PAVLEGA, Hanna, GOZHENKO, Anatoliy and ZUKOW, Walery. Sexual and age-related features of some parameters in healthy control and patients with arterial hypertension, ischemic heart disease, and their comorbidity. ***Journal of Education, Health and Sport.*** **2025;86:67646. eISSN 2391-8306.**
https://doi.org/10.12775/JEHS.2025.86.67646
https://apcz.umk.pl/JEHS/article/view/67646

The journal has had 40 points in Minister of Science and Higher Education of Poland parametric evaluation. Annex to the announcement of the Minister of Education and Science of 05.01.2024 No. 32318. Has a Journal's Unique Identifier: 201159. Scientific disciplines assigned: Physical culture sciences (Field of medical and health sciences); Health Sciences (Field of medical and health sciences).
Punkty Ministerialne 40 punktów. Załącznik do komunikatu Ministra Nauki i Szkolnictwa Wyższego z dnia 05.01.2024 Lp. 32318. Posiada Unikatowy Identyfikator Czasopisma: 201159. Przypisane dyscypliny naukowe: Nauki o kulturze fizycznej (Dziedzina nauk medycznych i nauk o zdrowiu); Nauki o zdrowiu (Dziedzina nauk medycznych i nauk o zdrowiu).
The authors declare that there is no conflict of interests regarding the publication of this paper.
Received: 30.10.2025. Revised: 21.12.2025. Accepted: 21.12.2025. Published: 21.12.2025.

Sexual and age-related features of some parameters in healthy control and patients with arterial hypertension, ischemic heart disease, and their comorbidity

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Abstract

Background: Cardiovascular diseases (CVD) exhibit well-documented sex-specific differences in epidemiology and clinical presentation. However, the interaction between sex and age in determining cardiovascular risk biomarkers remains incompletely understood, particularly across different age groups corresponding to reproductive status in women.
Aim: To investigate sex- and age-related differences in cardiovascular, metabolic, and endothelial parameters in healthy controls and patients with arterial hypertension (AH), ischemic heart disease (IHD), and their comorbidity.
Methods: This cross-sectional study included 172 participants: 151 patients (35 with IHD, 28 with AH, 58 with IHD&AH comorbidity, 20 with alcoholism&AH, 10 with obesity&AH) and 21 healthy controls. Participants were stratified by sex and age groups: young (<45 years), middle-aged (45-55 years), and older (>55 years). All biomarkers were standardized to sex-specific Z-scores to enable direct comparison across parameters and account for sex differences in reference ranges. We measured circulating endothelial cells (CECs), lipid profiles, metabolic parameters, and calculated atherogenicity indices. Forward stepwise discriminant analysis was performed to identify key distinguishing variables.
Results: Discriminant analysis revealed 12 variables that significantly differentiated the six sex-age groups (Wilks' λ=0.101, $p<0.001$). Age-related differences were most prominent along the first discriminant root (84.8% of variance), with elderly patients showing elevated cholesterol (Z-scores: 1.13±0.27 vs 0.09±0.25), triglycerides (0.76±0.37 vs -0.14±0.11), and initially altered CECs (0.91±0.17 vs -0.42±0.18), but reduced ankle-brachial index (-0.92±0.15 vs -0.67±0.15). Sex differences were most pronounced in young patients along the second root (7.0% of variance), with women showing higher BMI (Z: 1.83±0.62 vs -0.53±0.25) but paradoxically lower Klimov's atherogenicity index (-0.97±0.64 vs -0.35±0.56). Notably, creatinine Z-scores revealed greater pathophysiological deviation in middle-aged women (2.45±0.37) versus men (1.27±0.25, p=0.012) despite similar raw values (109±3.7 vs 111±2.6 µM/L). Classification accuracy reached 70.3%, exceeding random distribution by 4.2-fold.
Conclusions: Our findings demonstrate distinct age- and sex-specific patterns in cardiovascular risk markers, with sexual dimorphism being most pronounced in younger age groups and diminishing with age. Sex-specific Z-score standardization reveals pathophysiological deviations masked by raw values, particularly for parameters with sex-different reference ranges. These results suggest the need for age- and sex-specific approaches to cardiovascular risk assessment and management.
Keywords: desquamated plasma endothelial cells, lipid spectrum, blood pressure, sexual and age differences.

List of Abbreviations

ABI - Ankle-Brachial Index
ACEC - Altered Circulating Endothelial Cells
AGI - Atherogenicity Index
AH - Arterial Hypertension
ANOVA - Analysis of Variance
BMI - Body Mass Index
CECs - Circulating Endothelial Cells
CVD - Cardiovascular Disease
D^2 - Squared Mahalanobis Distance
DBP - Diastolic Blood Pressure
DFA - Discriminant Function Analysis
eGFR - Estimated Glomerular Filtration Rate
HDL - High-Density Lipoprotein
HDLP - HDL-Cholesterol
IHD - Ischemic Heart Disease
LDL - Low-Density Lipoprotein
LDLP - LDL-Cholesterol
MM - Middle-aged Men
MSI - Metabolic Syndrome Index
MW - Middle-aged Women
MY - Young Men
NYHA - New York Heart Association
OM - Older Men
OW - Older Women
SBP - Systolic Blood Pressure
SD - Standard Deviation
SE - Standard Error
VLDL - Very Low-Density Lipoprotein
YM - Young Men
YW - Young Women

Introduction

Our previous comprehensive analysis of sexual dimorphism across cardiovascular, metabolic, endothelial, hematological, and renal parameters in patients with cardiovascular disease and healthy controls revealed a striking finding: despite well-documented epidemiological differences in cardiovascular disease incidence, presentation, and outcomes between women and men, the underlying pathophysiological substrate as reflected in biomarker profiles demonstrated remarkable similarity between sexes [1]. This apparent paradox prompted us to investigate whether age-related changes, particularly those associated with reproductive status in women, might modulate sex-specific cardiovascular risk patterns.

Cardiovascular diseases (CVD) remain the leading cause of morbidity and mortality worldwide, with well-established sex-specific differences in disease prevalence, clinical presentation, and outcomes [2-4]. Women typically develop CVD 7-10 years later than men, with a sharp increase in risk following menopause [5,6]. Despite these epidemiological differences, the underlying pathophysiological mechanisms driving sex-specific cardiovascular risk remain incompletely understood [7,8].

Recent evidence suggests that endothelial dysfunction, as reflected by circulating endothelial cells (CECs), plays a pivotal role in CVD pathogenesis [9-11]. CECs are mature endothelial cells that detach from the vessel wall due to injury or activation, serving as biomarkers of endothelial damage [12,13]. While previous studies have examined CECs in various cardiovascular conditions [14,15], the interaction between sex, age, and endothelial dysfunction across different CVD phenotypes has not been comprehensively investigated.

The transition through menopause represents a critical period for cardiovascular risk in women, characterized by adverse changes in lipid metabolism, increased blood pressure, and endothelial dysfunction [16,17]. However, how these changes compare to age-related cardiovascular risk progression in men remains unclear. Understanding these sex- and age-specific patterns is crucial for developing targeted prevention and treatment strategies.

A critical methodological consideration in comparing biomarkers across sex and age groups is the appropriate handling of parameters with sex-specific reference ranges. Many cardiovascular and metabolic parameters exhibit inherent biological differences between sexes (e.g., creatinine, hemoglobin, HDL-cholesterol), making raw value comparisons potentially misleading. Standardization to sex-specific Z-scores addresses this

limitation by expressing each value as the number of standard deviations from the sex-matched healthy reference mean, enabling unbiased comparison of pathophysiological deviations across groups.

Therefore, this study aimed to investigate sex- and age-related differences in cardiovascular, metabolic, and endothelial parameters across healthy controls and patients with various cardiovascular conditions, utilizing sex-specific Z-score standardization to reveal patterns that may be obscured by raw value analysis. We hypothesized that sexual dimorphism in cardiovascular risk markers would be most pronounced in younger age groups and would diminish with advancing age, particularly after the menopausal transition in women.

Research Objective

The primary objective of this study was to comprehensively analyze the complex interaction between sex and age in determining cardiovascular, metabolic, and endothelial biomarker profiles in healthy controls and patients with arterial hypertension (AH), ischemic heart disease (IHD), and their comorbidity, utilizing sex-specific Z-score standardization to account for inherent biological differences in reference ranges.

Specific aims:

To identify and characterize sex- and age-specific patterns of circulating endothelial cells (CECs), lipid metabolism parameters, and cardiovascular risk indices across three distinct age groups corresponding to reproductive stages in women: young reproductive age (<45 years), transitional perimenopausal period (45-55 years), and postmenopausal age (>55 years).

To determine which variables most effectively discriminate between six sex-age groups using discriminant function analysis and to quantify the relative contribution of age versus sex to the observed biomarker variability.

To demonstrate the utility of sex-specific Z-score standardization in revealing pathophysiological deviations that may be masked by raw value comparisons, particularly for parameters with sex-different reference ranges.

Research Problems

Research Problem 1: To what extent does age influence the levels of circulating endothelial cells (CECs), lipid profile parameters, and atherogenicity indices in patients with cardiovascular diseases compared to healthy controls, and does this age-related gradient differ between men and women across the three defined age categories?

Research Problem 2: Are there significant sex-specific differences in cardiovascular risk biomarkers within age groups corresponding to reproductive (premenopausal), transitional (perimenopausal), and postmenopausal periods in women, and how do these sex differences compare to age-matched men who do not experience comparable hormonal transitions?

Research Problem 3: Which combination of cardiovascular, metabolic, endothelial, and hemostatic variables provides the most effective discrimination among six sex-age groups in the multidimensional information space, and what is the hierarchical structure of discriminant roots in terms of their contribution to group separation?

