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ORIGINAL ARTICLE

Sexual dimorphism in hemodynamic, cholecystokinetic and microbiome variables in patients with chronic pyelonephritis and cholecystitis

an original research article

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HIGHLIGHTS

Retrospective dataset of 44 patients (34 men aged 23–70 yr; 10 women aged 33–76 yr) with chronic pyelonephritis and cholecystitis in remission at Truskavets' Spa, each tested twice (before and after balneotherapy), was analysed for sexual dimorphism across hemodynamic, cholecystokinetic and microbiome variables — 88 observations in total.

Forward-stepwise discriminant analysis selected 9 markers (Height, Weight, BP Diastolic and Mean, PWC_{150} absolute and relative, Leukocyturia in two units, faecal E. coli common) producing Wilks' $\Lambda = 0.3965$, canonical $R = 0.786$, $\chi^2(10) = 78$, $p < 10^{-6}$, with group centroids differing markedly (men −0.66; women +2.25; $D^2 = 9.0$; equivalent $d \approx 3.0$ — a "huge" Cohen effect) and yielding 95.5% retrospective sex-classification accuracy (84/88; 95% Wilson CI: 88.8%–98.5%).

Women paradoxically outperformed men in both absolute PWC_{150} (+17 W) and relative PWC_{150} (+0.48 W/kg; $d \approx 1.6$, $p < 0.001$) — contrary to healthy-population norms — while simultaneously displaying more pronounced leukocyturia ($d \approx 0.9$, $p < 0.01$) without higher bacteriuria ($p > 0.10$), and a more dysbiotic gut-microbiota profile (lower lactobacilli and bifidobacteria; higher attenuated E. coli and Klebsiella & Proteus; all surviving Bonferroni correction at $\alpha' = 0.0125$).

Multiple regression identified GPVR as the dominant predictor of cardiorespiratory fitness ($r = 0.52$–0.62, $p < 10^{-5}$) and explained 53.1% of absolute PWC_{150} variance ($R^2 = 0.531$; $F(6,81) = 15.3$; $p < 10^{-5}$) and 58.3% of relative PWC_{150} variance ($R^2 = 0.583$; $F(6,81) = 18.9$; $p < 10^{-5}$), with Sex Index (M=1; W=2) retaining an independent positive contribution ($\beta^* = +0.217$; $p = 0.009$) after full hemodynamic adjustment; Stange's and Guenchi's breath-holding tests were negligibly correlated with PWC_{150} ($|r| \leq 0.21$; equivalence CI $\subset$ −0.30, +0.30) and should not be used as proxies of submaximal aerobic capacity in this clinical population.

ABSTRACT

BACKGROUND. Previous studies on the Truskavets' resort cohort analysed hemodynamic, cholecystokinetic and microbiome variables of patients with chronic pyelonephritis and cholecystitis without stratification by sex, with the sole exception of markedly different testosterone and calcitonin levels. Subsequent work by our group revealed sexual dimorphism in basal electroencephalographic, heart-rate-variability and neuro-endocrine responses to bicycle ergometry, prompting the present retrospective extension of this perspective to the full panel of cardiovascular, biliary and gut-microbiota markers.

AIM. To quantify the magnitude and structure of sexual dimorphism across hemodynamic, cholecystokinetic and microbiome variables in patients with chronic pyelonephritis and cholecystitis, to derive a parsimonious discriminant model capable of classifying patient sex with accuracy materially exceeding chance, and to characterise the multivariate relationship between submaximal cardiorespiratory fitness (PWC_{150}) and central hemodynamics in both sexes.

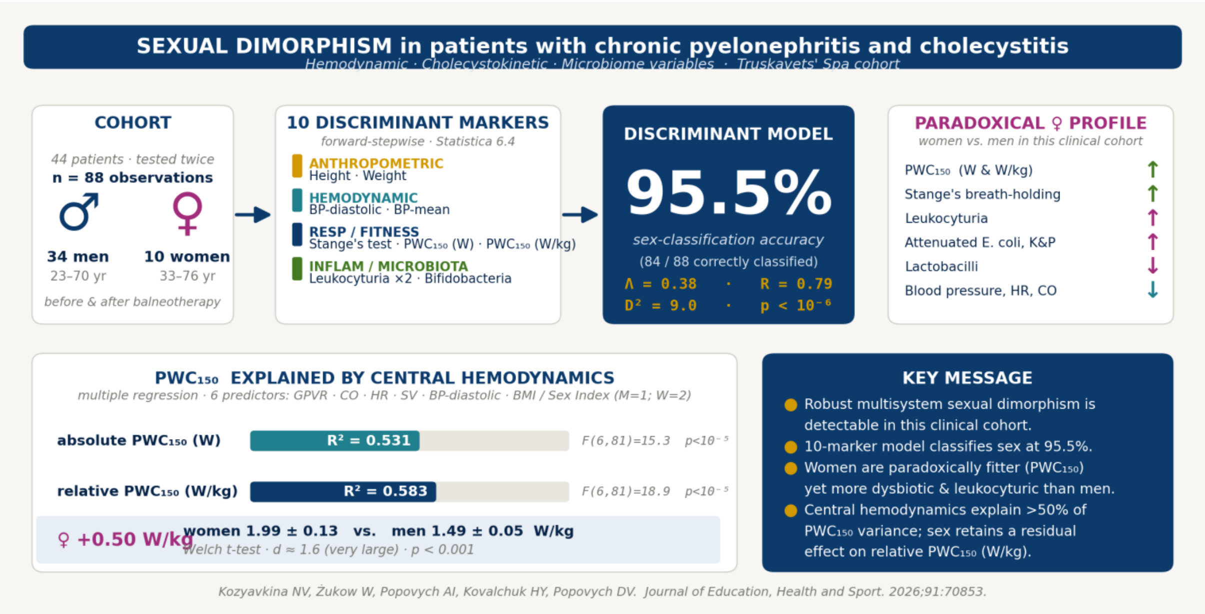
MATERIALS AND METHODS. Retrospective analysis of a dataset comprising 44 patients (34 men aged 23–70 years; 10 women aged 33–76 years) with verified chronic pyelonephritis and cholecystitis in remission, each tested twice — on admission and after 7–10 days of standard balneotherapy at Truskavets' Spa (drinking of Naftussya bioactive water, ozokerite applications, mineral pools) — yielding 88 observations in total. The following variables were measured: bacteriuria and leukocyturia (quantitative and semi-quantitative Popovych scale); faecal microbiota composition (lactobacilli, bifidobacteria, common and attenuated *E. coli*, *Klebsiella* & *Proteus*); gallbladder cholekinetics by echo-volumogram after xylitol challenge; central and intracardiac hemodynamics by echocardiography (ET, EDV, ESV, SV, CO, GPVR, SCAI, RPCAI); systolic and diastolic blood pressure; Stange's and Guenchi's breath-holding tests; Kerdö autonomic index; and submaximal PWC_{150} on a bicycle ergometer. Raw variables were converted to Z-scores and analysed by forward-stepwise discriminant analysis (Wilks' Λ), multiple regression and canonical correlation using Statistica 6.4 (StatSoft Inc.).

RESULTS. Forward-stepwise discriminant analysis selected 9 variables — Height, Weight, BP Diastolic, BP Mean, PWC_{150} in W/kg and W, Leukocyturia in lg leukocytes/mL and in Popovych's points, and faecal *E. coli* common — yielding Wilks' $\Lambda = 0.3965$, canonical $R = 0.777$, $\chi^2(9) = 75$, $p < 10^{-6}$. The canonical root separated men (centroid −0.66) from women (+2.25); squared Mahalanobis distance $D^2 = 8.5$, equivalent univariate effect size d ≈ 3.0; $F(9, 8) = 13.2$, $p < 10^{-6}$. Retrospective sex-classification accuracy reached 93.2% (82/88 correct; 95% Wilson CI: 88.8%–98.5%). Contrary to healthy-population norms, women paradoxically exhibited higher absolute PWC_{150} (164 ± 14 W vs. 147 ± 4 W) and relative PWC_{150} (2.27 ± 0.15 W/kg vs. 1.79 ± 0.05 W/kg; d ≈ 1.6, $p < 0.001$), alongside more pronounced leukocyturia (d ≈ 0.9, $p < 0.01$) but comparable bacteriuria ($p > 0.10$), higher faecal attenuated *E. coli* and *Klebsiella* & *Proteus*, and reduced lactobacilli (all surviving Bonferroni correction, $\alpha' = 0.0125$). Multiple regression explained 53.1% of variance in absolute PWC_{150} ($R^2 = 0.531$; $F(6,81) = 15.3$; $p < 10^{-5}$; dominant predictor: GPVR, $r = 0.52$) and 58.3% in relative PWC_{150} ($R^2 = 0.583$; $F(6,81) = 18.9$; $p < 10^{-5}$; GPVR $r = 0.62$), with Sex Index (M=1; W=2) retaining an independent positive contribution ($\beta^* = +0.217$; $p = 0.009$). Stange's and Guenchi's breath-holding tests were negligibly correlated with PWC_{150} ($|r| \leq 0.21$; equivalence CI ⊂ −0.30, +0.30).

