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Years since menopause and its metabolomic signature with biological aging in women at midlife: a population-based study

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This study analyzed UK Biobank data from 46,463 postmenopausal women to investigate metabolic changes linked to years since menopause (YSM) and their impact on aging biomarkers. Elastic net regression identified 115 YSM-associated metabolites, forming a metabolic signature strongly correlated with YSM ($r = 0.30$, $P < 0.001$). Each standard deviation increase in this metabolic signature was associated with decreased odds of long telomere length (0.94, 0.92–0.96), increased odds of high allostatic load (1.53, 1.50–1.56) and high PhenoAge (2.30, 2.17–2.44). Mediation analysis indicated that the metabolic signature explained 43.5% of the association between YSM and allostatic load, 9.09% between YSM and telomere length, and 89.3% between YSM and PhenoAge. These findings reveal how menopause-related metabolic shifts drive biological aging, highlighting potential intervention targets for postmenopausal health.

Menopause marks a key transition in women, characterized by the end of ovarian function and significant hormonal and metabolic changes[1]. Menopause induces an atherogenic metabolic shift, including increased low-density lipoprotein (LDL), remnant cholesterol, smaller LDL particles[2,3], and higher levels of certain amino acids, fatty acids, and inflammatory markers[3,4], all contributing to elevated cardiovascular risk. It also accelerates biological aging, as shown by faster telomere shortening[5], higher allostatic load (AL)[6], and increased PhenoAge (PA)[7], largely independent of chronological age and reflecting reproductive aging.

Ovarian aging and chronological aging are related but not identical. Ovarian aging is marked by loss of ovarian function and hormonal changes, with menopause as a key milestone. Extended postmenopausal duration does not directly correspond to chronological aging[8], as women experiencing early menopause will demonstrate longer postmenopausal intervals compared to age-matched peers with typical menopausal onset[9]. Distinguishing these processes is crucial for understanding menopause's unique impact on health and the metabolic pathways driving postmenopausal aging.

Metabolomics, as an emerging research approach, provides a comprehensive analysis of small-molecule metabolites in biological samples, capturing an individual's metabolic state and its interaction with environmental, lifestyle, and genetic factors[10]. Previous studies have demonstrated the utility of metabolomics in elucidating the links between metabolic alterations and health outcomes, such as all-cause mortality[11], cardiovascular disease risk[12], and dementia[13]. Biological aging is a complex process characterized by the progressive decline in physiological function and the accumulation of molecular damage. In recent years, several biomarkers have been developed to quantify biological aging, including telomere length (TL) (TL, reflecting cellular replicative senescence via chromosomal attrition), AL (AL, indicating cumulative physiological dysregulation from chronic stress), and PA (PA, predicting mortality risk through clinical biomarker integration)[14]. AL measures multisystem physiological stress, which increases after menopause[15]. PA estimates biological age using clinical and biochemical markers sensitive to menopause-related changes[16]. TL reflects cellular aging and is affected by oxidative stress and inflammation, both heightened postmenopause[16]. These biomarkers collectively span molecular, systemic, clinical, and multisystem dimensions of aging. However, studies specifically examining postmenopausal metabolic changes and their impact on biological aging are scarce.

Metabolomics and aging biomarkers are closely related. Certain metabolites, such as amino acids and lipids, are associated with aging biomarkers like TL and epigenetic clocks, and can predict biological aging rates[3,17,18]. Similar patterns are observed across various organ systems and diseases, where both metabolomic changes and aging biomarkers indicate disease progression and organ aging[19]. Thus, metabolomic changes not only

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parallel but may also drive or mediate the alterations captured by established biomarkers of biological aging.

This study integrates metabolomics and aging biomarkers to investigate postmenopausal metabolic changes and their impact on aging. The objectives are: (1) to identify years since menopause (YSM) associated metabolites and develop a metabolomic signature score; (2) to explore the links between YSM, metabolomic signature, and aging biomarkers (TL, AL, and PA); and (3) to evaluate the metabolomic signature's mediating role in the YSM-aging relationship. This research will provide insights into promoting healthy aging in women and identify potential targets for early intervention and personalized health management.

Results
Characteristics of the participants
This study included 46,463 participants at baseline and 3072 participants at first repeat assessment. Participants at baseline were 96.7% white, and mean (SD) age was 59.8 (5.4) years. Participants with lower educational attainment, lower income level, and higher Townsend deprivation index had higher prevalence of high PA (2.8%), and high AL (45.4%), and lower prevalence of long TL (50.1%). Also, those who were overweight or obese, smokers, and those with poor diet or sleep showed similar trends. People with longer menopause duration had higher prevalence of high PA (4.1%), high AL (57.8%) and lower prevalence of long TL (40.3%) (Table 1). The characteristics of participants in first repeat assessment were showed in Supplementary Table S7.

Metabolic signature in response to YSM
Menopause causes hormonal and metabolic changes, but YSM-related metabolites were not well characterized. Elastic net regressions on 251 metabolites in baseline data were performed to determine the metabolic signature in response to YSM. A total of 115 metabolites were selected from the model to calculate the total metabolic signature, spanned various metabolic classes including lipids, lipoprotein subclass, amino acids, fatty acids, and inflammation-related metabolites et al. (Supplementary Fig. S1). These metabolites were primarily enriched in four metabolic pathways: glyoxylate and dicarboxylate metabolism; valine, leucine, and isoleucine biosynthesis; alanine, aspartate, and glutamate metabolism; and phenylalanine, tyrosine, and tryptophan biosynthesis (Supplementary Table S8). Most metabolites remained significantly associated with YSM after adjustment for age (Supplementary Table S9). The metabolic signature was significantly correlated with YSM (baseline data: $r = 0.30$, $P < 0.001$; the first repeat assessment: $r = 0.26$, $P < 0.001$; Fig. 1). As per the metabolites' coefficients (weights) in the signature (Fig. 2), the most pronounced contribution to the positive coefficient of the metabolic signature came from triglycerides (TG) in large LDL, TG in large high-density lipoprotein (HDL), and phospholipids to total lipids in small LDL percentage. Conversely, cholines, albumin, free cholesterol to total lipids in small very-low-density lipoprotein (VLDL) percentage, phospholipids in medium HDL played a significant role in influencing the reverse coefficient of the metabolic signature. Figure 2 also illustrated the associations between YSM and the 115 metabolites comprising the metabolic signature, as well as the relationships between these metabolites and three aging biomarkers. These findings defined a metabolic profile that tracks postmenopausal progression, offering a molecular basis to study how menopause duration affects aging.

