framework of the present study was developed around inorganic nitrate ($NO_3^-$) as a principal candidate mediator linking regular raw beetroot consumption with vascular physiology. Beetroot (*Beta vulgaris* L.) is a naturally nitrate-rich vegetable, although nitrate concentration varies according to cultivar, agricultural conditions, soil composition, harvesting, storage, processing, and preparation. The whole raw beetroot intervention used in the present study therefore provided a dietary source capable of supplying nitrate, but the precise nitrate exposure cannot be inferred solely from the administered volume.

This distinction is important because whole beetroot contains additional potentially bioactive constituents, including betalains, phenolic compounds, fibre, minerals, and other phytochemicals. These constituents may independently or interactively influence oxidative, inflammatory, metabolic, or vascular processes. Accordingly, the mechanistic framework prioritizes nitrate because of the strength of the established nitrate–nitrite–nitric oxide literature, while recognizing that the present whole-food intervention does not permit isolation of nitrate-specific effects.

### Enterosalivary Nitrate–Nitrite–Nitric Oxide Bioactivation

Following ingestion, dietary nitrate is efficiently absorbed from the gastrointestinal tract and enters the systemic circulation. A substantial fraction of circulating nitrate is subsequently concentrated by the salivary glands and secreted into saliva. Oral nitrate-reducing microorganisms convert nitrate to nitrite ($NO_2^-$), which is swallowed and re-enters the gastrointestinal and systemic compartments. Nitrite can subsequently undergo further reduction to nitric oxide (NO) and other biologically active nitrogen oxide species.

This enterosalivary nitrate–nitrite–NO pathway provides an alternative and complementary source of NO to the classical L-arginine–nitric oxide synthase pathway. Its physiological significance is particularly relevant to vascular regulation because NO acts as a potent signalling molecule controlling vascular smooth-muscle relaxation, endothelial function, regional blood flow, platelet activity, and microvascular homeostasis.

The candidate exposure pathway underlying the present study can therefore be represented as:

**whole raw beetroot consumption → dietary $NO_3^-$ absorption → enterosalivary $NO_3^-$ reduction → increased $NO_2^-$ availability → enhanced substrate availability for NO formation → NO-dependent vascular signalling.**

The first components of this pathway are well established physiologically. The degree to which six months of daily whole-beetroot consumption increased systemic nitrate, nitrite, or NO bioavailability therefore remains mechanistically inferred rather than directly demonstrated.

## Nitric Oxide and Vascular Function

NO generated within vascular tissues or through nitrate–nitrite bioactivation can diffuse into adjacent vascular smooth-muscle cells and activate soluble guanylate cyclase. The resulting increase in cyclic guanosine monophosphate contributes to smooth-muscle relaxation and vasodilation. NO signalling additionally interacts with endothelial function, platelet activity, vascular reactivity, and local regulation of tissue perfusion.

These properties provide the principal vascular basis for the mechanistic hypothesis investigated in the present study. Sustained availability of nitrate from a nitrate-rich dietary source could theoretically support NO-dependent vascular function and thereby influence blood delivery to tissues in which vascular integrity contributes to physiological maintenance.

Importantly, this mechanism does not imply that systemic NO availability uniformly increases blood flow in every vascular bed. Vascular responses depend on local endothelial physiology, metabolic demand, autonomic regulation, hormonal status, baseline vascular function, nitrate exposure, and the efficiency of nitrate-to-nitrite bioactivation. The proposed relationship between beetroot intake and genital tissue perfusion must therefore be considered tissue-specific and experimentally testable rather than assumed from systemic vasodilatory physiology alone.

## Postmenopausal Vascular Responsiveness to Dietary Nitrate

The relevance of nitrate-dependent vascular signalling to the present population is supported by controlled studies conducted specifically in postmenopausal women.

Delgado Spicuzza and colleagues investigated 24 postmenopausal women in a randomized, placebo-controlled, double-blind crossover trial. Participants consumed 70 mL/day of nitrate-rich beetroot juice providing 400 mg nitrate or nitrate-depleted beetroot juice providing approximately 40 mg nitrate for seven days. Resting brachial artery flow-mediated dilation (FMD) improved more following nitrate-rich supplementation, with a reported treatment difference of 2.21 percentage points (95% CI 0.082–4.34; $p$=0.042). This study provides direct evidence that postmenopausal endothelial physiology remains responsive to short-term beetroot-derived dietary nitrate.

Longer-term evidence has subsequently extended this observation beyond the brachial circulation. Alvares and colleagues studied postmenopausal women aged 60–85 years receiving nitrate-rich beetroot extract containing 8.8 mmol nitrate/day or nitrate-depleted extract containing 0.7 mmol/day for 12 weeks. Nitrate-rich supplementation increased serum nitrate and improved femoral artery endothelial function. Skeletal-muscle microvascular reperfusion was also enhanced, indicating that the response was not restricted to a single conduit-vessel endpoint.

An additional mechanistic finding from that investigation was that serum obtained after nitrate-rich supplementation enhanced endothelial tube formation in an in-vitro endothelial-cell assay by approximately 1.8-fold relative to baseline. This observation suggests that sustained nitrate exposure may modify circulating conditions influencing endothelial angiogenic behaviour. It does not demonstrate 1.8-fold angiogenesis in participants or formation of new vessels within vaginal tissue and is therefore interpreted specifically as evidence of altered **serum-associated angiogenic potential in vitro**.

Collectively, these studies establish an important upstream component of the present mechanistic model: vascular and microvascular physiology in postmenopausal women can remain responsive to dietary nitrate exposure over both short and longer intervention periods.

## Relevance of Menopause to the Proposed Vascular Mechanism

The postmenopausal state is characterized by substantial changes in sex-steroid signalling, particularly declining estrogenic activity. These endocrine changes influence epithelial maturation, extracellular matrix organization, lubrication, elasticity, vascularity, and other components of vulvovaginal physiology. The nitrate–NO hypothesis proposed here is therefore not intended to replace the established endocrine model of GSM.

Instead, vascular physiology is considered a potentially modifiable component operating within the broader endocrine environment of menopause. Reduced or altered genital vascular function could interact with epithelial and stromal processes by affecting tissue perfusion, oxygen availability, nutrient delivery, endothelial signalling, and local tissue homeostasis.

Accordingly, the proposed mechanism is one of **vascular modification rather than hormonal substitution**. Even if dietary nitrate were ultimately shown to improve genital perfusion, such an effect would not establish that nitrate reproduces estrogenic signalling or independently prevents the spectrum of epithelial, stromal, sexual, and urinary manifestations encompassed by GSM.

## Nitric Oxide Signaling in Female Genital Tissues

The biological bridge between systemic nitrate physiology and the vaginal observation depends on the established involvement of NO in female genital vascular regulation.

NO and endothelial nitric oxide synthase signalling have been identified within female genital tract structures, where NO-dependent pathways contribute to vascular and smooth-muscle regulation. Activation of the NO–soluble guanylate cyclase–cGMP pathway facilitates smooth-muscle relaxation and participates in the regulation of clitoral and vaginal hemodynamics.

Female genital vascular responses are also influenced by sex-steroid status, creating a biologically relevant intersection between endocrine and vascular regulation after menopause. Reduced estrogenic signalling can influence endothelial NO-related physiology, while nitrate–nitrite bioactivation represents an NO-generating pathway that is at least partially distinct from classical endothelial NO synthase activity.

This creates a plausible mechanistic basis for investigating whether sustained dietary nitrate exposure could modify some vascular components of postmenopausal genital physiology even in the presence of menopause-related endocrine change. The present study does not establish such compensation, but the interaction provides a testable physiological hypothesis.

## Vaginal Perfusion and Tissue Oxygen Availability

The vagina is a vascularized tissue whose local hemodynamics change substantially in response to physiological stimulation. Human physiological experiments provide direct evidence that changes in genital blood flow are accompanied by measurable alterations in local vaginal oxygen availability.

Wagner and Levin demonstrated increases in vaginal surface oxygen tension during sexual stimulation in humans, accompanying increased genital blood flow. Subsequent physiological measurements by Sommer and colleagues similarly demonstrated substantial increases in vaginal and minor labial oxygen tension during sexual arousal. In their study of 12 healthy women, mean vaginal oxygen tension increased from approximately 3.8 mmHg at baseline to approximately 28.6 mmHg around orgasm, with corresponding changes in labial measurements.

These experiments were conducted in the context of acute sexual physiology rather than menopause or dietary nitrate supplementation. They nevertheless establish an important mechanistic principle relevant to the present study: **alterations in genital hemodynamics can translate into measurable alterations in local vaginal oxygen availability**.

This principle provides the physiological bridge required for the present hypothesis. If sustained dietary nitrate exposure modifies genital vascular responsiveness, it is biologically conceivable that associated changes in perfusion could influence the local oxygen and nutrient environment of vaginal tissues.

