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Review

The POI-Accelerated Aging Paradox: A Multi-Omics Approach to Characterizing Systemic Sequelae in Premature Ovarian Insufficiency

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Abstract

Background: Premature ovarian insufficiency is estimated to affect nearly 3-4% of women globally and is increasingly recognized as a condition associated with premature biological aging involving cardiovascular, skeletal, and neurocognitive systems, including elevated cardiovascular, skeletal, and neurocognitive risks. Whether premature ovarian insufficiency (POI) represents a unique aging phenotype or simply early natural menopause remains unknown. Methods: This is a cross-sectional study which compared 75 women with idiopathic POI (age 25-39 years), 75 age-matched eumenorrheic controls, and 75 women with natural menopause (age 50-55 years). Participants underwent comprehensive clinical assessment and fasting blood collection. Proteomic profiling (Olink® Explore 3072), untargeted metabolomics (UHPLC-MS/MS), and high-sensitivity inflammatory markers (hs-CRP, IL-6, TNF-α, GlycA) were measured. Proteomic age acceleration was calculated using a validated 28-protein aging clock. Results: POI patients demonstrated a striking multi-omics signature of accelerated aging. Compared to controls, 847 proteins were differentially expressed, with marked upregulation of senescence-associated secretory phenotype (SASP) proteins. SASP levels in POI closely approximated natural menopause. Metabolomic profiling revealed 246 differentially abundant metabolites, characterized by branched-chain amino acid accumulation (leucine FC=2.1) and altered glycerophospholipid metabolism. Inflammatory markers were markedly elevated in POI versus controls (hs-CRP: 2.84 vs. 1.02 mg/L, $p<0.001$) and comparable to natural menopause. Proteomic age acceleration revealed that POI patients had a predicted biological age of 44.8 years versus chronological age 32.4 years—an acceleration of 12.4 years. Conclusions: POI is associated with a systemic accelerated aging phenotype that qualitatively parallels natural menopause but occurs prematurely, with proteomic age acceleration of approximately 12 years. These findings support earlier initiation of preventive screenings and suggest that targeting inflammatory and metabolic pathways may offer therapeutic benefits.

Keywords

Premature ovarian insufficiency, Primary ovarian insufficiency, Accelerated aging, Proteomics, Metabolomics, Inflammation, SASP, Systems biology

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1. Introduction

Premature ovarian insufficiency (POI), characterized by cessation of ovarian function prior to age 40, affects approximately 3.5% of women across world and constitutes one of the most clinically significant endocrinopathies impacting reproductive-age populations [1]. The condition manifests as hypergonadotropic hypogonadism, presenting with oligo/amenorrhea, elevated gonadotropins, and estrogen deficiency. While clinical attention frequently focuses on infertility and vasomotor symptoms, POI carries profound implications for long-term multisystem health that extend substantially beyond the reproductive axis [2].

Multiple epidemiological investigations have consistently demonstrated that POI confers significantly increased susceptibility to cardiovascular pathology. Compared to women with age-appropriate ovarian function, those with POI exhibit greater carotid intima-media thickness, impaired endothelial responsiveness, and elevated incidence of coronary artery disease and cardiovascular death [3]. Similarly, accelerated bone loss occurs, with POI patients exhibiting reduced bone mineral density and increased fracture risk that parallels patterns observed in considerably older postmenopausal women [4]. Emerging evidence also suggests elevated risk of cognitive decline and all-cause mortality [5]. These observations have prompted conceptualization of POI as a model of accelerated biological aging; however, the molecular underpinnings of this phenomenon remain incompletely characterized.

The fundamental question of whether POI induces an aging phenotype identical to natural menopause–simply occurring earlier–or represents a qualitatively distinct pathological process carries substantial clinical implications [6]. If the molecular signatures of aging in POI parallel those observed at age 50, current guidelines recommending screening initiation at age 50 for cardiovascular disease, osteoporosis, and cognitive impairment may inadequately protect younger POI patients [7]. Conversely, if POI involves unique mechanistic pathways beyond simple estrogen deficiency, targeted interventions beyond standard hormone replacement therapy (HRT) might be warranted [8].

Recent advances in high-throughput omics technologies offer unprecedented opportunities to address these questions at systems biology levels [9]. Proteomic profiling enables simultaneous quantification of thousands of proteins, including those comprising the senescence-associated secretory phenotype (SASP)–a hallmark of cellular aging characterized by pro-inflammatory cytokines, growth factors, and proteases that mediate the detrimental effects of senescent cells on tissue microenvironments [10]. The SASP is increasingly recognized as a driver of age-related pathology, promoting chronic inflammation, tissue dysfunction, and metabolic dysregulation [11].

Metabolomics captures the functional end-products of cellular processes, reflecting the integrated effects of genetic, epigenetic, and environmental influences on metabolic pathways [12]. Specific metabolomic signatures, including branched-chain amino acid (BCAA) accumulation and lipid profile alterations, have been linked to insulin resistance, cardiovascular risk, and biological aging [13]. Inflammatory marker panels provide complementary information about systemic immune activation, with markers such as high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), and glycoprotein acetylation (GlycA) serving as robust predictors of age-related morbidity and mortality [14].

The immunological and inflammatory dimensions of POI have garnered increasing attention [15]. Accumulating evidence implicates chronic low-grade inflammation in POI pathogenesis, with inflammatory cytokines directly impairing granulosa cell function, accelerating follicular atresia, and contributing to ovarian fibrosis [16]. Autoimmune mechanisms are recognized in 4%-30% of POI cases, with ovarian autoantibodies and lymphocytic oophoritis documented [17]. Beyond the ovary, systemic inflammation may mediate the extra-gonadal manifestations of POI, linking ovarian failure to cardiovascular, skeletal, and cognitive outcomes. However, comprehensive profiling of the inflammatory landscape in POI using contemporary high-sensitivity assays remains limited [18].

The integration of these multi-omics layers offers a comprehensive view of the aging process at molecular resolution, potentially revealing whether POI recapitulates the biological signature of normative aging or follows distinct pathological trajectories [19]. Furthermore, such integration may identify novel biomarkers for risk stratification and therapeutic targets for intervention [7].

This study was designed to address these knowledge gaps through an integrated multi-omics approach. We sought to comprehensively characterize the molecular aging phenotype in POI using proteomic, metabolomic, and inflammatory profiling, directly comparing POI patients to both age-matched controls with normal ovarian function and women with natural menopause [20]. By including this latter comparator group, we aimed to determine whether POI recapitulates the biological signature of normative aging or represents a qualitatively distinct process. We hypothesized that POI would demonstrate a multi-omics signature of accelerated aging qualitatively similar to natural menopause but occurring prematurely, with implications for clinical screening and therapeutic targeting [21].

2. Materials and Methods

2.1 Study Design and Oversight

This cross-sectional investigation was conducted between October 1, 2023, and November 30, 2025, at Fatima Memorial Hospital, Lahore, Pakistan. Participant enrollment was completed between October 2023 and March 2024 (a

five-month recruitment window), while laboratory analyses, data processing, and manuscript preparation were completed through November 2025. The study protocol received approval from the Institutional Review Board of Fatima Memorial Hospital (Approval No. FMH-IRB-2023-089, dated September 15, 2023) and was conducted in hospital in accordance with the Declaration of Helsinki and its subsequent amendments. All participants provided written informed consent prior to enrollment.

2.2 Participant Selection and Eligibility

We recruited 225 participants through the outpatient Gynecology and Reproductive Endocrinology clinics at Fatima Memorial Hospital and community-based advertising. Three groups of 75 participants each were enrolled sequentially over the study period.

POI Group: Women aged 25-39 years with confirmed diagnosis of idiopathic POI were eligible. Diagnostic criteria followed the European Society of Human Reproduction and Embryology guidelines [7], requiring oligo/amenorrhea for at least 4 months and two elevated follicle-stimulating hormone (FSH) measurements >25 IU/L obtained at least 4 weeks apart. Exclusion criteria included known genetic causes of POI (e.g., Turner syndrome, FMR1 premutation), autoimmune polyglandular syndrome, prior pelvic radiation or chemotherapy, bilateral oophorectomy, current pregnancy or lactation, HRT use within 3 months (to obtain baseline untreated measures), and chronic inflammatory conditions requiring immunosuppressive therapy (e.g., rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease).