Associations of YSM, metabolic signature with three aging biomarkers
While menopause accelerates biological aging, the links between YSM-related metabolic changes and aging biomarkers (TL, AL, and PA) remain unclear. In the fully adjusted model, each 1-unit increase in YSM (representing a 5-year interval) was associated with a decreased likelihood of long TL (OR: 0.92, 95% CI: 0.90–0.94) and an increased likelihood of high AL (1.07, 1.05–1.10). Before adjusting for the metabolic signature, each 1-unit increase in YSM was linked to higher odds of high PA (1.14,1.07–1.20); however, this association became nonsignificant after further adjustment for

the metabolic signature (Model 4). Dose–response curves showed a positive linear relationship between YSM and high AL odds, and a negative linear relationship with long TL odds (Table 2).

In the fully adjusted model, we observed that each 1-SD increase in metabolic signature was associated with decreased odds of long TL (0.94, 0.92–0.96), increased odds of high AL (1.53, 1.49–1.56) and high PA (2.30, 2.17–2.44) (Table 2). Dose–response curves showed a positive relationship between metabolic signature and high AL, high PA, and negative relationship with long TL (Fig. 3). Replication analysis using metabolites at first repeat assessment showed similar findings to the primary analysis using metabolites at baseline (Supplementary Table S10). These results showed that the identified metabolic changes reflected menopause duration and were strongly linked to accelerated biological aging, highlighting their potential role in postmenopausal health risks.

Subgroup analysis and mediation analysis
After the analysis was stratified by baseline age and menopause hormone therapy (MHT) status, we observed consistent findings as the primary analysis (Table 3). Mediation analysis indicated that the metabolic signature partially mediated the association between YSM and long TL (8.5%) as well as high AL (43.5%), while the associations of YSM with PA (89.3%) were predominantly mediated by the metabolomic signature, with no direct link observed (Fig. 4). To clarify how YSM affects aging, we used mediation analyses to assess how much the metabolomic signature explains the link between YSM and aging biomarkers. This helped quantify indirect effects and reveal causal pathways. It was found that for HPA, mediation was mainly through lipoprotein subclasses, relative lipoprotein lipid concentrations, fatty acids, and amino acids. For HAL, the main mediators were lipoprotein subclasses, relative lipoprotein lipid concentrations, fluid balance metabolites, fatty acids, and amino acids. For LTL, mediation was primarily driven by relative lipoprotein lipid concentrations, lipoprotein subclasses, and amino acids (Supplementary Fig. S2). These findings suggested metabolic changes mediate the link between menopause duration and biological aging, especially for indices like PA, highlighting metabolic pathways as potential targets to slow aging in postmenopausal women.

Discussions
This study identified 115 metabolites significantly associated with YSM using elastic net regression, which were primarily involved in lipid metabolism, amino acid metabolism, and inflammatory pathways. A metabolomic signature was constructed based on these metabolites. We found that each SD increase in the YSM-related metabolomic signature was significantly associated with an increased odds of high AL, high PA and decreased odds of long TL. The metabolomic signature was found to mediate the relationship between YSM and aging biomarkers of TL and AL, highlighting its potential role as a key mechanism driving accelerated aging in postmenopausal women.

Menopause, marked by the cessation of ovarian function and estrogen depletion, represents a pivotal period associated with accelerated biological aging. A growing body of epidemiological research has investigated the relationship between menopause and various biomarkers of biological aging, including TL, PA and epigenetic clocks. Our findings corroborate and expand upon prior evidence. An earlier age at menopause has been consistently associated with shorter leukocyte TL, a well-established marker of cellular senescence. For instance, Schuermans et al. analyzed data from 130,254 postmenopausal women and observed a significant association between earlier menopause and shorter TL (per 5-year earlier menopause: $\beta = -0.02$ SD, 95% CI: -0.03 to -0.02, $P < 2.2 \times 10^{-16}$)[5]. Similarly, Crestol et al. reported that later natural menopause was associated with longer TL ($\beta = 0.030$, pFDR = 1.50×10^{-20}), with longer reproductive duration also positively correlated with TL ($\beta = 0.034$, pFDR = 1.34×10^{-4})[20]. These findings suggest that menopause may contribute to biological aging through accelerated telomere shortening. Epigenetic clocks, particularly DNA methylation-based measures, have emerged as robust tools for assessing biological aging at the molecular level. Evidence consistently indicates that

