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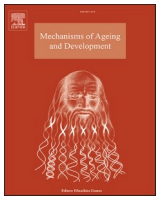

Lifestyle factors and metabolomic aging biomarkers: Meta-analysis of cross-sectional and longitudinal associations in three prospective cohorts

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ABSTRACT

Biological age uses biophysiological information to capture a person's age-related risk of adverse outcomes. MetaboAge and MetaboHealth are metabolomics-based biomarkers of biological age trained on chronological age and mortality risk, respectively. Lifestyle factors contribute to the extent chronological and biological age differ. The association of lifestyle factors with MetaboAge and MetaboHealth, potential sex differences in these associations, and MetaboAge's and MetaboHealth's sensitivity to lifestyle changes have not been studied yet.

Linear regression analyses and mixed-effect models were used to examine the cross-sectional and longitudinal associations of scaled lifestyle factors with scaled MetaboAge and MetaboHealth in 24,332 middle-aged participants from the Doetinchem Cohort Study, Rotterdam Study, and UK Biobank. Random-effect meta-analyses were performed across cohorts. Repeated metabolomics measurements had a ten-year interval in the Doetinchem Cohort Study and a five-year interval in the UK Biobank.

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In the first study incorporating longitudinal information on MetaboAge and MetaboHealth, we demonstrate associations between current smoking, sleeping ≥8 hours/day, higher BMI, and larger waist circumference were associated with higher MetaboHealth, the latter two also with higher MetaboAge. Furthermore, adhering to the dietary and physical activity guidelines were inversely associated with MetaboHealth. Lastly, we observed sex differences in the associations between alcohol use and MetaboHealth.

1. Introduction

With advancing age, human susceptibility to functional decline and disease increases (Rutledge et al., 2022). Yet, the onset and pace of individual susceptibility vary beyond chronological age (Rutledge et al., 2022). Multiple omics-based biomarkers of biological age, such as telomere length or epigenetic biomarkers (Vyas et al., 2021; Horvath and Raj, 2018; Jylhävä et al., 2017), were identified in the past decade. Recently, biomarkers of biological age utilizing metabolomics data trained on either chronological age (MetaboAge) (Van Den Akker et al., 2020) or mortality (MetaboHealth) (Deelen et al., 2019) were introduced. These omics-based aging biomarkers are suggested to be sensitive to individual health status and be predictive of adverse health outcomes (Rutledge et al., 2022; Van Den Akker et al., 2020; Deelen et al., 2019). Furthermore, metabolomics-based aging biomarkers are intended to serve as dynamic measures, responding to physiological, environmental, and lifestyle changes while distinguishing between "fast" and "slow" agers.

Individual health status, risk of adverse health outcomes, and the inter-individual disparity in chronological and biological age are influenced by lifestyle factors (Jylhävä et al., 2017). Lifestyle intervention studies aim to improve the individual aging trajectory, and dynamic aging biomarkers are used as early-response outcome measures in such interventions (Fitzgerald et al., 2021; Johnson et al., 2022; Fiorito et al., 2021; Gensous et al., 2020). In past studies, lifestyle factors have shown varying associations with biomarkers of biological age from different origins, i.e., epigenetic markers and telomere length (Vyas et al., 2021; Quach et al., 2017; García-Calzón et al., 2014).

Limited attention has been given to the relationship between lifestyle factors and metabolomics-based aging biomarkers (Van Den Akker et al., 2020; Smit et al., 2023). In particular longitudinal studies on metabolomics-based aging biomarkers are currently lacking. Consequently, it remains unclear which lifestyle changes are picked up by metabolomics-based biomarkers, pivotal information when using these biomarkers as early-response outcomes in lifestyle intervention studies. Moreover, lifestyle effects on aging biomarkers can vary by sex (Vyas et al., 2021; Smit et al., 2023; Hägg and Jylhävä, 2021). However, no previous study has determined sex-specific associations between lifestyle factors and metabolomics-based aging biomarkers. This study investigates the cross-sectional and longitudinal associations between lifestyle factors and metabolomics-based aging biomarkers and examines variation by sex.