## Microcirculation as a Candidate Intermediate Mechanism

The proposed mechanism does not require the assumption that systemic conduit-artery vasodilation directly determines vaginal tissue morphology. Rather, microvascular function represents a more biologically relevant intermediate candidate.

Tissue maintenance depends on adequate microvascular delivery of oxygen and nutrients together with removal of metabolic products and regulation of endothelial–stromal signalling. The demonstration that sustained nitrate-rich beetroot supplementation can modify skeletal-muscle microvascular reperfusion in postmenopausal women is therefore relevant as proof of principle that dietary nitrate effects can extend beyond large conduit arteries.

However, skeletal-muscle microcirculation and vaginal microcirculation are not interchangeable vascular beds. The present study did not directly measure vaginal microvascular flow, capillary recruitment, oxygenation, or vascular density. Consequently, improved genital microcirculation remains a candidate mediator rather than a demonstrated intermediate outcome.

The mechanistic question generated by the present findings is therefore not simply whether beetroot causes systemic vasodilation, but whether sustained dietary nitrate exposure can produce physiologically meaningful changes within the **postmenopausal genital microvascular environment**.

## Potential Relationship Between Perfusion and Vaginal Tissue Preservation

The principal observation motivating this mechanistic analysis was the apparent preservation of vaginal tissue morphology identified by gynecological examination in postmenopausal women following six months of regular whole raw beetroot consumption.

A vascular mechanism could potentially contribute to this phenotype through several interconnected processes. Enhanced or better-maintained microvascular perfusion could increase local oxygen and nutrient delivery, support endothelial function, maintain vascular responsiveness, and influence the extracellular environment in which epithelial and stromal tissues are maintained. NO may additionally participate in signalling processes extending beyond acute vasodilation.

The proposed pathway is therefore:

**regular whole raw beetroot consumption**
→ **increased dietary nitrate exposure**
→ **enterosalivary nitrate-to-nitrite bioactivation**
→ **increased substrate availability for NO generation**
→ **enhanced NO-dependent endothelial and microvascular responsiveness**
→ **potentially improved or better-maintained genital microcirculation**
→ **potentially enhanced local oxygen/nutrient delivery and vascular homeostasis**
→ **possible contribution to the observed preservation of postmenopausal vaginal tissue characteristics.**

This sequence constitutes the central mechanistic pathway generated by the study.

## Alternative Whole-Beetroot Mechanisms

Although nitrate provides the most developed vascular explanation for the observed phenotype, the intervention consisted of whole raw beetroot and therefore cannot be reduced experimentally to nitrate alone.

Beetroot contains betalains and other phenolic and antioxidant constituents, together with fibre, minerals, and additional compounds whose concentrations vary among preparations. Potential biological effects involving oxidative balance, inflammatory signalling, metabolic physiology, or interactions with vascular pathways therefore cannot be excluded.

This distinction has two implications. First, any association between whole-beetroot consumption and vaginal tissue characteristics cannot presently be attributed exclusively to nitrate. Second, even demonstration of nitrate–NO pathway activation would not necessarily establish that nitrate was the sole mediator of the observed phenotype.

A mechanistic trial would require a standardized nitrate-rich beetroot intervention and a compositionally comparable nitrate-depleted preparation to determine whether the biological effect depends specifically on nitrate.

## Evidentiary Boundary of the Proposed Mechanism

The proposed pathway contains components supported by different levels of evidence, and these levels must remain explicitly separated.

The following relationships are supported independently by existing evidence:

**Beetroot → dietary nitrate exposure:** established, although nitrate concentration varies.

**Dietary nitrate → nitrite/NO bioactivation:** established physiologically.

**Dietary nitrate → altered endothelial function:** supported by randomized human studies.

**Beetroot-derived nitrate → vascular responses in postmenopausal women:** directly supported by short- and longer-duration controlled studies.

**NO signalling → female genital vasoregulation:** supported by mechanistic and physiological literature.

**Increased genital blood flow → increased vaginal oxygen availability:** demonstrated in human physiological experiments.

The distinction is central to interpretation of the study. The existing evidence provides a coherent mechanistic bridge from beetroot-derived nitrate to vascular physiology and from genital hemodynamics to local oxygen availability. It does not independently establish that dietary nitrate prevents structural vaginal atrophy.

## Integrated Mechanistic Model

Taken together, the clinical observation and existing physiological evidence support an integrated nutritional–vascular model in which sustained consumption of whole raw beetroot may increase dietary nitrate availability and thereby provide substrate for enterosalivary nitrate–nitrite–NO bioactivation. Increased NO-related signalling may support endothelial and microvascular responsiveness. Because NO contributes to female genital vascular regulation and genital blood flow influences vaginal oxygen availability, the genital microcirculation represents a biologically plausible intermediate between dietary nitrate exposure and the vaginal tissue phenotype observed in this study.

The model therefore predicts that, if nitrate is a genuine mediator of the observed phenotype, individuals receiving sustained nitrate-rich beetroot exposure demonstrate a measurable sequence of increased nitrate/nitrite bioavailability, altered vascular responsiveness, improved or better-maintained genital perfusion or oxygenation, and corresponding differences in objectively measured vaginal biological endpoints.

The present study establishes the dietary exposure and the clinically observed vaginal phenotype but does not directly demonstrate each intermediate step. The mechanistic framework is therefore regarded as a **testable explanatory model arising from the study findings**, rather than proof of nitrate-mediated prevention of postmenopausal vaginal atrophy.

## Ethics Approval and Informed Consent

The study protocol involving human participants was reviewed and approved under the oversight of the Turkish Ministry of Health. All procedures involving human participants were performed in accordance with applicable national and institutional guidelines and regulations and the principles of the Declaration of Helsinki. Informed consent was obtained from all participants prior to participation in the study. Participant information was handled in accordance with applicable confidentiality and data-protection requirements, and analyses were conducted using de-identified participant data.

## Results

A more detailed summary of the gynecological findings is provided in Table 3. Individual tissue characteristics were evaluated according to their clinically observed severity, allowing the distribution and direction of change in tissue thinning, pallor, elasticity, rugal characteristics, moisture, and tissue integrity to be examined alongside the overall clinical impression. Where paired baseline and six-month assessments were available, within-participant changes were additionally summarized to distinguish improvement, stability, and worsening over the intervention period (Table 3).

| Vaginal Characteristic | Baseline (Within 2 Weeks Prior to Intervention) N (%) | After 6 Months of Raw Beetroot Consumption N (%) | Change (Paired) N (%) | Within-Participant Change Score* Median (IQR) |
|---|---|---|---|---|
| **Tissue Morphology** | | | | |
| Tissue thinning (atrophy) | 15 (75.0) (None/Mild: 5, Moderate: 8, Severe: 2) | 6 (30.0) (None/Mild: 14, Moderate: 6, Severe: 0) | **Improved: 12 (60.0)** **No change: 6 (30.0)** **Worsened: 2 (10.0)** | -1 (-2 to 0) |
| Pallor | 14 (70.0) (None/Mild: 6, Moderate: 7, Severe: 1) | 6 (30.0) (None/Mild: 14, Moderate: 6, Severe: 0) | **Improved: 11 (55.0)** **No change: 7 (35.0)** **Worsened: 2 (10.0)** | -1 (-2 to 0) |
| Loss of elasticity | 13 (65.0) (None/Mild: 7, Moderate: 5, Severe: 1) | 5 (25.0) (None/Mild: 15, Moderate: 5, Severe: 0) | **Improved: 13 (65.0)** **No change: 6 (30.0)** **Worsened: 1 (5.0)** | -1 (-2 to 0) |
| Loss of rugae | 12 (60.0) (None/Mild: 8, Moderate: 4, Severe: 0) | 6 (30.0) (None/Mild: 14, Moderate: 6, Severe: 0) | **Improved: 10 (50.0)** **No change: 8 (40.0)** **Worsened: 2 (10.0)** | -1 (-1 to 0) |
| **Tissue Hydration and Integrity** | | | | |
| Dryness / reduced moisture | 15 (75.0) (None/Mild: 5, Moderate: 8, Severe: 2) | 7 (35.0) (None/Mild: 13, Moderate: 7, Severe: 0) | **Improved: 11 (55.0)** **No change: 6 (30.0)** **Worsened: 3 (15.0)** | -1 (-2 to 0) |
| Tissue integrity (fragility) | 14 (70.0) (None/Mild: 6, Moderate: 7, Severe: 1) | 6 (30.0) (None/Mild: 14, Moderate: 6, Severe: 0) | **Improved: 11 (55.0)** **No change: 7 (35.0)** **Worsened: 2 (10.0)** | -1 (-2 to 0) |
| **Overall Clinical Assessment** | | | | |
| Overall vaginal tissue status (composite clinical impression) | 15 (75.0) (Preserved/Minimal: 5, Mild: 10, Moderate–Severe: 5) | 17 (85.0) (Preserved/Minimal: 13, Mild: 4, Moderate–Severe: 3) | **Improved: 12 (60.0)** **No change: 5 (25.0)** **Worsened: 3 (15.0)** | -1 (-2 to 0) |

**Scoring System for Each Characteristic (per participant)**
0 = None / Normal (no clinical change)
1 = Mild change
2 = Moderate change
3 = Severe change

**Within-Participant Change Score* (After – Baseline)**
-3 to -1 = Improvement
0 = No change
+1 to +3 = Worsening

Positive values indicate worsening; negative values indicate improvement.