Table 1 | Characteristics of participants at baseline by aging biomarkers status

	N	PA		AL		TL	
		High PA (N, %)	P	High AL (N, %)	P	Long TL (N, %)	P
Total	46,463	1321 (2.8)		21,078 (45.4)		23,282 (50.1)	
Age at baseline, mean (SD)	59.8 (5.4)	60.2 (5.6)	0.004	60.7 (5.2)	<0.001	59.2 (5.4)	<0.001
Race			<0.001		<0.001		<0.001
White	44,935	1233 (2.7)		20,219 (45)		22,373 (49.8)	
Non white	1528	88 (5.8)		859 (56.2)		909 (59.5)	
Education (years)			<0.001		<0.001		<0.001
≤10	23,806	757 (3.2)		11,784 (49.5)		11,318 (47.5)	
11–12	5412	151 (2.8)		2308 (42.6)		2818 (52.1)	
>12	17,245	413 (2.4)		6986 (40.5)		9146 (53)	
Income			<0.001		<0.001		<0.001
Less than 18,000	12,774	486 (3.8)		6624 (51.9)		6013 (47.1)	
18,000 to 30,999	13,089	384 (2.9)		6131 (46.8)		6423 (49.1)	
31,000 to 51,999	11,429	274 (2.4)		4862 (42.5)		5970 (52.2)	
Greater than 52,000	9171	177 (1.9)		3461 (37.7)		4876 (53.2)	
Townsend			<0.001		<0.001		0.131
1	11,767	230 (2)		5015 (42.6)		5947 (50.5)	
2	11,622	292 (2.5)		5182 (44.6)		5787 (49.8)	
3	11,650	323 (2.8)		5205 (44.7)		5909 (50.7)	
4	11,424	476 (4.2)		5676 (49.7)		5639 (49.4)	
BMI status (kg/m²)			<0.001		<0.001		<0.001
≤24.9	18763	365 (1.9)		5820 (31)		9750 (52)	
25.0–29.9	17725	374 (2.1)		8489 (47.9)		8753 (49.4)	
≥30.0	9975	582 (5.8)		6769 (67.9)		4779 (47.9)	
Smoking status			<0.001		<0.001		<0.001
Never	27,132	635 (2.3)		11,874 (43.8)		13,719 (50.6)	
Previous	15,753	447 (2.8)		7219 (45.8)		7885 (50.1)	
Current	3578	239 (6.7)		1985 (55.5)		1678 (46.9)	
Alcohol intake			<0.001		<0.001		0.017
Never	2388	109 (4.6)		1338 (56)		1166 (48.8)	
Previous	1539	96 (6.2)		833 (54.1)		723 (47)	
Current	42,536	1116 (2.6)		18,907 (44.4)		21,393 (50.3)	
Diet			<0.001		<0.001		0.157
Poor	28,939	888 (3.1)		13,684 (47.3)		14,427 (49.9)	
Good	17,524	433 (2.5)		7394 (42.2)		8855 (50.5)	
Sleep			<0.001		<0.001		0.717
Poor	31,133	990 (3.2)		14,718 (47.3)		15,582 (50)	
Good	15,330	331 (2.2)		6360 (41.5)		7700 (50.2)	
Physical activity			<0.001		<0.001		0.274
Light	10,415	413 (4)		5003 (48)		5147 (49.4)	
Moderate	19,556	527 (2.7)		8987 (46)		9847 (50.4)	
Vigorous	16,492	381 (2.3)		7088 (43)		8288 (50.3)	
MHT			0.382		<0.001		<0.001
No use	26,818	747 (2.8)		11,820 (44.1)		13,922 (51.9)	
Used	19,645	574 (2.9)		9258 (47.1)		9360 (47.6)	
YSM			<0.001		<0.001		<0.001
1	11,823	287 (2.4)		4330 (36.6)		6692 (56.6)	
2	13,263	350 (2.6)		5748 (43.3)		6865 (51.8)	
3	11,569	327 (2.8)		5656 (48.9)		5575 (48.2)	
4	6688	230 (3.4)		3541 (52.9)		2894 (43.3)	
5	3120	127 (4.1)		1803 (57.8)		1256 (40.3)	
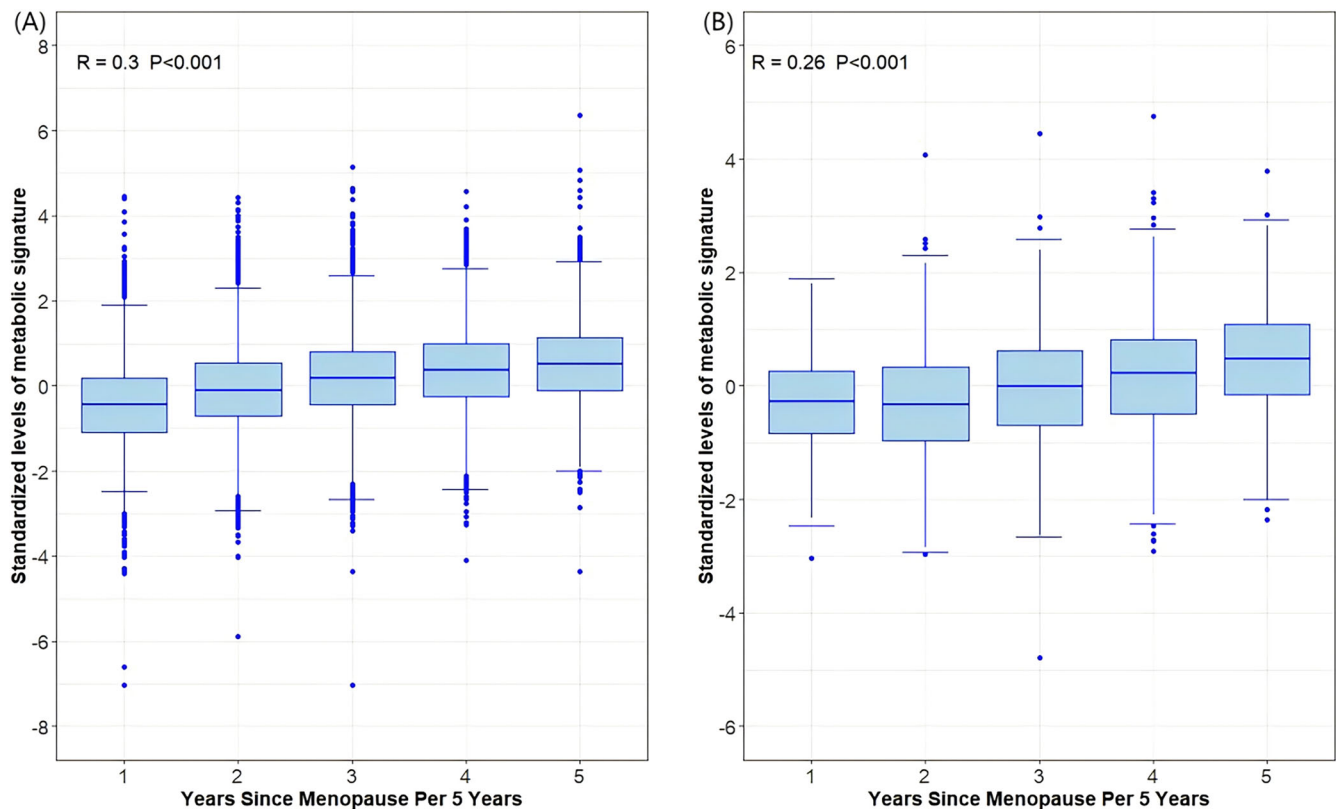
Fig. 1 | Correlation between years since menopause and metabolic signature. A Correlation between years since menopause and metabolite signature at baseline. **B** Correlation between years since menopause and metabolite signature at first repeat assessment.

menopause accelerates epigenetic aging. For instance, Morgan et al. found that earlier menopause was significantly associated with increased epigenetic age acceleration ($P = 8.32 \times 10^{-4}$) after adjusting for age, race/ethnicity, smoking status, age at menarche, and MHT use. Furthermore, the duration of time since menopause was positively associated with greater epigenetic age acceleration in blood ($\beta = 0.038$, $P = 0.007$), suggesting that postmenopausal physiological changes may drive epigenetic aging[18]. To date, direct studies examining the associations between menopause and AL remain limited. Consistent with previous findings[5], our analysis suggests that a longer duration since menopause is associated with a decreased likelihood of long TL, an increased likelihood of high AL, and elevated odds of advanced PA.

These findings underscore the critical role of menopause as a biological aging accelerator and highlight the need for further investigation into its mechanistic pathways. Menopause and biological aging share several molecular pathways, notably chronic low-grade inflammation ("inflammaging,") mitochondrial dysfunction, and increased oxidative stress. The decline in estrogen after menopause is associated with elevated inflammatory markers (such as GlycA), impaired mitochondrial bioenergetics, and reduced antioxidant capacity, all of which accelerate biological aging and disease risk[2–4].