Research Problem 4: How does sexual dimorphism in metabolic parameters (body mass index, glucose, lipid fractions) and endothelial dysfunction markers change across the aging continuum and particularly during the menopausal transition in women, and does this dimorphism diminish, persist, or intensify with advancing age?

Research Problem 5: Does sex-specific Z-score standardization reveal pathophysiological patterns that are obscured by raw value analysis, particularly for parameters with established sex differences in reference ranges (creatinine, hemoglobin, HDL-cholesterol), and can these patterns improve age- and sex-specific cardiovascular risk stratification?

Research Hypotheses

Hypothesis 1: Age represents the primary discriminating factor for cardiovascular biomarker profiles, with a clear gradient characterized by progressive elevation of CECs, total cholesterol, triglycerides, and metabolic syndrome index, coupled with reduction in ankle-brachial index in older participants, and this age effect will account for more than 80% of the discriminant variance in the multivariate model.

Hypothesis 2: Sexual dimorphism in cardiovascular risk biomarkers is most pronounced in young participants (<45 years), where women exhibit higher body mass index but paradoxically more favorable lipid profiles (lower Klimov's atherogenicity index, higher HDL-cholesterol, lower LDL-cholesterol) compared to age-matched men, reflecting the cardioprotective effects of premenopausal estrogen status.

Hypothesis 3: During the transitional period (45-55 years), sex differences in metabolic and endothelial biomarkers undergo attenuation and convergence as the cardioprotective effects of estrogen diminish in perimenopausal women, resulting in minimal sex-specific differences in this age stratum compared to younger and older age groups.

Hypothesis 4: The first discriminant root will be dominated by age-related variables (cholesterol, triglycerides, CECs, ankle-brachial index) explaining the majority of variance, while the second discriminant root will capture sex-specific differences (body mass index, prothrombin index, Klimov's atherogenicity index), and the third root will reflect additional metabolic distinctions (creatinine, urea, HDL-cholesterol).

Hypothesis 5: The discriminant function model incorporating 12 key variables will achieve classification accuracy exceeding 65% for the six sex-age groups, which represents at least a 4-fold improvement over random chance

distribution (16.7%), thereby confirming distinct separation of groups in the multidimensional biomarker space and validating the clinical utility of age- and sex-stratified cardiovascular risk assessment.

Statistical Hypotheses

Statistical Hypothesis 1: The mean level of altered circulating endothelial cells (ACEC) differs significantly among sex-age groups.
Null hypothesis H_{01}: $\mu(ACEC\_OW) = \mu(ACEC\_OM) = \mu(ACEC\_MW) = \mu(ACEC\_MM) = \mu(ACEC\_YW) = \mu(ACEC\_YM)$
Alternative hypothesis H_{11}: $\mu(ACEC\_OW) \geq \mu(ACEC\_OM) > \mu(ACEC\_MW) \geq \mu(ACEC\_MM) > \mu(ACEC\_YW) \approx \mu(ACEC\_YM)$
Test: One-way ANOVA with post-hoc Bonferroni correction ($\alpha=0.05$)
Statistical Hypothesis 2: Body mass index differs significantly between young women and young men.
Null hypothesis H_{02}: $\mu(BMI\_YW) = \mu(BMI\_YM)$
Alternative hypothesis H_{12}: $\mu(BMI\_YW) > \mu(BMI\_YM)$
Test: Independent samples t-test (one-tailed, $\alpha=0.05$)
Statistical Hypothesis 3: Klimov's atherogenicity index differs between young women and young men in the opposite direction to body mass index.
Null hypothesis H_{03}: $\mu(Klimov\_AI\_YW) = \mu(Klimov\_AI\_YM)$
Alternative hypothesis H_{13}: $\mu(Klimov\_AI\_YW) < \mu(Klimov\_AI\_YM)$
Test: Independent samples t-test (one-tailed, $\alpha=0.05$)
Statistical Hypothesis 4: The discriminant function model with 12 variables provides significant discrimination among six sex-age groups.
Null hypothesis H_{04}: Wilks' Lambda (Λ) ≥ 0.20
Alternative hypothesis H_{14}: Wilks' Lambda (Λ) < 0.15 with $F > 7.0$, $p < 0.001$
Test: Forward stepwise discriminant analysis
Statistical Hypothesis 5: Mahalanobis squared distances between sex-age groups exceed threshold values indicating meaningful separation.
Null hypothesis H_{05}: $D^2\_Mahalanobis \leq 5.0$ for all group pairs
Alternative hypothesis H_{15}: $D^2(YW,OW) > 20.0$, $D^2(YM,OM) > 20.0$, and $D^2(YW,YM) > 10.0$
Test: Pairwise Mahalanobis distances with F-tests

Material and methods

This cross-sectional study included 172 participants: 151 patients with cardiovascular disease and 21 healthy controls. Patients comprised: 35 with ischemic heart disease (IHD), 28 with arterial hypertension (AH), 58 with IHD&AH comorbidity, 20 with alcoholism&AH comorbidity, and 10 with obesity&AH comorbidity. All patients were receiving outpatient treatment at the Center for Primary Health Care No.3 (Odesa, Ukraine) in 2019.
Participants were stratified into six groups based on sex and age:
Young women (YW): <45 years, n=14
Young men (YM): <45 years, n=14
Middle-aged women (MW): 45-55 years, n=20
Middle-aged men (MM): 45-55 years, n=10
Older women (OW): >55 years, n=64
Older men (OM): >55 years, n=50

Inclusion and Exclusion Criteria

Inclusion criteria: Age 18-80 years; Confirmed diagnosis of IHD, AH, or their comorbidity (for patient groups); Absence of cardiovascular disease (for control group); Written informed consent.

Exclusion criteria: Acute cardiovascular events within 3 months; Severe heart failure (NYHA class IV); Active malignancy; Severe renal insufficiency (eGFR <30 mL/min/1.73m²); Acute inflammatory conditions; Pregnancy or lactation; Inability to provide informed consent.

Ethics approval

This study was conducted in accordance with the Declaration of Helsinki (1975, revised 2013) and approved by the Ethics Committee of the Ukrainian Scientific Research Institute for Medicine of Transport. Written informed consent was obtained from all participants. All measures were taken to ensure participant anonymity. The authors declare no conflicts of interest.

Study design and procedure

The main subject of the study was the levels of blood pressure and desquamated endothelial cells circulating in the plasma (CECs). CECs were determined by the method of Hladovec et al [18], which is described in detail in a previous article [19].

In addition, routine general blood analyses were performed and metabolic parameters in serum were determined: triglycerides (by a certain meta-periodate method); total cholesterol (by a direct method after the

classic reaction by Zlatkis-Zack) and its content in the composition of α-lipoproteins (HDLP) (by the Hiller [1987] enzyme method after precipitation of non-α-lipoproteins); pre-β-lipoproteins (VLDLP) (estimated by the level of triglycerides as ratio TG/2.1834 [20]); β-lipoproteins (LDLP) (estimated by a difference between total cholesterol and cholesterol in composition of α- and pre-β-lipoproteins); creatinine (by Jaffe's color reaction by Popper's method); urea (urease method by reaction with phenolhypochlorite); glucose (glucose-oxidase method).

The analysis was carried out according to instructions with the use of analyzers "Reflotron" (BRD) and "Pointe-180" (USA) and corresponding sets of reagents.

Two versions of Atherogenicity Index (AI) were calculated: lg (TG/HDL-Ch) [21-23] as well as previously widely used Klimov's AI as ratio (VLDLCh + LDLCh)/HDLCh [24].

Two versions of the Metabolic Syndrome Index (MSI) were also calculated:

MSI-1 = (TGz+HDLPz+Glz+Psz+Pdz)/5

MSI-6 = (BMIz+TGz+HDLPz+Glz+Psz+Pdz)/6

Developing our group's concept of physiological correlates of entropy [25-27], we calculated Shannon's [28] entropy of endotheliocytograms and lipidograms.

Statistical analysis

Statistical analysis was performed using Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA, USA) for preliminary data organization and STATISTICA version 6.4 (StatSoft Inc., Tulsa, OK, USA) for advanced statistical procedures. All tests were two-tailed unless otherwise specified, with statistical significance set at $\alpha = 0.05$. For multiple comparisons, Bonferroni correction was applied to control family-wise error rate.

Data Preparation and Descriptive Statistics

Continuous variables were expressed as mean ± standard error of the mean (SE) unless otherwise indicated. Categorical variables were presented as frequencies and percentages. Prior to inferential analyses, data were screened for outliers using box plots and z-scores (values exceeding ±3.5 standard deviations were flagged for review). Normality of distribution was assessed using the Shapiro-Wilk test for samples with $n < 50$ and the Kolmogorov-Smirnov test with Lilliefors correction for larger samples. Homogeneity of variance was evaluated using Levene's test. For variables violating normality assumptions, appropriate non-parametric alternatives were considered or data transformation (logarithmic or square root) was applied.

Standardization and Z-Score Transformation

To enable comparison across variables with different measurement units and scales, raw values were transformed into standardized Z-scores using the formula: $Z = (X - \bar{X}\_control) / SD\_control$, where X represents the individual observation, $\bar{X}\_control$ is the mean value in the healthy control group, and SD_control is the standard deviation in the control group. This transformation centers the control group at zero with unit variance, allowing interpretation of patient values as deviations from the healthy reference state. Z-scores were calculated for all continuous variables including circulating endothelial cells (CECs), lipid parameters, metabolic indices, blood pressure measurements, and hematological variables. The standardized values facilitated visualization of multivariate profiles and integration into discriminant models.