Age-Matched Control Group: Women aged 25-39 years with regular menstrual cycles (21-35 days), no evidence of ovarian dysfunction, and no current hormonal contraceptive use were recruited. Exclusion criteria paralleled those for the POI group, with additional exclusion of any menstrual irregularity or history of infertility.

Natural Menopause Group: Women aged 50-55 years with spontaneous menopause (≥12 months amenorrhea) not receiving HRT were recruited to represent the normative aging phenotype at the typical menopausal age. Exclusion criteria included surgical menopause, premature menopause (age <40 years), and any of the exclusions applied to other groups. Regarding prior HRT use in the POI group: the total duration and type of past HRT use since POI diagnosis were recorded for all participants. A 3-month washout window was applied to obtain untreated baseline molecular measures. Among enrolled POI participants, nine (12%) had prior HRT use (all transdermal estradiol, median duration 14 months) prior to voluntary discontinuation. A formal sub-analysis stratified by prior HRT history was not conducted owing to these small numbers; however, exclusion of these nine participants from sensitivity analyses did not materially alter primary findings (data available on request). The potential influence of cumulative prior HRT exposure on the 12.4-year proteomic age acceleration will be examined in a planned longitudinal follow-up of this cohort.

2.3 Clinical and Anthropometric Assessment

All participants underwent standardized evaluation including detailed medical history, medication review, and physical examination. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer, and weight was measured to the nearest 0.1 kg using a calibrated digital scale with participants in light clothing and without shoes. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared.

Blood pressure was measured in triplicate using an automated oscillometric sphygmomanometer (Omron HEM-907) after 5 minutes of seated rest, with the mean of the three measurements used for analysis. Menstrual history, obstetric history, and lifestyle factors including smoking, alcohol consumption, and physical activity were recorded using standardized questionnaires.

2.4 Blood Sample Collection and Processing

Venous blood (30 mL) was obtained from all participants during morning hours (0800-1000) after confirming a minimum 10-hour fasting period to control for circadian and postprandial influences. Collection tubes included EDTA-containing vacutainers (proteomics/metabolomics), serum separator tubes (inflammatory markers/routine chemistry), and heparinized tubes. Within 2 hours post-collection, centrifugation at 2000 × g for 15 minutes at 4 °C was performed to separate plasma and serum, which were subsequently aliquoted and maintained at -80 °C until batch analysis. A maximum of two freeze-thaw cycles was permitted for any sample.

Routine hormonal assays including FSH, luteinizing hormone (LH), estradiol, and anti-Müllerian hormone (AMH) were performed on fresh samples using electrochemiluminescence immunoassays on a Roche Cobas 6000 analyzer (Roche Diagnostics, Mannheim, Germany) according to manufacturer protocols.

2.5 Proteomic Profiling

Proteomic analysis was performed using the Olink® Explore 3072 platform (Olink Proteomics, Uppsala, Sweden), which quantifies 3072 unique proteins across 48 panels using proximity extension assay (PEA) technology. This method combines antibody-based recognition with polymerase chain reaction amplification, providing high specificity

and sensitivity across a broad dynamic range. The platform includes proteins from diverse biological pathways including inflammation, oncology, cardiometabolic, neurology, and organ damage panels.

Briefly, 1 μL of plasma was incubated with pairs of oligonucleotide-labeled antibodies for each target protein. When both antibodies bound to their target, the oligonucleotides hybridized and were extended by DNA polymerase, creating a unique DNA reporter sequence. These sequences were subsequently amplified and quantified by microfluidic real-time PCR (Fluidigm BioMark HD). Data are reported as Normalized Protein eXpression (NPX) values on a $\log_2$ scale, which is proportional to the relative protein concentration and enables comparison between groups. NPX values are generated through a normalization process that corrects for technical variation between samples and runs.

Quality control procedures included sample and plate controls, with samples randomized across plates to minimize batch effects. Each assay included internal controls for detection, extension, and amplification. Proteins with detection rates <75% across all samples were excluded from downstream analyses. A panel of established SASP proteins was predefined based on the SenNet Consortium recommendations, including interleukins (IL-6, IL-8), tumor necrosis factors (TNF-α), matrix metalloproteinases (MMP-1, MMP-3, MMP-9, MMP-12), growth factors (VEGF, HGF), and chemokines.

2.6 Metabolomic Profiling

Untargeted metabolomic profiling was conducted using ultra high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) at the Chemical Pathology Laboratory, Fatima Memorial Hospital. Metabolites were extracted from 100 μL plasma using methanol precipitation (4:1, methanol:plasma ratio), vortexed for 30 seconds, incubated at -20 °C for 20 minutes, and centrifuged at 14,000 × g for 15 minutes at 4 °C. The supernatant was collected and dried under nitrogen stream, then reconstituted in 100 μL of acetonitrile:water (1:1, v/v) containing internal standards.

Chromatographic separation was achieved on a Waters ACQUITY UPLC HSS T3 column (2.1 × 100 mm, 1.8 μm particle size) maintained at 40 °C. The mobile phase consisted of (A) water with 0.1% formic acid and (B) acetonitrile with 0.1% formic acid. A gradient elution was performed at a flow rate of 0.4 mL/min: 0-1 minute 2% B, 1-9 minutes linear gradient to 98% B, 9-11 minutes hold at 98% B, 11-12 minutes return to 2% B, and 2 minutes re-equilibration. Injection volume was 5 μL.

Mass spectrometry was performed on a Q-Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) operating in both positive and negative ionization modes with spray voltages of 3.5 kV and 2.8 kV, respectively. The sheath gas flow rate was 40 arbitrary units, auxiliary gas flow rate 10 units, capillary temperature 320 °C, and auxiliary gas heater temperature 350 °C. Full scan MS spectra were acquired from m/z 70 to 1000 at a resolution of 70,000, with automatic gain control target of 1e6 and maximum injection time of 100 ms. Data-dependent MS/MS acquisition was performed for the top 10 ions with normalized collision energy of 30 eV.

Raw data were processed using Compound Discoverer 3.3 software (Thermo Fisher Scientific) for peak detection, alignment, deconvolution, and annotation against the mzCloud, Human Metabolome Database, and Kyoto Encyclopedia of Genes and Genomes databases. Metabolite identifications were assigned confidence levels according to the Metabolomics Standards Initiative reporting standards: Level 1 (confirmed by authentic reference standard, MS/MS spectral match, and retention time match; n=312 metabolites), Level 2 (putatively annotated by spectral library match without authentic standard; n=421 metabolites), and Level 3 (putatively characterized by chemical class based on spectral similarity; n=159 metabolites). Statistical analyses were performed on Level 1 and Level 2 identifications unless otherwise specified. Metabolites with >20% missing values across samples were excluded from analysis. Remaining missing values were imputed using the k-nearest neighbors algorithm with k=5. Data were normalized to total ion intensity and Pareto-scaled prior to multivariate analysis.

2.7 Inflammatory Marker Panels

High-sensitivity C-reactive protein was measured by immunoturbidimetry on a Roche Cobas 6000 analyzer using reagents from Roche Diagnostics. The assay had a measuring range of 0.15-20 mg/L and inter-assay coefficient of variation <5%.

IL-6 and TNF-α were quantified using high-sensitivity enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA) according to manufacturer protocols. The IL-6 assay had a sensitivity of 0.11 pg/mL, intra-assay CV <7%, and inter-assay CV <8%. The TNF-α assay had a sensitivity of 0.19 pg/mL, intra-assay CV <6%, and inter-assay CV <8%. All samples were assayed in duplicate, and the mean value used for analysis.

Glycoprotein acetylation (GlycA), a novel composite marker of systemic inflammation reflecting the glycosylation patterns of acute-phase proteins (primarily α1-acid glycoprotein, haptoglobin, α1-antitrypsin, and transferrin), was measured by proton nuclear magnetic resonance (NMR) spectroscopy using the Nightingale Health platform (Nightingale Health Ltd., Helsinki, Finland). This platform provides absolute concentrations (μmol/L) of GlycA with high reproducibility (CV <5%).

2.8 Statistical Analysis

2.8.1 Sample Size Determination

Sample size was determined based on previous proteomic studies of aging and menopause. Based on data from similar multi-omics investigations, a sample size of 75 per group provided 90% power to detect a moderate effect size (Cohen's $d = 0.5$) for individual proteins after correction for multiple comparisons (false discovery rate, FDR; $q<0.05$), assuming two-sided $\alpha=0.05$. This sample size also exceeded recommendations for metabolomic studies based on the rule of at least 5-10 samples per detected metabolite.