Metabolism plays an important role in regulating aging at several levels, and metabolic reprogramming is the main driving force of aging. Menopause marked by a significant decline in circulating estrogen levels, which profoundly influences metabolic pathways and contributes to biological aging. The cascading impact of these metabolic alterations drive biological aging through inflammation, mitochondrial dysfunction, and metabolic instability. One of the most notable metabolic changes following menopause is dysregulated lipid metabolism, characterized by an increase in LDL and VLDL particles, accompanied by a reduction in HDL. These alterations promote an atherogenic lipid profile, predisposing postmenopausal women to cardiovascular diseases[2]. Additionally, TG levels tend to rise, with small

LDL and intermediate-density lipoprotein particles increasing in circulation, further exacerbating the risk of atherosclerosis[3]. The decline in estrogen also influences phospholipid metabolism, leading to reductions in phosphatidylcholine, a key component of cell membranes, which may compromise cellular integrity and accelerate aging-related processes[21].

Beyond lipid alterations, amino acid metabolism undergoes substantial shifts post-menopause. Elevated branched-chain amino acids (BCAAs), including isoleucine, leucine, and valine, are associated with insulin resistance, chronic inflammation, and metabolic syndrome[22]. BCAAs activate the mTOR pathway, inhibiting autophagy, promoting cellular senescence, and driving metabolic dysfunction[23]. Additionally, increased glutamine and tyrosine levels in postmenopausal women suggest a shift toward insulin resistance and systemic inflammation, contributing to age-related diseases like type 2 diabetes[24]. Impairments in glucose metabolism have also been observed in postmenopausal women, largely driven by a reduction in estrogen's regulatory effects on insulin sensitivity. A decrease in glycolytic intermediates (e.g., pyruvate) and tricarboxylic acid cycle metabolites (e.g., citrate) suggests a decline in mitochondrial function, leading to inefficient ATP production and metabolic inflexibility. This mitochondrial dysfunction, further amplifies oxidative stress, promoting DNA damage and cellular senescence[25].

Investigating the links between metabolomic signature and aging biomarkers will help identify therapeutic targets to mitigate postmenopausal metabolic decline[23]. The metabolomic signature partially elucidates the relationship between YSM and accelerated aging, highlighting distinct biological pathways revealed through metabolomic profiling. This signature provides a holistic representation of metabolic homeostasis as it adapts to the duration of YSM. Utilizing this approach offers a more precise and objective understanding of the cumulative metabolic effects associated with postmenopausal progression.

One strength of this study lies in using untargeted metabolomics to uncover diverse metabolic changes beyond predefined pathways, enabling
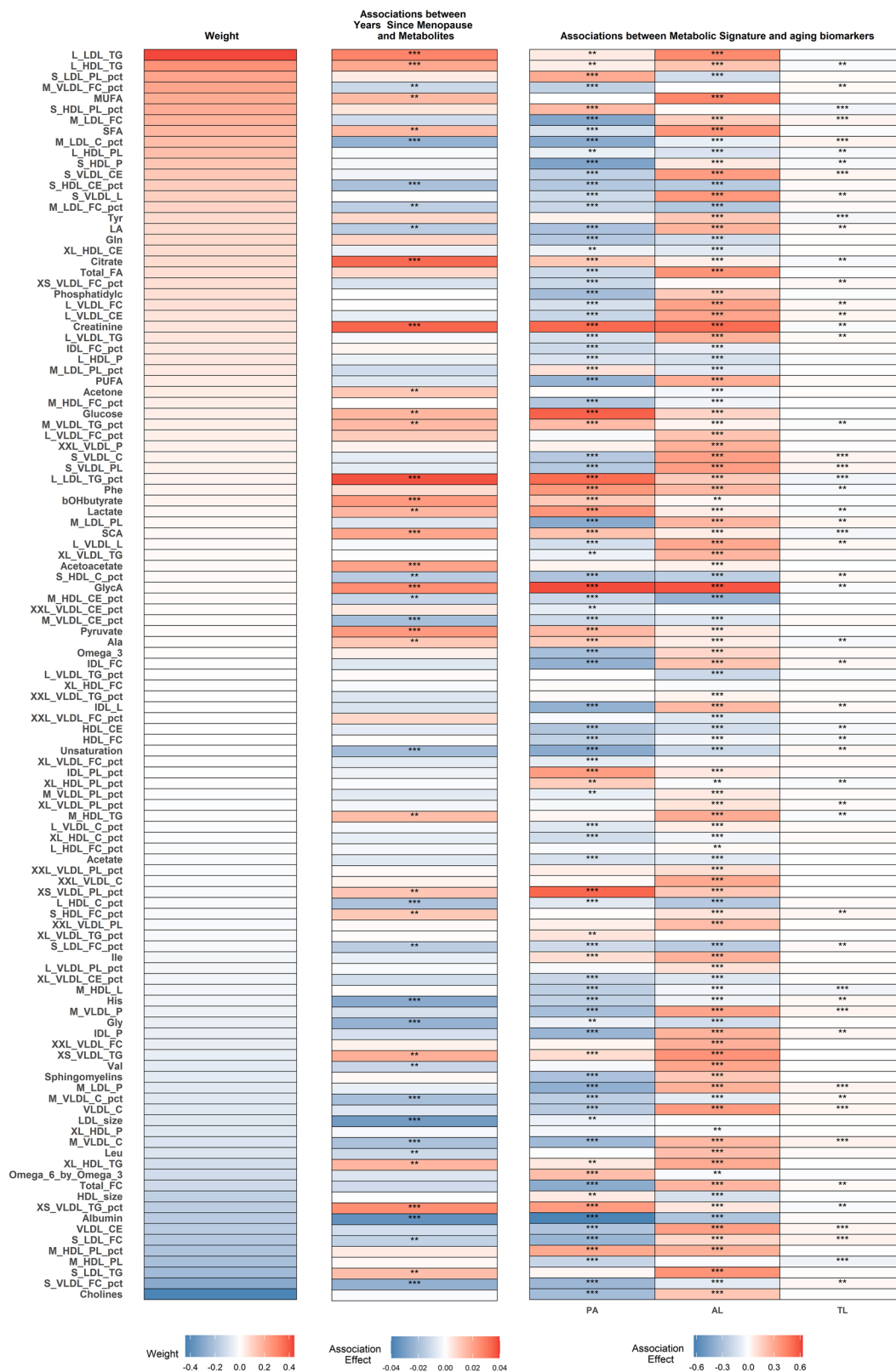
Fig. 2 | Association of 115 metabolites constituting the metabolomic signature with years since menopause and aging biomarkers. Presented from left to right are metabolite weights in the metabolic signature, regression coefficients for YSM (changes in metabolites per YSM increment), and regression coefficients for aging biomarkers (ln(HR) per SD increase in metabolites). Elastic net analysis, multivariable linear models and logistic regression models were used respectively. Colors denote the association directions (red-positive and blue-inverse) and magnitudes (the darker the color, the stronger the magnitude); asterisks represent association significance (two stars indicated Bonferroni-corrected $p < 0.05$, and three stars indicated $p < 0.001$), and $p < 0.05$ was considered statistically significant. Telomere length (TL), allostatic load (AL), PhenoAge (PA).