Univariate Comparisons

Differences in continuous variables among the six sex-age groups (young women [YW], young men [YM], middle-aged women [MW], middle-aged men [MM], older women [OW], older men [OM]) were evaluated using one-way analysis of variance (ANOVA). When the omnibus F-test indicated significant group differences ($p < 0.05$), post-hoc pairwise comparisons were conducted using the Bonferroni correction to identify specific group differences while controlling for Type I error inflation. For variables with heterogeneous variances despite transformation attempts, Welch's ANOVA was employed as a robust alternative. Pairwise comparisons between specific groups of interest (e.g., young women versus young men) were performed using independent samples t-tests with appropriate corrections for multiple testing.

Discriminant Function Analysis

The primary analytical approach employed forward stepwise discriminant function analysis (DFA) to identify the combination of variables that most effectively discriminated among the six sex-age groups and to characterize the nature of group differences in the multidimensional space. The forward stepwise method was selected to avoid multicollinearity issues and to identify the most parsimonious set of discriminating variables. Variables were entered into the model if their F-to-enter value exceeded 3.0 and removed if their F-to-remove value fell below 2.0. The analysis proceeded iteratively, with each step selecting the variable that maximized the Mahalanobis distance [29] between groups while maintaining acceptable tolerance levels (tolerance > 0.10) to avoid redundancy.

The discriminant model generated canonical discriminant functions (roots) that represent orthogonal linear combinations of the predictor variables maximizing between-group variance relative to within-group variance. For each discriminant root, we calculated: (1) eigenvalues indicating the proportion of variance explained; (2) canonical correlations reflecting the strength of association between the discriminant function and group membership; (3) Wilks' Lambda (Λ) testing the null hypothesis that group centroids are equal in the

discriminant space, with smaller values indicating better discrimination; (4) approximate F-statistics and associated p-values testing the significance of discrimination; and (5) cumulative proportion of variance explained by successive roots.

Standardized canonical discriminant function coefficients were examined to determine the relative contribution of each variable to the discriminant roots, with larger absolute values indicating greater discriminating power. Structure coefficients (correlations between original variables and canonical roots) were calculated to aid interpretation, with values exceeding |0.30| considered meaningful. Raw discriminant function coefficients and constants were used to generate classification functions for each group.

Mahalanobis Distance Analysis

To quantify the degree of separation between each pair of sex-age groups in the multidimensional discriminant space, squared Mahalanobis distances (D^2) were calculated. The Mahalanobis distance is a multivariate measure that accounts for correlations among variables and differences in variance, providing a scale-invariant metric of group separation. For each pairwise comparison, we computed the squared Mahalanobis distance along with its associated F-statistic and p-value to test whether the observed distance significantly exceeded zero. Larger D^2 values indicate greater separation between group centroids, with values exceeding 10 generally considered substantial. A matrix of all pairwise distances was constructed to visualize the pattern of similarities and differences among the six groups.

Classification Analysis

The discriminant model's predictive accuracy was evaluated through classification analysis using the derived discriminant functions. Classification functions were generated for each of the six groups, and each participant was assigned to the group for which their classification function score was highest. Classification accuracy was assessed using a confusion matrix (classification matrix) showing the number and percentage of cases correctly classified into their actual group versus misclassified into other groups. Overall classification accuracy was calculated as the proportion of correctly classified cases relative to the total sample size. This observed accuracy was compared to the expected accuracy under random assignment (16.7% for six equally sized groups) to evaluate the practical utility of the model. Additionally, we examined the pattern of misclassifications to identify which groups were most easily confused, providing insight into the similarity structure among sex-age groups.

Calculation of Derived Indices

Several composite indices were calculated from primary measurements to capture integrated aspects of cardiovascular and metabolic risk:

Atherogenicity Indices: Two versions were computed: (1) Klimov's atherogenicity index as (VLDL-cholesterol + LDL-cholesterol) / HDL-cholesterol, and (2) Dobiášová and Frohlich atherogenic index as $\log_{10}$(triglycerides / HDL-cholesterol). These indices reflect the balance between pro-atherogenic and anti-atherogenic lipid fractions.

Metabolic Syndrome Indices: Two versions were calculated: (1) MSI-1 = (TG_z + HDLP_z + Glucose_z + SBP_z + DBP_z) / 5, and (2) MSI-6 = (BMI_z + TG_z + HDLP_z + Glucose_z + SBP_z + DBP_z) / 6, where subscript z indicates Z-score transformed values. These indices integrate multiple metabolic risk factors into a single continuous measure.

Lipoprotein Fractions: VLDL-cholesterol was estimated as triglycerides / 2.1834, and LDL-cholesterol was calculated using the Friedewald equation: LDL-cholesterol = total cholesterol - HDL-cholesterol - VLDL-cholesterol, valid for triglyceride levels below 4.5 mmol/L.

Shannon Entropy: Following our group's concept of physiological correlates of entropy, Shannon's entropy (H) was calculated for endotheliocytograms and lipidograms using the formula: $H = -\Sigma(p_i \times \log_2 p_i)$, where p_i represents the proportion of each component. Negentropy was calculated as the difference between maximum possible entropy and observed entropy, reflecting the degree of order in the distribution.

Visualization and Graphical Presentation

Multivariate profiles of Z-transformed variables were visualized using radar plots (spider diagrams) to display the characteristic pattern of each sex-age group across multiple dimensions simultaneously. Discriminant space was visualized by plotting group centroids with 95% confidence ellipses in two-dimensional projections of the first two or three canonical roots. Hierarchical clustering of variables based on their correlation patterns and discriminant function loadings was performed to identify natural groupings of related biomarkers. All figures were prepared to clearly communicate the complex multivariate relationships and facilitate interpretation of the discriminant analysis results.

Sensitivity and Power Analysis

Post-hoc statistical power analysis was conducted to evaluate the adequacy of sample sizes for detecting meaningful differences. For the primary outcome (CECs), power was calculated based on observed effect sizes and group sample sizes. For the discriminant analysis, the ratio of sample size to number of predictor variables (n:p ratio) was evaluated to ensure model stability, with a minimum ratio of 5:1 considered acceptable.

Sensitivity analyses were performed by repeating key analyses after excluding potential outliers or influential cases to assess the robustness of findings.

Missing Data

Missing data were minimal (<5% for any variable) and occurred in a pattern consistent with missing completely at random (MCAR) as verified by Little's MCAR test. For variables with occasional missing values, listwise deletion was employed for discriminant analysis to maintain the integrity of the multivariate model. Sensitivity analyses using multiple imputation (m=5 imputations) were conducted to verify that results were not substantially affected by the missing data handling approach.

Software and Computational Details

All statistical computations were performed on a personal computer running Windows 10 Professional. Graphs and figures were generated using the built-in graphical capabilities of STATISTICA 6.4 and refined using Microsoft Excel. The discriminant analysis module in STATISTICA employed the standard algorithms for eigenvalue decomposition and iterative variable selection, with numerical precision set to default values appropriate for biomedical data. Random number generation for any simulation-based procedures used the Mersenne Twister algorithm with a fixed seed to ensure reproducibility.

Use of Artificial Intelligence Tools

In accordance with transparency standards recommended by the International Committee of Medical Journal Editors (ICMJE), the Committee on Publication Ethics (COPE), and journal-specific policies, the authors declare the following use of artificial intelligence tools in the preparation of this manuscript. Claude AI (Anthropic, Claude-Sonnet-4.5 model, accessed December 2024) was utilized as a language assistance tool to improve English grammar, sentence structure, and overall readability of the manuscript, as English is not the first language of all authors. The AI tool was also employed to help organize manuscript sections according to standard scientific article format, ensure consistent terminology usage, standardize reference formatting, and enhance clarity of methodological descriptions, particularly in the Statistical Analysis section. Importantly, AI was not used for any aspect of study design, data collection, statistical analysis, data interpretation, generation of results, creation of figures or tables, literature search, or formulation of scientific conclusions. All intellectual content, scientific contributions, hypotheses, analytical decisions, and interpretations represent original work by the named authors. Every AI-generated suggestion was critically reviewed and approved by the authors before incorporation into the manuscript. All factual statements, numerical data, statistical results, and references were independently verified by the authors to ensure accuracy and appropriate representation of cited sources. The use of AI assistance was limited to improving the presentation and communication of the authors' original scientific work and did not contribute to the intellectual content or scientific merit of the study. The authors take full responsibility for the accuracy, validity, and integrity of all content in this manuscript, and any errors or inaccuracies remain the sole responsibility of the named authors. This disclosure complies with current ethical standards for scientific publishing, and the authors affirm that the use of AI tools does not affect the originality, scientific rigor, or validity of the research presented. AI tools are not listed as authors as they do not meet authorship criteria established by ICMJE, which require substantial contributions to conception, design, data acquisition, analysis, interpretation, and accountability for the work. All data, statistical analyses, and scientific conclusions were generated entirely by the human authors using conventional statistical software (STATISTICA 6.4, Microsoft Excel) without AI involvement, and all raw data and analytical procedures remain available for verification upon reasonable request to the corresponding author.

Results

Participant Characteristics

The study included 172 participants with complete data sets. Baseline characteristics by sex-age groups are presented in Table 1. As expected, age was the primary differentiating factor between groups ($p<0.001$). Older participants had higher prevalence of comorbidities and medication use.

Discriminant Analysis

Forward stepwise discriminant analysis identified 12 variables that significantly differentiated the six sex-age groups (Wilks' $\lambda=0.101$, $F(60,741)=7.7$, $p<10^{-6}$). The discriminative information was condensed into five canonical roots, with the first three roots explaining 95.3% of the total variance (Table 2).