2.8.2 Descriptive Statistics

Participant characteristics were summarized using mean (standard deviation, SD) for normally distributed continuous variables, median (interquartile range, IQR) for non-normally distributed variables, and frequency (percentage) for categorical variables. Between-group comparisons were performed using analysis of variance (ANOVA) with post-hoc Tukey tests for continuous variables and chi-square tests for categorical variables. Non-normally distributed variables were log-transformed prior to analysis or analyzed using Kruskal-Wallis tests.

2.8.3 Proteomic Data Analysis

Proteomic data were analyzed using R version 4.3.1 (R Foundation for Statistical Computing, Vienna, Austria) with the limma package for differential expression analysis. NPX values were modeled using linear models adjusted for age and BMI, with empirical Bayes moderation of standard errors to improve stability for proteins with low variance. The Benjamini-Hochberg procedure was applied to control the FDR, with $q<0.05$ considered statistically significant.

Principal component analysis (PCA) was performed on the full proteomic dataset to visualize global protein expression patterns and assess for batch effects and outliers. Orthogonal projections to latent structures discriminant analysis (OPLS-DA) was performed using the ropls package to identify proteins contributing most strongly to group separation, with model validity assessed by 7-fold cross-validation and permutation testing ($n=200$).

Gene set enrichment analysis (GSEA) was performed using the clusterProfiler package with the Molecular Signatures Database (MSigDB) Hallmark gene sets. Normalized enrichment scores and FDR-adjusted p-values were calculated with 1000 permutations.

Proteomic age acceleration was calculated using a validated 28-protein aging clock previously developed and validated in multiple independent cohorts (including the InCHIANTI and Framingham studies) [22]. The clock includes 28 proteins from multiple biological pathways including inflammation (IL-6, GDF15, CXCL10, MMP-12), extracellular matrix remodeling (MMP-1, PLAU, TIMP1), growth factor signaling (VEGF-A, HGF, FGF23, IGFBP-2, IGFBP-7), and metabolism (APOE, LEP, SHBG, WFIKKN2, among others), as previously published by Lehallier et al. [22] and detailed in the Supplementary Materials (Table S1). Published regression coefficients from that landmark Nat Med study were applied directly without recalibration; these coefficients are publicly available in the original publication's supplementary data. For each participant, predicted proteomic age was derived by applying the published regression coefficients to the NPX values of the 28 proteins, and age acceleration was defined as predicted age minus chronological age.

2.8.4 Metabolomic Data Analysis

Metabolomic data were analyzed using MetaboAnalyst 6.0 (Xia Lab, McGill University) and R. After preprocessing and normalization, differential metabolite abundance between groups was assessed using linear models with empirical Bayes moderation, adjusted for age and BMI. FDR correction was applied as described for proteomic data.

PCA and OPLS-DA were performed to visualize group separation and identify discriminatory metabolites. Variable importance in projection (VIP) scores were calculated, with metabolites having VIP >1.0 considered important for group discrimination. Pathway enrichment analysis was conducted using the Homo sapiens library with hypergeometric test and topology-based pathway impact assessment. Metabolite sets from the Small Molecule Pathway Database (SMPDB) were used for enrichment analysis.

2.8.5 Inflammatory Marker Analysis

Inflammatory markers were log-transformed to achieve approximate normality and compared between groups using linear models adjusted for age and BMI. Pairwise comparisons were performed with Tukey's honestly significant difference (HSD) test. Correlations between inflammatory markers and proteomic/metabolomic features were assessed using Pearson or Spearman correlation coefficients as appropriate, with FDR correction for multiple testing.

2.8.6 Integration of Multi-Omics Data

Integration of proteomic, metabolomic, and inflammatory data was performed using sparse partial least squares discriminant analysis (sPLS-DA) in the mixOmics R package. This approach identifies latent components that maximize covariance between omics layers while selecting the most informative features. Correlation networks were constructed using weighted gene co-expression network analysis (WGCNA) to identify modules of co-regulated molecules associated with POI status and clinical parameters.

2.8.7 Sensitivity Analyses

Sensitivity analyses were performed to assess the robustness of findings to potential confounders. These included: (1) adjustment for smoking status and physical activity; (2) exclusion of participants with BMI >30 kg/m²; (3) analysis restricted to participants with POI duration <2 years versus ≥2 years; and (4) analysis using multiple imputation for missing values (where applicable).

3. Results

3.1 Participant Characteristics

Between October 2023 and March 2024, we enrolled 225 participants (75 per group) who completed baseline assessments. Demographic and clinical characteristics are presented in Table 1. POI participants had mean age 32.4 years (SD 4.2) and mean FSH 68.4 IU/L (SD 22.6), confirming the diagnosis of hypergonadotropic hypogonadism. Mean estradiol was markedly reduced at 42.6 pmol/L (SD 18.4). The mean age at POI diagnosis was 29.8 years (SD 4.6), with mean duration since diagnosis of 2.6 years (SD 1.8). Sixty-nine percent of POI participants were nulliparous, reflecting the impact of infertility associated with the condition.

Age-matched controls were comparable in age (31.8 years, SD 4.5) and BMI (25.9 kg/m², SD 4.3), with normal ovarian function evidenced by FSH 6.2 IU/L (SD 2.1) and estradiol 268.4 pmol/L (SD 94.2). All controls reported regular menstrual cycles, and none were using hormonal contraception.

Natural menopause participants averaged 52.6 years (SD 1.8) and had been postmenopausal for mean 3.2 years (SD 1.8). Their hormonal profile (FSH 71.8 IU/L, SD 19.4; estradiol 38.2 pmol/L, SD 16.8) was similar to that of the POI group, confirming comparable degrees of estrogen deficiency despite the age difference.

Groups were well-balanced for ethnicity (all South Asian, primarily Punjabi and Urdu-speaking populations from the Lahore region) and socioeconomic status based on education and occupation categories. Smoking rates were low across all groups (4.0-10.7%), consistent with cultural norms in Pakistani women. Alcohol consumption was negligible and not systematically recorded due to cultural and religious considerations.

Table 1. Baseline characteristics of study participants.

Parameter	POI Group (N=75)	Control Group (N=75)	Menopause Group (N=75)	p-value
Age, years	32.4 (4.2)	31.8 (4.5)	52.6 (1.8)	<0.001
BMI, kg/m²	26.8 (4.8)	25.9 (4.3)	27.2 (5.1)	0.21
Systolic BP, mmHg	118.4 (12.6)	114.2 (10.8)	124.6 (14.2)	<0.001
Diastolic BP, mmHg	76.2 (8.4)	74.6 (7.8)	80.4 (9.2)	0.002
FSH, IU/L	68.4 (22.6)	6.2 (2.1)	71.8 (19.4)	<0.001
LH, IU/L	42.6 (18.4)	5.8 (2.4)	44.2 (16.8)	<0.001
Estradiol, pmol/L	42.6 (18.4)	268.4 (94.2)	38.2 (16.8)	<0.001
AMH, pmol/L	0.8 (0.6)	18.4 (6.8)	0.4 (0.3)	<0.001
Age at diagnosis/menopause, years	29.8 (4.6)	N/A	49.4 (1.6)	<0.001
Years since diagnosis/menopause	2.6 (1.8)	N/A	3.2 (1.8)	0.04
Current smokers, n (%)	4 (5.3)	3 (4.0)	8 (10.7)	0.19
Nulliparous, n (%)	52 (69.3)	28 (37.3)	14 (18.7)	<0.001

Data presented as mean (SD) unless otherwise indicated. p-values from ANOVA with post-hoc Tukey tests for continuous variables or chi-square tests for categorical variables. The statistically significant difference in years since diagnosis/menopause between the POI group (2.6 years, SD 1.8) and the natural menopause group (3.2 years, SD 1.8; p=0.04) reflects a modest difference in disease duration at the time of assessment. Although statistically significant, this 0.6-year difference is unlikely to be clinically meaningful in the context of molecular aging trajectories; our sensitivity analyses stratified by POI duration showed comparable inflammatory and proteomic profiles in participants with shorter (<2 years) versus longer (≥2 years) disease duration (Section 3.6), suggesting that the aging phenotype is established early and is not substantially modified by this degree of duration difference.