Table 2 | Associations of YSM one unit, metabolomic signature per SD increment with aging biomarkers in UK Biobank baseline data

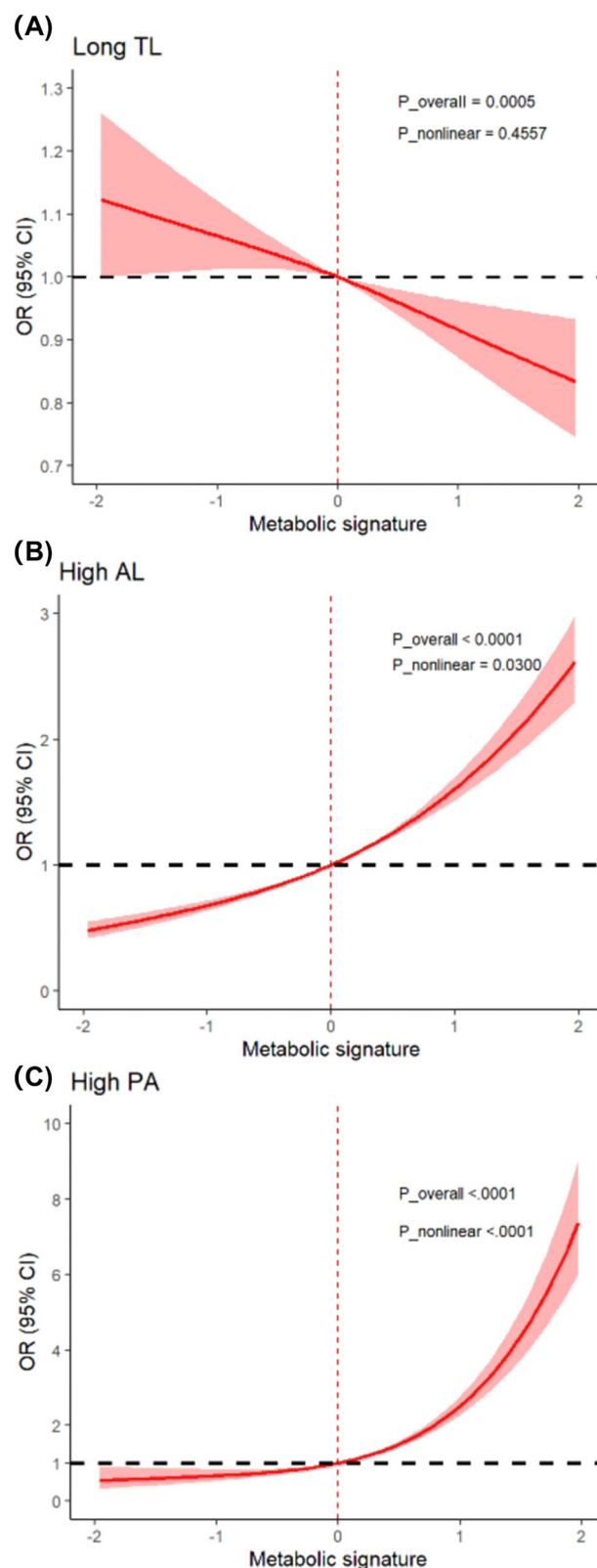|	YSM*		Metabolic signature	
	OR (95%CI)	*P*	OR (95%CI)	*P*
Long TL				
Model 1	0.90 (0.88, 0.92)	<0.001	0.92 (0.90, 0.93)	<0.001
Model 2	0.91 (0.89, 0.93)	<0.001	0.92 (0.90, 0.94)	<0.001
Model 3	0.92 (0.90, 0.93)	<0.001	0.93 (0.91, 0.95)	<0.001
Model 4	0.92 (0.90, 0.94)	<0.001	0.94 (0.92, 0.96)	<0.001
High AL				
Model 1	1.13 (1.11, 1.15)	<0.001	1.66 (1.63, 1.70)	<0.001
Model 2	1.11 (1.09, 1.13)	<0.001	1.65 (1.61, 1.68)	<0.001
Model 3	1.11 (1.09, 1.14)	<0.001	1.54 (1.50, 1.57)	<0.001
Model 4	1.07 (1.05, 1.10)	<0.001	1.53 (1.49, 1.56)	<0.001
High PA				
Model 1	1.14 (1.07, 1.20)	<0.001	2.54 (2.40, 2.69)	<0.001
Model 2	1.10 (1.04, 1.17)	0.001	2.48 (2.34, 2.63)	<0.001
Model 3	1.09 (1.03, 1.15)	0.005	2.30 (2.17, 2.44)	<0.001
Model 4	1.02 (0.96, 1.08)	0.617	2.30 (2.17, 2.44)	<0.001

CI Confidence Interval, *OR* Odds Ratio, *TL* Telomere Length, *AL* Allostatic Load, *PA* PhenoAge.
*One unit representing a 5-year interval.

the discovery of novel biomarkers linked to postmenopausal aging. By integrating aging markers like TL, AL, and PA, it offers a multidimensional perspective on how metabolic changes contribute to biological aging. Another strength is the longitudinal aspect of postmenopausal metabolic changes, capturing the cumulative metabolic effects over different YSM. Also, the study benefits from a well-characterized cohort with detailed clinical and biochemical assessments, allowing for robust adjustments of potential confounders, including lifestyle factors, comorbidities, and MHT status.