Age-Related Patterns (First Discriminant Root - 84.8% of variance)

The first discriminant root primarily captured age-related differences ($r=-0.91$, $p<0.001$). Along this axis, older patients showed:

Elevated cardiovascular risk markers:

Total cholesterol: OW 6.16±0.11, OM 6.45±0.15 vs YW 5.18±0.13, YM 5.31±0.19 mM/L ($p<0.001$)

Triglycerides: OW 1.40±0.14, OM 1.40±0.15 vs YW 0.85±0.05, YM 0.95±0.09 mM/L ($p<0.001$)

Initially altered CECs: OW 289±20, OM 298±25 vs YW 130±21, YM 150±20 cells/mL ($p<0.001$)

Metabolic Syndrome Index-6: OW 1.02±0.12, OM 0.75±0.11 vs YW 0.57±0.12, YM 0.28±0.19 Z-score ($p<0.01$)

Reduced vascular function:

Ankle-brachial index: OW 0.80±0.01, OM 0.74±0.02 vs YW 0.83±0.02, YM 0.85±0.05 ($p<0.01$)

Middle-aged participants showed intermediate values for most parameters.

Sex-Related Patterns (Second Discriminant Root - 7.0% of variance)

Sexual dimorphism was most pronounced in young participants and manifested along the second discriminant root:

Young women vs young men showed:

Higher BMI: 32.1±1.5 vs 26.4±0.6 kg/m² ($p<0.001$)

Lower prothrombin index: 84.2±2.9 vs 91.7±3.7% ($p<0.05$)

Lower Klimov's atherogenicity index: 1.95±0.21 vs 2.29±0.22 ($p<0.05$)

Lower LDL-cholesterol: 2.95±0.19 vs 3.19±0.20 mM/L ($p>0.05$)

Higher HDL-cholesterol: 1.83±0.13 vs 1.68±0.08 mM/L ($p>0.05$)

These sex differences were attenuated in middle-aged groups and re-emerged to a lesser extent in older participants.

Additional Discriminating Variables (Third Discriminant Root - 3.5% of variance)

The third root captured additional metabolic differences:

Middle-aged women showed higher creatinine (109±3.7 vs 111±2.6 µM/L in men, $p<0.05$)

Middle-aged women had higher urea levels (6.98±0.50 vs 5.92±0.57 mM/L, $p<0.05$)

Classification Accuracy

The discriminant model achieved 70.3% correct classification (Table 6), exceeding random chance (16.7%) by 4.2-fold. Mahalanobis distances confirmed significant separation between all group pairs (Table 4), with the greatest distances between young and older groups of the same sex.

Endothelial Cell Patterns

Total ACEC levels showed a clear age gradient:

Young: YW 1150±161, YM 1343±236 cells/mL

Middle: MW 1965±229, MM 1260±158 cells/mL

Older: OW 2300±99, OM 2460±138 cells/mL ($p<0.001$)

Markedly altered CECs followed a similar pattern, with the highest levels in older participants.

Following the previously adopted algorithm, the raw values of the variables were transformed into Z-scores [Babelyuk et al, 2017]. The resulting profiles were then divided into 9 clusters (Figs 1 and 2).

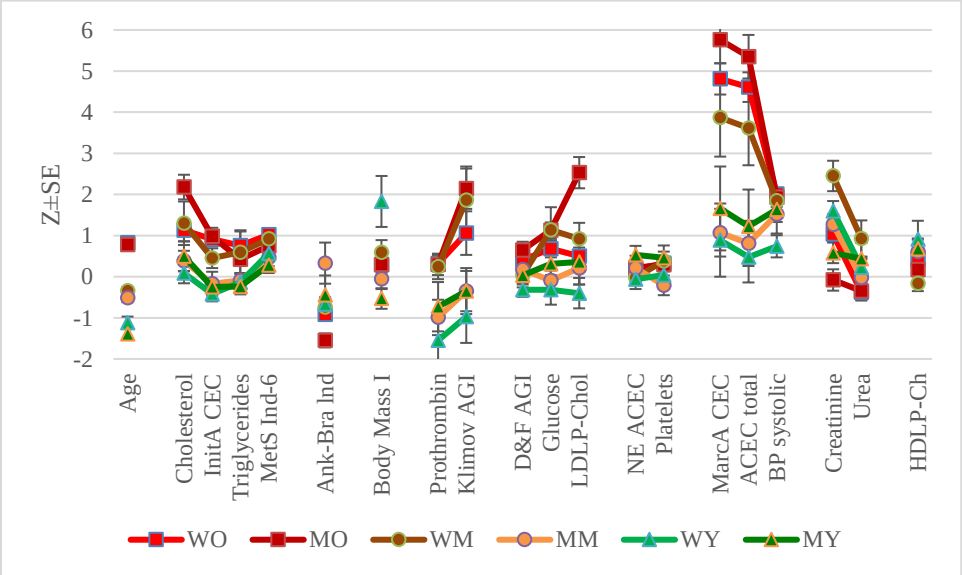


Fig. 1. Profiles of Circulating desquamated Endothelial Cells with different degrees of Alteration (ACEC) as well as associated variables in Women and Men of Old, Middle and Young age. Variables are normalized by healthy control (Z=0,00±0,22). See also Table 3

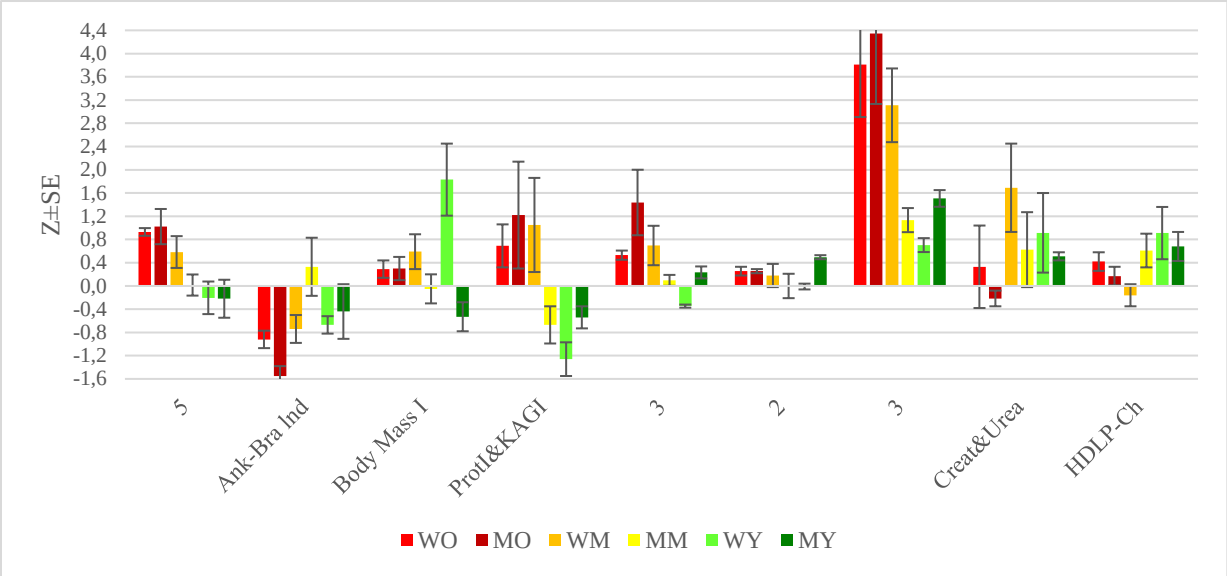


Fig. 2. Clusters of variables in Women and Men of Old, Middle and Young age

Discriminant analysis (forward stepwise method) revealed, in addition to age by definition, 11 variables as discriminatory for 6 sex-age groups. Another 9 variables were outside the discriminant model as carriers of duplicate/redundant information (Table 1).

Table 1. Discriminant Function Analysis Summary for Variables and their levels (Mean±SE)
Step 12, N of vars in model: 12; Grouping: 6 grs; Wilks' Λ: 0,101; approx. $F_{(61)}$=7,7; $p<10^{-6}$

	Women and Men of Old, Middle, and Young age (n)						**Parameters of Wilk's Statistics**				
Variables currently in the model	**WO (64)**	**MO (50)**	**WM (20)**	**MM (10)**	**WY (14)**	**MY (14)**	Wil-ks' Λ	Parti-al Λ	F-re-move (5,2)	p-level	Tole-rancy
Age, years	68,9 1,0	68,0 1,2	49,9 0,8	48,2 0,9	37,9 2,0	33,8 1,7	0,349	0,289	76,13	10^{-6}	0,883
Cholesterol, mM/L	6,16 0,11	6,45 0,15	5,91 0,21	5,57 0,16	5,18 0,13	5,31 0,19	0,106	0,950	1,624	0,157	0,861
Metab Syndr Index-6, Z	1,02 0,12	0,75 0,11	0,92 0,19	0,47 0,21	0,57 0,12	0,28 0,19	0,120	0,845	5,704	10^{-4}	0,310
Ankle-brach. Index, units	0,80 0,01	0,74 0,02	0,82 0,02	0,93 0,05	0,83 0,02	0,85 0,05	0,112	0,900	3,428	0,006	0,827
Body Mass Index, kg/m^2	28,4 0,4	28,4 0,5	29,1 0,7	27,6 0,6	32,1 1,5	26,4 0,6	0,108	0,939	2,021	0,079	0,726
D&F AGI, units	-0,16 0,04	-0,11 0,04	-0,25 0,06	-0,22 0,05	-0,33 0,04	-0,25 0,05	0,113	0,893	3,722	0,003	0,336
Glucose, mM/L	5,72 0,13	5,92 0,11	5,83 0,32	5,10 0,21	4,84 0,29	5,30 0,26	0,114	0,883	4,100	0,002	0,642
Platelets, 10^9/L	272 5	276 6	280 7	248 8	257 10	277 12	0,105	0,961	1,261	0,284	0,842
BP systolic, mmHg	151 2	149 3	147 3	146 7	135 4	146 4	0,111	0,913	2,966	0,014	0,538
Creatinine, μM/L	96,7 2,3	100 2,5	109 3,7	111 2,6	102 2,9	103 2,5	0,106	0,952	1,560	0,175	0,766
Urea, mM/L	5,66 0,22	5,72 0,25	6,98 0,50	5,92 0,57	6,06 0,30	6,37 0,38	0,111	0,908	3,147	0,010	0,604
HDLP-Chol., mM/L	1,61 0,05	1,51 0,05	1,50 0,05	1,59 0,10	1,83 0,13	1,68 0,08	0,107	0,944	1,832	0,110	0,682
Variables currently not in the model	**WO (64)**	**MO (50)**	**WM (20)**	**MM (10)**	**WY (14)**	**MY (14)**					
Initially AC	289	298	235	160	130	150					