2. Methods

2.1. Study design and population

The study population consisted of participants from three prospective cohorts: the Doetinchem Cohort Study (DCS) (Verschuren et al., 2008) (n=4,644), the Rotterdam Study (RS) (Ikram et al., 2024) (n=4,719), and the UK Biobank (UKBB) (Sudlow et al., 2015) (n=14,969). In DCS, we included participants with metabolomics information on the fourth (2003–2007) and/or the sixth (2013–2017) examination rounds. Metabolomics data were measured only once per participant in RS from

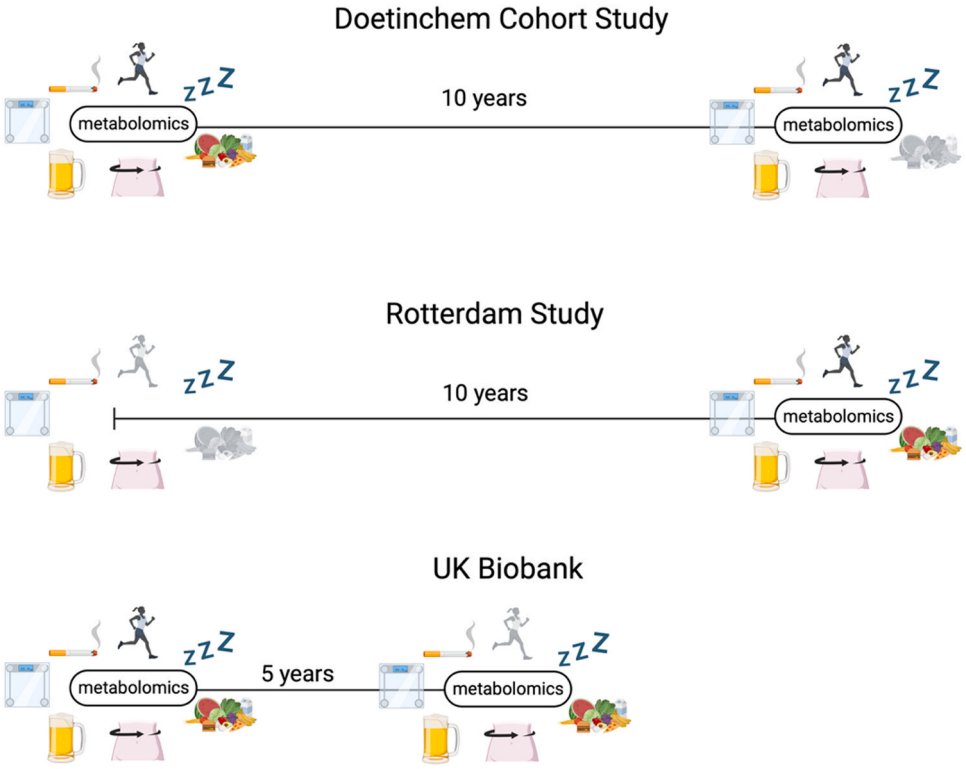

Fig. 1. Schematic outline of data availability per cohort.

the fourth visit of the first sub-cohort (2002–2004), the third visit of the second sub-cohort (2011–2012), or the second visit of the third sub-cohort (2012–2014) (Fig. 1). We included UKBB participants with information on metabolomics-based aging biomarkers at both the baseline and follow-up visits with an interval of roughly five years. A more detailed description is available in Appendix A.

Grey icons indicate that a lifestyle factor is not available at that time point. Ovals with metabolomics indicate metabolomics data availability. The years above the line indicate the years between measurements.