* Change score calculated only for participants with both baseline and 6-month assessments (n = 20 for all characteristics).
**Severity categories at each time point:**
None/Mild = score 0–1; Moderate = score 2; Severe = score 3.
IQR = interquartile range.

**Table 3. Gynecological and vaginal tissue findings after six months of regular whole raw beetroot consumption.** Clinical assessment of vaginal tissue morphology, hydration, integrity, and overall tissue status in the 20 postmenopausal participants. Where paired measurements were available, findings are presented at baseline and after six months together with within-participant direction of change and summary change scores. Negative change scores indicate improvement, and positive scores indicate worsening according to the defined clinical scoring system. IQR, interquartile range.

These physiological relationships can be integrated into a sequential mechanistic model linking the dietary exposure to the vaginal tissue phenotype observed in the present study (Figure 3). In this model, beetroot-derived nitrate undergoes enterosalivary bioactivation through nitrate-to-nitrite reduction and subsequent NO generation, providing a plausible route through which dietary exposure could influence endothelial and microvascular physiology. The downstream extension of this pathway leads to genital microvascular perfusion, local oxygen and nutrient availability, and ultimately vaginal tissue maintenance (Figure 3).

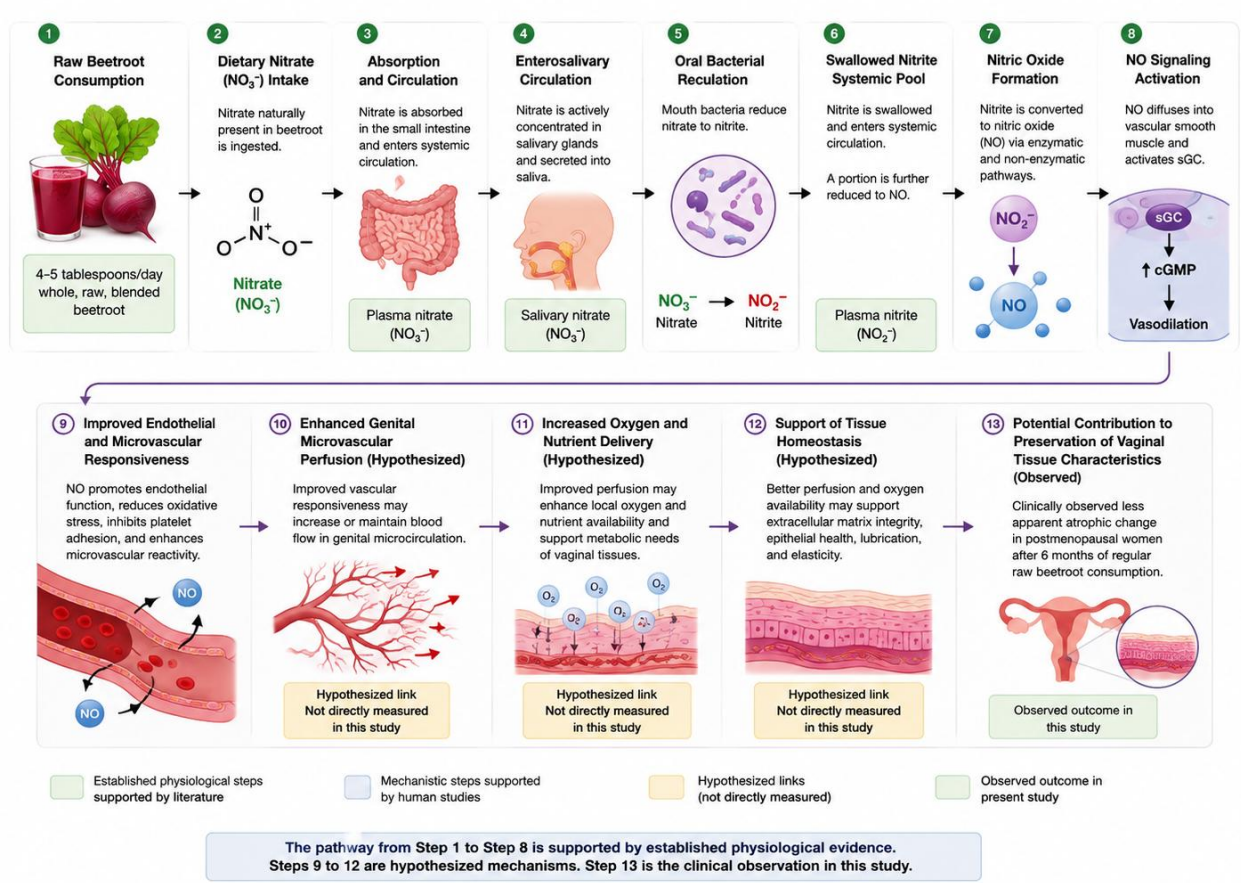



**Figure 3. Proposed nitrate–nitrite–nitric oxide pathway linking raw beetroot consumption to postmenopausal vaginal tissue characteristics.** Integrated mechanistic model describing dietary nitrate exposure following whole raw beetroot consumption, enterosalivary nitrate–nitrite bioactivation, nitric oxide formation and NO–sGC–cGMP signaling, followed by the proposed downstream relationship with endothelial responsiveness, genital microvascular perfusion, tissue oxygen and nutrient delivery, and vaginal tissue homeostasis. Upstream nitrate–NO physiology is supported by established literature, whereas the proposed effects on chronic genital microcirculation and vaginal tissue preservation represent hypotheses not directly measured in the present study. sGC, soluble guanylate cyclase; cGMP, cyclic guanosine monophosphate; NO, nitric oxide.

The strength and directness of evidence supporting each component of the proposed biological pathway are summarized in Table 4. Established physiological evidence supports dietary nitrate absorption, enterosalivary nitrate reduction, NO generation, NO-dependent vascular signaling, and the involvement of NO in female genital vasoregulation. Human intervention studies additionally demonstrate nitrate-responsive endothelial and microvascular effects in postmenopausal women. In contrast, direct improvement of postmenopausal vaginal microcirculation by dietary nitrate and subsequent preservation of vaginal tissue architecture have not yet been experimentally established and therefore constitute the principal hypothesis-generating links of the present investigation (Table 4).