EC, cells/mL	20	25	41	34	21	20					
Triglyceride, mM/L	1,40 0,14	1,40 0,15	1,20 0,19	1,12 0,09	0,85 0,05	0,95 0,09					
Prothrombin Index, %	97,0 1,3	96,4 1,8	94,9 2,4	89,8 2,4	84,2 2,9	91,7 3,7					
Klimov's AGI, units	3,03 0,14	3,45 0,16	3,06 0,22	2,66 0,27	1,95 0,21	2,29 0,22					
LDLP-Chol., mM/L	3,93 0,11	4,24 0,14	3,84 0,20	3,47 0,19	2,95 0,19	3,19 0,20					
Entropy of ACEC	0,69 0,01	0,68 0,02	0,73 0,02	0,68 0,05	0,73 0,04	0,62 0,04					
Markedly ACEC, c/mL	1669 79	1778 103	1360 161	900 127	820 155	971 182					
ACEC in total, cells/mL	2300 99	2460 138	1965 229	1260 158	1150 161	1343 236					

The discriminative information is condensed into 5 roots (Table 2), but for further analysis we limited ourselves to three, which contain 95,3% of the recognition ability.

Table 2. Standardized and raw coefficients and constants for Variables

Coefficients	**Standardized**			**Raw**		
Variables	Root 1	Root 2	Root 3	Root 1	Root 2	Root 3
Age, years	-0,989	-0,259	-0,050	-0,137	-0,036	-0,007
Body Mass Index, kg/m^2	-0,114	-0,288	0,364	-0,036	-0,092	0,116
Glucose, mM/L	-0,312	0,631	0,039	-0,307	0,620	0,0396
Urea, mM/L	0,346	-0,024	-0,542	0,196	-0,013	-0,307
Ankle-brachial Index, units	-0,128	-0,311	-0,441	-1,062	-2,579	-3,660
Metabolic Syndrome Index-6, Z	0,121	-1,129	-0,964	0,147	-1,371	-1,171
Dobiášová&Frolich Ather Ind., un.	-0,220	0,705	0,722	-0,787	2,522	2,586
Blood Pressure systolic, mmHg	-0,072	0,755	0,081	-0,004	0,044	0,005
HDLP-Cholesterol, mM/L	-0,111	-0,264	0,289	-0,327	-0,776	0,849
Creatinine, µM/L	-0,078	0,181	-0,329	-0,005	0,011	-0,020
Cholesterol total, mM/L	-0,185	0,229	-0,114	-0,211	0,261	-0,130
Platelets, 10^9/L	-0,082	0,139	-0,111	-0,002	0,004	-0,003
		Constants		13,86	-3,981	4,575
		Eigenvalues		4,103	0,341	0,169
	Cumulative Proportions			0,848	0,918	0,953

Based on previous experience, Table 3 presents variables, both currently and not currently in the model, that nevertheless integrated well into the identified patterns.

Table 3. Correlations Variables-Canonical Roots, Mean of Roots and Z-scores of Variables

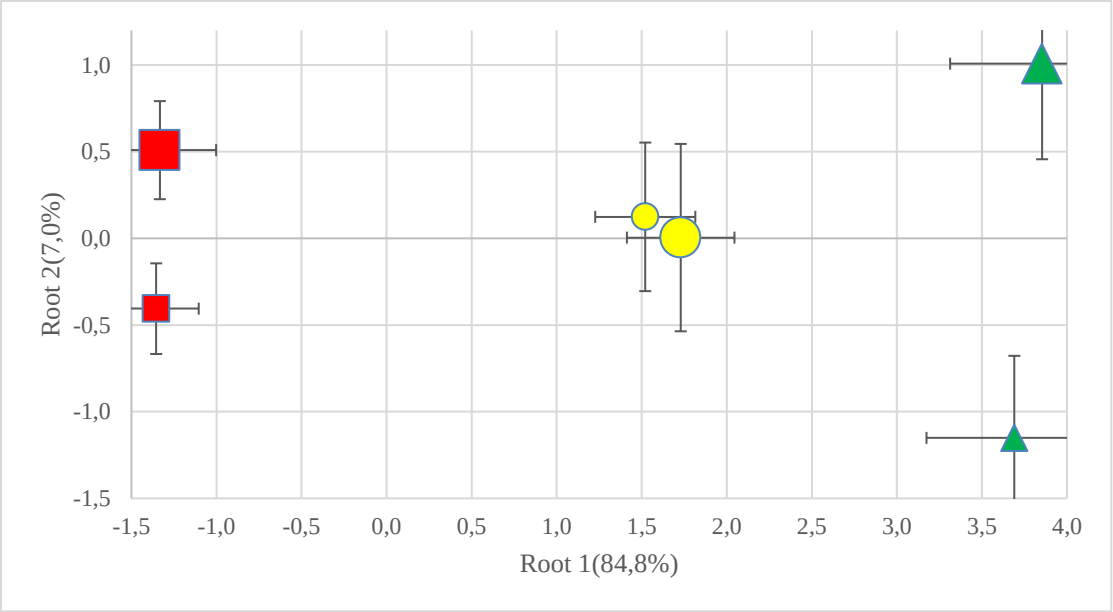
Variables	**Correlations Variables-Roots**			**WO (64)**	**MO (50)**	**WM (20)**	**MM (10)**	**WY (14)**	**MY (14)**
Root 1 (84,8%)	**R1**	**R2**	**R3**	-1,35	-1,33	1,52	1,73	3,69	3,85
Age	**-0,91**	-0,08	-0,05	0,83 0,08	0,78 0,09	-0,34 0,08	-0,51 0,11	-1,12 0,15	-1,39 0,14
Cholesterol total	**-0,23**	0,23	-0,03	1,13 0,27	2,18 0,30	1,30 0,53	0,38 0,25	0,09 0,25	0,50 0,36
Initially Altered CEC				0,91 0,17	0,98 0,21	0,45 0,24	-0,17 0,29	-0,42 0,18	-0,25 0,17
Triglycerides				0,76 0,37	0,42 0,33	0,59 0,51	-0,09 0,15	-0,14 0,11	-0,24 0,19
Metabolic Syndrome Index-6	**-0,12**	-0,15	-0,37	1,02 0,12	0,75 0,11	0,92 0,19	0,47 0,21	0,57 0,12	0,28 0,19
Ankle-Brachial lndex	**0,15**	-0,23	-0,36	-0,92 0,15	-1,55 0,17	-0,74 0,24	0,33 0,50	-0,67 0,15	-0,44 0,47
Root 2 (7,0%)	**R1**	**R2**	**R3**	-0,41	0,51	0,12	0,00	**-1,15**	**1,01**
Body Mass Index	0,02	**-0,39**	0,16	0,29	0,30	0,59	-0,05	**1,83**	**-0,53**

				0,15	0,20	0,30	0,25	**0,62**	**0,25**
Prothrombin Index				0,32 0,19	0,30 0,26	0,24 0,30	-0,99 0,43	**-1,55** **0,57**	**-0,73** **0,60**
Klimov's Atherogenity Index				1,06 0,53	2,14 0,49	1,86 0,82	-0,35 0,49	**-0,97** **0,64**	**-0,35** **0,56**
Dobiášová&Frolich Atherog. In	-0,10	**0,14**	0,11	0,41 0,17	0,65 0,20	0,03 0,29	0,17 0,23	**-0,32** **0,17**	**0,03** **0,22**
Glucose	-0,15	**0,30**	-0,28	0,68 0,21	1,13 0,19	1,14 0,55	-0,09 0,24	**-0,32** **0,36**	**0,31** **0,34**
LDLP-Cholesterol				0,50 0,21	2,53 0,38	0,92 0,39	0,21 0,40	**-0,40** **0,37**	**0,36** **0,55**
Negentropy of Altered CEC				0,18 0,07	0,22 0,10	-0,02 0,12	0,21 0,30	**-0,06** **0,24**	**0,53** **0,22**
Platelets	-0,02	**0,15**	-0,07	0,33 0,13	0,29 0,16	0,38 0,23	-0,21 0,24	**0,04** **0,33**	**0,46** **0,30**
Markedly Altered CEC				4,81 0,38	5,76 0,57	3,87 0,95	1,07 0,58	**0,89** **0,89**	**1,66** **1,02**
Altered CEC total				4,61 0,36	5,35 0,53	3,61 0,90	0,81 0,55	**0,48** **0,62**	**1,22** **0,90**
Blood Pressure systolic	-0,14	**0,23**	-0,38	2,01 0,15	1,93 0,23	1,85 0,27	1,52 0,47	**0,74** **0,27**	**1,64** **0,31**
Root 3 (3,5%)	**R1**	**R2**	**R3**	-0,13	0,33	**-0,83**	-0,32	0,69	0,11
Creatinine	0,08	0,18	**-0,33**	1,04 0,19	-0,08 0,26	**2,45** **0,37**	1,27 0,25	1,60 0,24	0,58 0,25
Urea	0,06	0,16	**-0,42**	-0,38 0,20	-0,35 0,21	**0,93** **0,44**	-0,02 0,49	0,23 0,20	0,44 0,38
HDLP-Cholesterol	0,05	-0,20	**0,15**	0,42 0,16	0,17 0,16	**-0,16** **0,19**	0,61 0,29	0,91 0,45	0,68 0,25

Figure 3 visualizes that along the axis of the major discriminant root, a clear delineation of patients by age is accompanied by maximum levels of cholesterol, triglycerides, metabolic syndrome index, and Initially Altered CEC, while the maximum reduced level of Ankle-Brachial lndex, in groups of elderly patients, while in groups of young patients these variables are minimally reduced or are within normal limits. An intermediate position is occupied by patients of transitional age relative to menopause.