2.2. Metabolomic age

Metabolomic biomarkers from EDTA plasma were determined using the Nightingale platform. In DCS and UKBB, the Nightingale 2020 assay was used, while in RS, we used the 2016 assay, which was subsequently re-quantified to match the Nightingale 2020 assay. In RS, we calculated 56-metabolites-based MetaboAge on the 2016 data using the dedicated website (Van Den Akker et al., 2020). MetaboHealth (14-metabolites-based) (Deelen et al., 2019) and 2020-data-MetaboAge (63-metabolites-based) (Bizzarri et al., 2023a) were calculated in all cohorts using the *MiMIR* R-package. The metabolites comprised in MetaboAge and MetaboHealth are detailed in Appendix A. In DCS and UKBB, we calibrated the metabolomic measures of the second blood draw to minimize inter-visit bias, assuming that participants of the same age, sex, and BMI would have similar metabolomic profiles (Mäkinen et al., 2022; Bizzarri et al., 2023b). Calibration involved matching samples between visits by these factors and normalizing the metabolites from the second measurement to the first measurement (Appendix A).

2.3. Lifestyle factors

Data on smoking status (current; not), the number of cigarettes smoked per day (smoking quantity) [per 10 cigarettes], regular alcohol consumption (at least once a week; not), daily alcohol consumption [alcoholic units/day], diet, physical activity [METhours/week], and sleep duration were collected using interviews or questionnaires (Appendix A). The Dutch cohorts also assessed adherence to the Dutch physical activity guideline, requiring at least 30 minutes of moderate to vigorous physical activity per day $\geq$5 days per week (Kemper et al., 2000). The UK Biobank assessed adherence to the UK Biobank physical activity guidelines requiring 150 minutes of moderate or 75 minutes of vigorous activity per week. Average sleep duration per 24 hours was categorized into short (<7 hours), reference (7-8 hours), and long (>8 hours). Dietary guideline adherence was categorized as high, medium, or low per cohort based on tertiles. To get more insight into the associations between diet and metabolomic aging, we also included individual components of the dietary indices, i.e., food group intakes (Appendix A) as determinants. Waist circumference [cm], height [m], and weight [kg] were measured, and body mass index (BMI) [kg/m^2] was calculated.

2.4. Covariates

Sex was based on self-reported sex. Chronological age was defined as the time between birth and the blood collection. Access to precise birthdates in the UKB was limited to the month and year only. Therefore, we set the birth dates of all participants to the 15th of each month. Socioeconomic status was defined as the highest attained education level and assessed by questionnaires. Cell counts were measured as the percentage of lymphocytes and monocytes; cell count information was unavailable in DCS. The season of blood collection was based on the meteorological seasons. Observation time was defined as the time elapsed since the first metabolomics measurement, with a value of 0 for the initial measurement and the actual time for subsequent measurement. Only RS data were sent to Nightingale in three batches; therefore, we adjusted analyses in RS for these batches.

2.5. Cross-sectional analysis (DCS, RS, UKBB)

Mixed-effect models *lmer* and *lmerTest* R-functions in DCS and UKBB and linear regression analyses in RS were used to study the association between lifestyle factors and metabolomic aging. Our choice of analysis in the RS dataset differed because metabolomics data was available for only one time point, while we aimed to consider all available information from the two visits in the DCS and UKBB datasets. We performed a sensitivity analysis using linear regression across all cohorts to assess the potential impact of using different models.

In all analyses, we used the raw residuals of a linear regression between chronological age and MetaboAge and MetaboHealth as outcome measures. These residuals provide the chronological age-independent part of the aging biomarkers and indicate whether an individual is biologically "older" or "younger" than their chronological age's population average. We standardized both the continuous lifestyle factors and age-independent metabolomic aging biomarkers using Z-scores to improve the comparability of effect estimates across the lifestyle factors and biomarkers. In the first model, the associations between lifestyle factors and metabolomics-based aging biomarkers were adjusted for sex, socioeconomic status, and season of blood collection, -in the RS- batch, and -in the case of the mixed-effect models- observation time, as the time between visits differed up to three years between participants from the same cohort. In the second model, we additionally adjusted for potential confounding effects of other lifestyle factors (Appendix A) to determine the independent impact of individual lifestyle factors. In RS and UKBB, we performed sensitivity analyses, additionally adjusting for cell counts to improve the comparability of our results with other omics-based aging biomarkers. We additionally performed a sensitivity analysis using linear regression in all cohorts to determine whether using a different statistical approach impacted the results.