Despite these strengths, several limitations should be acknowledged. First, although this study shows links between YSM-related metabolic profiles and aging biomarkers, the inherent correlation between YSM and age may cause residual confounding even after adjusting for age, especially in mediation analyses. Therefore, these associations should be interpreted with caution, and future studies using alternative methods (such as instrumental variable analysis) are needed to delineate the independent effects of YSM. Second, menopausal status and age at natural menopause were ascertained through self-reported questionnaires, which may lead to misclassification and should be considered when interpreting our findings. Third, we were unable to account for women with a history of irregular menses or gynecologic disorders, such as PCOS, which can affect hormone levels and metabolic profiles. These conditions are associated with persistent metabolic abnormalities and increased cardiovascular risk that may continue after menopause[26]. Including these individuals may introduce heterogeneity and confound associations between menopausal duration, metabolomic changes, and aging biomarkers. Further studies with more detailed reproductive and gynecologic histories are needed to clarify these effects. Fourth, while metabolomic profiling provides valuable insights into systemic metabolic alterations, the cross-sectional nature of the metabolomic data limits the ability to infer causality from the mediation analysis. Finally, The UK Biobank cohort is primarily white, well-educated, and relatively healthy, which may limit the generalizability of our findings to broader populations.

This study highlights the critical association between YSM, metabolomic signature, and aging biomarkers, demonstrating that metabolic alterations following menopause are closely linked to accelerated aging

Fig. 3 | The dose–response relationships between metabolic signature and aging biomarkers. A The dose–response relationships between metabolic signature and telomere length (TL). **B** The dose–response relationships between metabolic signature and allostatic load (AL). **C** The dose–response relationships between metabolic signature and PhenoAge (PA).

Table 3 | Associations of YSM one unit, metabolomic signature per SD increment with aging biomarkers by baseline age and MHT use status

	YSM		Metabolic signature	
	OR (95%CI)	*P*	OR (95%CI)	*P*
Baseline age <= 60				
Long TL	0.94 (0.92, 0.97)	<0.001	0.94 (0.92, 0.97)	<0.001
High AL	1.09 (1.05, 1.12)	<0.001	1.52 (1.47, 1.57)	<0.001
High PA	0.96 (0.88, 1.06)	0.429	2.40 (2.20, 2.61)	<0.001
Baseline age > 60				
Long TL	0.89 (0.87, 0.91)	<0.001	0.93 (0.90, 0.96)	<0.001
High AL	1.07 (1.04, 1.10)	<0.001	1.53 (1.48, 1.58)	<0.001
High PA	1.07 (0.99, 1.15)	0.095	2.22 (2.05, 2.42)	<0.001
MHT (No)				
Long TL	0.90 (0.88, 0.93)	<0.001	0.95 (0.93, 0.98)	<0.001
High AL	1.10 (1.06, 1.13)	<0.001	1.53 (1.48, 1.57)	<0.001
High PA	1.03 (0.95, 1.12)	0.521	2.33 (2.15, 2.53)	<0.001
MHT (Yes)				
Long TL	0.94 (0.92, 0.97)	<0.001	0.93 (0.90, 0.95)	<0.001
High AL	1.05 (1.02, 1.09)	<0.001	1.52 (1.47, 1.58)	<0.001
High PA	1.00 (0.92, 1.09)	0.987	2.27 (2.08, 2.48)	<0.001

OR Odds Ratio, *CI* Confidence Interval, *TL* Telomere Length, *AL* Allostatic Load, *PA* PhenoAge, *MHT* menopausal hormone therapy.

pathways. By identifying a metabolomic signature reflective of post-menopausal progression, we provide evidence that specific metabolic shifts-particularly in lipoprotein subclasses, relative lipoprotein lipid concentrations, amino acids-mediate the relationship between YSM and aging biomarkers. These findings underscore the importance of metabolomics in understanding postmenopausal health and aging mechanisms, offering potential applications in clinical risk stratification, early intervention, and personalized health management for aging women. Future longitudinal studies and research in more ethnically and socioeconomically diverse populations are warranted to further validate the generalizability and robustness of the identified metabolomic signatures associated with YSM and biological aging.
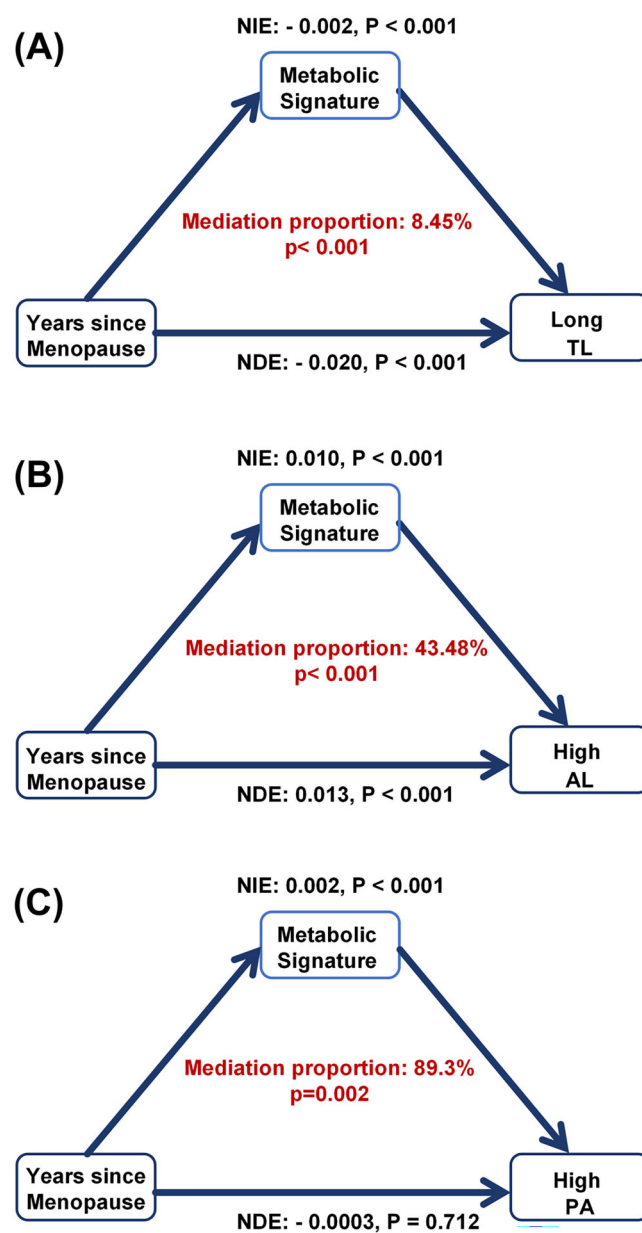
Methods
Study design and participants
The UK Biobank is a large-scale, population-based prospective cohort study that recruited over 500,000 volunteers aged 40–69 years between 2006 and 2010. Participants attended one of 22 assessment centers across England, Scotland, and Wales, where they underwent a comprehensive baseline evaluation. Written informed consent was obtained from all individuals for the collection of questionnaire responses, biological samples, and other health-related data[27]. UK Biobank has approval from the North West Multicenter Research Ethics Committee (Ref: 21/NW/0157) (https://www.ukbiobank.ac.uk/learn-more-about-uk-biobank/about-us/ethics). This study was performed in line with the principles of the Declaration of Helsinki. This research was conducted under UK Biobank application number 227947. Women who were postmenopausal at baseline and had no missing key covariates were included. Figure 5 provides an overview of the study design and participant selection. In this study, we used clinical traits at baseline to define three aging biomarkers. This study is reported as per the strengthening the reporting of observational studies in epidemiology guidelines (Supplementary Table S1).