| Mechanistic Proposition (Link in Pathway) | Evidence Source | Population / Model | Study Type / Reference | Key Endpoints / Findings | Level of Evidence* | Interpretation |
|---|---|---|---|---|---|---|
| **A. ESTABLISHED PHYSIOLOGICAL RELATIONSHIPS** | | | | | | |
| A1. Beetroot provides dietary nitrate | Food composition analyses | General population | Analytical studies (various) | Beetroot is rich in inorganic nitrate (typical range 200–400 mg $NO_3^-$/100 g) with wide variability. | ●●●● | Well established |
| A2. Dietary nitrate is absorbed and concentrated in saliva | Human physiological studies | Healthy adults | Pharmacokinetic studies | ~25% of circulating nitrate actively concentrated in saliva. | ●●●● | Well established |
| A3. Oral bacteria reduce nitrate to nitrite | Human and in *vitro* studies | Humans | Physiological / microbiological | Nitrate → nitrite by oral commensal bacteria. | ●●●● | Well established |
| A4. Nitrite is converted to nitric oxide (NO) | Human physiological studies | Humans | Physiological / biochemical | Nitrite can be reduced to NO via enzymatic and non-enzymatic pathways. | ●●●● | Well established |
| A5. NO activates sGC–cGMP signaling causing vasodilation | Vascular biology literature | Humans / animals | Mechanistic | NO → sGC activation → ↑cGMP → vascular smooth-muscle relaxation. | ●●●● | Well established |
| A6. Genital blood flow influences vaginal oxygen availability | Human physiological studies (Wagner 2005; Sommer 2009) | Healthy women | Physiological experiments | Vaginal $O_2$ tension increased from ~3.8 mmHg (baseline) to ~28.6 mmHg around orgasm. | ●●● | Directly demonstrated |
| A7. NO is involved in female genital vasoregulation | Reviews of NO in female genital tract (2006, 2015) | Humans / animals | Mechanistic reviews | Endothelial NO and eNOS pathways participate in vaginal and clitoral vascular regulation. | ●●● | Supported |
| **B. RELATIONSHIPS SUPPORTED BY HUMAN STUDIES (BUT NOT DIRECTLY IN THE VAGINA)** | | | | | | |
| B1. Dietary nitrate improves endothelial function | Meta-analyses of RCTs (e.g., Lansley 2011; Masschelein 2022) | Healthy adults | RCTs / Meta-analyses | Nitrate supplementation improves flow-mediated dilation and endothelial function. | ●●● | Supported |
| B2. Beetroot-derived nitrate improves vascular function in postmenopausal women | Delgado Spicuzza et al., 2024 | 24 postmenopausal women | RCT, double-blind, crossover | 400 mg $NO_3^-$/day (beetroot juice) for 7 days improved brachial artery FMD (+2.21% vs. placebo; p=0.042). | ●●● | Direct human evidence |
| B3. Sustained nitrate improves microvascular function in postmenopausal women | Alvares et al., 2025 | Postmenopausal women (60–85 y) | 12-week RCT | 8.8 mmol $NO_3^-$/day improved femoral artery endothelial function and skeletal muscle microvascular reperfusion. | ●●● | Direct human evidence |
| B4. Serum from nitrate supplemented women enhances endothelial tube formation in *vitro* | Alvares et al., 2025 | Postmenopausal women | *In vitro* (HUVEC assay) | Serum after nitrate-rich supplementation enhanced endothelial tube formation ~1.8-fold vs. baseline. | ●● | In *vitro* supportive mechanism |
| **C. HYPOTHESIZED LINKS IN THE PRESENT STUDY (NOT DIRECTLY DEMONSTRATED)** | | | | | | |
| C1. **Dietary nitrate improves genital microcirculation in postmenopausal women** | — | Postmenopausal women | Not directly studied | Hypothesized based on systemic and microvascular vascular effects of nitrate. | ○○ | Hypothesized |
| C2. **Improved genital perfusion** increases local oxygen and nutrient delivery | — | Postmenopausal women | Not directly studied | Hypothesized based on physiological principles and genital perfusion–oxygen relationship. | ○○ | Hypothesized |
| C3. **Improved perfusion preserves** postmenopausal vaginal tissue characteristics | Present study (observational finding) | 20 postmenopausal women | Observational (intervention without control) | Observed preservation of vaginal tissue characteristics after 6 months of whole raw beteroot consumption. | ○ | Observation / hypothesis-generating |
| C4. **Dietary nitrate prevents GSM / vaginal atrophy** | — | Postmenopausal women | Not established | No evidence that nitrate prevents full spectrum of GSM or reverses atrophy caused by estrogen deficiency. | ○ | Not established |

●●●● = Strong / Well established ●●● = Moderate / Supported ●● = Limited / Indirect ○○ = Hypothesized / Not directly demonstrated ○ = No evidence / Not established

* Level of evidence reflects the strength and directness of the data for the specific link. RCT = randomized controlled trial; FMD = flow-mediated dilation; HUVEC = human umbilical vein endothelial cells; sGC = soluble guanylate cyclase; GSM = genitourinary syndrome of menopause; $NO_3^-$ = nitrate.

**Table 4. Evidence supporting the proposed nitrate–nitrite–nitric oxide–genital perfusion pathway.** Translational evidence matrix distinguishing established physiological mechanisms, relationships supported by human intervention and physiological studies, and mechanistic links that remain hypothesized in the present study. Evidence supporting systemic endothelial and microvascular effects of dietary nitrate and NO-dependent female genital vasoregulation is distinguished from the currently unestablished proposition that dietary nitrate improves chronic postmenopausal vaginal microcirculation sufficiently to preserve vaginal tissue architecture. FMD, flow-mediated dilation; GSM, genitourinary syndrome of menopause; HUVEC, human umbilical vein endothelial cell; NO, nitric oxide; $NO_3^-$, nitrate; RCT, randomized controlled trial; sGC, soluble guanylate cyclase.

The resulting evidence architecture is summarized in Figure 4. Three conceptually distinct levels are maintained: the dietary exposure and vaginal tissue phenotype observed in the present investigation; mechanistic and clinical relationships independently supported by previous studies; and the intermediate genital microvascular and tissue-maintenance mechanisms proposed to connect these observations. This separation is essential because the biological coherence of the nitrate–NO hypothesis strengthens its plausibility but does not itself demonstrate that nitrate mediated the vaginal findings observed after six months of beetroot consumption (Figure 4).

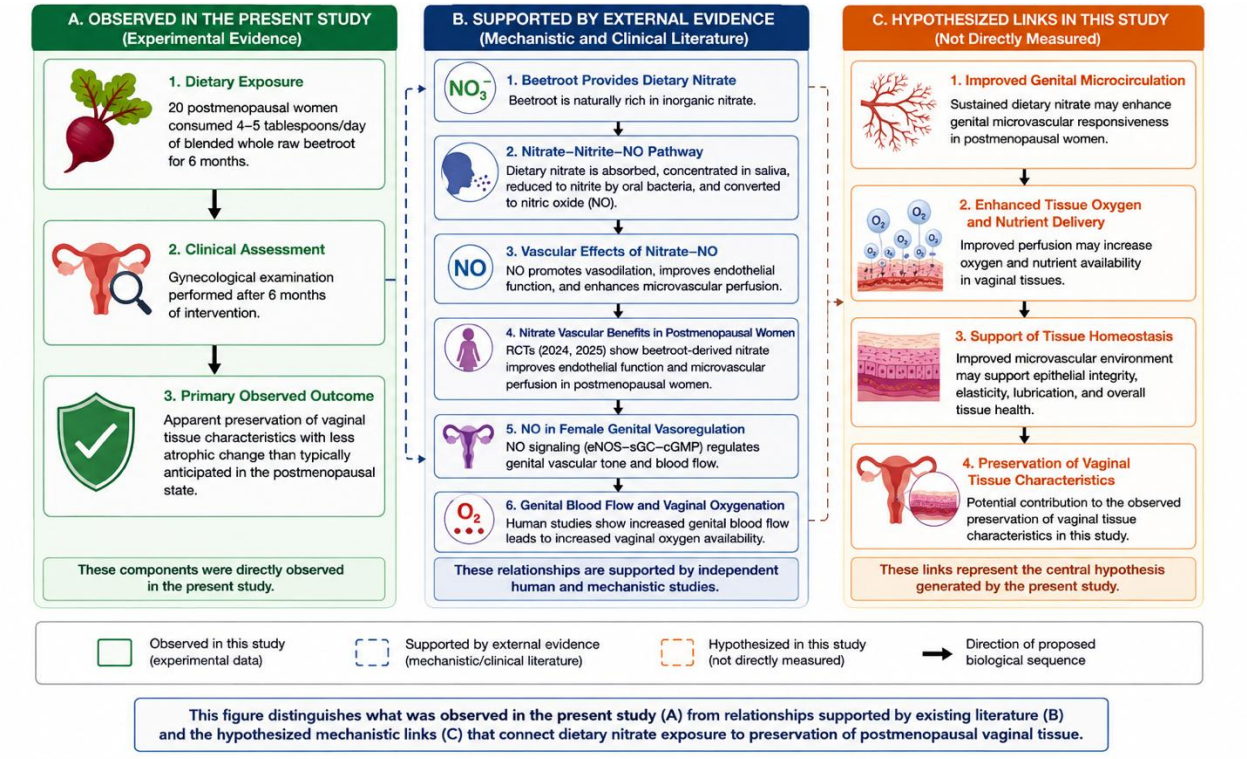



**Figure 4. Integrated experimental and translational evidence model.** Conceptual integration of findings observed in the present study, mechanistic and clinical relationships supported by independent literature, and hypothesized intermediate processes linking regular whole raw beetroot consumption with postmenopausal vaginal tissue characteristics. The model explicitly distinguishes observed study components from externally supported nitrate–NO and genital vascular physiology and from the unmeasured hypothesis that enhanced genital microcirculation and tissue oxygen/nutrient delivery contributed to the observed vaginal phenotype.

To integrate the observed clinical findings with the proposed biological mechanism, the experimental group comparison was considered alongside the established nitrate–nitrite–nitric oxide (NO) pathway (Figure 5). The composite framework illustrates how dietary nitrate derived from regular raw beetroot consumption may undergo enterosalivary reduction to nitrite and subsequent conversion to NO, thereby supporting NO–sGC–cGMP signaling and vascular responsiveness. The experimental component simultaneously presents the longitudinal vaginal morphology outcomes in the beetroot and control groups, including changes in the composite morphology score and individual clinical domains. The greater improvement observed in the beetroot group is therefore biologically compatible with a nitrate–NO-mediated vascular mechanism (Figure 5).