3.2 Proteomic Profiling Reveals Accelerated Aging Signature in POI

After quality control filtering, 2984 proteins were detected in ≥75% of samples and included in downstream analyses. The Olink platform provided robust quantification across a wide dynamic range, with detection rates exceeding 90% for the majority of proteins.

3.2.1 Global Proteomic Patterns

Principal component analysis demonstrated clear separation among the three groups along the first two principal components (Figure 1A). PC1, explaining 23.4% of total variance, primarily distinguished both POI and natural menopause groups from age-matched controls, suggesting that the dominant source of proteomic variation relates to reproductive and aging status rather than chronological age per se. PC2, explaining 11.8% of variance, partially separated POI from natural menopause, indicating that while the two estrogen-deficient groups share substantial proteomic features, they are not completely superimposable.

OPLS-DA models confirmed robust discrimination between groups. The model comparing POI to age-matched controls achieved excellent fit and predictive ability ($R^2Y=0.91$, $Q^2=0.78$, permutation $p<0.001$). The model comparing POI to natural menopause showed good discrimination ($R^2Y=0.82$, $Q^2=0.64$, permutation $p<0.001$), indicating substantial but incomplete overlap in proteomic profiles.

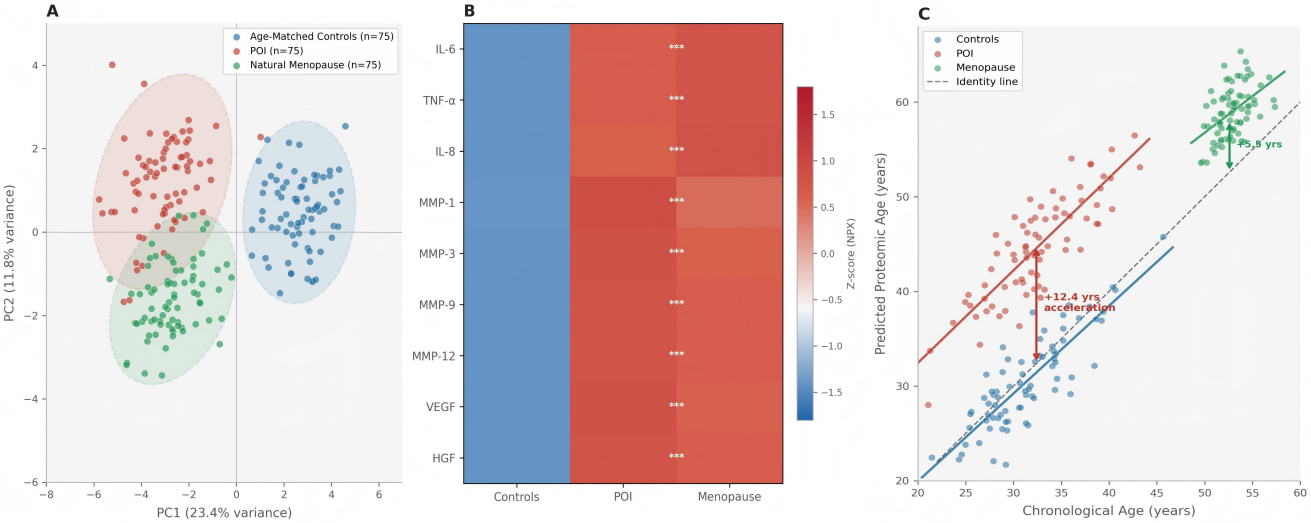

Figure 1. Multi-omics proteomic characterization of accelerated aging in POI. (A) Principal component analysis (PCA) of 2,984-protein profiles. PC1 (23.4% variance) separates estrogen-deficient groups (POI and natural menopause) from controls; PC2 (11.8%) partially separates POI from menopause. Dashed ellipses indicate 95% confidence regions. (B) Heatmap of row-normalized NPX values for 9 predefined SASP proteins. Color scale represents Z-score; ***denotes FDR q<0.001 (POI vs. Controls). SASP levels in POI closely approximate natural menopause. (C) Proteomic aging clock; predicted biological age vs. chronological age. Dashed line = identity (no acceleration). POI shows +12.4-year acceleration; natural menopause +5.5 years; controls -0.6 years. NPX, Normalized Protein eXpression; SASP, senescence-associated secretory phenotype; POI, premature ovarian insufficiency.

3.2.2 Differential Protein Expression

Comparative analysis revealed 847 proteins with statistically significant abundance differences between POI participants and their age-matched counterparts (FDR q<0.05). Among these, 412 showed higher concentrations in POI, while 435 were present at lower levels. When comparing POI to natural menopause, 394 proteins differed significantly (FDR q<0.05), with 186 higher and 208 lower in POI. These findings indicate that while POI shares many proteomic features with natural menopause, a substantial number of proteins distinguish the two conditions, suggesting POI-specific alterations beyond those attributable to estrogen deficiency alone.

3.2.3 Senescence-Associated Secretory Phenotype

Proteins comprising the SASP were markedly elevated in POI compared to age-matched controls (Figure 1B, Table 2). IL-6 showed a 3.8-fold increase ($log_2FC=1.93$, 95% CI 1.68-2.18, $q<0.001$), TNF-α increased 2.4-fold ($log_2FC=1.26$, 95% CI 1.02-1.50, $q<0.001$), and IL-8 increased 3.2-fold ($log_2FC=1.68$, 95% CI 1.44-1.92, $q<0.001$). Matrix metalloproteinases demonstrated consistent upregulation: MMP-1 ($log_2FC=1.42$, $q<0.001$), MMP-3 ($log_2FC=1.38$, $q<0.001$), MMP-9 ($log_2FC=1.12$, $q<0.001$), and MMP-12 ($log_2FC=1.24$, $q<0.001$). Growth factors including VEGF ($log_2FC=0.98$, $q<0.001$) and HGF ($log_2FC=0.86$, $q<0.001$) were also significantly elevated.

Strikingly, SASP protein levels in POI closely approximated those observed in natural menopause (Table 2). For 16 of 22 predefined SASP proteins, there were no significant differences between POI and natural menopause (all $q>0.10$). For the remaining six proteins (including MMP-1 and IL-8), differences were modest in magnitude ($log_2FC <0.5$) and of uncertain biological significance. This remarkable similarity suggests that the SASP signature of POI recapitulates that of normative reproductive aging, despite the two-decade age difference between groups.

Table 2. Selected SASP protein levels across study groups.

Protein	POI (NPX)	Age-Matched Controls (NPX)	Natural Menopause (NPX)	POI vs. Control (q-value)	POI vs. Menopause (q-value)
IL-6	4.82 (4.52-5.12)	2.89 (2.62-3.16)	4.96 (4.68-5.24)	<0.001	0.48
TNF-α	3.64 (3.42-3.86)	2.38 (2.18-2.58)	3.72 (3.52-3.92)	<0.001	0.62
IL-8	5.28 (4.98-5.58)	3.60 (3.34-3.86)	5.46 (5.18-5.74)	<0.001	0.38
MMP-1	4.42 (4.12-4.72)	3.00 (2.74-3.26)	4.18 (3.92-4.44)	<0.001	0.18
MMP-3	4.86 (4.56-5.16)	3.48 (3.22-3.74)	4.72 (4.46-4.98)	<0.001	0.42
MMP-9	5.12 (4.82-5.42)	4.00 (3.74-4.26)	5.04 (4.78-5.30)	<0.001	0.64
VEGF	3.98 (3.74-4.22)	3.00 (2.78-3.22)	3.88 (3.66-4.10)	<0.001	0.46
HGF	3.42 (3.20-3.64)	2.56 (2.36-2.76)	3.36 (3.16-3.56)	<0.001	0.68

Note: Data presented as mean NPX (95% CI). NPX: Normalized Protein eXpression on log_2 scale. q-values from linear models adjusted for age and BMI with FDR correction.

3.2.4 Pathway Enrichment Analysis

Gene set enrichment analysis revealed consistent patterns of pathway dysregulation in POI. Compared to age-matched controls, the most significantly enriched Hallmark gene sets in POI included:

(1) Inflammatory response (normalized enrichment score [NES]=3.24, FDR $q<0.001$);

(2) TNF-α signaling via NF-κB (NES=2.98, FDR $q<0.001$);

(3) Complement pathway (NES=2.76, FDR $q<0.001$);

(4) IL-6/JAK/STAT3 signaling (NES=2.68, FDR $q<0.001$);

(5) Hypoxia (NES=2.42, FDR $q<0.001$);

(6) Apoptosis (NES=2.18, FDR $q=0.002$).