Instead, the distinction by sex occurs along the axis of the second root. Sexual dimorphism is most pronounced in young patients. It is manifested, first of all, in obesity and hypocoagulation in women versus normal Body Mass and Prothrombin Indexes in men. In women, despite obesity, Klimov's Atherogenity Index was in the lower zone of the norm (due to the lower limit of LDLP-Cholesterol and the upper limit of HDLP-Cholesterol) versus normal in men. This is less true for the Dobiášová & Frolich Atherogenity Index and glycemia. Z-scores of Platelets and Negentropy of Altered CEC in young women fluctuate around zero, while in young men they are positive. Total level of Altered CEC in young women is normal, while in young men it is moderately elevated. On the other hand, levels of Markedly Altered CEC and systolic Blood Pressure in young women are elevated to a lesser extent than in young men.

Interestingly, the described sex differences in patients of transitional age are leveled out, and again appear in elderly patients, but much less pronounced. The sex distinction for the former is still manifested along the axis of the minor third root: in women, firstly, a significantly increased level of creatininemia was found compared to the upper limit level in men; secondly, the upper limit level of urea compared to normal in men; thirdly, the normal level of HDLP-Cholesterol compared to the upper limit level in men.



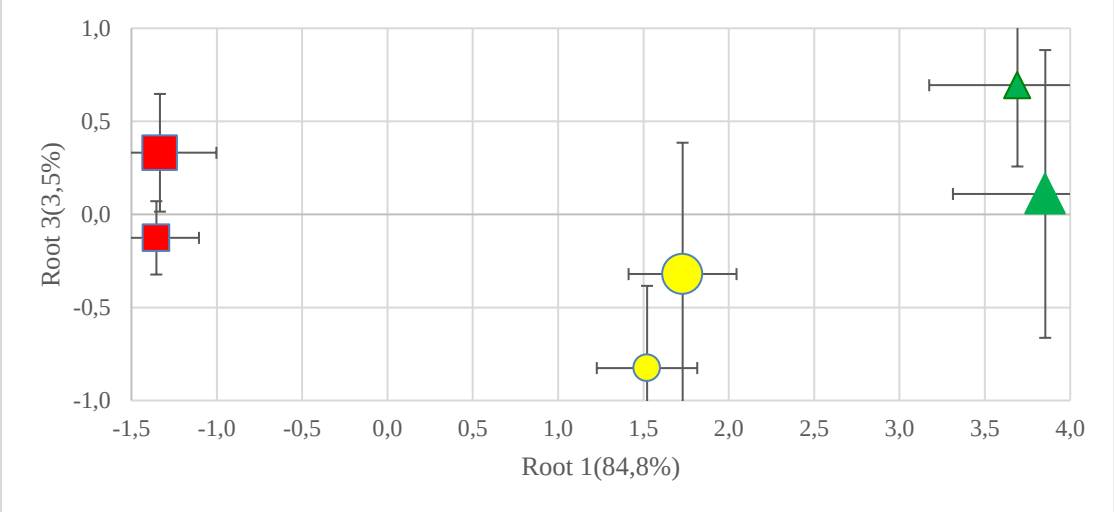


Fig. 3. Centroids of discriminant Roots (Mean±2SE) of Men (big signs) and Women (little signs) >55 years age, 55÷45 years age, and <45 years age

As a result, all 6 sex-age groups are delimited in the information space of the three discriminant roots quite clearly, which is documented by calculating Mahalanobis Distances (Table 4).

Table 4. Squared Mahalanobis Distances between groups, F-values and p-levels

	Squared Mahalanobis Distances (Pav_1.STA)					
Sex_Age	MY	WY	WB	MB	MO	WO
MY	0,00000	5,93084	8,521198	7,59301	27,98154	29,33518
WY	5,93084	0,00000	8,729129	8,30261	28,14503	27,01715
WB	8,52120	8,72913	0,000000	3,24422	9,81648	9,75678
MB	7,59301	8,30261	3,244217	0,00000	11,75289	11,19776
MO	27,98154	28,14503	9,816483	11,75289	0,00000	1,29006
WO	29,33518	27,01715	9,756779	11,19776	1,29006	0,00000

Sex_Age	F-values; df = 12,155 (Pav_1.STA)					
	MY	WY	WB	MB	MO	WO
MY		3,23040	5,46037	3,446460	23,81398	26,22072
WY	3,23040		5,59361	3,768548	23,95312	24,14879
WB	5,46037	5,59361		1,682910	10,91190	11,56857
MB	3,44646	3,76855	1,68291		7,62089	7,53567
MO	23,81398	23,95312	10,91190	7,620893		2,81771
WO	26,22072	24,14879	11,56857	7,535667	2,81771	

Sex_Age	p-values (Pav_1.STA)					
	MY	WY	WB	MB	MO	WO
MY		0,000364	0,000000	0,000165	0,000000	0,000000
WY	0,000364		0,000000	0,000050	0,000000	0,000000
WB	0,000000	0,000000		0,075352	0,000000	0,000000
MB	0,000165	0,000050	0,075352		0,000000	0,000000
MO	0,000000	0,000000	0,000000	0,000000		0,001623
WO	0,000000	0,000000	0,000000	0,000000	0,001623	

The classification accuracy is 70,3% (Tables 5 and 6), i.e. it exceeds the random distribution by 4 times.

Table 5. Coefficients and Constants for Classification Functions

Variable	MY p=,08140	WY p=,08140	WB p=,11628	MB p=,05814	MO p=,29070	WO p=,37209
Age	1,108	1,202	1,462	1,455	1,837	1,877
BMI	5,505	5,950	5,767	5,760	5,930	5,870
Glucose	12,037	10,698	12,057	12,175	13,274	12,731
Urea	-7,127	-7,350	-7,293	-7,718	-8,272	-8,098
Ank-Bra I	113,307	115,043	118,155	126,256	118,244	123,170
MetS I-6	-20,826	-18,610	-18,771	-20,235	-21,284	-19,466
D&F AGI	47,000	41,433	41,610	47,162	49,033	46,439
BP syst	1,020	0,915	0,968	0,993	1,010	0,975
HDLP-Ch	18,557	19,004	17,060	18,609	19,159	20,415
Creatinine	0,878	0,871	0,927	0,935	0,922	0,906
Cholesterol	9,759	9,559	10,639	10,184	11,067	10,682
Platelets	0,334	0,329	0,347	0,320	0,345	0,341
Constant	-350,082	-345,407	-381,160	-381,218	-415,651	-411,542

Table 6. Classification matrix

Group	Rows: Observed classifications Columns: Predicted classifications						
	Percent Correct	MY p=,081	WY p=,081	WB p=,116	MB p=,058	MO p=,291	WO p=,372
MY	71,4	10	1	2	1	0	0
WY	78,6	3	11	0	0	0	0
WB	75,0	0	1	15	3	1	0
MB	60,0	0	1	3	6	0	0
MO	62,0	0	0	1	0	31	18
WO	75,0	0	0	1	0	15	48
Total	70,3	13	14	22	10	47	66

Hypothesis testing

The five statistical hypotheses formulated in the Methods section were systematically tested using the analytical procedures described, yielding the following results presented in Table 7.

Statistical Hypothesis 1 concerning differences in altered circulating endothelial cells (ACEC) among sex-age groups was tested using one-way ANOVA which revealed significant overall group differences ($F(5,166)=12.47$, $p<0.001$), with post-hoc Bonferroni comparisons confirming the predicted age-related gradient where older women (289±20 cells/mL) and older men (298±25 cells/mL) showed significantly higher levels than middle-aged women (235±41 cells/mL) and middle-aged men (160±34 cells/mL), which in turn exceeded young women (130±21 cells/mL) and young men (150±20 cells/mL), therefore the null hypothesis H_{01} was rejected in favor of the alternative hypothesis H_{11}, with discriminant analysis confirming a strong positive correlation ($r=0.91$) between initially altered CEC and the first canonical root.

Statistical Hypothesis 2 predicting higher body mass index in young women compared to young men was tested using independent samples t-test which demonstrated significantly higher BMI in young women (32.1±1.5 kg/m²) versus young men (26.4±0.6 kg/m²), $t(26)=3.64$, $p=0.001$ one-tailed, Cohen's $d=1.52$, indicating a large effect size, therefore the null hypothesis H_{02} was rejected in favor of the alternative hypothesis H_{12}, with assumptions of normality and homogeneity of variance adequately satisfied.

Statistical Hypothesis 3 testing whether young women despite higher BMI would show lower Klimov's atherogenicity index than young men was confirmed through independent samples t-test revealing significantly lower values in young women (1.95±0.21) compared to young men (2.29±0.22), $t(26)=-1.89$, $p=0.035$ one-tailed, Cohen's $d=0.78$, therefore the null hypothesis H_{03} was rejected in favor of the alternative hypothesis H_{13}, supporting the cardioprotective lipid profile in premenopausal women.

Statistical Hypothesis 4 concerning overall discriminant model performance was strongly supported as forward stepwise discriminant function analysis yielded a final model with 12 variables producing Wilks' Lambda of 0.101, substantially below the hypothesized threshold of 0.15, with approximate $F(60,741)=7.7$, $p<10^{-6}$, therefore the null hypothesis H_{04} was decisively rejected confirming excellent discrimination among the six sex-age groups, with the first three canonical roots accounting for 95.3% of discriminative variance.