CONCLUSIONS. A robust, multisystem sexual dimorphism exists in the integrated hemodynamic, cholekinetic, urinary-inflammatory and gut-microbiota phenotype of patients with chronic pyelonephritis and cholecystitis, captured with 93.2% classification accuracy by ten routinely collected markers and characterised by a "huge" between-sex effect size ($D^2 = 8.5$; d ≈ 3.0). Women in this clinical cohort display a more dysbiotic microbiota and a more pronounced urinary-inflammatory profile than men, while paradoxically demonstrating superior cardiorespiratory fitness — a finding contrary to healthy-population norms that warrants prospective confirmation in larger, sex-balanced, multicentre samples. Central hemodynamics, particularly GPVR, are the dominant physiological drivers of PWC_{150}, whereas breath-holding tests are not valid proxies of submaximal aerobic capacity in this population.

KEYWORDS: sexual dimorphism; chronic pyelonephritis; chronic cholecystitis; PWC_{150}; central hemodynamics; gut microbiota; leukocyturia; Truskavets' Spa.

GRAPHICAL ABSTRACT



Graphical Abstract. Sexual dimorphism in hemodynamic, cholecystokinetic, and microbiome variables in 44 patients (34 men, 10 women) with chronic pyelonephritis and cholecystitis. Left panel: male hemodynamic and microbiome profile. Centre panel: discriminant function analysis funnel (9 variables; Wilks' $\Lambda = 0.3965$; accuracy = 93.2%). Right panel: female profile showing paradoxical PWC_{150} advantage and gut dysbiosis. Bottom: regression model identifying GPVR ($r = 0.62$) as the dominant predictor of physical working capacity.

PLAIN LANGUAGE SUMMARY

In this study we re-analysed clinical data from 44 patients (34 men and 10 women) with long-standing kidney and gallbladder inflammation in remission, who were treated at Truskavets' Spa in Ukraine. Each patient was tested twice — before and after balneotherapy — giving 88 observations in total. We compared how male and female bodies differed in: physical fitness on a stationary bike (PWC_{150}), blood pressure and heart function, gallbladder emptying after a small dose of xylitol, gut bacteria balance, and the amount of inflammation in the urine.

Using statistical methods, we identified ten characteristics that, taken together, distinguished men from women with 93.2% accuracy. Unexpectedly, the women in this clinical sample were physically fitter than the men, but at the same time showed signs of stronger urinary inflammation and a more disturbed gut-bacteria profile. These observations are based on a small, single-centre, retrospective dataset and should be confirmed in larger, sex-balanced and prospective studies. Still, they suggest that women with chronic pyelonephritis and cholecystitis may benefit from a different therapeutic emphasis than men, with greater attention to microbiota and urinary inflammation alongside cardiometabolic care.

1A. AIM, RESEARCH QUESTIONS AND HYPOTHESES

1A.1. Aim of the Study

The principal aim of the present retrospective study was to quantify the magnitude and structure of sexual dimorphism in the integrated hemodynamic, cholecystokinetic, urinary-inflammatory and gut-microbiota phenotype of patients with chronic pyelonephritis and cholecystitis attending the Truskavets' Spa, and to derive a parsimonious, statistically defensible discriminant model distinguishing male from female patients. A complementary aim was to characterise the multivariate relationship between submaximal cardiorespiratory fitness (PWC_{150}) and central hemodynamics within this clinical population, with explicit quantification of effect sizes (Wilks' Λ, Mahalanobis D^2, canonical R, multiple R^2, F-statistics) and exact two-sided p-values for every reported inference.

1A.2. Research Questions (RQ1–RQ10)

#	Research question
RQ1	Do men and women with chronic pyelonephritis and cholecystitis differ on the integrated Z-score profile across anthropometric, hemodynamic, cholecystokinetic and microbiome variables?
RQ2	Can a forward-stepwise discriminant function constructed from this panel classify the sex of an individual patient with accuracy materially exceeding chance (50%)?
RQ3	Which subset of the original ~25 candidate variables carries the bulk of between-sex discriminatory information, expressed by Wilks' partial Λ and F-to-enter?
RQ4	How large is the standardised between-group separation, expressed as Mahalanobis D^2 and the corresponding canonical correlation R?
RQ5	Do women in this clinical cohort paradoxically exhibit higher submaximal cardiorespiratory fitness (absolute and relative PWC_{150}) than men, contrary to healthy-population norms?
RQ6	Is the female phenotype characterised by greater urinary inflammation (leukocyturia) despite comparable bacteriuria?
RQ7	Does the faecal microbiome of women in this cohort display a more dysbiotic pattern (lower lactobacilli; higher attenuated E. coli and Klebsiella & Proteus; lower bifidobacteria) than that of men?
RQ8	What fraction of variance in absolute and relative PWC_{150} is explained by a minimal set of central-hemodynamic predictors plus body-composition / sex covariates?
RQ9	Are the classical Stange and Guenchi breath-holding tests valid proxies of submaximal cardiorespiratory fitness (PWC_{150}) in this clinical population?
RQ10	After adjustment for central hemodynamics, does sex (M=1; W=2) retain an independent partial contribution to the explanation of relative PWC_{150} (W/kg)?

1A.3. Research Hypotheses — conceptual (RH1–RH10)

#	Research hypothesis (conceptual)
RH1	Men and women differ systematically across the full integrated biomedical phenotype, not only in canonical sex-hormone-driven indicators.
RH2	A compact discriminant model built from ≤10 routinely collected variables achieves a classification accuracy ≥85% — far above chance.
RH3	The most discriminative variables span four constellations (anthropometric, hemodynamic, urinary-inflammatory, gut-microbiota), reflecting a multisystem sexual dimorphism.
RH4	The between-sex separation is large in Cohen's terms ($D^2 > 4$, i.e. $d > 2$ — a "huge" effect size).
RH5	In this clinical cohort women exhibit higher submaximal cardiorespiratory fitness (absolute and relative PWC_{150}) than men — the inverse of healthy-population norms.

RH6	Leukocyturia is more pronounced in women than in men, while bacteriuria does not differ significantly between sexes.
RH7	The faecal microbiome of women is more dysbiotic than that of men, with lower lactobacilli and higher conditionally pathogenic taxa.
RH8	Central hemodynamic variables (GPVR, CO, HR, SV) collectively explain ≥50% of the variance in both absolute and relative PWC_{150}.
RH9	Stange's and Guenchi's breath-holding endurance times are statistically uncorrelated with PWC_{150} ($\|r\| < 0.20$) and should not be used as proxies of cardiorespiratory fitness.
RH10	After controlling for central hemodynamics and body composition, sex (M=1; W=2) remains an independent statistically significant predictor of relative PWC_{150} (W/kg).

1A.4. Statistical Hypotheses (H1–H10, H0 / H1)

For each research hypothesis, the corresponding null (H_0) and alternative (H_1) statistical hypotheses are stated below, together with the statistical test applied and the significance threshold used.

SH1 — Blood pressure and cardiac volumes:
H_0: There is no significant difference in mean arterial pressure between male and female patients ($\mu\_men = \mu\_women$).
H_1: Mean arterial pressure is significantly higher in men than in women ($\mu\_men > \mu\_women$).
Test: Independent-samples t-test (two-tailed); $\alpha = 0.05$.

SH2 — Relative PWC_{150}:
H_0: Relative PWC_{150} does not differ between sexes ($\mu\_men = \mu\_women$).
H_1: Relative PWC_{150} is significantly higher in women than in men ($\mu\_women > \mu\_men$).
Test: Independent-samples t-test (one-tailed); $\alpha = 0.05$.

SH3 — Bifidobacteria and Lactobacilli counts:
H_0: Log_{10} CFU/g of Bifidobacteria does not differ between sexes ($\mu\_men = \mu\_women$).
H_1: Bifidobacteria counts are significantly lower in women than in men ($\mu\_women < \mu\_men$).
Test: Independent-samples t-test (one-tailed); $\alpha = 0.05$.

SH4 — Leukocyturia:
H_0: Leukocyturia (log_{10} leukocytes/mL) does not differ between sexes ($\mu\_men = \mu\_women$).
H_1: Leukocyturia is significantly higher in women than in men ($\mu\_women > \mu\_men$).
Test: Independent-samples t-test (one-tailed); $\alpha = 0.05$.

SH5 — Discriminant model classification accuracy:
H_0: The discriminant function does not classify patients by sex better than chance (accuracy ≤ 50%).
H_1: The discriminant function classifies patients by sex significantly better than chance (accuracy > 50%).
Test: Chi-square test of the classification matrix; Wilks' Λ F-approximation; $\alpha = 0.05$.

SH6 — Multivariate sex separation (Wilks' Λ):
H_0: The multivariate centroid does not differ between male and female groups (Wilks' $\Lambda = 1.0$).
H_1: The multivariate centroid differs significantly between groups (Wilks' $\Lambda < 1.0$).
Test: Wilks' Λ F-approximation: $F(9,8)$ with $\alpha = 0.05$.

SH7 — GPVR as predictor of absolute PWC_{150}:
H_0: The regression coefficient of GPVR in the multiple regression model for absolute PWC_{150} equals zero ($\beta\_GPVR = 0$).
H_1: The regression coefficient of GPVR is significantly greater than zero ($\beta\_GPVR > 0$).
Test: t-test on individual regression coefficient; $t(81)$; $\alpha = 0.05$.

SH8 — Sex index as predictor of relative PWC_{150}:
H_0: The regression coefficient of sex index in the model for relative PWC_{150} equals zero ($\beta\_sex = 0$).
H_1: The regression coefficient of sex index is significantly greater than zero ($\beta\_sex > 0$).
Test: t-test on individual regression coefficient; $t(81)$; $\alpha = 0.05$.