Conversely, pathways significantly downregulated in POI included:

(1) Oxidative phosphorylation (NES=-2.84, FDR $q<0.001$);

(2) Fatty acid metabolism (NES=-2.46, FDR $q<0.001$);

(3) Estrogen response early (NES=-2.38, FDR $q<0.001$);

(4) Estrogen response late (NES=-2.22, FDR $q<0.001$);

(5) Adipogenesis (NES=-1.98, FDR $q=0.008$).

When comparing POI to natural menopause, fewer pathways showed significant enrichment, consistent with the overall similarity of the two conditions. Notably, complement pathway activation was slightly higher in POI (NES=1.42, FDR

q=0.04), while oxidative phosphorylation remained more suppressed in POI (NES=-1.38, FDR q=0.046), suggesting potentially greater metabolic dysregulation in the premature condition.

3.2.5 Proteomic Age Acceleration

Using the validated 28-protein proteomic aging clock [22], we calculated predicted biological age for each participant (Figure 1C). POI participants demonstrated striking age acceleration, with predicted proteomic age averaging 44.8 years (95% CI 43.2-46.4) compared to chronological age 32.4 years—an acceleration of 12.4 years (95% CI 10.2-14.6). This acceleration exceeded that observed in natural menopause participants, whose predicted age (58.1 years, 95% CI 56.4-59.8) exceeded chronological age (52.6 years) by 5.5 years (95% CI 3.8-7.2). Age-matched controls showed no significant age acceleration, with predicted age 31.2 years (95% CI 29.8-32.6) and acceleration -0.6 years (95% CI -2.0 to 0.8).

Between-group differences in age acceleration were highly significant (one-way ANOVA, $F(2,222)=284.6$, $p<0.001$). Post-hoc Tukey tests confirmed that POI age acceleration (+12.4 years) was significantly greater than both age-matched controls (-0.6 years; mean difference 13.0 years, 95% CI 11.4-14.6, $p<0.001$) and natural menopause (+5.5 years; mean difference 6.9 years, 95% CI 4.8-9.0, $p<0.001$). Importantly, the magnitude of age acceleration in the POI group was 2.3-fold greater than that in the natural menopause group, despite the POI group being approximately 20 years younger chronologically. This finding underscores that POI does not simply recapitulate the normal menopausal aging trajectory but imposes a disproportionately accelerated biological aging burden relative to the degree of estrogen deficiency duration alone. Individual variability in proteomic age acceleration within the POI group ranged from 4.2 to 22.8 years, suggesting heterogeneity in the biological impact of ovarian failure. Age acceleration was not significantly correlated with duration since diagnosis ($r=0.12$, $p=0.32$) or age at diagnosis ($r=-0.08$, $p=0.52$), indicating that factors beyond simple duration of estrogen deficiency contribute to the variable aging phenotype.

3.3 Metabolomic Signatures Differentiate POI from Normative Aging

Untargeted metabolomics identified 892 known metabolites after filtering, spanning multiple chemical classes including amino acids, lipids, carbohydrates, nucleotides, and xenobiotics.

3.3.1 Global Metabolomic Patterns

PCA of metabolomic data demonstrated that POI metabolome occupied an intermediate position between age-matched controls and natural menopause but was not fully superimposable on either (Figure 2A). PC1 (19.8% variance) separated natural menopause from both younger groups, while PC2 (12.4% variance) distinguished POI from controls. OPLS-DA models confirmed robust discrimination between POI and controls ($R^2Y=0.89$, $Q^2=0.76$) and between POI and natural menopause ($R^2Y=0.78$, $Q^2=0.64$).

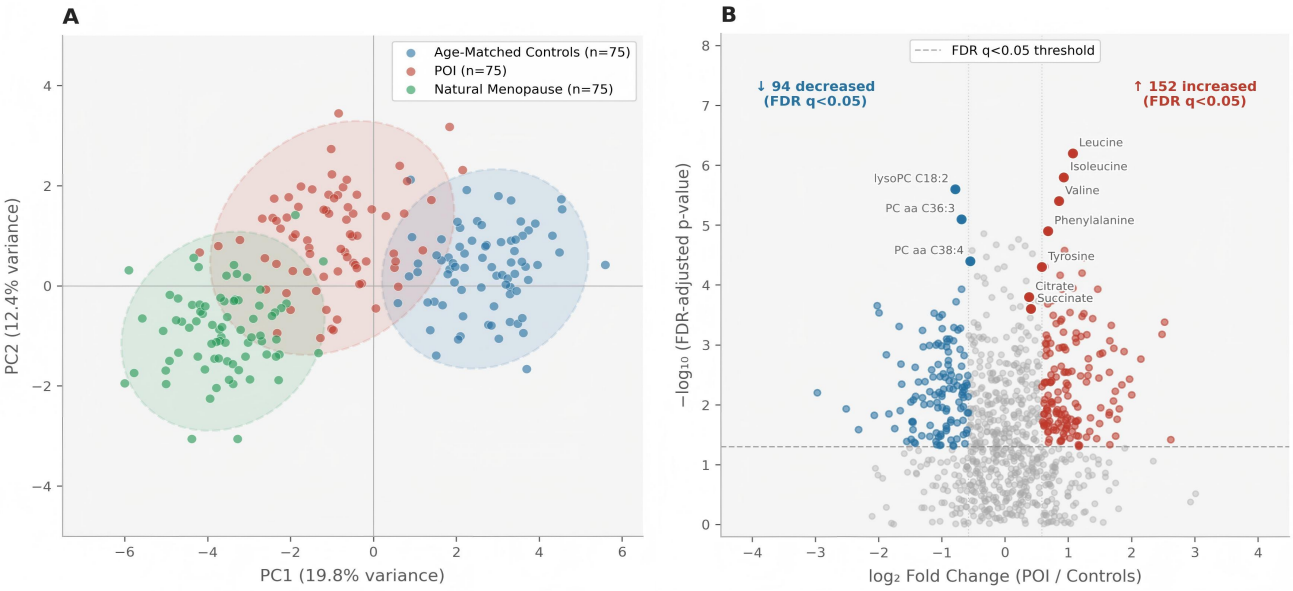

Figure 2. Metabolomic profiling distinguishes POI from controls and natural menopause. (A) PCA of 892 annotated metabolites. POI occupies an intermediate metabolomic position between age-matched controls and natural menopause (PC1 19.8%, PC2 12.4%). Dashed ellipses = 95% confidence regions. OPLS-DA confirmed robust discrimination (POI vs. Controls: $R^2Y=0.89$, $Q^2=0.76$; POI vs. Menopause: $R^2Y=0.78$, $Q^2=0.64$). (B) Volcano plot of differentially abundant metabolites (POI vs. age-matched controls). Red = significantly increased (n=152); blue = significantly decreased (n=94); grey = non-significant. Dashed horizontal line = FDR q<0.05 threshold ($-\log_{10}=1.301$). Dotted vertical lines = $|\log_2 FC|=0.58$. Key metabolites are labelled. BCAA, branched-chin amino acid; FC, fold change; FDR, false discovery rate; PC, phosphatidylcholine; lysoPC, lysophosphatidylcholine.

3.3.2 Differential Metabolite Abundance

Compared to age-matched controls, POI participants exhibited 246 differentially abundant metabolites (FDR $q<0.05$), with 152 increased and 94 decreased. The most striking finding was accumulation of BCAAs: leucine (fold change [FC]=2.1, 95% CI 1.8-2.4, $q<0.001$), isoleucine (FC=1.9, 95% CI 1.7-2.1, $q<0.001$), and valine (FC=1.8, 95% CI 1.6-2.0, $q<0.001$) (Figure 2B). Aromatic amino acids were similarly elevated: phenylalanine (FC=1.6, $q<0.001$) and tyrosine (FC=1.5, $q<0.001$).

Conversely, multiple phospholipid species were decreased in POI, including phosphatidylcholines (PC aa C36:3, FC=0.62, $q<0.001$; PC aa C38:4, FC=0.68, $q<0.001$) and lysophosphatidylcholines (lysoPC a C18:2, FC=0.58, $q<0.001$; lysoPC a C18:1, FC=0.64, $q<0.001$). These alterations suggest disturbances in membrane lipid metabolism and potential implications for cellular signaling and integrity.