Definition of YSM
Natural menopause was defined as the permanent cessation of menstrual cycles for a consecutive 12-month period, excluding individuals with a history of hysterectomy and/or oophorectomy prior to this timeframe[28]. The

(A)

NIE: - 0.002, P < 0.001

Metabolic Signature

Mediation proportion: 8.45% p< 0.001

Years since Menopause → Long TL

NDE: - 0.020, P < 0.001

(B)

NIE: 0.010, P < 0.001

Metabolic Signature

Mediation proportion: 43.48% p< 0.001

Years since Menopause → High AL

NDE: 0.013, P < 0.001

(C)

NIE: 0.002, P < 0.001

Metabolic Signature

Mediation proportion: 89.3% p=0.002

Years since Menopause → High PA

NDE: - 0.0003, P = 0.712

Fig. 4 | Proportions mediated by the constructed metabolic signature in the association between years since menopause and aging biomarkers. A Proportions mediated by the constructed metabolic signature in the association between years since menopause and telomere length (TL). **B** Proportions mediated by the constructed metabolic signature in the association between years since menopause and allostatic load (AL). **C** Proportions mediated by the constructed metabolic signature in the association between years since menopause and PhenoAge (PA).

duration of the postmenopausal phase, quantified in YSM, was calculated by subtracting the age at natural menopause from the age at study enrollment. For analytical purposes, YSM was normalized by dividing it by five, with each unit corresponding to a 5-year interval.

Metabolomics profiling
This research utilized a high-throughput nuclear magnetic resonance metabolomics platform (Nightingale Health Ltd, Finland) to assess EDTA plasma samples from nearly 280,000 UK Biobank participants. A total of 251 metabolic biomarkers were evaluated, comprising 170 metabolites measured in absolute concentrations and 81 composite ratio indices (Supplementary Table S2). These biomarkers capture a broad spectrum of metabolic pathways, including 14 lipoprotein lipid
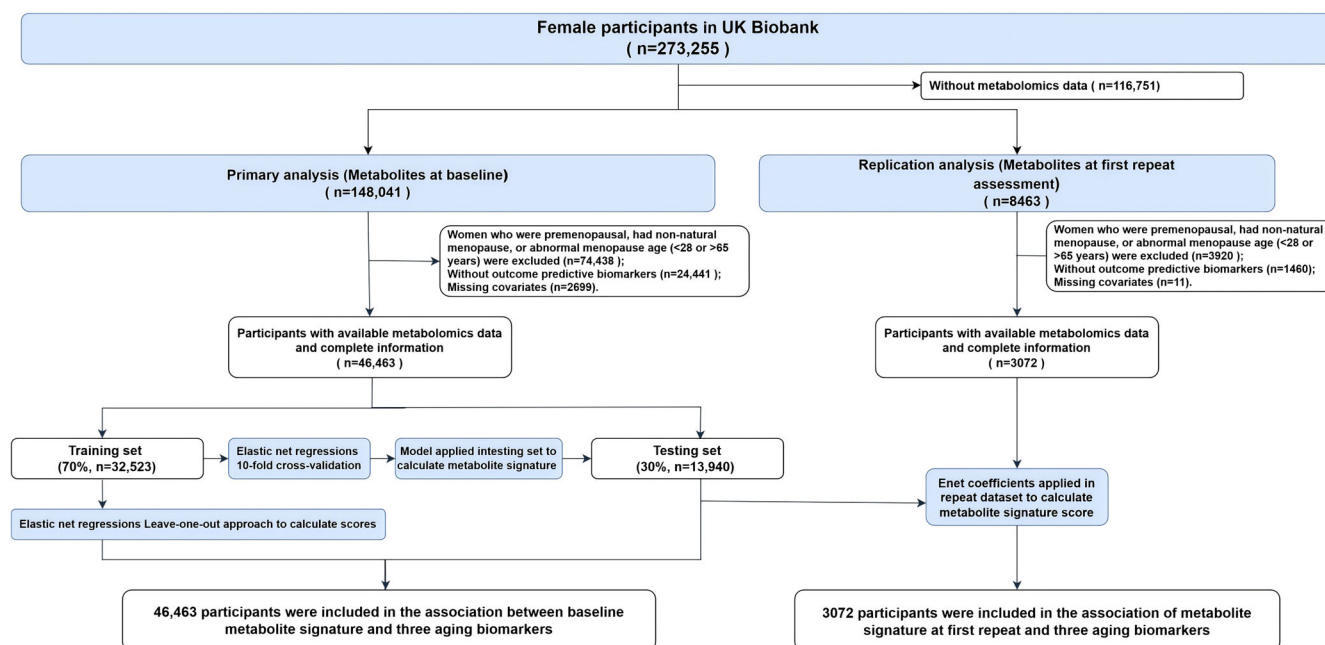
Fig. 5 | Flow chart of study design and analytical approach.

subclasses, fatty acids and their compositions, as well as small-molecule metabolites such as amino acids, ketone bodies, and glycolysis-related compounds[29]. To ensure data accuracy, strict quality control procedures were applied. Technical variation in these data was removed using the "ukbnmr" R package (for further details see https://github.com/srtichie73/ukbnmr). Briefly, technical variation removed included: (1) time between sample preparation and measurement, (2) batch effects from sample position on the 96-well plate, (3) measurement drift, (4) inter-spectrometer differences, and (5) plates with systematically abnormal concentrations of non-biological origin. Metabolite concentrations underwent natural logarithmic transformation[30] and z-score standardization (mean 0, SD 1) to address systemic and technical variability.

The data analyzed originated from phases 1 (baseline) and 2 (first repeat assessment) of the UK Biobank study, with ~16,000 participants completing a follow-up assessment. All metabolic biomarkers were expressed in molar concentration units, providing an in-depth depiction of participants' metabolic profiles. Further details on the biomarker measurement methods and quality control protocols can be found in the relevant literature[31].