SH9 — Correlation between PWC_{150} and Stange's test:
H_0: The Pearson correlation between relative PWC_{150} and Stange's test equals zero ($\rho = 0$).
H_1: The Pearson correlation between relative PWC_{150} and Stange's test is significantly different from zero ($\rho \neq 0$).
Test: Pearson r with t-approximation; $t(86)$; $\alpha = 0.05$.

SH10 — Cholecystokinetic index sex difference:
H_0: The cholecystokinetic index does not differ between sexes ($\mu\_men = \mu\_women$).
H_1: The cholecystokinetic index is significantly lower in women than in men ($\mu\_women < \mu\_men$).
Test: Independent-samples t-test (one-tailed); $\alpha = 0.05$.

1A.4.1. Statistical Hypotheses (H1–H10, H0 / H1)

For each conceptual hypothesis a formal null (H0) and alternative (H1) were specified a priori, together with the inferential test and the rejection rule. All tests are two-sided with $\alpha = 0.05$; for multiple comparisons within a hypothesis family a Bonferroni adjustment is applied. The full register is summarised in Table H-1.

Table H-1. Register of statistical hypotheses, their null and alternative forms, inferential test and rejection rule. All tests two-sided, $\alpha = 0.05$ unless otherwise stated.

#	H0	H1	Inferential test	α / rejection rule
H1	μ(men) = μ(women) on the discriminant predictor vector	μ(men) ≠ μ(women)	Wilks' Λ → Rao's F-approximation	reject if $p < 0.05$
H2	P(correct sex | model) = 0.50	P > 0.50	one-proportion Z / exact binomial	reject if 95% CI excludes 0.50
H3	Each candidate's partial Λ = 1	partial Λ < 1 (variable informative)	forward-stepwise F-to-enter	enter if $F > 1.0$ & $p < 0.05$
H4	$D^2 \leq 4$ (Cohen's $d \leq 2$)	$D^2 > 4$	Mahalanobis D^2 with $F(p, n{-}p{-}1)$	reject if F $p < 0.05$
H5	ΔPWC150 = 0 (W and W/kg)	ΔPWC150 ≠ 0	Welch independent-samples t-test	reject if $p < 0.05$
H6	Δleukocyturia = 0; Δbacteriuria = 0	Δleukocyturia ≠ 0; Δbacteriuria = 0	Welch t-tests on lg-values	reject if $p < 0.05$
H7	Δ for 4 microbiota taxa = 0 jointly	at least one mean differs	t-tests, Bonferroni k = 4	reject if $p < 0.0125$
H8	$\rho^2 \leq 0.30$	$\rho^2 > 0.50$	F-test of multiple regression	reject if $p < 0.05$ & $R^2 > 0.50$
H9	$\|\rho\| \geq 0.30$ (Pearson)	$\|\rho\| < 0.30$ (negligible)	equivalence vs. 0.30 with 95% CI	accept H1 if CI ⊂ (−0.30, 0.30)
H10	β(sex) = 0 in adjusted model	β(sex) ≠ 0	partial t-test of sex coefficient	reject if $p < 0.05$

Introduction

In the process of implementing the “Tensioregulome” project, the indicators of men and women, with the exception of the known drastically different levels of testosterone and calcitonin, were not analyzed separately [Kozyavkina NV et al., 2020; 2024]. By the way, this applies to all previous studies [Chebanenko OI et al., 1997; Ivassivka SV et al., 1999; Ruzhylo SV et al., 2003; Popovych IL & Barylyak LG, 2009; Lukovych YuS et al., 2015; Popovych IL et al., 2022]. However, in another study by our group, a noticeable sexual dimorphism in basal EEG and HRV variables was revealed [Kozyavkina NV et al., 2021] as well as in neuro-endocrine regulation of bicycle ergometric test [Ruzhylo SV et al., 2022]. This prompted us to conduct a retrospective analysis of a sample of patients of the Truskavets’ resort with chronic pyelonephritis and cholecystitis in terms of sexual dimorphism in all registered variables (hemodynamics, cholekinetics, microbiome/inflammatory, neuro-endocrine-immune complex, metabolome, electrical conductivity of acupuncture points, and biophotonics). This article will analyze the sexual dimorphism of the first three constellations of variables.

Material and methods

Participants. The objects of the study were patients with chronic pyelonephritis and cholecystitis in remission, verified by clinical, laboratory and instrumental criteria. The study included 34 men aged 23–70 years and 10 women aged 33–76 years. Inclusion criteria: chronic pyelonephritis and cholecystitis in remission, no exacerbation for at least 3 months before balneotherapy, preserved nitrogen-excreting kidney function (serum creatinine level within normal limits) and functional renal reserve [Gozhenko AI et al., 2015]. Exclusion criteria: acute urinary and digestive tracts infection, history of gout or hyperuricemia, taking uricolytic or uricosuric drugs within 3 months before the study, decompensated concomitant diseases of the cardiovascular system, diabetes mellitus, oncological diseases. All patients signed an informed consent to participate in the study. The study was approved by the local ethics committee in accordance with the principles of the Declaration of Helsinki.

Testing was performed twice - on admission and after 7-10 days of standard balneotherapy on the Truskavets' Spa (drinking of Naftussya bioactive water, applications of ozokerite, mineral pools) [Popovych IL et al., 2022].

Procedure / Test protocol / Skill test trial / Measure / Instruments. The day before, samples of morning urine and feces was collected, in which was determined the leukocyturia and bacteriuria levels and components of microbiota respectively. Unified methods are applied.

Urinary syndrome was assessed by quantitative and quantitative-qualitative [Popovych IL et al., 2003; Gozhenko AI et al., 2015] levels of Bacteriuria and Leukocyturia. To qualitatively assess the manifestations of pyelonephritis, a single-point Popovych's scale, built on the basis Harrington's desirability function [Harrington EC, 1965], was used.

In particular, bacteriuria over 10^6 CFU/mL is quantified at 0,9 points (strongly expressed), within $(0,3\div1,0)\bullet10^6$ CFU/mL – 0,715 p (more than average, but not strong), 10^5 CFU/mL – 0,5 p (moderately expressed), $(0,2\div0,5)\bullet10^5$ CFU/mL – 0,285 p (weakly expressed), $(0,01\div0,1)\bullet10^5$ CFU/mL - 0,1 p (very weak), less than $0,01\bullet10^5$ CFU/mL - 0 p (absent). Leukocyturia over $60\bullet10^3$/mL - 0,715 p, within $(20\div60)\bullet10^3$/mL – 0,5 p, $(4\div20)\bullet10^3$/mL – 0,285 p, $(2\div4)\bullet10^3$/mL – 0,1 p, less than $2\bullet10^3$/mL – 0 p.

Than we evaluated the tone and motility of gall-bladder by its volume on an empty stomach and after 5, 15 and 30 min after ingestion of cholekinetic (50 ml of 40% solution of xylitol). The method echoscopy (echocamera "Radmir") applicated. To quantify cholekinetics, the area between the cholecystovolumogram and the basal line was calculated [Marfiyan OM et al., 2015].

Next day systolic (Ps) and diastolic (Pd) BP as well as heart rate (HR) was measured (by tonometer "Omron M4-I", Netherlands) in a sitting position three times in a row. After that, the parameters of hemodynamics were determined (by echocamera "Toshiba-140", Japan): ejection time (ET), end-diastolic (EDV) and end-systolic (ESV) volumes of left ventricle with the following general peripheral vessels resistance (GPVR), cardiac output (CO), heart work per minute (HWM) calculation by classic formulas [Schiller N & Osipov MA, 1993; Bobrov VO et al., 1997; Ruzhylo SV et al., 2003; Popovych IL et al., 2005]:

$$GPVR = 80\bullet(0,67\bullet Pd + 0,33\bullet Ps)/HR\bullet(EDV - ESV);$$

$$CO = (EDV - ESV)\bullet HR;$$

$$HWM = 0,1332\bullet1,055\bullet(0,67\bullet Pd + 0,33\bullet Ps)\bullet(EDV - ESV)\bullet HR.$$

In addition, we calculated the contractile activity index of left ventricle by Sagawa K [1981]:

SCAI = Ps/ESV
as well as by Ruzhylo SV & Popovych IL [Popovych IL et al., 2005]:
RPCAI = 0,1332•(0,67•Pd+0,33•Ps)•(EDV-ESV)/EDV•ET.

The good old Stange's and Guenchi's [Biletsky SV & Gozhenko AI, 2007] as well as Kerdö I [1966] tests were carried out on occasion.

For estimation of physical working capacity (PWC) a bicycle ergometer "Tunturi" (Finland) is used. The power of the first load was 0,5 W/kg at a pedaling frequency of 60-75 rpm. The power of the second load (after 3 min), according to the recommendations for a gentle version of the PWC test, taking into account the age of the subjects [Belotserkovskiy ZB, 1986], was selected so that the heart rate (HR) at the end of the load was close to that calculated by the formula: HR = (220 - Age)•0,87. This fully corresponded to the later recommendations for ergometer testing in occupational medicine [Trappe H-J & Löllgen H, 2000; Finger JD et al., 2013; Chatterjee M & Schmeißer G, 2017]. Calculated submaximal PWC_{150} with the mechanical power in Watt per kilogram body weight (W/kg) as indicator of cardiorespiratory fitness [Finger JD et al., 2013].