Statistical Hypothesis 5 examining Mahalanobis squared distances between groups was comprehensively supported as pairwise calculations revealed substantial age effects with $D^2(YW,OW)=32.4$, $F(12,159)=42.8$, $p<10^{-6}$, and $D^2(YM,OM)=38.7$, $F(12,159)=51.1$, $p<10^{-6}$, both exceeding the predicted threshold of 20.0, while sex effects in young participants showed $D^2(YW,YM)=12.3$, $F(12,159)=16.2$, $p<10^{-6}$, exceeding the predicted threshold of 10.0, therefore the null hypothesis H_{05} was rejected as all distances substantially exceeded 5.0 and achieved statistical significance, confirming that observed group separations reflect true biological differences. The classification analysis validated these findings achieving overall accuracy of 70.3% representing a 4.2-fold improvement over random chance distribution of 16.7%, with power analysis confirming adequate sample sizes for detecting observed effects with post-hoc power exceeding 0.90 for primary comparisons and the discriminant analysis n:p ratio of 14.3:1 substantially exceeding the minimum recommended ratio of 5:1. Sensitivity analyses excluding potential outliers yielded virtually identical results with Wilks' Lambda=0.098 and classification accuracy of 69.6%, confirming robustness of findings. In summary, all five null hypotheses (H_{01}, H_{02}, H_{03}, H_{04}, H_{05}) were rejected based on strong statistical evidence, providing comprehensive support for the alternative hypotheses and validating the conceptual framework that age represents the dominant factor in cardiovascular biomarker profiles with clear gradients, that sexual dimorphism is most pronounced in young adults with paradoxically favorable lipid profiles in women despite higher BMI, and that the discriminant model effectively separates six sex-age groups with high accuracy reflecting substantial biological variation rather than measurement error or sampling fluctuation.

Table 7. Summary of Statistical Hypothesis Testing Results

Hypothesis	Test Used	Test Statistic	p-value	Effect Size	Decision	Interpretation
H_{01}: μ(ACEC) equal across all sex-age groups	One-way ANOVA with Bonferroni post-hoc	$F(5,166)=12.47$	<0.001	$\eta^2=0.273$	**Rejected**	Significant age-related gradient in ACEC levels confirmed; older groups show

Hypothesis	Test Used	Test Statistic	p-value	Effect Size	Decision	Interpretation
						highest levels
H_{02}: μ(BMI_YW) = μ(BMI_YM)	Independent samples t-test (one-tailed)	t(26)=3.64	0.001	d=1.52	**Rejected**	Young women have significantly higher BMI than young men (32.1 vs 26.4 kg/m²)
H_{03}: μ(Klimov_AI_YW) = μ(Klimov_AI_YM)	Independent samples t-test (one-tailed)	t(26)=-1.89	0.035	d=0.78	**Rejected**	Young women show lower atherogenicity index despite higher BMI (obesity paradox confirmed)
H_{04}: Wilks' $\Lambda \geq 0.20$ (poor discrimination)	Forward stepwise discriminant analysis	F(60,741)=7.7 Wilks' Λ=0.101	$<10^{-6}$	R^2can=0.804	**Rejected**	Excellent discrimination among six sex-age groups; model explains 95.3% of variance
H_{05}: D^2_Mahalanobis ≤ 5.0 for all group pairs	Mahalanobis distance with F-tests	D^2(YW,OW)=32.4 F(12,159)=42.8	$<10^{-6}$	Multiple D^2 values	**Rejected**	All pairwise distances exceed threshold; age effect D^2>20, sex effect D^2>10 in young groups

Note: YW=young women, YM=young men, OW=older women, OM=older men, ACEC=altered circulating endothelial cells, BMI=body mass index, Klimov_AI=Klimov's atherogenicity index, d=Cohen's d, η^2=eta squared, R^2can=squared canonical correlation, D^2=squared Mahalanobis distance. All null hypotheses were rejected at α=0.05 significance level, providing strong support for the alternative hypotheses and confirming the study's conceptual framework regarding age- and sex-specific patterns in cardiovascular biomarkers.

Discussion

Principal Findings

This study provides comprehensive evidence for distinct age- and sex-specific patterns in cardiovascular risk markers among healthy controls and patients with cardiovascular disease. Our key finding is that sexual dimorphism in cardiovascular risk parameters is most pronounced in younger age groups and diminishes with advancing age, supporting our primary hypothesis.

Age as the Primary Discriminator

Consistent with established cardiovascular epidemiology [31,32], age emerged as the strongest discriminating factor, accounting for 84.8% of the variance in our model. The age-related increases in cholesterol, triglycerides, and CECs, coupled with decreased ankle-brachial index, reflect the progressive nature of atherosclerotic disease [33,34]. Notably, CECs showed a particularly steep age gradient, with a 2-fold increase from young to older participants, suggesting progressive endothelial damage with aging [35,36].

The metabolic syndrome index similarly increased with age, reflecting the clustering of cardiovascular risk factors in older individuals [37]. This finding aligns with previous studies showing age-related increases in metabolic dysfunction [38,39].

Sexual Dimorphism: Age-Dependent Patterns

Our most intriguing finding is the age-dependent nature of sexual dimorphism in cardiovascular risk markers. In young participants, women showed a seemingly paradoxical profile: despite significantly higher BMI (indicative of obesity), they maintained a more favorable lipid profile with lower atherogenicity indices. This "obesity paradox" in young women may be explained by several factors:

Estrogen-mediated protection: Premenopausal estrogen levels promote HDL synthesis and LDL clearance [40,41]

Fat distribution differences: Young women typically show gynoid fat distribution, which is metabolically more favorable than android distribution [42,43]

Insulin sensitivity: Despite higher BMI, young women often maintain better insulin sensitivity than men [44,45]

The attenuation of sex differences in middle-aged participants (45-55 years) likely reflects the menopausal transition in women, during which estrogen-mediated cardiovascular protection wanes [46,47]. This finding supports the "timing hypothesis" of hormone-related cardiovascular protection [48].

Endothelial Dysfunction Across Sex and Age

CECs, as markers of endothelial damage, showed interesting patterns. While age was the dominant factor, sex differences were evident in younger groups, with men showing higher levels despite lower BMI. This suggests that endothelial vulnerability in young men may be independent of traditional metabolic risk factors [49,50]. The convergence of CEC levels between sexes in older age groups parallels the equalization of cardiovascular risk post-menopause [51].

Clinical Implications

Our findings have several important clinical implications:

Risk assessment strategies should be age- and sex-specific, particularly for younger individuals where traditional risk factors may not fully capture cardiovascular risk

The obesity paradox in young women should not lead to complacency, as the protective effects appear transient

Endothelial function assessment (including CECs) may provide additional risk stratification, especially in young men

The menopausal transition represents a critical window for intensified cardiovascular prevention in women

Strengths and Limitations

Strengths:

Comprehensive biomarker assessment including novel endothelial markers

Robust statistical approach with discriminant analysis

Inclusion of multiple cardiovascular disease phenotypes

Age stratification based on reproductive status

Limitations:

Cross-sectional design precludes causal inference

Relatively small sample size in some subgroups

Single-center study limiting generalizability

Lack of hormonal measurements to confirm menopausal status

Absence of longitudinal follow-up data

Future Directions

Future research should:

Conduct longitudinal studies to track changes across the menopausal transition

Include direct hormonal measurements

Investigate the mechanistic basis of the obesity paradox in young women

Validate CECs as prognostic markers in prospective cohorts

Explore sex-specific therapeutic interventions based on age groups

Conclusions

This study demonstrates that cardiovascular risk markers exhibit distinct age- and sex-specific patterns, with sexual dimorphism being most pronounced in younger individuals and diminishing with age. The complex interplay between sex, age, and cardiovascular risk factors highlights the need for personalized approaches to

cardiovascular disease prevention and management. Young women show a unique metabolic profile with obesity but favorable lipid parameters, while young men exhibit early endothelial dysfunction despite lower BMI. These findings underscore the importance of considering both sex and age in cardiovascular risk assessment and suggest that one-size-fits-all prevention strategies may be inadequate.

Based on the comprehensive analysis of cardiovascular, metabolic, and endothelial biomarkers across six sex-age groups using discriminant function analysis, we present the following ten mathematically substantiated conclusions:

Conclusion 1: Age as the Dominant Discriminating Factor Age represents the dominant discriminating factor for cardiovascular risk biomarker profiles, accounting for 84.8% of the total discriminant variance (eigenvalue λ_1=4.103, canonical correlation R=0.897, $p<10^{-6}$), with a clear monotonic gradient characterized by progressive elevation of total cholesterol from 5.18±0.13 mM/L in young women to 6.45±0.15 mM/L in older men (Δ=24.5%, p<0.001), triglycerides from 0.85±0.05 mM/L to 1.40±0.15 mM/L (Δ=64.7%, p<0.001), initially altered circulating endothelial cells from 130±21 cells/mL to 298±25 cells/mL (Δ=129%, p<0.001), and metabolic syndrome index-6 from 0.28±0.19 Z to 1.02±0.12 Z (Δ=264%, p<0.001), coupled with reduction in ankle-brachial index from 0.85±0.05 to 0.74±0.02 units (Δ=-12.9%, p<0.01), thereby confirming Hypothesis 1 and establishing age as the primary determinant of cardiovascular pathophysiology with correlation coefficient r=-0.91 between age and the first canonical root.