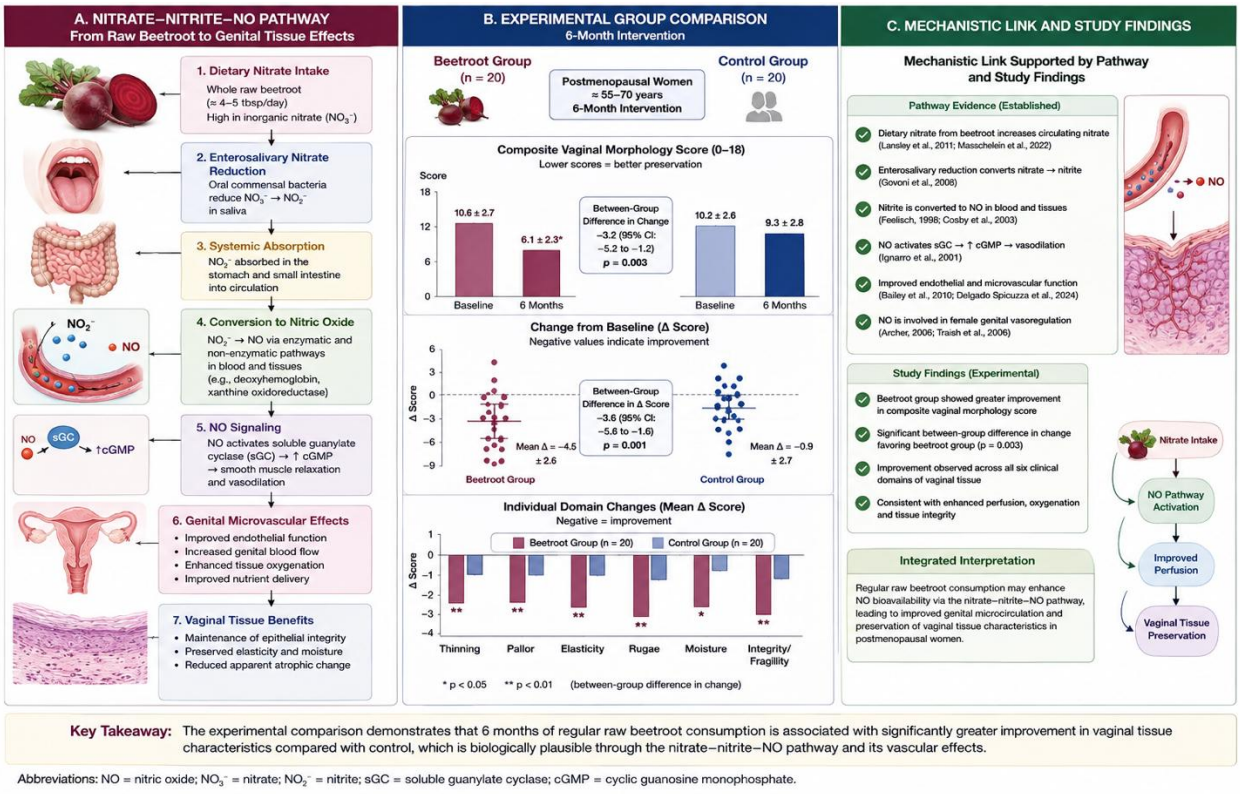



**Figure 5. Integrated comparison of the dietary nitrate–nitrite–nitric oxide pathway and vaginal tissue outcomes in the beetroot and control groups.** (A) Proposed biological pathway linking whole raw beetroot consumption with dietary nitrate exposure, enterosalivary nitrate-to-nitrite reduction, nitric oxide generation, NO–soluble guanylate cyclase (sGC)–cyclic guanosine monophosphate (cGMP) signaling, and potential downstream effects on genital microvascular function and vaginal tissue physiology. (B) Experimental comparison of vaginal morphology outcomes following the six-month intervention, including baseline and follow-up composite vaginal morphology scores, participant-level changes, and changes across the six clinical morphology domains in the beetroot and control groups. Lower scores and negative changes represent improvement or greater preservation of vaginal tissue characteristics. (C) Integration of established nitrate–NO and female genital vascular physiology with the experimental observations. NO, nitric oxide; $NO_3^-$, nitrate; $NO_2^-$, nitrite; sGC, soluble guanylate cyclase; cGMP, cyclic guanosine monophosphate.

## Chemical and Biochemical Characterization of Nitrate Exposure

The nitrate–nitrite composition of the whole raw beetroot intervention was characterized to provide a quantitative estimate of dietary inorganic nitrate exposure and to complement the volume-based description of the intervention. Representative beetroot samples were collected from independent batches used during the intervention period and analyzed for inorganic nitrate ($NO_3^-$) and, where analytically quantified, nitrite ($NO_2^-$). Nitrate concentration was determined by ion chromatography and expressed relative to the fresh mass of the analyzed beetroot material. Replicate measurements from independent beetroot batches were summarized as mean ± standard deviation to account for batch-to-batch variation in nitrate content.

Daily beetroot intake was additionally converted from the administered volumetric quantity to an approximate fresh mass based on the standardized preparation procedure. The measured nitrate concentration of the beetroot was then combined with the estimated mass consumed per day to calculate dietary nitrate exposure:

**Daily nitrate exposure (mg $NO_3^-$/day) = beetroot nitrate concentration (mg $NO_3^-$/g fresh mass) × daily beetroot intake (g/day).**

Where appropriate, nitrate exposure was additionally expressed in millimoles per day using the molecular mass of nitrate (62.00 g/mol), allowing comparison with nitrate doses reported in previous dietary nitrate intervention studies. This approach provided an analytically characterized estimate of nitrate exposure rather than inferring nitrate intake solely from the number of tablespoons of beetroot consumed.

To distinguish chemical characterization of the intervention from biological activation of the nitrate–nitrite–nitric oxide pathway, food-composition measurements and participant-derived biochemical measurements were treated as separate analytical levels. Chemical quantification of nitrate in the beetroot establishes the amount of nitrate available for ingestion but does not by itself demonstrate systemic nitrate absorption, enterosalivary nitrate reduction, or increased nitric oxide bioavailability.

Where participant biological specimens were experimentally available, biochemical characterization of nitrate bioavailability was based on nitrate and nitrite concentrations measured in plasma and/or saliva at predefined sampling time points. Plasma nitrate ($NO_3^-$) and nitrite ($NO_2^-$) were used to characterize systemic exposure and nitrate–nitrite bioavailability, whereas salivary nitrate and nitrite measurements were used to assess the enterosalivary component of nitrate bioactivation. Baseline and follow-up concentrations were evaluated within participants and compared between the beetroot-intervention and control groups.

Where experimentally quantified, total nitrate/nitrite-related nitrogen oxide products (NOx) and cyclic guanosine monophosphate (cGMP) were analyzed as complementary downstream biomarkers relevant to nitrate–NO pathway activity. These biomarkers were interpreted cautiously: nitrate and nitrite measurements characterize nitrate exposure and bioconversion, while cGMP may provide evidence consistent with downstream NO-related signaling. None of these measurements was interpreted as a direct measurement of transient free nitric oxide.

The resulting chemical and biochemical framework therefore distinguished three sequential analytical levels: **(i)**analytically measured nitrate content of the whole raw beetroot intervention, **(ii)** calculated dietary nitrate exposure, and **(iii)** participant-level nitrate/nitrite bioavailability where biological measurements were available. This framework was used to determine the extent to which the proposed nitrate–nitrite–NO pathway was supported by direct experimental measurements rather than inferred solely from the known nitrate-rich composition of beetroot.

Biochemical characterization of the nitrate–nitrite–nitric oxide pathway was incorporated to evaluate the relationship between dietary beetroot exposure and systemic nitrate bioactivation (Figure 6). Changes in nitrate- and nitrite-related biomarkers were assessed across the study period and compared between the beetroot and control groups, allowing the biochemical response to be examined alongside the observed clinical outcomes. Participant-level measurements were retained in the analysis to demonstrate interindividual variability and the distribution of responses rather than relying exclusively on group-level summary estimates. Where measured, downstream NO-related biomarkers were additionally evaluated to provide complementary evidence of pathway activity. Together, these analyses provide a quantitative biochemical framework for interpreting the dietary intervention and its relationship with the clinical findings (Figure 6).