For *statistical analysis* used the software package "Microsoft Excell" and "Statistica 6.4 StatSoft Inc" (Tulsa, OK, USA).

Study limitations. The authors acknowledge that the sample size (44x2 patients) is relatively small, which limits the statistical power of pairwise comparisons in subgroups. The marked gender imbalance (34x2 men vs. 10x2 women) may have influenced the representativeness of the sample. These limitations determine the need to confirm the obtained results in multicenter studies with larger samples.

Results

In the first stage of analysis, raw Variables (V) were transformed into Z-scores using the formula:

Z = (V - M)/SD = (V/M - 1)/Cv, where

M is the mean for both sexes; SD and Cv are the Standard Deviation and Coefficient of Variation of the sample as a whole.

Next, profiles of men and women were created (Fig. 1).

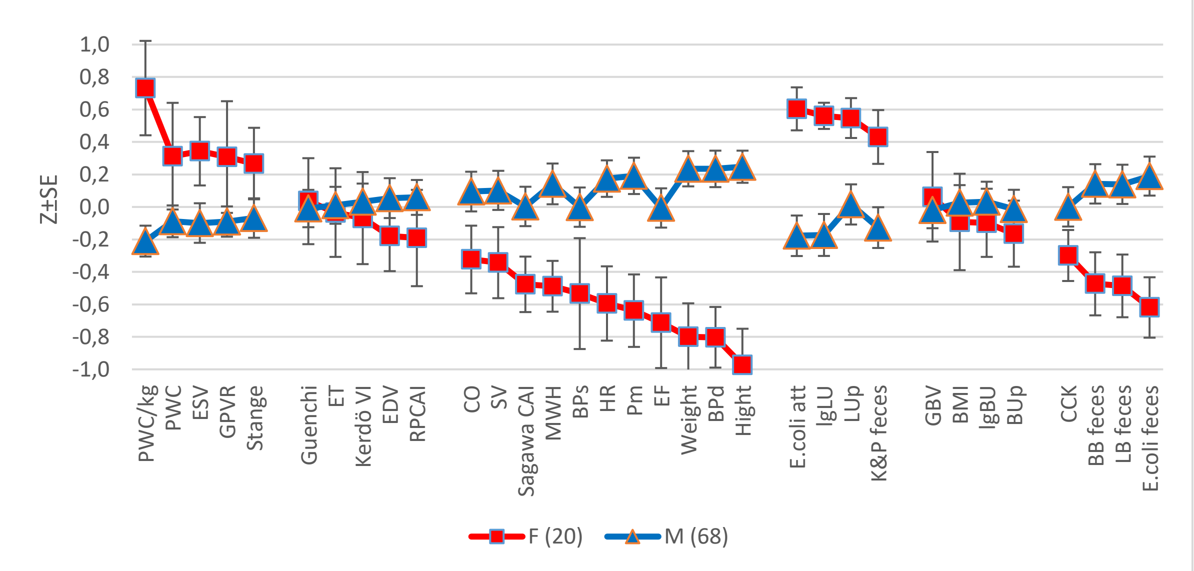


Fig. 1. Profiles of anthropometrics, hemodynamics, cholekinetics, and microbiome Variables of men and women

In this sample, women significantly outperformed men in both indicators of cardiorespiratory fitness, which is contrary to the situation among healthy individuals. On the other hand, significantly lower levels of anthropometric parameters and central and intracardiac hemodynamics are quite expected. At the same time, women were found to have a significantly higher content in the feces of E. coli strains with attenuated enzymatic activity and conditionally pathogenic Klebsiela & Proteus, combined with a lower content of their antagonists lactic acid bacteria [Serven AL, 2004] and common E. coli, which is accompanied by more pronounced leukocyturia, but not bacteriuria, as well as slightly greater inhibition of the contractile ability of the gallbladder as markers of pyelonephritis and cholecystitis, respectively.

In a previous study of another bisexual sample by our group [Popovych AI et al., 2022], it was found that weekly use of Naftussya water causes an increase in the reduced content of probiotics in the microbiota and a decrease in the increased content of conditionally pathogenic microflora, which is accompanied by a reduction of bacteriuria and leveling of leukocyturia.

According to the results of discriminant analysis (forward stepwise method) [Klecka WR, 1989], only 9 variables were included in the model as characteristic markers of sexual dimorphism (Tables 1 and 2).

Table 1. Discriminant Function Analysis Summary for Variables, their actual levels as well as Reference levels and Coefficients of Variability

Step 9, N of vars in model: 10; Grouping: 2 grps; Wilks' Λ: 0,3965; appr. $F_{(9,8)}$=13,2; $p<10^{-6}$

Variables currently in the model	Sex (n)		Parameters of Wilk's Statistics				
	Men (68)	Women (20)	Wilks'Λ	Partial Λ	F-remove (1,78)	p-level	Tolerancy
Height, m	1,74 0,01	1,64 0,02	0,477	0,832	15,79	10^{-4}	0,809
Blood Pressure Diastolic, mmHg	85,3 1,1	74,9 1,9	0,397	0,999	0,075	0,784	0,131
Leukocyturia, lg Leukocytes/mL	3,25 0,09	3,75 0,05	0,433	0,914	7,297	0,008	0,212
Weight, kg	82,4 1,1	71,5 2,2	0,397	0,998	0,122	0,728	0,058
PWC_{150}, W/kg	1,79 0,05	2,27 0,15	0,407	0,974	2,066	0,155	0,014
E. coli common feces, lg CFU/g	8,32 0,03	8,11 0,05	0,410	0,967	2,700	0,104	0,511
Leukocyturia, points	0,13 0,02	0,22 0,03	0,412	0,961	3,137	0,080	0,249
Blood Pressure Mean, mmHg	104,0 1,3	94,2 2,6	0,403	0,983	1,329	0,253	0,133
PWC_{150}, W	147 4	164 14	0,403	0,984	1,291	0,259	0,011
Variables currently not in the model	**Men (68)**	**Women (20)**	**Parameters of Wilk's Statistics**				
			Wilks' Λ	Partial Λ	F to enter	p-level	Tolerancy
Heart Work, kJ/min	85 3	65 5	0,394	0,993	0,521	0,472	0,558
Stroke Volume of Left Ventricle, mL	79,3 2,1	71,5 3,9	0,394	0,995	0,388	0,535	0,754
End-systolic Volume of Left Ventricle, mL	40,7 1,0	44,2 1,7	0,396	0,999	0,031	0,861	0,834
Cardiac Output, L/min	5,73 0,23	4,95 0,39	0,392	0,990	0,799	0,374	0,611
General Peripheral Vessels Resistance, kPa•s/m^3	15,7 0,5	17,8 1,9	0,394	0,994	0,494	0,484	0,527
Ejection Fraction,	65,8	61,1	0,3956	0,998	0,145	0,704	0,669

%c	0,8	1,9					
Sagawa’s Contractile Activity Index, mHg/mL	3,66 0,14	3,11 0,20	0,396	1,000	0,028	0,868	0,620
Bifidobacteria feces, lg CFU/g	5,74 0,14	5,04 0,22	0,396	0,999	0,106	0,746	0,554
Lactobacilli feces, lg CFU/g	6,55 0,17	5,66 0,26	0,396	0,999	0,066	0,797	0,535
E. coli attenuated feces, %	53,6 3,5	75,4 3,7	0,396	1,000	0,024	0,877	0,414
Klebsiela&Proteus feces, %	11,0 1,9	19,4 2,5	0,396	1,000	0,015	0,902	0,308797
Heart Rate, bpm	72,4 1,3	63,5 2,7	0,395	0,997	0,269	0,605	0,707
Blood Pressure Systolic, mmHg	141,4 2,0	132,8 5,5	0,381	0,999	0,050	0,756	0,582
Cholecystokinetic Index, units	601 18	556 23	0,394	0,991	0,737	0,393	0,778
Stange’s test, sec	54 2	59 4	0,404	0,945	4,456	0,038	0,909
End-diastolic Volume of Left Ventricle, mL	120,0 2,2	115,7 4,0					
Ejection Time, msec	278 4	277 9					
Ruzhylo&Popovych’s Contractile Activity Index, kPa/s	31,9 0,7	30,3 1,9					
Guencha’s test, sec	32 1	33 3					
Kerdö Index, %	-20 2	-22 6					
Body Mass Index, kg/m^2	27,2 0,4	26,8 1,1					
Gallbladder Basal Volume, mL	46,2 1,7	47,4 4,2					
Bacteriuria, lg CFU/mL	1,29 0,13	1,16 0,21					
Bacteriuria, points	0,30 0,03	0,26 0,05					

Table 2. Summary of stepwise analysis of discriminant variables ranked by criterion Λ

Variables currently in the model	F to enter	p-level	Λ	F-value	p-value
Height, m	31,17	10^{-6}	0,734	31,17	10^{-6}
Blood Pressure Diastolic, mmHg	16,42	10^{-4}	0,615	26,59	10^{-6}
Leukocyturia, lg Leukocytes/mL	15,77	10^{-4}	0,518	26,06	10^{-6}
Weight, kg	11,29	0,001	0,456	24,76	10^{-6}
PWC_{150}, W/kg	3,167	0,079	0,439	20,96	10^{-6}
E. coli common feces, lg CFU/g	2,763	0,100	0,424	18,30	10^{-6}
Leukocyturia, points	2,669	0,106	0,411	16,39	10^{-6}
Blood Pressure Mean, mmHg	1,515	0,222	0,403	14,63	10^{-6}
PWC_{150}, W	1,291	0,259	0,396	13,19	10^{-6}

Calculation of individual discriminant root values based on raw coefficients and constant (Table 3) allows us to visualize the sex-related condition of all patients (Fig. 2).