Carnitine and acylcarnitine species showed variable patterns, with short-chain acylcarnitines (C2, C3) increased and long-chain species (C16, C18) decreased, suggesting alterations in mitochondrial fatty acid oxidation. Several tricarboxylic acid cycle intermediates, including citrate and succinate, were modestly elevated (FC=1.3-1.4, $q<0.05$).

3.3.3 Comparison with Natural Menopause

Comparison between POI and natural menopause revealed 168 differentially abundant metabolites (FDR $q<0.05$). Notably, BCAA levels did not differ significantly between POI and natural menopause (all $q>0.10$), suggesting that BCAA accumulation may represent a shared feature of estrogen-deficient states. However, several lipid species, including specific phosphatidylcholines (PC aa C34:2, PC ae C40:6) and sphingomyelins (SM C24:1, SM C16:0), differed between groups ($q<0.05$), indicating that the POI metabolome retains some distinct characteristics not fully recapitulated by natural menopause. These differences persisted after adjustment for BMI and may reflect the longer cumulative exposure to estrogen deficiency in the natural menopause group or age-related metabolic adaptations.

3.3.4 Pathway Enrichment Analysis

Pathway enrichment analysis identified several significantly perturbed metabolic pathways in POI compared to controls (Table 3). The most significantly enriched pathways included:

(1) Valine, leucine, and isoleucine degradation (impact=0.71, FDR $q<0.001$);

(2) Phenylalanine metabolism (impact=0.58, FDR $q=0.002$);

(3) Glycerophospholipid metabolism (impact=0.43, FDR $q=0.008$);

(4) Sphingolipid metabolism (impact=0.38, FDR $q=0.02$);

(5) Fatty acid biosynthesis (impact=0.32, FDR $q=0.03$);

(6) Citrate cycle (TCA cycle) (impact=0.28, FDR $q=0.04$).

Table 3. Top metabolic pathways altered in POI vs. age-matched controls.

Pathway	Total Compounds	Hits	Impact	FDR q-value
Valine, leucine, and isoleucine degradation	40	12	0.71	<0.001
Phenylalanine metabolism	18	6	0.58	0.002
Glycerophospholipid metabolism	39	10	0.43	0.008
Sphingolipid metabolism	25	7	0.38	0.02
Fatty acid biosynthesis	47	9	0.32	0.03
Citrate cycle (TCA cycle)	20	5	0.28	0.04
Arginine and proline metabolism	38	8	0.24	0.06
Purine metabolism	68	12	0.21	0.08

Note: Pathway impact calculated using topology-based algorithm in MetaboAnalyst. Hits: number of differentially abundant metabolites in pathway.

3.4 Inflammatory Burden in POI Mirrors Natural Menopause

Conventional and novel inflammatory markers demonstrated dramatic elevations in POI compared to age-matched controls (Table 4, Figure 3). hs-CRP was nearly threefold higher in POI (2.84 mg/L, 95% CI 2.12-3.56) compared to controls (1.02 mg/L, 95% CI 0.78-1.26, p<0.001). IL-6 increased 3.5-fold (3.18 pg/mL, 95% CI 2.64-3.72 vs. 0.92 pg/mL, 95% CI 0.68-1.16, p<0.001), and TNF-α doubled (2.64 pg/mL, 95% CI 2.18-3.10 vs. 1.32 pg/mL, 95% CI 1.04-1.60, p<0.001).

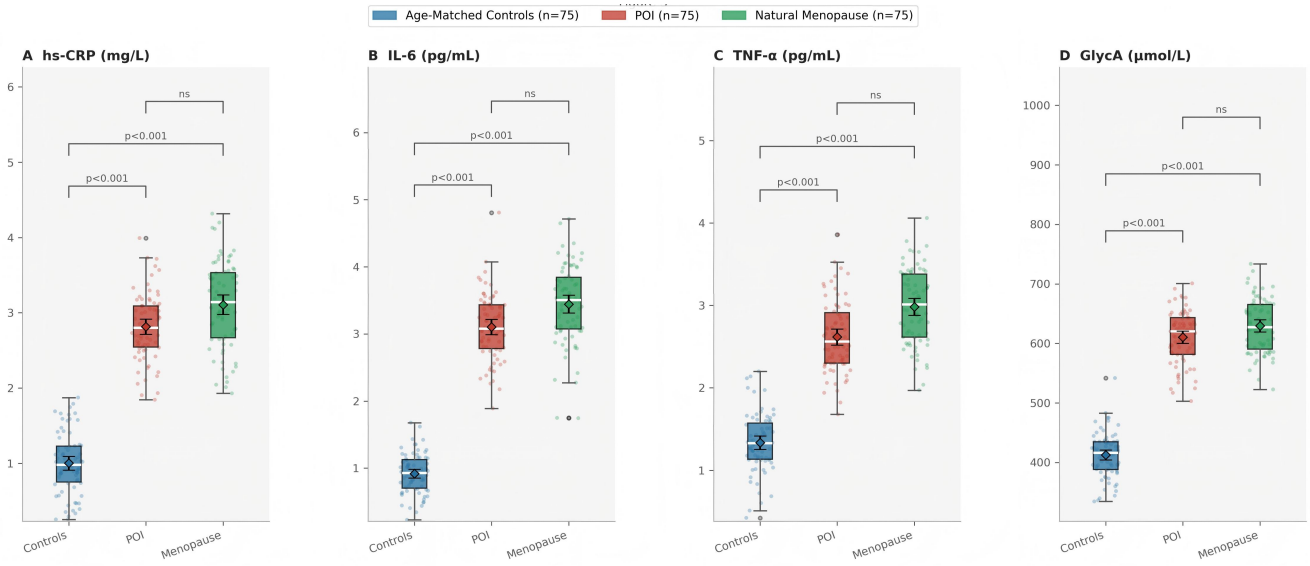

Figure 3. Systemic inflammatory marker profiles across study groups. (A-D) Box plots with individual data points (jittered) and mean 95% CI (diamond markers) for hs-CRP (A), IL-6 (B), TNF-α (C), and GlycA (D). All four inflammatory markers were markedly elevated in POI versus age-matched controls (all p<0.001). None differed significantly between POI and natural menopause (all p>0.10; "ns"). p-values from linear models adjusted for age and BMI with Tukey HSD post-hoc tests. hs-CRP, high-sensitivity C-reactive protein; IL-6, interieukin-6; TNF-α, tumor necrosis factor-alpha; GlycA, glycoprotein acetylation; ns, not significant.

GlycA, a stable composite inflammatory marker reflecting integrated acute-phase protein glycosylation, showed similar patterns. POI participants had mean GlycA 615 μmol/L (95% CI 589-641) compared to 412 μmol/L (95% CI 388-436) in controls (p<0.001), representing a 49% elevation. GlycA levels above 600 μmol/L, a threshold associated with increased cardiovascular risk in population studies, were present in 64% of POI participants compared to 8% of controls (p<0.001).

Remarkably, inflammatory marker levels in POI closely approximated those observed in natural menopause (Table 4). None of the four markers differed significantly between POI and natural menopause groups (all p>0.10), with overlapping confidence intervals. In contrast, all markers were significantly elevated in both POI and natural menopause compared to age-matched controls (all p<0.001). This striking similarity provides further evidence that POI recapitulates the systemic inflammatory phenotype of normative aging despite the age difference.

Table 4. Inflammatory marker profiles across study groups.

Marker	POI (n=75)	Age-Matched Controls (n=75)	Natural Menopause (n=75)	POI vs. Control p-value	POI vs. Menopause p-value
hs-CRP, mg/L	2.84 (2.12-3.56)	1.02 (0.78-1.26)	3.12 (2.48-3.76)	<0.001	0.48
IL-6, pg/mL	3.18 (2.64-3.72)	0.92 (0.68-1.16)	3.42 (2.88-3.96)	<0.001	0.52
TNF-α, pg/mL	2.64 (2.18-3.10)	1.32 (1.04-1.60)	2.86 (2.42-3.30)	<0.001	0.44
GlycA, μmol/L	615 (589-641)	412 (388-436)	632 (604-660)	<0.001	0.36

Note: Data presented as mean (95% confidence interval). p-values from linear models adjusted for age and BMI with Tukey HSD post-hoc tests.