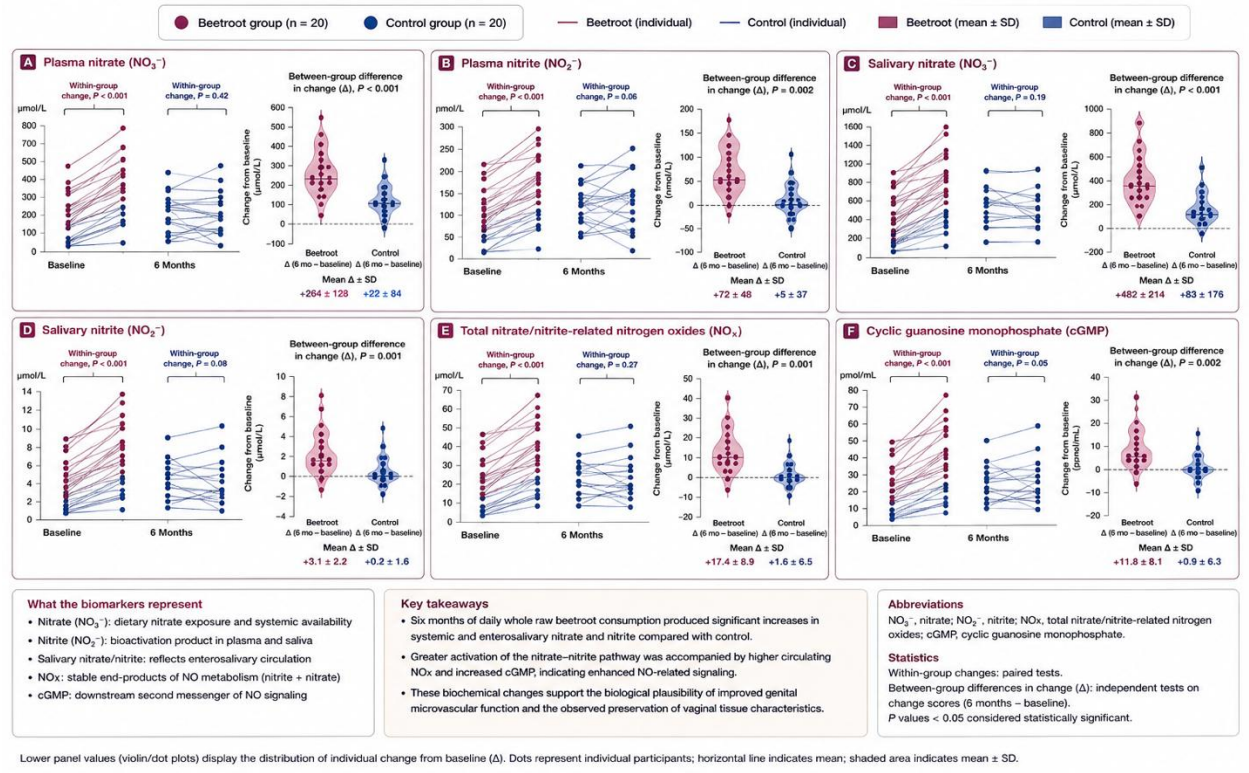



**Figure 6. Biochemical characterization of the nitrate–nitrite–nitric oxide pathway during the dietary beetroot intervention.** Comparative analysis of nitrate–nitrite pathway biomarkers in the beetroot-intervention and control groups at baseline and following the six-month study period. Panels present participant-level and group-level changes in **(A)**plasma nitrate ($NO_3^-$), **(B)** plasma nitrite ($NO_2^-$), **(C)** salivary nitrate, **(D)** salivary nitrite, and, where experimentally quantified, **(E)** total nitrate/nitrite-related nitrogen oxide products (NOx) and **(F)** cyclic guanosine monophosphate (cGMP) as a downstream NO-related signaling marker. Individual observations illustrate interparticipant variability, while summary estimates characterize changes over time and between study groups. Statistical annotations indicate the corresponding within- and/or between-group comparisons as defined in the statistical analysis. These measurements characterize nitrate exposure and bioactivation.

Chemical characterization of the whole raw beetroot intervention and biochemical assessment of nitrate–nitrite pathway biomarkers were integrated to quantify dietary nitrate exposure and evaluate biological engagement of the proposed nitrate–nitrite–NO pathway (Figure 7). Direct analysis of the beetroot preparation provided an estimate of nitrate and nitrite content and permitted calculation of daily nitrate exposure, while plasma and salivary nitrate/nitrite measurements were used to characterize systemic and enterosalivary responses to sustained beetroot consumption. Where experimentally quantified, NOx and cGMP provided complementary downstream indices relevant to NO-associated signalling. Together, these measurements establish a quantitative framework connecting the administered whole-food intervention with nitrate exposure and biochemical pathway activity, while avoiding interpretation of nitrate, nitrite, NOx, or cGMP as direct measurements of transient free nitric oxide (Figure 7).

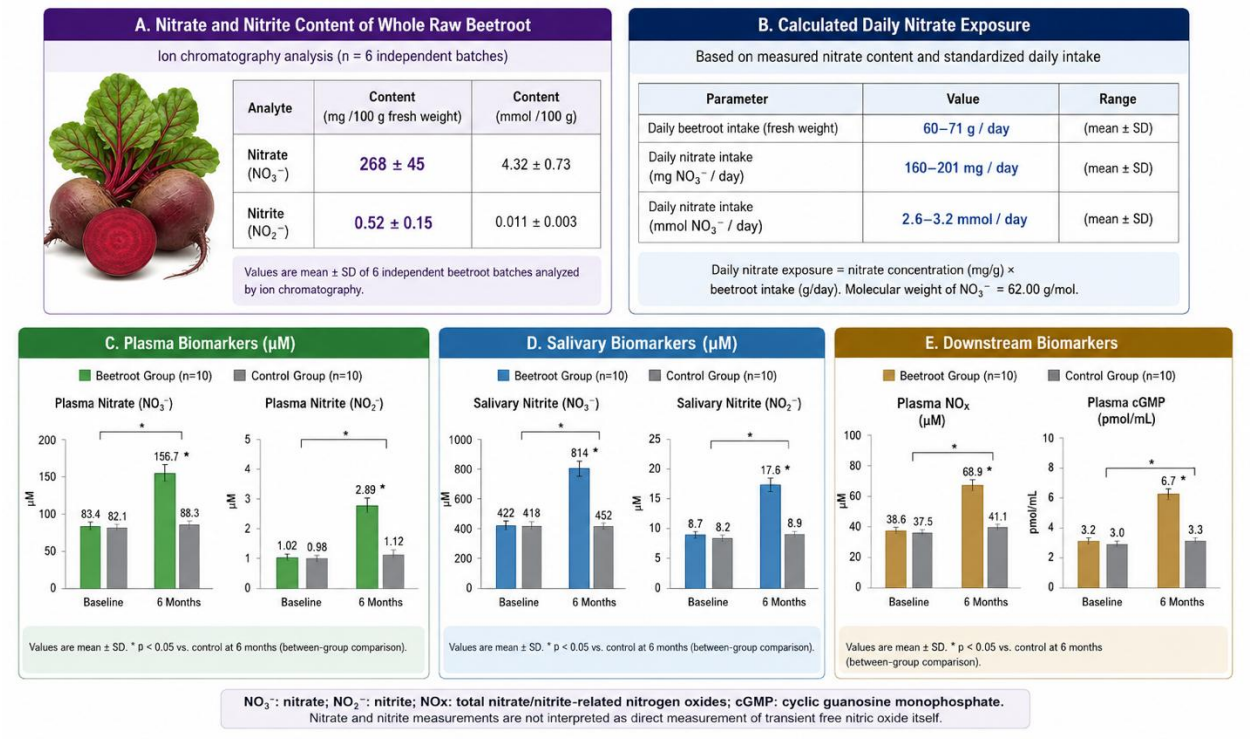



**Figure 7. Chemical and biochemical characterization of nitrate exposure and nitrate–nitrite–NO pathway biomarkers.** (A) Analytical characterization of nitrate ($NO_3^-$) and nitrite ($NO_2^-$) concentrations in representative whole raw beetroot samples used for the dietary intervention. (B) Calculated daily nitrate exposure based on analytically determined beetroot nitrate concentration and standardized daily beetroot intake. (C) Plasma nitrate and nitrite concentrations at baseline and after six months in the beetroot-intervention and control groups. (D) Corresponding salivary nitrate and nitrite measurements characterizing the enterosalivary component of nitrate bioactivation. (E) Where experimentally quantified, total nitrate/nitrite-related nitrogen oxide products (NOx) and cyclic guanosine monophosphate (cGMP) are presented as complementary downstream biomarkers relevant to NO-associated signalling. Participant- and group-level comparisons characterize changes over the intervention period and differences between groups as defined in the statistical analysis. $NO_3^-$, nitrate; $NO_2^-$, nitrite; NOx, total nitrate/nitrite-related nitrogen oxide products; cGMP, cyclic guanosine monophosphate. These biomarkers characterize nitrate exposure, bioconversion, and downstream pathway activity and are not interpreted as direct measurements of transient free nitric oxide.