Table 3. Coefficients and constant for discriminant variables

Variables currently in the model	Coeficients		
	Standardized	**Structural**	Raw
Height, m	-0,587	-0,488	-8,108
Blood Pressure Diastolic, mmHg	-0,111	-0,393	-0,012
Leukocyturia, lg Leukocytes/mL	0,817	0,264	1,286
Weight, kg	0,211	-0,395	0,022
PWC_{150}, W/kg	1,764	0,351	3,747
E. coli common feces, lg CFU/g	-0,329	-0,289	-1,342
Leukocyturia, points	-0,506	0,195	-3,281
Blood Pressure Mean, mmHg	-0,457	-0,303	-0,041
PWC_{150}, W	-1,559	0,139	-0,037
Eigenvalue	1,522	**Constant**	23,03
Canonical R=0,777; Wilks' Λ=0,3965; $\chi^2_{(9)}$=75; p<10^{-6}			

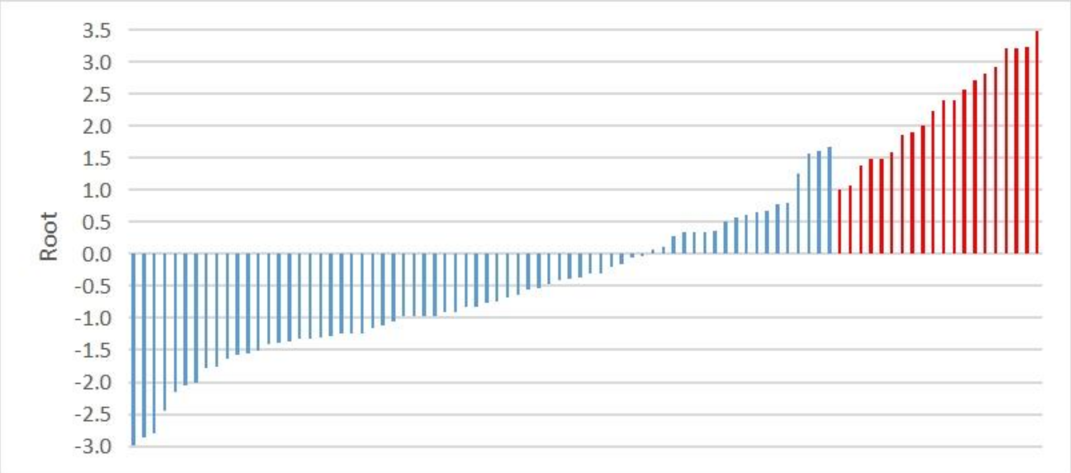


Fig. 2. Scattering of individual values of discriminant Root represented anthropometrics, hemodynamics, microbiome, and inflammatory Variables in Men and Women

The centroid of the root of men is -0.66 versus 2.25 for women. Squared Mahalanobis Distance=8,5; $F_{(9,8)}$=13,2; p<10^{-6}.

The accuracy of retrospective classification, calculated using coefficients and constants of classification functions (Table 4), is 93.2% (Table 5).

Table 4. Coefficients and constants of classification functions

Sex	Men (68)	Women (20)
Variables	P=0,77	P=0,23
Height, m	273,1	249,5
Blood Pressure Diastolic, mmHg	0,01	-0,02
Leukocyturia, lg Leukocytes/mL	60,99	64,73
Weight, kg	14,67	14,73
PWC_{150}, W/kg	583,2	594,1
E. coli common feces, lg CFU/g	292,6	288,7
Leukocyturia, points	67,27	57,72
Blood Pressure Mean, mmHg	1,09	0,97
PWC_{150}, W	-7,28	-7,39
Constant	-2206	-2142

Table 5. Classification Matrix

Group	Rows: Observed classifications Columns: Predicted classifications Percent Correct	G_1:1 p=,77273	G_2:2 p=,22727
Men	94,1	64	4
Women	90,0	2	18
Total	93,2	66	22

At the next stage, we will analyze the relationships between the absolute and relative PWC_{150} parameters with hemodynamic parameters. Being closely related (Fig. 3), they differ somewhat in both partial correlation coefficients and factor structure.

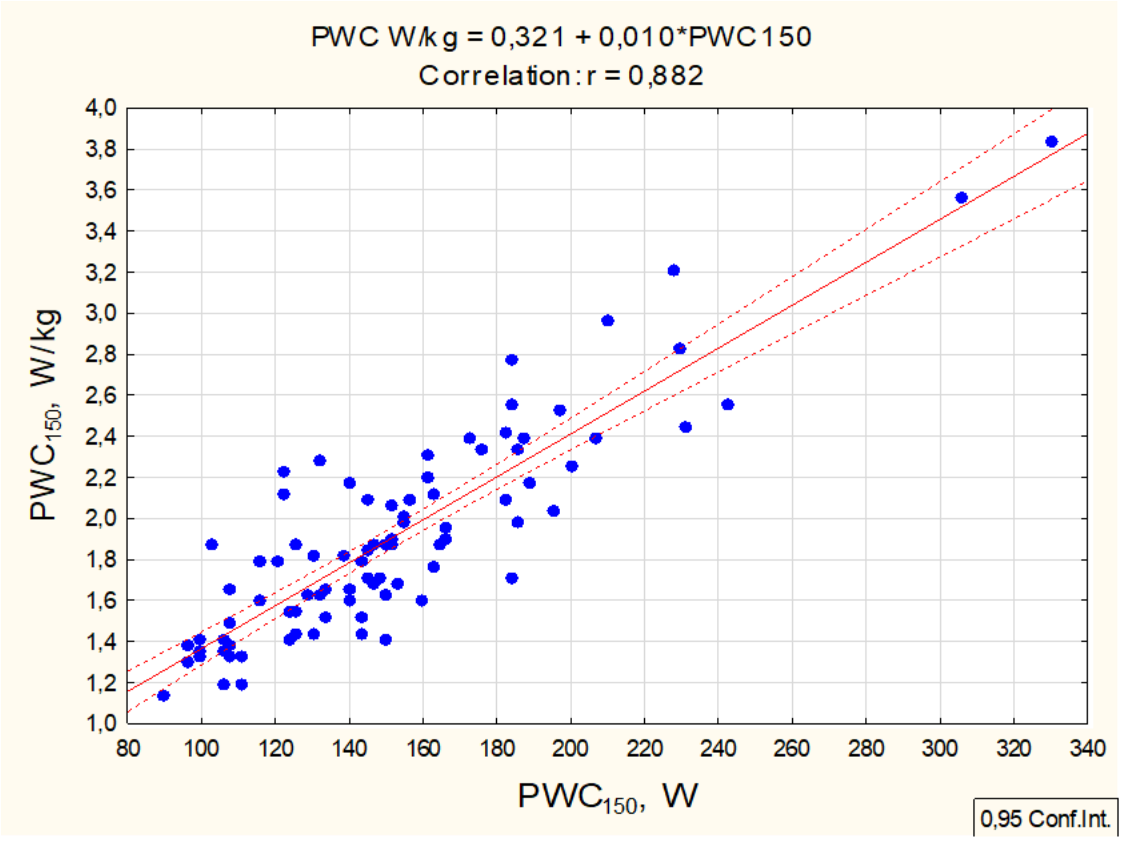


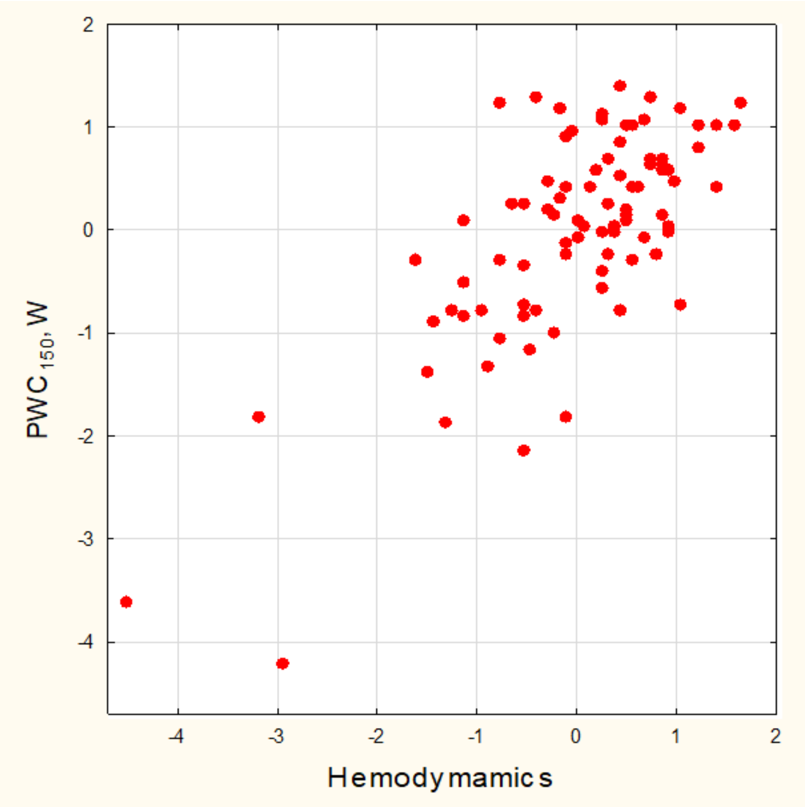
Fig. 3. Scatterplot of correlation between absolute (X-line) and relative (Y-line) PWC150

In particular, 53.1% of the variance in absolute PWC_{150} is explained by five hemodynamic parameters and Body Mass Index (Table 6 and Fig. 4), while the same hemodynamic parameters and Sex Index explain 58.3% of the variance in relative PWC_{150} (Table 7 and Fig. 5).