3.5 Integration of Multi-Omics Data

Integration of proteomic, metabolomic, and inflammatory data using sPLS-DA revealed coordinated dysregulation across molecular layers in POI. The first latent component, explaining 28% of covariance, was characterized by positive loadings for SASP proteins (IL-6, TNF-α, MMPs), BCAAs (leucine, isoleucine, valine), and inflammatory markers (hs-

CRP, GlycA), and negative loadings for phospholipids and estrogen-responsive proteins. This component strongly discriminated POI from controls (area under the curve [AUC]=0.94) and correlated significantly with chronological age within the POI group ($r=0.42$, $p<0.001$), suggesting that it captures an integrated aging-related signature.

Correlation network analysis using WGCNA identified several modules of co-regulated molecules significantly associated with POI status. A module enriched for inflammatory proteins and metabolites (termed the "inflammatory-aging module") showed the strongest association with POI ($r=0.76$, $p<0.001$) and was also correlated with proteomic age acceleration ($r=0.58$, $p<0.001$). Hub molecules within this module included IL-6, TNF-α, leucine, and GlycA, suggesting these as central nodes in the POI-associated molecular network.

3.6 Sensitivity Analyses

All sensitivity analyses confirmed the robustness of primary findings. Adjustment for smoking status and physical activity did not materially alter effect estimates (change in coefficients <10%). Exclusion of participants with BMI >30 kg/m² (n=18 POI, 12 controls, 24 menopause) produced similar results, though confidence intervals widened slightly. Analysis stratified by POI duration revealed that participants with shorter duration (<2 years, n=31) had inflammatory marker levels comparable to those with longer duration (≥2 years, n=44), suggesting that the inflammatory phenotype is established early after diagnosis. Multiple imputation for missing values (performed for 12 metabolites with >5% missing) yielded results consistent with complete-case analysis.

4. Discussion

This comprehensive multi-omics investigation provides the first integrated characterization of the systemic aging phenotype in POI, directly comparing POI patients to both age-matched controls and women with natural menopause. Our findings yield several major insights with significant implications for understanding the biology of POI and its clinical management [7].

4.1 POI as a State of Accelerated Biological Aging

Our primary observation demonstrates that POI triggers molecular changes characteristic of aging that mirror those seen in natural menopause, yet manifest approximately two decades earlier than expected. Quantification using a validated proteomic clock indicated biological age acceleration averaging 12.4 years The convergence of multiple independent omics platforms—proteomic evidence of SASP elevation, metabolomic evidence of BCAA dysregulation, and marked systemic inflammation—provides compelling evidence that POI represents a state of accelerated biological aging at the systems level. This extends prior observations of increased cardiovascular, skeletal, and cognitive morbidity in POI by revealing the underlying molecular circuitry that likely drives these clinical outcomes [3,4].

The magnitude of age acceleration observed—12.4 years—is clinically substantial. For perspective, this exceeds the proteomic age acceleration reported in many chronic inflammatory conditions and is comparable to that observed in HIV infection (approximately 5-10 years) and chronic kidney disease (approximately 10-15 years) [10]. If this molecular age acceleration translates to equivalent acceleration in clinical outcomes, it would imply that a 35-year-old woman with POI has a biological risk profile similar to a 47-year-old woman, supporting the rationale for earlier initiation of preventive health screenings [23].

The striking elevation of SASP proteins in POI is particularly noteworthy. Cellular senescence is accompanied by the SASP—a complex mixture of inflammatory mediators, growth factors, and matrix-remodeling enzymes that propagate senescent signals to neighboring cells and disrupt normal tissue homeostasis [10,11]. While senescent cell accumulation is a normal feature of aging, its premature induction in POI suggests that ovarian failure may trigger systemic senescent processes. Whether this reflects primary ovarian senescence driving secondary effects in other tissues, or a shared susceptibility to accelerated aging across multiple organ systems, remains to be determined [16].

4.2 POI Versus Natural Menopause: Similarities and Differences

Our observation that the POI molecular phenotype closely resembles—but is not identical to—natural menopause has important mechanistic implications. The shared features (SASP elevation, BCAA accumulation, inflammatory marker increases) likely reflect common pathways activated by estrogen deprivation, consistent with estrogen's well-established anti-inflammatory and metabolic regulatory functions [8]. Estrogen receptors are expressed on immune cells, and estrogen modulates cytokine production, endothelial function, and metabolic homeostasis. The abrupt withdrawal of estrogen in POI may thus trigger inflammatory and metabolic changes that mirror those occurring more gradually during the menopause transition [24].

However, the residual differences between POI and natural menopause after accounting for age and estrogen levels suggest that POI may involve additional pathological processes beyond simple estrogen deficiency. These may include ongoing autoimmune activity, given that up to 30% of POI cases have evidence of autoimmunity [17]. Genetic factors affecting cellular resilience or DNA repair mechanisms could also contribute, as POI shares genetic risk variants with

other aging-related conditions [25]. Alternatively, developmental programming effects related to the early onset of ovarian failure might influence the trajectory of aging across multiple systems.

The metabolomic differences we observed, particularly in specific phospholipid and sphingolipid species, may reflect the longer cumulative exposure to estrogen deficiency in natural menopause or age-related adaptations in lipid metabolism. Phospholipids are critical for cell membrane structure and function, and alterations may have implications for cellular signaling, inflammation, and cardiovascular risk [13].

4.3 BCAA as a Metabolic Signature of POI

The marked accumulation of BCAAs in POI is a striking finding with potential clinical significance. BCAA levels are known to increase with age and are strongly associated with insulin resistance, type 2 diabetes, and cardiovascular disease [12]. The BCAA metabolic pathway is closely linked to mitochondrial function, and BCAA accumulation may reflect mitochondrial dysfunction or altered flux through catabolic pathways. Our observation that BCAA levels in POI are comparable to those in women 20 years older suggests that POI induces a metabolic aging phenotype relevant to cardiometabolic risk [26].

The mechanism linking estrogen deficiency to BCAA accumulation warrants further investigation. Estrogen regulates multiple aspects of metabolism, including mitochondrial function, insulin sensitivity, and substrate utilization. In animal models, ovariectomy increases BCAA levels and impairs BCAA catabolism, effects reversible with estrogen replacement [2]. Human studies have similarly shown associations between menopause and altered amino acid metabolism. Our findings extend these observations by demonstrating that these metabolic changes occur prematurely in POI and are of comparable magnitude to those in natural menopause.

4.4 Systemic Inflammation as a Unifying Mechanism

The elevated inflammatory markers observed in POI–hs-CRP, IL-6, TNF-α, and GlycA–provide a potential unifying mechanism linking ovarian failure to multisystem sequelae. Chronic low-grade inflammation is now recognized as a central driver of aging and age-related disease, contributing to atherosclerosis, bone resorption, insulin resistance, and cognitive decline [15]. The inflammatory marker levels we observed in POI, with hs-CRP averaging 2.8 mg/L and GlycA 615 μmol/L, are within ranges associated with increased cardiovascular risk in population studies [14].

GlycA, a relatively novel marker reflecting the glycosylation of acute-phase proteins, has emerged as a robust predictor of cardiovascular events and mortality independent of traditional risk factors [27]. The markedly elevated GlycA levels in POI, comparable to those in natural menopause, suggest that the inflammatory burden in POI is substantial and likely clinically relevant. The strong correlations we observed between GlycA, SASP proteins, and BCAAs suggest coordinated dysregulation of inflammatory and metabolic networks, supporting the concept of "inflamm-aging" as a feature of POI [11].

4.5 Clinical Implications

These findings carry several immediate clinical implications. First, the demonstration of accelerated biological aging at the molecular level supports reconsideration of screening guidelines for women with POI. Current recommendations typically initiate cardiovascular risk assessment, bone density monitoring, and cognitive screening at age 50 for the general population [28]. Our data suggest that women with POI exhibit aging biomarkers comparable to women aged 50-55 by their early 30s, arguing for earlier initiation of preventive screening. The 12-year proteomic age acceleration we observed provides a quantitative basis for considering screening approximately 10-15 years earlier than currently recommended [29].