Analyses were corrected for multiple testing by Benjamini-Hochberg false discovery rates (FDR) correction. Spearman's ρ was used to determine the correlation between chronological age and the different aging biomarkers.

2.6. Retrospective: Ten-year lifestyle changes and metabolomics-based aging biomarkers (RS, DCS)

To assess ten-year lifestyle changes, we subtracted the lifestyle information recorded ten years before blood collection from the data recorded at the time of blood collection. In the DCS analysis, information from round 6 served as the reference point for blood collection. This lifestyle change was used to determine whether lifestyle change was associated with metabolomics-based biomarkers of biological age beyond cross-sectional lifestyle measurements. The analyses followed the same procedure as the cross-sectional analyses in RS, incorporating the 10-year-prior lifestyle factor and the observation time into all models.

2.7. Prospective: Five/ten-year-changes in lifestyle and five/ten-year-change in aging biomarker (DCS, UKBB)

Linear mixed-effect models were used for the longitudinal analyses. To examine the dynamics of the crude metabolomic-based biomarkers of biological age at individual and population levels, we calculated the within-change-to-total-change ratio and minimal detectable change (Appendix A). In short, the within-change-to-total-change was the complement of the intra-class correlation between the biomarker at two visits divided by the total variance. The minimal detectable change represents the minimal difference between measurements that can be attributed to actual change instead of measurement error (Appendix A).

The same models were used for the longitudinal association of lifestyle factors with metabolomics-based aging biomarkers as in the cross-sectional analyses with the incorporation of an interaction term between observation time and the lifestyle factor of interest to capture the

longitudinal effect of the lifestyle factor on the metabolomics-based aging biomarkers. We again used Z-transformed continuous lifestyle factors and biomarkers to improve the comparability of effect estimates. As BMI was used in the metabolite calibration, we used the uncalibrated metabolite biomarkers for the association with BMI. In UKBB, we performed sensitivity analyses adjusting for cell counts to improve the comparability of the results to other biomarkers of biological age.

2.8. Meta-analyses

We random-effect meta-analyzed the results from both the linear regression analysis and the linear mixed model analysis using the *metafor* R-package. We opted for random-effect meta-analysis as both the Doetinchem Cohort Study and Rotterdam Study are population-based cohort studies (Verschuren et al., 2008; Ikram et al., 2024), whereas the UK Biobank is based on active participation (Sudlow et al., 2015; Fry et al., 2017). For the cross-sectional sensitivity analysis using linear regression in all cohorts, we used the results from the sixth examination round of DCS and round 1 of the UKBB, as the age distribution of this visit was closest to the age distribution in RS. Dietary information was not available in DCS round 6 and physical activity information was not available in UKBB round 1. Therefore, we excluded those cohorts for those specific variables in this sensitivity analysis.

2.9. Sex-specific analyses

To investigate the association between lifestyle and metabolomic aging biomarkers by sex, we conducted sex-stratified analyses across all cohorts. We then performed a random-effects meta-analysis on the sex-specific results, following the previously described method. Subsequently, we used the metafor R-package to conduct fixed-effects meta-analyses for men and women separately. We assessed heterogeneity between sexes using the Wald test.

Table 1
Baseline characteristics of the study population.