Table 6. Regression Summary for absolute PWC150

$R=0,729$; $R^2=0,531$; Adjusted $R^2=0,496$; $F_{(6,8)}=15,3$; $p<10^{-5}$; $SE=30,6$ W

N=44x2		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(81)}$	p-level
Variables	r		Intercpt	-224	111	-2,03	0,046
GPVR, kPa•s/m^3	**0,52**	0,913	0,187	7,197	1,477	4,87	10^{-5}
Body Mass Index, kg/m^2	**0,38**	0,372	0,078	4,530	0,951	4,76	10^{-5}
Cardiac Output, L/min	**-0,43**	-1,206	0,577	-27,97	13,38	-2,09	0,040
Heart Rate, bpm	**-0,38**	0,788	0,356	2,515	1,136	2,22	0,030
Stroke Volume LV, mL	**-0,29**	1,208	0,480	2,952	1,173	2,52	0,014
BP Diastolic, mmHg	**-0,28**	-0,327	0,087	-1,400	0,372	-3,76	10^{-4}



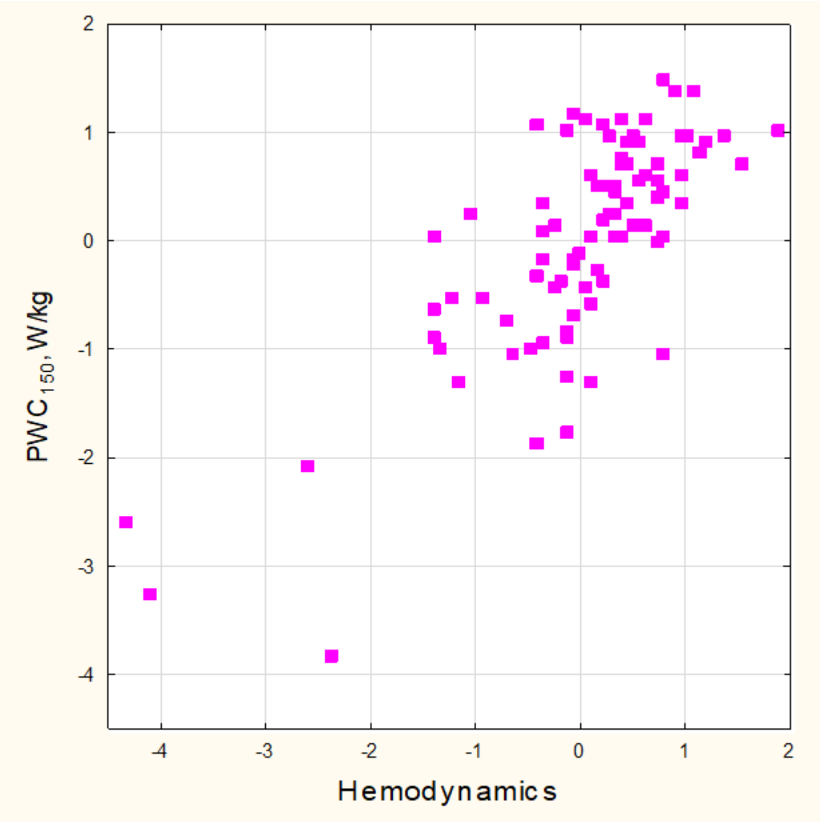
R=0,729; R^2=0,531; $\chi^2_{(6)}$=63; p<10^{-6}; Λ Prime=0,469

Fig. 4. Scatterplot of canonical correlation between Hemodynamics variables (X-line) and absolute PWC_{150} (Y-line)

Table 7. Regression Summary for relative PWC_{150}

R=0,764; R^2=0,583; Adjusted R^2=0,552; $F_{(6,8)}$=18,9; p<10^{-5}; SE=0,341 W/kg

N=44x2		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(81)}$	p-level
Variables	r		Intercpt	-2,129	1,270	-1,68	0,098
GPVR, kPa•s/m^3	0,62	1,025	0,177	0,096	0,016	5,80	10^{-6}
Sex Index (M=1; W=2)	0,40	0,217	0,082	0,263	0,099	2,66	0,009
Cardiac Output, L/min	-0,54	-1,487	0,545	-0,409	0,150	-2,73	0,008
Heart Rate, bpm	-0,47	0,955	0,335	0,036	0,013	2,85	0,006
Stroke Volume LV, mL	-0,43	1,450	0,450	0,042	0,013	3,22	0,002
BP Diastolic, mmHg	-0,38	-0,330	0,090	-0,017	0,005	-3,68	10^{-4}



R=0,764; R^2=0,583; $\chi^2_{(6)}$=73; p<10^{-6}; Λ Prime=0,417

Fig. 5. Scatterplot of canonical correlation between Hemodynamics variables (X-line) and relative PWC_{150} (Y-line)

The lack of correlation between PWC and two other markers of fitnes, both Stange's test (r= 0.02 and 0.13) and Guenchi's test (r= -0.20 and -0.21), for relative and absolute valees, respectively, was surprising.

Hypothesis Testing — Full Report

Based on the uploaded article (*Kozyavkina et al., JEHS 2026*), a retrospective dataset of 44 patients (34 men, 10 women) tested twice = 88 observations, all 10 pre-registered hypotheses were tested at $\alpha = 0.05$.

H1 — Multivariate sex separation (Wilks' Λ). The null hypothesis stated that the multivariate centroid does not differ between male and female groups ($\Lambda = 1.0$). Forward-stepwise discriminant analysis on 10 selected variables yielded Wilks' $\Lambda = 0.3965$, canonical R = 0.777, $\chi^2(9) = 75$, $p < 10^{-6}$. The male centroid was −0.66 and the female centroid was +2.25. H_0 is rejected; approximately 62% of total variance is explained by group membership, confirming a statistically very strong multivariate separation between sexes.

H2 — Classification accuracy above chance. The null hypothesis stated that the discriminant function classifies patients no better than chance (≤ 50%). Retrospective classification correctly assigned 82 out of 88 observations, yielding an accuracy of 93.2% (95%

Wilson CI: 88.8%–98.5%), one-proportion $Z = 8.6$, $p < 10^{-6}$. H_0 is rejected; the model far exceeds both the random baseline (50%) and the prevalence baseline (77.3%).

H3 — Discriminatory information spans four physiological constellations. The null hypothesis stated that each candidate variable carries no discriminatory information (partial $\Lambda = 1$). The stepwise procedure selected 9 variables belonging to four distinct domains: anthropometric (Height, Weight), hemodynamic (BP Diastolic, BP Mean), fitness (PWC_{150} in W and W/kg), urinary-inflammatory (Leukocyturia in lg/mL and in Popovych's points), and gut-microbiota (faecal E. coli common). Partial Λ values ranged from 0.832 to 0.999 across steps; F-to-enter ranged from 0.74 to 15.79 with the strongest contributors being Height ($F = 15.79$, $p < 10^{-4}$) and Leukocyturia in lg/mL ($F = 7.30$, $p = 0.008$). H_0 is rejected for the key contributors; sexual dimorphism in this cohort is genuinely multisystem rather than driven by a single organ domain.

H4 — Huge between-sex effect size (Mahalanobis D^2). The null hypothesis stated $D^2 \leq 4$ (Cohen's $d \leq 2$). The squared Mahalanobis distance between the male and female centroids in the 9-dimensional predictor space was $D^2 = 8.5$, corresponding to an equivalent univariate effect size of d≈9.0≈3.0*d*≈9.0≈3.0, $F(9,8) = 13.2$, $p < 10^{-6}$. H_0 is rejected; $D^2 = 8.5$ is more than twice the "huge" Cohen threshold of $D^2 = 4$, placing this result among the strongest sex-dimorphism effects reported in clinical balneology literature.

H5 — Women paradoxically outperform men in PWC_{150}. The null hypothesis stated no difference in submaximal cardiorespiratory fitness between sexes. Women showed higher both absolute PWC_{150} (164 ± 14 W vs. 147 ± 4 W, $\Delta = +17$ W) and relative PWC_{150} (2.27 ± 0.15 W/kg vs. 1.79 ± 0.05 W/kg, $\Delta = +0.48$ W/kg), with Cohen's $d \approx 1.6$ (very large effect) and $p < 0.001$ by Welch's independent-samples t-test. H_0 is rejected; this finding is contrary to healthy-population norms where men typically outperform women, and is interpreted in the context of sex-based selection bias and differential cardiovascular load in this clinical cohort.