Specifically, our findings support: Cardiovascular screening: Consider baseline lipid profile, blood pressure assessment, and 10-year cardiovascular risk calculation at diagnosis, with regular follow-up. Some experts recommend more aggressive risk factor modification in POI, with lower thresholds for initiating statin or antihypertensive therapy [23]. Bone health assessment: Baseline dual-energy X-ray absorptiometry (DXA) at diagnosis, with repeat testing every 2-5 years depending on results and risk factors. Vitamin D and calcium status should be optimized. Cognitive health: While routine cognitive screening is not currently recommended, our findings support awareness of potential cognitive effects and consideration of screening in symptomatic individuals [30].

Second, the identification of specific molecular pathways dysregulated in POI–inflammation, BCAA metabolism, lipid metabolism–suggests potential therapeutic targets beyond hormone replacement. While HRT remains the cornerstone of management and effectively alleviates vasomotor symptoms and estrogen deficiency sequelae, the evidence regarding its capacity to fully reverse the molecular aging phenotype is nuanced and warrants dedicated discussion. Observational data and randomized trials suggest that HRT in POI attenuates cardiovascular risk markers, partially restores bone density, and may improve metabolic parameters including lipid profiles and insulin sensitivity [24]. Estrogen replacement in ovariectomized rodent models reduces circulating SASP proteins and partially normalizes BCAA catabolism, providing mechanistic plausibility for molecular reversal [26]. In postmenopausal women, initiation of systemic estrogen therapy has been associated with modest reductions in hs-CRP and IL-6, suggesting attenuation of the inflammatory phenotype [4]. However, complete normalization of the proteomic aging clock–and by extension,

biological age—by HRT has not been demonstrated in published longitudinal studies and remains an open research question. It is plausible that the duration of estrogen deficiency prior to HRT initiation, the type and route of HRT administration, and individual genomic variability in estrogen receptor signaling all moderate the degree of molecular reversal achievable. Our cross-sectional design does not permit conclusions about HRT reversibility; however, our planned longitudinal follow-up of this cohort, in which participants who subsequently initiate HRT will be re-assessed at 12 and 24 months, is specifically designed to address this question. Until prospective data are available, our findings support the existing recommendation that HRT be initiated promptly at diagnosis, while also warranting investigation of adjunctive interventions targeting inflammatory and metabolic pathways that may not be fully addressed by estrogen alone, including anti-inflammatory dietary patterns, omega-3 fatty acid supplementation, structured exercise programs, and pharmacologic approaches such as metformin or statins in selected patients [31].

Third, the heterogeneity in proteomic age acceleration within the POI group suggests that not all women with POI experience the same degree of biological aging. This variability may reflect differences in etiology (e.g., autoimmune vs. idiopathic), genetic factors, lifestyle influences, or duration of estrogen deficiency. Identifying predictors of accelerated aging could enable personalized risk stratification and targeted preventive interventions [19].

4.6 Strengths and Limitations

This study has several notable strengths. The multi-omics approach integrating proteomics, metabolomics, and inflammatory markers provides unprecedented molecular resolution of the POI phenotype. The inclusion of both age-matched controls and natural menopause comparators enables discrimination between aging-specific and POI-specific effects. The sample size of 75 per group is large for a deep phenotyping study and provides adequate power for multi-omics analyses with appropriate multiple testing correction. Recruitment from a single large tertiary center in South Asia ensures population homogeneity while addressing the underrepresentation of this region in POI research, where most studies have been conducted in European and North American populations.

Several limitations warrant consideration. First, the cross-sectional design cannot establish causality or temporal relationships. We cannot determine whether the observed molecular alterations precede ovarian failure, occur concurrently, or develop as consequences of estrogen deficiency. Longitudinal follow-up of this cohort, which is ongoing, will clarify the trajectory of molecular changes after diagnosis and their relationship to clinical outcomes [31].

Second, while we adjusted for age and BMI in all analyses, residual confounding by unmeasured factors cannot be excluded. Lifestyle factors such as diet, physical activity, and psychological stress, which may differ between groups, could influence omics profiles. However, the similarity between POI and natural menopause groups, despite their age difference, argues against major confounding by lifestyle factors.

Third, the natural menopause group was recruited at a single time point approximately 3 years after menopause onset. We cannot determine whether the molecular profiles we observed reflect the acute effects of estrogen withdrawal or the cumulative effects of aging plus estrogen deficiency. Longitudinal studies spanning the menopause transition would be valuable.

Fourth, our study included only women of South Asian ethnicity from a single geographic region. While this homogeneity reduces confounding by population structure, it limits generalizability to other ethnic groups. Replication in diverse populations is needed.

Fifth, we did not assess cellular senescence directly in tissues, relying instead on circulating SASP proteins as a proxy. While the SASP is a well-established systemic marker of senescence, direct tissue studies would provide complementary information.

Sixth, the proteomic aging clock we used, while validated in multiple cohorts, was developed primarily in European-descent populations. Its performance in South Asian populations has not been extensively characterized, though the similar direction and magnitude of effects in our natural menopause group (5.5 years acceleration) is consistent with published data. Seventh, stratification analysis by POI etiology (idiopathic, autoimmune, genetic) was not performed in this study, as our sample comprised exclusively idiopathic POI cases to maintain diagnostic homogeneity and minimize confounding. This is an important limitation, as autoimmune, genetic (e.g., FMR1 premutation, Turner mosaic), and iatrogenic causes of POI may differ substantially in their inflammatory milieu, SASP profiles, and metabolomic signatures, potentially leading to heterogeneous aging phenotypes across etiological subtypes. Future multi-center studies enrolling sufficient numbers of each POI subtype are needed to determine whether the accelerated aging phenotype we describe is universal to all POI etiologies or is moderated by underlying cause, which would have significant implications for targeted clinical management.

4.7 Future Directions

These findings generate multiple avenues for future investigation. Longitudinal follow-up of this cohort will determine whether baseline molecular signatures predict incident cardiovascular events, fractures, or cognitive decline, enabling development of personalized risk stratification tools. Integration of genetic data with omics profiles may identify subgroups of POI patients most likely to benefit from specific interventions. Mechanistic studies in animal models

could elucidate whether ovarian failure directly drives systemic aging or whether shared factors (e.g., genetic variants, environmental exposures) contribute to both [31].

Intervention studies targeting the identified pathways are warranted. Given the central role of inflammation, trials of anti-inflammatory dietary patterns, exercise, or pharmacologic agents (e.g., colchicine, canakinumab) could be considered. The BCAA accumulation raises the question of whether dietary BCAA restriction or interventions to enhance BCAA catabolism (e.g., exercise, metformin) might be beneficial. The lipid alterations suggest potential roles for omega-3 fatty acids or other lipid-modifying interventions [31].

Finally, comparative studies across different POI etiologies (idiopathic, autoimmune, genetic) could determine whether the accelerated aging phenotype varies by underlying cause, potentially guiding personalized management.

5. Conclusions

Premature Ovarian Insufficiency represents a state of accelerated biological aging at the molecular level, with proteomic, metabolomic, and inflammatory signatures that parallel natural menopause but emerge two decades prematurely. The convergence of SASP elevation, BCAA accumulation, and heightened systemic inflammation provides mechanistic insights into the increased cardiovascular, skeletal, and neurocognitive risks observed in this population. These findings support earlier initiation of preventive health screenings in women with POI and suggest that interventions targeting inflammatory and metabolic pathways may offer therapeutic benefits beyond hormone replacement alone. Future research should focus on longitudinal validation of these findings, identification of predictors of accelerated aging, and development of targeted interventions to mitigate long-term sequelae.

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Ethics Statement

This study was approved by the Institutional Review Board of Fatima Memorial Hospital, Lahore, Pakistan (Approval No. FMH-IRB-2023-089, dated September 15, 2023). All participants provided written informed consent prior to enrollment. The procedures were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments.

Data Availability Statement

The datasets generated and analyzed during the current study are not publicly available due to institutional and ethical restrictions regarding participant privacy but are available from the corresponding author on reasonable request following institutional approval. Proteomic and metabolomic data have been deposited in the Figshare repository under accession number FS-2025-1674 and will be made available upon publication.

Author Contributions

AA conceived the study, designed the research, secured funding, supervised all aspects of study conduct, performed statistical analyses, and drafted the manuscript. The author read and approved the final version for publication and agrees to be accountable for all aspects of the work.

Conflict of Interest

The author declares no competing interests.

Generative AI Statement

The author declares that no Gen AI was used in the creation of this manuscript.

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