Definitions of three aging biomarkers
In this study, baseline clinical traits were used to define three aging biomarkers. We used the best-validated algorithms to construct PA and AL using blood-chemistry-derived biomarkers from the UK Biobank[32–34] (Supplementary Table S3). The R package BioAge (https://github.com/dayoonkwon/BioAge) was used for computation[35]. PA was derived from a multivariate mortality hazard model[36]. AL was calculated as the proportion of ten biomarkers classified as "at risk", defined as values in the highest quartile for nine biomarkers and the lowest quartile for albumin, resulting in an AL score ranging from 0 to 1[37]. DNA was extracted from participants' peripheral blood leukocytes at baseline, and TL was measured via multiplex quantitative polymerase chain reaction, using the telomere amplification products to single-copy genes (T/S) ratio as an estimate. The T/S ratio was log-transformed and z-standardized for analysis[34].

We set PA, AL, and TL as binary variables for analysis. Participants were categorized for each aging marker as follows: those with PA exceeding chronological age were classified as accelerated aging (i.e., high PA), while others were non-accelerated[38]. For AL, individuals with higher levels of AL

were at greater risk of physiological stress; thus, values above the median indicated physiological dysregulation (i.e., high AL), and those below indicated no dysregulation. TL above the median was classified as long telomeres (i.e., long TL), while values below were short telomeres[34].

Covariates
We included the following factors as covariates based on evidence from prior studies: baseline age, race/ethnicity, years of education, income levels, Townsend Deprivation Index, smoking status, alcohol consumption, BMI, sleep patterns, diet, and medication use, including menopausal hormone therapy, aspirin, and statins. Race/ethnicity was categorized as White, Asian or Asian British, Black or Black British, and Other. Years of education were grouped into ≤10 years, 11–12 years, and >12 years. Annual household income was divided into four categories: Level 1 (<£18,000), Level 2 (£18,000–£30,999), Level 3 (£31,000–£51,999), and Level 4 (>£52,000). The Townsend Deprivation Index, which reflects area-level socioeconomic status, was derived from participants' residential postal codes at recruitment and categorized into quartiles, with higher values indicating greater deprivation. Smoking status was classified as current, former, or never smokers. Alcohol consumption was grouped into daily, 3–4 times per week, 1–2 times per week, occasional, and never. BMI was classified according to World Health Organization criteria as <18.5, 18.5–24.9, 25.0–29.9, and ≥30 kg/m². Diet consumption was classified into ideal or poor according to whether adequate intake of at least half of the ten diet components[39]. Sleep quality was defined by the criteria recommended by the National Sleep Foundation, which integrates five sleep behaviors (sleep duration, chronotype preference, insomnia, snoring, and daytime sleepiness)[40]. Participants were divided into two groups: good sleep quality and poor sleep quality. Medication use was categorized as use or non-use. For further details on the collection and definitions of covariates, please refer to Supplementary Table S4–6.

Statistical analysis
Using baseline metabolomics data from the UK Biobank, we investigated metabolites associated with YSM in postmenopausal women. Metabolite levels exceeding four interquartile ranges from median were excluded as outliers. Before performing the analyses, all 251 metabolites were log-transformed and standardized to z-scores to ensure comparability across scales. Pearson correlation coefficients were used to assess correlations

between metabolites. The relationships between each metabolite and YSM were analyzed using multivariable linear regression, with statistical significance determined by an FDR-adjusted p-value threshold of 0.05.

To identify a metabolomic signature linked to YSM, we utilized an elastic net regression model. This method integrates the advantages of both Lasso and Ridge regression, effectively managing multicollinearity, minimizing overfitting, and selecting key features[41]. In our analysis, YSM was regressed on 251 standardized plasma metabolites. The optimal penalty parameter (Lambda) was determined through a ten-fold cross-validation process, selecting the largest lambda value that yielded a mean squared error within one standard error of the minimum. The derived metabolomic signature was calculated as a weighted sum of metabolites with nonzero coefficients, where the weights corresponded to the coefficients estimated by the elastic net model. Finally, the metabolomic signature was standardized using z-scores (mean of 0 and standard deviation of 1), with each unit reflecting the cumulative contribution of the selected metabolites' weighted effects[42].We assessed the Spearman correlation between YSM, and the derived metabolomic signature at both the baseline period (2006–2010) and the first follow-up assessment (2012–2013).

Logistic regression analyses were conducted to test the association between YSM (each 5-year increase), metabolic signature (each SD increase) and high PA, high AL, and long TL, with non-accelerated, no dysregulation, and short telomeres as the reference, respectively. Four nested models were developed, sequentially including four sets of covariates to account for potential confounders. In model 1, age was adjusted; in model 2, ethnicity, income level, years of education and Townsend index of deprivation were further adjusted based on model 1; in model 3, BMI, smoking status, alcohol consumption, diet, sleep and hormone therapy were further adjusted based on model 2; in model 4 (full adjusted model), we further included mutual adjustments for both YSM and metabolic signature to assess their independent associations. We also examined potential non-linear associations between metabolic signature, YSM and aging biomarkers using restricted cubic spline analysis.

To distinguish the effects of chronological age from postmenopausal duration, we conducted a stratified analysis based on baseline age (<60 years and ≥60 years), as these two factors are closely intertwined. We also stratified the analyses by MHT status.

We conducted mediation analyses to examine whether the association between YSM and the three aging biomarkers—TL, AL, and PA—was mediated by metabolomic signature. We used mediation models via the "mediation" R package. The proportion of mediation was determined by dividing the indirect effect by the total effect, with 95% confidence intervals estimated via bootstrapping. Covariates from the logistic regression model were included in the mediation analysis to account for potential confounders. We further analyzed the mediation proportions of various metabolite types within the metabolic signature in the relationship between YSM and aging biomarkers to identify the key metabolite types serving as primary mediators.

Statistical significance was defined as two-sided $P < 0.05$, with p values adjusted for multiple comparisons using the Benjamini-Hochberg FDR method. Elastic net regression was conducted with the R package 'glmnet."

Data availability
The data described in the manuscript will be made available for researchers who apply to use the UK Biobank data set by registering and applying at https://www.ukbiobank.ac.uk/enable-your-research/register.

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B.X. and D.S.Z.: Conceptualization and methodology; Q.W., C.Y.F., X.Y.W.: Data curation and formal analysis; B.X., M.L.L. and D.S.Z.: Proofread and revise; D.S.Z. and Q.W.: Data curation; D.S.Z.: Project administration and supervision. All authors have read and agreed to the published version of the manuscript.

Competing interests
The authors declare no competing interests.

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