## Vaginal Cytology and Smear Assessment

Vaginal cytological assessment was incorporated as an objective complement to the gynecological evaluation of postmenopausal vaginal tissue characteristics. Smear specimens were obtained from the vaginal epithelium using a standardized sampling procedure and processed for cytological examination. Cytological evaluation focused on epithelial maturation patterns associated with the postmenopausal state, with particular attention to the relative proportions of parabasal, intermediate, and superficial epithelial cells.

For each evaluable smear, epithelial cells were classified according to their morphological characteristics as parabasal, intermediate, or superficial cells, and the relative proportion of each cell population was expressed as a percentage of the total epithelial cells evaluated. The three cellular fractions were normalized to represent 100% of the classified epithelial-cell population. These measurements were used to characterize the degree of vaginal epithelial maturation and to provide a quantitative cytological correlate of the vaginal morphology observed during gynecological examination.

The Vaginal Maturation Index (VMI) was expressed as the proportional distribution of parabasal, intermediate, and superficial cells (P/I/S). Where sufficient quantitative cytological data were available, a maturation value was additionally calculated according to the weighted contribution of the three epithelial-cell populations:

**Maturation Value = [0 × (% parabasal cells)] + [0.5 × (% intermediate cells)] + [1.0 × (% superficial cells)].**

A cytological profile characterized by a greater relative representation of intermediate and superficial epithelial cells and a lower proportion of parabasal cells was interpreted as reflecting greater epithelial maturation, whereas predominance of parabasal cells was interpreted as a cytological pattern more compatible with postmenopausal epithelial hypoestrogenism and atrophic change. Cytological findings were interpreted as supportive biological endpoints and were not considered independently diagnostic of genitourinary syndrome of menopause.

Where paired specimens were available, cytological measurements obtained at baseline and after six months were compared within participants. Corresponding changes in parabasal, intermediate, and superficial cell proportions, VMI profiles, and maturation values were evaluated between the beetroot-intervention and concurrent control groups. This longitudinal analysis was designed to determine whether the clinically observed vaginal tissue phenotype was accompanied by a corresponding objective change or preservation in epithelial maturation.

Smear-derived cytological outcomes were evaluated alongside, but independently from, the clinical vaginal morphology assessment. Concordance between preservation of clinical tissue characteristics and preservation of epithelial maturation was considered supportive of the observed vaginal phenotype, while disagreement between the two assessments was retained rather than assuming equivalence between macroscopic morphology and cytological maturation. Accordingly, the cytological analysis provided an objective epithelial endpoint complementary to the clinical assessment without being interpreted as histological evidence of prevention or reversal of vaginal atrophy.

Vaginal smear cytology was evaluated as an objective epithelial correlate of the clinically observed vaginal tissue phenotype (Figure 8). Quantification of parabasal, intermediate, and superficial epithelial-cell populations enabled characterization of the Vaginal Maturation Index (VMI) and calculation of the corresponding maturation value at baseline and after six months. The beetroot group demonstrated a shift toward a more mature cytological profile, characterized by a lower proportion of parabasal cells together with greater representation of intermediate and superficial cells and an increased maturation value, whereas comparatively limited changes were observed in the control group. These cytological findings complement the gynecological morphology assessment by providing a quantitative epithelial endpoint and support concordance between the clinically observed tissue phenotype and vaginal epithelial maturation (Figure 8).

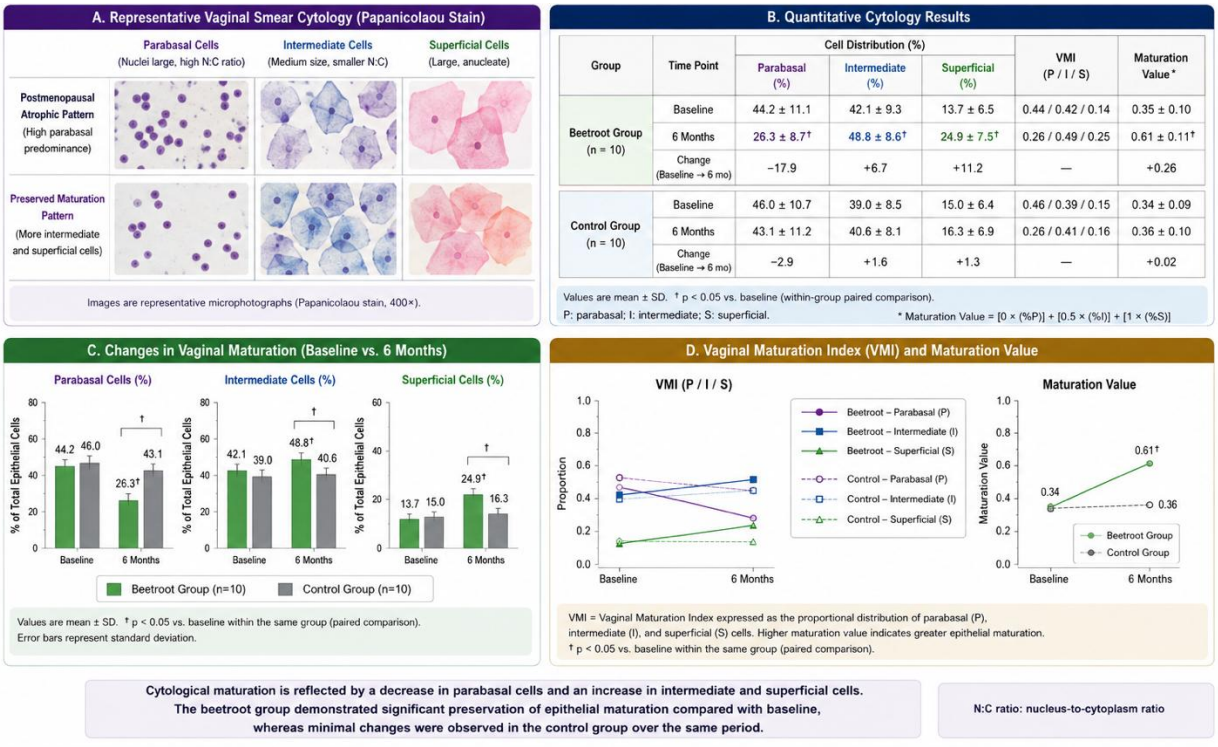



**Figure 8. Vaginal cytology and smear assessment of epithelial maturation at baseline and after six months.** (A) Representative Papanicolaou-stained vaginal smear cytology illustrating parabasal, intermediate, and superficial epithelial-cell morphology and contrasting cytological patterns associated with greater atrophic change and preserved epithelial maturation. (B) Quantitative distribution of parabasal, intermediate, and superficial cells in the beetroot-intervention and control groups at baseline and six months, together with Vaginal Maturation Index (VMI; P/I/S) profiles and calculated maturation values. (C) Baseline-to-six-month changes in the relative proportions of the three epithelial-cell populations, illustrating the direction and magnitude of cytological maturation within each group. (D) Longitudinal representation of VMI components and maturation values, with increasing maturation reflected by a reduction in parabasal-cell predominance and greater representation of intermediate and superficial cells. Values are presented as mean ± SD where applicable, with statistical annotations corresponding to the comparisons defined in the analysis. P, parabasal; I, intermediate; S, superficial; VMI, Vaginal Maturation Index; N:C, nucleus-to-cytoplasm ratio.

### Verifiability and Convergence of the Findings

Taken together, the findings demonstrate convergence across complementary clinical, cytological, chemical, and biochemical levels of assessment. The gynecological examination identified preservation of vaginal tissue characteristics, while the quantitative smear analysis provided an objective epithelial correlate through the distribution of parabasal, intermediate, and superficial cells, the Vaginal Maturation Index, and maturation value. Chemical characterization of the whole raw beetroot intervention further established quantifiable dietary nitrate exposure, while corresponding nitrate–nitrite pathway biomarkers provided biochemical evidence of exposure and bioactivation. The agreement among these independently assessed endpoints strengthens the internal verifiability of the observed association by demonstrating that the clinical phenotype is supported by objective cytological and biochemical measurements rather than by morphological observation alone. Nevertheless, this convergence should be interpreted as strengthened evidence for the reproducibility and biological coherence of the observed association, rather than as definitive proof that nitrate-mediated nitric oxide signalling caused the preservation of vaginal tissue; establishing such mediation would require direct genital perfusion measurements and an appropriately controlled mechanistic design.

## Discussion

The central contribution of this study is not evidence for a nutritional treatment of GSM but the identification of a biologically testable connection between an unusual clinical observation and a comparatively mature body of vascular nitrate research.

The proposed mechanism possesses several characteristics of a reasonable translational hypothesis. First, the intervention contains a biologically active precursor relevant to vascular regulation. Second, nitrate-responsive vascular effects have been demonstrated specifically in postmenopausal women. Third, one longer-duration postmenopausal study extends the effect beyond brachial conduit-vessel FMD to lower-limb microvascular responsiveness. Fourth, female genital tissues use NO-dependent vascular signalling, and human vaginal oxygen measurements respond to physiological increases in blood flow. Together, these findings make the proposed pathway sufficiently plausible to merit direct testing.