	Doetinchem Cohort Study				Rotterdam Study		UK Biobank			
	First moment of blood collection		Second moment of blood collection				First moment of blood collection		Second moment of blood collection	
	N		N		N		N		N	
Chronological age (years)	4446	55.6 ± 9.9	3236	64.0 ± 9.2	4695	71.0 ± 8.1	14968	57.7 ± 7.4	14969	61.9 ± 7.4
Women	4446	2344 (52.7 %)	3238	1722 (53.2 %)	4695	2726 (58.1 %)	14968	7527 (50.3 %)	14969	7528 (50.3 %)
Highest attained education										
Primary education	4435	357 (8.1 %)	3081	153 (5.0 %)	4669	536 (11.5 %)	14906	1292 (8.7 %)	14924	1252 (8.4 %)
Lower vocational or intermediate general education	4435	1792 (40.4 %)	3081	1186 (38.5 %)	4669	1882 (40.3 %)	14906	3444 (23.1 %)	14924	3488 (23.4 %)
Intermediate vocational or secondary education	4435	1267 (28.6 %)	3081	949 (30.8 %)	4669	1385 (29.7 %)	14906	3606 (24.2 %)	14924	3513 (23.5 %)
Higher vocational education	4435	1019 (23.0 %)	3081	793 (25.7 %)	4669	866 (18.6 %)	14906	6564 (44.0 %)	14924	6671 (44.7 %)
Season of blood collection										
Winter	4446	1007 (22.6 %)	3236	937 (29.0 %)	4695	1262 (26.9 %)	14968	3220 (21.5 %)	14969	4195 (28.0 %)
Spring	4446	1176 (26.4 %)	3236	886 (27.4 %)	4695	1298 (27.6 %)	14968	3861 (25.8 %)	14969	5724 (38.2 %)
Summer	4446	1086 (24.4 %)	3236	570 (17.6 %)	4695	724 (15.4 %)	14968	4367 (29.2 %)	14969	761 (5.1 %)
Fall	4446	1177 (26.5 %)	3236	843 (26.1 %)	4695	1411 (30.1 %)	14968	3520 (23.5 %)	14969	4289 (28.6 %)
Lymphocytes (%)					4683	33.2 ± 8.4	14624	29.0 ± 7.2	14547	28.5 ± 7.7
Monocytes (%)					4683	6.5 ± 2.1	14624	7.2 ± 2.5	14547	6.7 ± 3.0
Adhering to physical activity guidelines	4494	2617 (58.2 %)	3421	1990 (58.2 %)	1718	1535 (89.3 %)	12637	6783 (53.7 %)		
Total METhours/week	4445	7.1 ± 9.9	3235	6.6 ± 8.5	1718	56.7 ± 44.5	12637	25.3 ± 31.2		
Body mass index (kg/m2)	4435	26.5 ± 4.1	3232	26.8 ± 4.2	4602	27.4 ± 4.2	14932	26.9 ± 4.5	14943	26.9 ± 4.5
Waist circumference	4443	94.9 ± 11.8	3233	96.6 ± 12.3	4675	93.5 ± 12.1	14946	88.9 ± 13.1	14958	90.6 ± 13.2
Current smoking	4472	949 (21.2 %)	3389	410 (12.1 %)	4627	562 (12.1 %)	14964	932 (6.2 %)	14962	669 (4.5 %)
Smoking quantity (per 10 cigarettes a day)	4422	0.8 ± 1.6	3200	0.4 ± 1.2	4621	0.3 ± 1.0	14772	0.1 ± 0.5	14836	0.1 ± 0.4
Adherence to dietary guidelines	4435	67.1 ± 13.9			1611	7.0 ± 1.8	13860	3.5 ± 1.2	13910	3.5 ± 1.2
Drinking alcohol at least once a week	4490	3000 (66.8 %)	3414	2147 (62.9 %)	4624	3250 (70.3 %)	14959	11269 (75.3 %)	14963	10742 (71.8 %)
Alcoholic beverages per day	4445	1.1 ± 1.4	3180	0.9 ± 1.1	4624	1.0 ± 1.2	14927	2.6 ± 3.0	14945	2.2 ± 2.8
Sleeping more than 8 hours	3592	283 (7.9 %)	2619	215 (8.2 %)	3162	362 (11.4 %)	11713	1083 (9.2 %)	11716	1264 (10.8 %)
Sleeping less than 7 hours	4463	871 (19.5 %)	3396	777 (22.9 %)	4204	1404 (33.4 %)	13829	3199 (23.1 %)	13665	3213 (23.5 %)
$MetaboAge_{2020}$	4446	-0.1 [-5.4; 5.3]	3236	-0.3 [-6.3; 6.1]	4695	-0.2 [-5.0; 4.8]	14968	-0.1 [-4.8; 4.7]	14969	-0.2 [-5.6; 5.2]
Crude $MetaboAge_{2020}$ (years)	4446	58.4 [52.1; 64.6]	3238	61.7 [54.9; 68.5]	4695	63.2 [57.5; 68.9]	14968	54.2 [49.1; 59.5]	14969	54.4 [48.5; 60.1]
$MetaboHealth_{2020}$	4446	-0.0 [-0.3; 0.2]	3236	-0.0 [-0.3; 0.2]	4695	-0.0 [-0.3; 0.3]	14968	-0.0 [-0.3; 0.3]	14969	-0.0 [-0.3; 0.2]
Crude $MetaboHealth_{2020}$ (arbitrary units)	4446	-0.0 [-0.3; 0.2]	3238	-0.0 [-0.3; 0.2]	4695	0.0 [-0.3; 0.3]	14969	-0.0 [-0.3; 0.3]	14969	-0.0 [-0.3; 0.2]
$MetaboAge_{2016}$					4695	-0.1 [-4.5; 4.4]				
Crude $MetaboAge_{2016}$ (years)					4695	64.5 [59.9; 69.3]				
$MetaboHealth_{2016}$					4695	-0.0 [-0.3; 0.2]				
Crude $MetaboHealth_{2016}$ (arbitrary units)					4695	-0.0 [-0.3; 0.3]				