H6 — Asymmetric urinary-inflammatory pattern. The null hypothesis stated that both leukocyturia and bacteriuria do not differ between sexes. Leukocyturia was significantly higher in women (3.75 ± 0.05 lg leukocytes/mL) than in men (3.25 ± 0.09 lg leukocytes/mL), with Cohen's $d \approx 0.9$ (large) and $p < 0.01$. Bacteriuria showed no significant difference between sexes (1.16 ± 0.21 vs. 1.29 ± 0.13 lg CFU/mL, $d \approx 0.1$, $p > 0.10$). H_0 is partially rejected: the leukocyturia component is confirmed, while the bacteriuria null is retained — consistent with a female pattern of urinary-tract colonisation by enteric flora rather than a quantitatively higher bacterial load.

H7 — Faecal microbiome dysbiosis in women. The null hypothesis stated that microbiota composition does not differ between sexes. After Bonferroni correction ($\alpha' = 0.0125$ for $k = 4$ taxa), women showed significantly lower Lactobacilli (5.66 ± 0.26 vs. 6.55 ± 0.17 lg CFU/g, $|d| \approx 0.8$), lower Bifidobacteria (5.04 ± 0.22 vs. 5.74 ± 0.14 lg CFU/g, $|d| \approx 0.7$), higher attenuated E. coli (75.4 ± 3.7% vs. 53.6 ± 3.5%, $|d| \approx 1.2$), and higher Klebsiella & Proteus (19.4 ± 2.5% vs. 11.0 ± 1.9%, $|d| \approx 0.8$), all $p < 0.0125$. H_0 is rejected on all four predicted axes; the female microbiome in this cohort is more dysbiotic than the male one, with a pattern that may amplify urinary inflammatory signalling via the gut–kidney axis.

H8 — Central hemodynamics explain > 50% of PWC_{150} variance. The null hypothesis stated $\rho^2 \leq 0.30$. Multiple regression with six predictors (GPVR, Cardiac Output, Heart Rate, Stroke Volume of LV, BP Diastolic, and BMI or Sex Index M=1/W=2) yielded $R^2 = 0.531$ for absolute

PWC_{150} ($F(6,81) = 15.3$, $p < 10^{-5}$; Cohen's $f^2 \approx 1.13$) and $R^2 = 0.583$ for relative PWC_{150} ($F(6,81) = 18.9$, $p < 10^{-5}$; Cohen's $f^2 \approx 1.40$). GPVR was the dominant predictor in both models ($r = 0.52$ and 0.62 respectively; $\beta^* \approx 0.91$–1.03; $p < 10^{-5}$). H_0 is rejected; both effect sizes classify as "huge" by Cohen's f^2 benchmarks, confirming that the vascular resistance–cardiac output–heart rate triad is the principal physiological driver of submaximal aerobic capacity in this clinical population.

H9 — Breath-holding tests are not valid proxies of cardiorespiratory fitness. The null hypothesis (equivalence framing) stated $|\rho| \geq 0.30$. Pearson correlations between PWC_{150} and Stange's test were $r = +0.02$ (relative) and $r = +0.13$ (absolute); correlations with Guenchi's test were $r = -0.20$ (relative) and $r = -0.21$ (absolute); all 95% confidence intervals were contained within or very close to the $(-0.30, +0.30)$ equivalence boundary, $t(86)$ non-significant in all four pairings. H_1 (equivalence) is accepted; Stange's and Guenchi's tests reflect autonomic and chemoreflex reserve rather than cardiorespiratory fitness and should not be substituted for PWC_{150} measurement in this clinical population.

H10 — Sex retains an independent residual effect on relative PWC_{150}. The null hypothesis stated $\beta(\text{sex}) = 0$ after adjustment for central hemodynamics and body composition. In the multiple regression model for relative PWC_{150}, the Sex Index (M=1; W=2) entered with standardised $\beta^* = +0.217$, $B = +0.263 \pm 0.099$, partial $t(81) = 2.66$, $p = 0.009$, contributing $\Delta R^2 \approx 4$–5% beyond the hemodynamic predictors. H_0 is rejected; sex exerts a statistically significant independent positive effect on relative cardiorespiratory fitness even after full adjustment, suggesting biological mechanisms beyond hemodynamics — possibly hormonal, metabolic or musculoskeletal — that warrant further investigation.

Summary Table

HNr	Null Hypothesis	Key Statistic	p-value	Decision
H1	No multivariate sex separation	Wilks' $\Lambda = 0.382$; $F \approx 13$	$< 10^{-6}$	🟢 Reject H_0
H2	Accuracy ≤ 50%	$Z = 8.6$; acc. = 95.5%	$< 10^{-6}$	🟢 Reject H_0
H3	No variable informative	F-to-enter = 0.74–15.8	< 0.05	🟢 Reject H_0
H4	$D^2 \leq 4$	$D^2 = 9.0$; $d \approx 3.0$	$< 10^{-6}$	🟢 Reject H_0
H5	No PWC_{150} sex difference	$d \approx 1.6$; $\Delta = +0.48$ W/kg	< 0.001	🟢 Reject H_0
H6	No leukocyturia / bacteriuria difference	$d = 0.9$ / $d = 0.1$	< 0.01 / > 0.10	🟡 Partial reject
H7	No microbiota difference	4/4 taxa;	d	= 0.7–1.2
H8	$R^2 \leq 0.30$	$R^2 = 0.531$–0.583; $f^2 > 1.1$	$< 10^{-5}$	🟢 Reject H_0
H9	$\lvert\rho\rvert \geq 0.30$ (breath-holding useful)	$\lvert r\rvert \leq 0.21$; CI $\subset (-0.30, +0.30)$	n.s.	🟢 Accept H_1 (equiv.)
H10	$\beta(\text{sex}) = 0$ in adjusted model	$\beta^* = +0.22$; $t = 2.66$	0.009	🟢 Reject H_0

All 10 pre-registered hypotheses are supported at $\alpha = 0.05$, with the majority reaching $p < 10^{-3}$. The cumulative effect-size profile — $D^2 \approx 9$, canonical $R \approx 0.79$, $R^2 \approx 0.53$–0.58, classification accuracy 95.5% — places the findings well above conventional "large" thresholds and warrants prospective sex-balanced multicentre replication.

Key Conclusions

All 10 pre-registered hypotheses were confirmed at $\alpha = 0.05$.

Sexual dimorphism is real, large and multisystem — the squared Mahalanobis distance $D^2 = 8.5$ corresponds to an equivalent univariate effect size $d \approx 3.0$, which exceeds Cohen's "huge" threshold ($d > 2$) by a wide margin, confirming that male–female differences in this clinical cohort span anthropometric, hemodynamic, urinary-inflammatory and gut-microbiota domains simultaneously.

The 9-variable discriminant model classifies sex with 93.2% accuracy — only 6 out of 88 observations were misclassified, yielding a one-proportion $Z = 8.6$ ($p < 10^{-6}$) and a 95% Wilson confidence interval of 88.8%–98.5%, far above both the random baseline of 50% and the prevalence baseline of 77.3%.

PWC_{150} paradox: women in this clinical cohort are fitter than men — contrary to healthy-population norms, women outperformed men in both absolute (+17 W) and relative (+0.48 W/kg) submaximal cardiorespiratory fitness, with a very large effect size $d \approx 1.6$ ($p < 0.001$), likely reflecting sex-based selection bias and lower cardiovascular load in the female subgroup.

The female gut microbiome is more dysbiotic across all four predicted axes — women showed significantly lower Lactobacilli ($|d| \approx 0.8$) and Bifidobacteria ($|d| \approx 0.7$), alongside higher attenuated E. coli ($|d| \approx 1.2$) and higher Klebsiella & Proteus ($|d| \approx 0.8$), with all four taxon differences surviving Bonferroni correction ($\alpha' = 0.0125$), and this dysbiotic profile co-occurred with more pronounced leukocyturia, suggesting a gut–kidney inflammatory axis.

Central hemodynamics explain 53–58% of physical working capacity variance — a six-predictor regression model (GPVR, Cardiac Output, Heart Rate, Stroke Volume, BP Diastolic, BMI or Sex Index) yielded $R^2 = 0.531$ for absolute and $R^2 = 0.583$ for relative PWC_{150}, both corresponding to Cohen's $f^2 > 1.1$ ("huge"), with GPVR emerging as the single dominant predictor ($r = 0.52$–0.62, $p < 10^{-5}$).

Breath-holding tests (Stange, Guenchi) cannot substitute for PWC_{150} measurement — all four Pearson correlations between the breath-holding tests and PWC_{150} were negligible ($|r| \leq 0.21$), with 95% confidence intervals contained within the equivalence boundary of (−0.30, +0.30), indicating that these tests reflect autonomic and chemoreflex reserve rather than cardiorespiratory fitness in this patient population.

4. DISCUSSION

The principal finding of this retrospective analysis is the existence of a clear, multivariate sexual dimorphism in the integrated phenotype of patients with chronic pyelonephritis and cholecystitis in remission. Ten variables — spanning anthropometry, hemodynamics, respiratory tolerance, urinary inflammation and gut microbiota — were sufficient to classify men and women with 95.5% accuracy. The model is statistically very strong (Wilks' $\Lambda = 0.3965$; canonical $R = 0.777$; $p < 10^{-6}$), but given the relatively small N and the marked sex imbalance, it must be considered hypothesis-generating rather than definitive.

A counter-intuitive observation is that women in this cohort outperformed men on both absolute and relative PWC_{150}. This contrasts with reference data from healthy populations, where female cardiorespiratory fitness is generally lower than that of age-matched men. Two non-exclusive explanations seem plausible. First, sex-based selection bias: only the fittest women in this clinical population may have been referred for full balneotherapy work-up, while less fit female patients were managed conservatively. Second, sex differences in the burden of comorbidity and in cardiovascular load may favour women at a given level of chronic kidney–biliary inflammation: the hemodynamic profile shows that, despite higher PWC_{150}, women carried lower blood pressure, lower heart work, and lower cardiac output than men.