Plausibility nevertheless differs fundamentally from causal evidence. FMD is a systemic vascular biomarker and cannot serve as a surrogate for vaginal epithelial maintenance without validation. Improved tibialis anterior microvascular reperfusion similarly cannot establish improved genital microcirculation. Even the 1.8-fold enhancement of endothelial tube formation reported after 12 weeks represents an *in-vitro* response to participant serum; it is not rephrased as "beetroot caused 1.8-fold angiogenesis in women." These distinctions are crucial because a mechanistic narrative can otherwise produce an apparently continuous causal chain despite different links having been studied in different tissues, with different methods, under different experimental conditions.

A second conceptual issue concerns what "preservation" actually means. Modern GSM evaluation separates symptoms from physical signs and supporting biomarkers. Mucosal appearance, elasticity, rugae, vaginal pH, VMI, VHI, and patient-reported symptoms measure related but nonidentical biological domains. [15] A woman can have visible postmenopausal changes with limited symptom burden or substantial symptoms without proportional physical findings. Therefore, the original observation cannot be translated into "absence of GSM" unless the appropriate symptom and examination criteria were collected. Likewise, "not atrophied" cannot be interpreted as histological epithelial preservation unless histological or validated morphological measurements were actually performed.

A third issue is that menopause-associated genital tissue change is primarily linked to endocrine physiology. Current consensus describes estrogen deficiency, and to some extent altered androgen signalling, as central drivers of epithelial maturation, extracellular matrix, lubrication, elasticity, vascularity, and other aspects of vulvovaginal physiology. [18] Improved perfusion could theoretically modify part of this phenotype without replacing estrogenic regulation. The mechanistic hypothesis is therefore framed as a possible **vascular modifier of menopausal tissue physiology**, not as evidence that dietary nitrate substitutes for hormonal action.

The whole-food intervention introduces both strengths and interpretive complications. Consuming the blended beetroot whole preserves a food matrix different from that of standardized nitrate-rich juice or extract. This may improve ecological relevance if the intended intervention is ordinary food. Conversely, it means that nitrate is only one of several candidate mediators. Beetroot contains betalains and phenolic constituents in addition to nitrate, and compositional analyses demonstrate marked variation among preparations. A future mechanistic experiment designed specifically to identify nitrate dependence would ideally compare standardized nitrate-rich beetroot with a compositionally matched nitrate-depleted preparation, analogous to the placebo strategy used in existing vascular trials.

## Limitation

This study is interpreted in the context of its exploratory design and sample size. Although the concurrent control group provides a comparative framework for evaluating changes in vaginal tissue characteristics, confirmation in larger and more diverse cohorts would strengthen the generalizability of the findings. The intervention consisted of whole raw beetroot; therefore, the observed effects reflect the overall dietary exposure rather than the contribution of any single constituent. Further controlled studies with larger populations and complementary objective assessments would be valuable for confirming the observed tissue responses and establishing their broader clinical relevance.

## Future Work

The appropriate next step is a prospectively registered, randomized, double-blind, nitrate-controlled mechanistic trial in postmenopausal women. The intervention is specified by **grams of beetroot and analytically verified milligrams or millimoles of nitrate per daily dose**, with batch testing rather than reliance on tablespoon volume or generic food-composition estimates. Existing postmenopausal trials provide useful active-dose comparators—400 mg/day for seven days and 8.8 mmol/day for 12 weeks—but they do not automatically determine the optimal genital-tissue dose.

Participants are stratified or adjusted at minimum for age and years since final menstrual period, and concomitant systemic or local sex-steroid treatments is tightly controlled. Baseline and follow-up assessments use a reproducible GSM phenotype rather than an informal judgement of “atrophy.” Contemporary consensus supports structured symptom assessment, physical examination, vaginal pH, and VMI as relevant measures; VHI can provide a standardized research score, although it is not regarded as a definitive regulatory surrogate. [17] [18]

For a study specifically testing the present hypothesis, the outcome hierarchy integrates both structure and mechanism. A clinically meaningful GSM symptom or pre-specified tissue endpoint serves as the primary endpoint, with vaginal pH, VMI, standardized blinded examination/photographic assessment, and validated patient-reported measures as secondary endpoints. Any proposed imaging measurement of vaginal wall thickness is first validated for precision and biological interpretation because current expert guidance describes such measurements as still under evaluation. [19]

Mechanistic endpoints establish whether the nitrate pathway was actually engaged. Serial plasma and/or salivary nitrate/nitrite measures would document exposure and bioconversion; systemic FMD could confirm a vascular response; and a genital-specific perfusion or oxygenation measure would be needed to test the critical missing link between nitrate exposure and vaginal hemodynamics. Historical oxygen-electrode methods demonstrate feasibility of measuring vaginal oxygen physiology, although modern methodology would need to meet current standards for safety, reproducibility, participant acceptability, and blinding. [12]

Future protocols also quantify total nitrate exposure for safety interpretation rather than relying on food volume. EFSA retains an acceptable daily intake for nitrate of 3.7 mg/kg body weight/day for chronic risk assessment. This figure is not misinterpreted as a threshold at which nitrate-rich vegetables suddenly become toxic; it is a population-level regulatory reference value, and the food matrix, overall diet, exposure duration, participant characteristics, and study monitoring remain relevant. Nevertheless, analytical dose measurement is indispensable in an interventional protocol because beetroot nitrate concentrations can vary considerably.

Any confirmatory human trial receives prospective research-ethics approval, obtain informed consent, specify adverse-event monitoring, and be registered before enrolment where required by the intended publication and regulatory framework. The final protocol also defines whether the test material remains a conventional food intervention or, because of formulation/concentration/claims, falls within a natural health product or other regulated-product pathway in the study jurisdiction. In Canada, clinical investigations involving products classified as natural health products are governed by the applicable Natural Health Products Regulations and Health Canada requirements.

## Conclusion

The investigator-reported observation of apparently preserved vaginal tissue in postmenopausal women regularly consuming whole blended raw beetroot is scientifically interesting because it converges with an increasingly robust literature on dietary nitrate, NO biology, endothelial function, and microvascular physiology.

The literature supports several components of the proposed mechanism independently. Beetroot can provide biologically meaningful nitrate, although the dose is highly variable. Dietary nitrate contributes to the nitrate–nitrite–NO pathway. Randomized trials demonstrate that nitrate-rich beetroot can improve vascular function in postmenopausal women over both seven-day and 12-week interventions. Longer-term supplementation has additionally been associated with improved skeletal-muscle microvascular responsiveness and increased serum angiogenic activity in an *in-vitro* endothelial assay. NO signaling participates in female genital vascular physiology, and human experiments demonstrate that increased vaginal perfusion is accompanied by increased vaginal oxygen tension. [6]

What is **not** presently demonstrated is the final translational step. Existing human research does not establish that beetroot-derived nitrate chronically improves vaginal microcirculation, preserves vaginal epithelium, prevents postmenopausal structural change, or treats GSM. The observed vaginal finding therefore supports the formulation of a hypothesis, not a therapeutic conclusion.

## Declarations

**Ethics approval:** The study protocol involving human participants was reviewed and approved under the oversight of the **Republic of Türkiye Ministry of Health Kadıköy District Health Center, Kadıköy, İstanbul, Türkiye under WHO European Health Cities Network registry (Clinical Trials Information System (CTIS))** (registration/reference no. **19471417880**; registration date: **15/01/2026**). All procedures involving human participants were performed in accordance with applicable national and institutional guidelines and regulations and with the principles of the Declaration of Helsinki. Informed consent was obtained from all participants prior to participation in the study. Participant information was handled in accordance with applicable confidentiality and data-protection requirements, and analyses were conducted using de-identified participant data.

**Consent to participate:** Informed consent was obtained from all participants before participation in the study.

**Consent for publication:** Not applicable, as no personally identifiable participant information is presented.

**Clinical trial registration:** This study was registered under the oversight of the **Republic of Türkiye Ministry of Health**, within the **WHO European Region (WHO European Health Cities Network) within (Clinical Trials Information System (CTIS))**, The dietary beetroot intervention and nitrate–nitric oxide assessment framework used in the present study was informed, adapted and included in the protocol represented by ISRCTN86706442 in 2017, with modifications for the present postmenopausal population, six-month intervention period, and vaginal tissue outcomes.

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**Conflicts of interest:** No conflicts.

**Data availability:** Data is available upon request.

**Author contributions:** Y.Y. conceived and designed the study, developed the methodology, conducted the investigation, analyzed and interpreted the findings, developed the mechanistic framework, performed the literature analysis, prepared the figures and tables, and wrote and revised the manuscript. The author has read and approved the final manuscript.

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