N indicates the number of participants with information on the factor present.
Presented values in the middle columns are n (%) binary or categorical variables; mean ± standard deviation for continuous variables; and median [Q1;Q3] for metabolomics-based aging biomarkers

2.10. Software

All analyses were performed in R. Session information is available in Supplement A. Fig. 1 was created using Biorender.

2.11. Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, report writing, or the decision to submit the paper.

3. Results

3.1. Baseline characteristics

Table 1 shows the descriptive statistics of the study participants from the three cohorts. RS participants were, on average, older than participants from DCS and UKBB. DCS participants were at the baseline blood collection more often current smokers than participants from other cohorts. Sex-stratified baseline characteristics can be found in Appendix B.

3.2. Comparison across Nightingale platforms

In the RS, MetaboHealth showed a Spearman correlation of 0.82 and MetaboAge a correlation of 0.63 between Nightingale assays (Appendix C). The moderate correlation of MetaboAge across assays raised concerns about the robustness of the results across them. Nonetheless, consistent results in RS were observed across Nightingale assays, only reporting stronger associations of BMI ($MetaboAge_{2020}$:adjusted β per standard deviation increase 0.23[95 %-confidence interval 0.21;0.26], $MetaboAge_{2016}$:0.12[0.09;0.15]) and waist circumference ($MetaboAge_{2020}$:0.26[0.23;0.29], $MetaboAge_{2016}$:0.15[0.12;0.18]) with $MetaboAge_{2020}$ compared to the $MetaboAge_{2016}$(Appendix D). As results were consistent across platforms and the correlation between MetaboAge and MetaboHealth was consistent across cohorts, we used the 2020 re-quantified data in RS.

The figure represents **a.** the adjusted betas and 95 %-confidence intervals of the meta-analyses of the cross-sectional results; **b.** the adjusted betas and 95 %-confidence intervals of the meta-analyses of the retrospective results; **c.** the adjusted interaction terms between lifestyle and observation time and 95 %-confidence intervals of the meta-analyses of the longitudinal results. An asterisk indicates that the result was statistically significant after false discovery rate adjustment, a hashtag indicates that only one cohort was used to obtain the result. Legends and labels apply to all three graphs.