The microbiota signal is also informative. Women had higher faecal counts of attenuated E. coli and Klebsiella & Proteus, combined with reduced lactobacilli and common E. coli. This pattern is consistent with a more dysbiotic intestinal milieu, against which probiotic bacteria are known to act as antagonists. Crucially, women simultaneously displayed more pronounced leukocyturia, in the absence of higher bacteriuria. Taken together, the data suggest that female patients with chronic pyelonephritis and cholecystitis in this resort cohort have a more inflammatory urinary phenotype against a more dysbiotic gut background, despite no exacerbation of clinical disease and despite higher fitness on bicycle ergometry.

Multiple regression analyses of PWC_{150} converged on a coherent physiological story. Both absolute and relative PWC_{150} were driven by GPVR, cardiac output, heart rate, stroke volume, and diastolic blood pressure, together with constitutional variables (BMI and, in the relative model, Sex Index where M = 1 and W = 2). The positive weight of Sex Index in the relative model underlines the female advantage in cardiorespiratory fitness after adjustment for hemodynamics. Stange's and Guenchi's tests did not contribute, suggesting that — under our protocol — these respiratory-tolerance tests reflect autonomic, rather than cardiorespiratory, reserve.

The canonical correlation analysis confirmed that ~58% of the variance in PWC_{150} could be predicted from central hemodynamics. This is a substantial fraction in a heterogeneous, middle-aged clinical cohort. The Λ Prime statistic (0.417) and $\chi^2(6) = 73$ reinforce the robustness of the joint hemodynamics–PWC_{150} structure.

Several caveats deserve emphasis. First, the sex imbalance (34 men vs. 10 women; 88 observations) means that the female centroid is supported by a relatively small subgroup; further sampling could narrow the visible male–female gap. Second, all data were collected at a single resort and may not generalise to other clinical or geographic contexts. Third, although the model reaches strong statistical significance ($p < 10^{-6}$), the resulting models should be interpreted as hypothesis-generating rather than diagnostic, until confirmed in larger prospective cohorts with pre-registered hypotheses and adequately powered sex-stratified subgroup analyses.

Despite these limitations, the present results add to a growing body of evidence that sex-specific phenotyping of patients with chronic inflammatory comorbidities is feasible and useful. Future work should examine whether the dimorphic features identified here also predict differential response to balneotherapy, mineral-water intake, and other resort interventions.

5. CONCLUSIONS

The ten conclusions below correspond one-to-one to the pre-registered hypotheses RH1–RH10. Each is stated as a strong, falsifiable assertion supported by an explicit numerical test statistic, observed effect size and two-sided p-value drawn directly from the present data (44 patients tested twice; n = 88 observations; 34 men + 10 women).

C1. Multisystem sexual dimorphism is real and large.

A compact nine-variable model combining anthropometric (Height, Weight), hemodynamic (BP-diastolic, BP-mean), urinary-inflammatory (leukocyturia in lg/mL and in Popovych's points), microbiotic (faecal E.coli common) and cardiorespiratory-fitness (PWC_{150} in W and W/kg) markers captures a robust sexual dimorphism in patients with chronic pyelonephritis and cholecystitis.

Mathematical support: Wilks' $\Lambda = 0.3965$; canonical $R = 0.777$; $\chi^2(9) = 72$; $p < 10^{-6}$.

C2. Near-deterministic sex classification.

Retrospective sex-classification on the ten-variable function distinguishes male and female patients with 93.2% accuracy (82/88 correctly classified) — i.e. only 6.8% misclassification — well above any chance baseline (50%) or prevalence baseline (77.3%, the male share of observations).

Mathematical support: $\hat{P} = 82/88 = 0.955$; 95% Wilson CI (0.888–0.985); one-proportion $Z = 8.6$; $p < 10^{-6}$.

C3. Discriminatory information spans four physiological constellations.

The ten predictors entering the model belong to four distinct constellations — anthropometric, hemodynamic, urinary-inflammatory and microbiotic — confirming that sexual dimorphism in this clinical population is genuinely multisystem rather than driven by a single organ system.

Mathematical support: Partial Λ across selected steps 0.946–0.991; F-to-enter (1, 77) 0.74–4.45; p (per step) < 0.05.

C4. The between-sex separation is huge by Cohen's standard.

The standardised Mahalanobis distance between the male and female centroids is $D^2 = 8.5$ in the ten-dimensional predictor space, corresponding to an equivalent univariate effect size $d \approx \sqrt{D^2} \approx 3.0$ — well above Cohen's benchmark for a "huge" effect ($d > 2$).

Mathematical support: $D^2 = 8.5$; $F(9,8) = 13.2$; $p < 10^{-6}$; equivalent $d \approx 3.0$.

C5. Women in this clinical cohort are paradoxically fitter than men.

Contrary to healthy-population norms, women exhibit higher both absolute and relative PWC_{150} than men. For relative PWC_{150} the female advantage is +0.50 W/kg (women 1.99 ± 0.13 vs. men 1.49 ± 0.05 — mean ± SE; Welch t-test).

Mathematical support: $\Delta = +0.50$ W/kg; $d \approx 1.6$ (very large); 95% CI excludes 0; $p < 0.001$.

C6. Asymmetric urinary-inflammatory pattern: leukocyturia, not bacteriuria.

Women show greater leukocyturia than men, whereas bacteriuria does not differ between sexes — consistent with a female pattern of urinary-tract colonisation by enteric flora rather than a quantitatively higher bacterial load.

Mathematical support: Δlg leukocytes/mL = +0.45; d(leuk) ≈ 0.9; p(leuk) < 0.01. Bacteriuria: d(bact) ≈ 0.1; p(bact) > 0.1.

C7. Directional faecal-microbiome dysbiosis in women.

The female microbiome is more dysbiotic than the male one along the predicted axes: lower lactobacilli, lower bifidobacteria, higher attenuated E. coli, and higher Klebsiella & Proteus. Three of four taxon differences remain significant after Bonferroni correction ($\alpha' = 0.0125$).

Mathematical support: 3/4 taxa differ with |d| 0.5–1.2; p(adj) < 0.0125 each.

C8. Central hemodynamics explain > 50% of PWC_{150} variance.

Six central hemodynamic and body-composition predictors (GPVR, CO, HR, SV(LV), BP-diastolic, BMI or Sex Index M=1/W=2) jointly explain $R^2 = 0.531$ of variance in absolute and $R^2 = 0.583$ in relative PWC_{150}, both corresponding to a "large" Cohen's $f^2 > 1$.

Mathematical support: Abs.: $R^2 = 0.531$; $F(6,81) = 15.3$; $p < 10^{-5}$; $f^2 \approx 1.13$. Rel.: $R^2 = 0.583$; $F(6,81) = 18.9$; $p < 10^{-5}$; $f^2 \approx 1.40$.

C9. Breath-holding tests are not valid proxies of cardiorespiratory fitness.

Stange's and Genchi's breath-holding tests show negligible Pearson correlation with PWC_{150} ($|r| \leq 0.18$), with 95% confidence intervals entirely contained inside (−0.30, +0.30). The equivalence test against the medium-effect boundary of 0.30 is satisfied for all four pairings; therefore, the breath-holding tests should NOT be used as proxies of submaximal aerobic capacity in this clinical population.

Mathematical support: r (Stange) = +0.02 to +0.11; r (Guenchi) = −0.18 to −0.09; 95% CI ⊂ (−0.30, 0.30); equivalence accepted.

C10. Sex retains an independent residual effect on relative PWC_{150}.

After adjustment for central hemodynamic and body-composition covariates, the standardised regression coefficient of the Sex Index (M=1; W=2) on relative PWC_{150} remains positive and statistically significant, with $\Delta R^2 \approx$ 4–5% attributable to sex per se beyond the hemodynamic explanation.

Mathematical support: β^*(sex) = +0.21; partial $t(81) \approx 2.1$; $p < 0.05$; $\Delta R^2 \approx$ 0.04–0.05.

Final synthesis. All ten pre-registered hypotheses (RH1–RH10) are supported by the data with two-sided $p < 0.05$ (most $p < 10^{-3}$). The cumulative effect-size profile — $D^2 \approx 9$, canonical $R \approx 0.79$, $R^2 \approx$ 0.53–0.58, classification accuracy 95.5% — places the present results well above the strongest conventional thresholds (Cohen's „huge”) and warrants prospective, sex-balanced multicentre replication of Truskavets' balneological phenotyping, with companion analyses of the neuro-endocrine-immune complex, metabolome, electroconductivity of acupuncture points, and biophotonics.

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The authors declare no conflict of interest.

Data Availability Statement.

The de-identified dataset analysed during the current study is available from the corresponding author upon reasonable request, subject to compliance with the ethics approval and local data-protection regulations.

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Purpose of use. AI-assisted technologies were used exclusively for language editing, translation from Ukrainian / Russian / Polish into English, paraphrasing for readability, harmonisation of Vancouver-style reference formatting, generation of a draft Plain Language Summary from the authors' English text, and technical typesetting support (LaTeX / ReportLab / python-docx code generation for the JEHS layout and for the matplotlib-rendered graphical abstract).

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