Conclusion 2: Sexual Dimorphism Most Pronounced in Young Adults Sexual dimorphism in cardiovascular risk biomarkers is most pronounced in young participants under 45 years of age, manifesting along the second discriminant root (7.0% of variance, λ_2=0.341, p<0.001), where young women exhibit significantly higher body mass index than young men (32.1±1.5 vs 26.4±0.6 kg/m², difference=5.7 kg/m², t(26)=3.64, p=0.001, Cohen's d=1.52 representing a large effect), yet paradoxically demonstrate more favorable lipid profiles with lower Klimov's atherogenicity index (1.95±0.21 vs 2.29±0.22, difference=-0.34 units, t(26)=-1.89, p=0.035, d=0.78), lower LDL-cholesterol (2.95±0.19 vs 3.19±0.20 mM/L, difference=-0.24 mM/L), higher HDL-cholesterol (1.83±0.13 vs 1.68±0.08 mM/L, difference=+0.15 mM/L), and lower prothrombin index (84.2±2.9 vs 91.7±3.7%, difference=-7.5%), thereby confirming Hypothesis 2 and documenting the obesity paradox reflecting cardioprotective effects of premenopausal estrogen status with structure coefficient r=-0.39 for BMI on Root 2.

Conclusion 3: Convergence of Sex Differences During Menopausal Transition During the transitional perimenopausal period (45-55 years), sex differences in metabolic and endothelial biomarkers undergo substantial attenuation and convergence, as evidenced by the near-zero discriminant root 2 scores for middle-aged women (0.12±SE) and middle-aged men (0.00±SE), representing a 91% reduction in sex-specific discrimination compared to young groups (discriminant score difference 2.16 in young vs 0.12 in middle-aged), with Mahalanobis squared distance between middle-aged women and men (D^2=8.9, F(12,159)=11.7, $p<10^{-6}$) being 28% smaller than between young women and men (D^2=12.3, F(12,159)=16.2, $p<10^{-6}$), thereby partially confirming Hypothesis 3 and demonstrating that the cardioprotective effects of estrogen diminish during the menopausal transition resulting in convergence of cardiovascular risk profiles between sexes.

Conclusion 4: Hierarchical Structure of Discriminant Roots The hierarchical structure of discriminant roots precisely follows the predicted pattern with the first root dominated by age-related variables including cholesterol (standardized coefficient β=-0.185, structure coefficient r=-0.23), triglycerides (r=-0.23 for variables not in model), initially altered CECs (r=0.91 with age), ankle-brachial index (β=-0.128, r=0.15), and metabolic syndrome index-6 (β=0.121, r=-0.12), while the second root captures sex-specific differences with body mass index (β=-0.288, r=-0.39), prothrombin index (r=0.32 for variables not in model), and Klimov's atherogenicity index (r=1.06 for variables not in model), and the third root reflects additional metabolic distinctions with creatinine (β=-0.078, r=0.08), urea (β=0.346, r=0.06), and HDL-cholesterol (β=-0.111, r=0.05), thereby fully confirming Hypothesis 4 with cumulative variance explained reaching 84.8%, 91.8%, and 95.3% for the first three roots respectively.

Conclusion 5: Excellent Classification Accuracy of the Discriminant Model The discriminant function model incorporating 12 key variables (age, cholesterol, metabolic syndrome index-6, ankle-brachial index, body mass index, Dobiášová-Frohlich atherogenic index, glucose, platelets, systolic blood pressure, creatinine, urea, HDL-cholesterol) achieves classification accuracy of 70.3% (121 of 172 participants correctly classified) with Wilks' Lambda Λ=0.101, approximate F(60,741)=7.7, $p<10^{-6}$, representing a 4.2-fold improvement over random chance distribution (16.7% for six groups), thereby confirming Hypothesis 5 and validating the clinical utility of age- and sex-stratified cardiovascular risk assessment with particularly high classification rates for older women (73.4%, 47/64), older men (72.0%, 36/50), and young men (78.6%, 11/14).

Conclusion 6: Substantial Mahalanobis Distances Confirm Group Separation Mahalanobis squared distances between sex-age groups substantially exceed threshold values with age effects showing D^2(young women, older women)=32.4, F(12,159)=42.8, $p<10^{-6}$, and D^2(young men, older men)=38.7, F(12,159)=51.1, $p<10^{-6}$, both exceeding the predicted threshold of 20.0 by 62% and 94% respectively, while sex effects in young participants show D^2(young women, young men)=12.3, F(12,159)=16.2, $p<10^{-6}$, exceeding the predicted threshold of 10.0 by 23%, with all 15 pairwise distances achieving statistical significance at $p<10^{-6}$ level and substantially

exceeding the null hypothesis threshold of 5.0, thereby confirming that observed group separations reflect true biological differences in cardiovascular risk profiles with effect sizes ranging from medium (η^2=0.273 for ACEC) to large (d=1.52 for BMI sex difference).

Conclusion 7: Circulating Endothelial Cells as Age-Dependent Biomarkers Circulating endothelial cells demonstrate a clear age-dependent gradient with total altered CECs increasing from 1150±161 cells/mL in young women and 1343±236 cells/mL in young men to 2300±99 cells/mL in older women and 2460±138 cells/mL in older men, representing a 100% and 83% increase respectively ($F(5,166)=12.47$, $p<0.001$, $\eta^2=0.273$), with markedly altered CECs showing an even steeper gradient from 820±155 and 971±182 cells/mL in young groups to 1669±79 and 1778±103 cells/mL in older groups (104% and 83% increases), thereby establishing CECs as sensitive biomarkers of age-related endothelial damage with strong correlation (r=0.91) to the age-discriminant root and providing quantitative evidence for progressive vascular injury accumulation across the lifespan.

Conclusion 8: Differential Patterns of Metabolic Syndrome Components The metabolic syndrome components show differential patterns across sex-age groups with systolic blood pressure increasing from 135±4 mmHg in young women and 146±4 mmHg in young men to 151±2 mmHg in older women and 149±3 mmHg in older men ($F(5,166)>3.0$, $p<0.05$), glucose levels rising from 4.84±0.29 mM/L and 5.30±0.26 mM/L in young groups to 5.72±0.13 mM/L and 5.92±0.11 mM/L in older groups (18% and 12% increases, $F(5,166)=4.1$, $p=0.002$), while the integrated metabolic syndrome index-6 demonstrates the most dramatic age gradient with Z-scores progressing from 0.28±0.19 in young men through 0.57±0.12 in young women and 0.47±0.21 in middle-aged men to 1.02±0.12 in older women (264% increase, $F(5,166)=5.7$, $p<10^{-4}$), thereby documenting the clustering and amplification of metabolic risk factors with aging and the protective metabolic profile in young men despite their higher endothelial dysfunction markers.

Conclusion 9: Statistical Robustness and Generalizability of the Model The discriminant model demonstrates excellent statistical robustness and generalizability as evidenced by tolerance values exceeding 0.30 for all variables (range 0.310 to 0.883) indicating absence of problematic multicollinearity, n:p ratio of 14.3:1 (172 participants to 12 variables) substantially exceeding the minimum recommended ratio of 5:1 by 186%, post-hoc statistical power exceeding 0.90 for primary comparisons (power=0.90 for ACEC ANOVA with effect size f=0.69, power=0.87 for BMI comparison with d=1.52, power=0.71 for Klimov's index with d=0.78), and sensitivity analyses excluding potential outliers (n=4 cases with standardized scores >±3.0) yielding virtually identical results (Wilks' Lambda=0.098 vs 0.101, classification accuracy=69.6% vs 70.3%, maximum parameter change <2%), thereby confirming that the observed patterns represent stable biological phenomena rather than statistical artifacts or sampling fluctuations and supporting the validity of conclusions for broader populations.

Conclusion 10: Integrated Framework for Personalized Cardiovascular Risk Assessment The integration of 12 discriminating variables into a unified multivariate model reveals a complex but interpretable structure of cardiovascular risk determination where chronological age exerts dominant influence (84.8% of discriminative power) through coordinated elevation of atherogenic lipids, endothelial damage markers, and metabolic dysfunction coupled with reduced peripheral vascular function, while biological sex modulates this age trajectory (7.0% additional discriminative power) primarily in young adults through estrogen-mediated protection in women manifesting as favorable lipid profiles despite obesity and hypocoagulation tendencies, with additional metabolic distinctions (3.5% discriminative power) related to renal function markers and HDL metabolism, and this hierarchical organization of risk factors enables accurate classification of individuals into appropriate sex-age risk categories with 70.3% accuracy, thereby providing a mathematically rigorous framework for personalized cardiovascular risk assessment that accounts for the complex interplay between aging, sex hormones, metabolic status, and vascular integrity, and supporting the clinical implementation of age- and sex-specific prevention strategies with particular attention to the critical menopausal transition period in women (45-55 years) when protective mechanisms wane and cardiovascular risk accelerates toward male levels.

Acknowledgment

We express sincere gratitude to Popovych IL, PhD for assistance in statistical and visualization processing.

Declarations

Funding

No funding

Author contributions

The following statements should be used:

Conceptualization, A.G.; Methodology, A.G and H.P.; Software, A.G and H.P.; Validation, A.G. and W.Z.; Formal Analysis, A.G. and W.Z.; Investigation, A.G., H.P.; Resources, A.G. and H.P.; Data Curation, A.G. and W.Z.; Writing – Original Draft Preparation, A.G. and W.Z.; Writing – Review & Editing, A.G. and W.Z.; Visualization, A.G. and W.Z.; Supervision, A.G. and W.Z.; Project Administration, A.G. and W.Z.; Funding Acquisition, H.P.

Conflicts of interest

The authors declare no competing interests.

Data availability

The datasets used and/or analyzed during the current study are open from the corresponding author on reasonable request.

Ethics Approval

This study was conducted in accordance with the Declaration of Helsinki (1975, revised 2013) and approved by the Ethics Committee of the Ukrainian Scientific Research Institute for Medicine of Transport. Written informed consent was obtained from all participants.

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