3.3. Cross-sectional associations between lifestyle and metabolomic age

Fig. 2a and Appendix D show the results of the cross-sectional analyses between lifestyle factors and age-independent metabolomics-based aging biomarkers. Given the overall consistency of findings across the three cohorts, we report results from the meta-analysis. However, an exception emerges for the association between waist circumference and MetaboAge, as larger waist circumference was linked to higher MetaboAge in DCS and RS (DCS:0.12[0.09;0.15], RS:0.26[0.23;0.29]), but not in UKBB (0.00[-0.02;0.01]). Study-specific results can be found in Appendix D. In all three cohorts, we observed associations between higher BMI with higher MetaboAge (0.14[0.03;0.24]) and MetaboHealth (0.16[0.14;0.17]). Additionally, larger waist circumference, current smoking, smoking quantity, sleeping long, and sleeping short were related to higher MetaboHealth, 0.19[0.15;0.22], 0.31[0.25;0.38], 0.11[0.06;0.15], 0.14[0.09;0.19], 0.05[0.02;0.09] respectively. Regular alcohol consumption was observed to correspond with lower MetaboAge (-0.04[-0.06;-0.02]) and MetaboHealth (-0.14[-0.19;-0.09]). Inverse associations with MetaboHealth were observed for adhering to the guidelines for physical activity (-0.15[-0.18;-0.13]), total physical activity (-0.05[-0.06;-0.04]), and per tertile of dietary guideline adherence (-0.07[-0.10;-0.04]). Information on the associations with the different elements of the dietary indexes can be found in Appendix D. All observed cross-sectional associations relationships but the association link between BMI and MetaboAge remained statistically significant after FDR-correction, and all but the association between regular alcohol intake and MetaboAge were independent of other lifestyle factors (Appendix D). A sensitivity analysis using linear regression in all cohorts did not notably change the results (Appendix E).

3.4. Cross-sectional: effects of sex

We observed heterogeneity by sex with higher effect sizes in the association between current smoking with MetaboHealth in men (0.38 [0.33;0.43]) compared to women:0.25 [0.16;0.34]). Furthermore, heterogeneity by sex was observed for the associations of adhering to the dietary guidelines with MetaboHealth with stronger inverse associations in men (-0.10[-0.16;-0.05] versus −0.04[-0.06;-0.01] in women). Lastly, we observed heterogeneity by sex for the association of daily alcohol consumption with MetaboHealth with only inverse associations in women (-0.03[-0.06;-0.01]) versus 0.01[-0.01;0.03] in men) (Appendix F).

Table 2
Meta-analyzed results from the cross-sectional analyses between scaled lifestyle factors and scaled metabolomics-based aging biomarkers.

	MetaboAge					MetaboHealth				
	B (CI)	pFDR	Q	Qp	I^2	B(CI)	pFDR	Q	Qp	I^2
Adhering to physical activity guidelines	-0.03 (-0.07;0.00)	0.14	3.93	0.14	32.69	-0.15 (-0.18;-0.13)	<0.0001	4.46	0.11	0.20
Total METhours/week	-0.01 (-0.05;0.02)	0.63	7.39	0.02	84.98	-0.05 (-0.06;-0.04)	<0.0001	3.75	0.15	0.04
BMI	0.14 (0.03;0.24)	0.04	155.77	<0.0001	98.65	0.16 (0.14;0.17)	<0.0001	3.30	0.19	31.66
Waist circumference	0.13 (-0.02;0.27)	0.24	259.02	<0.0001	99.18	0.19 (0.15;0.22)	<0.0001	10.60	4.99×10^{-3}	82.48
Current smoking	0.00 (-0.08;0.08)	0.99	8.60	0.01	77.46	0.31 (0.25;0.38)	<0.0001	5.17	0.08	64.88
Smoking quantity	0.00 (-0.01;0.02)	0.77	5.26	0.07	60.93	0.11 (0.06;0.15)	<0.0001	37.08	<0.0001	93.37
Adherence to dietary guidelines	-0.01 (-0.08;0.07)	0.91	22.03	<0.0001	94.33	-0.07 (-0.10;-0.04)	5.02×10^{-4}	7.64	0.02	73.49
Drinking alcohol at least once a week	-0.04 (-0.06;-0.02)	3.50×10^{-3}	0.76	0.68	0.00	-0.14 (-0.19;-0.09)	<0.0001	7.08	0.03	71.97
Alcoholic beverages per day	0.00 (-0.05;0.05)	0.97	34.81	<0.0001	92.84	-0.01 (-0.02;0.00)	0.40	2.12	0.35	0.79
Sleeping more than 8 hours	0.00 (-0.04;0.03)	0.97	2.10	0.35	0.13	0.14 (0.09;0.19)	<0.0001	2.27	0.32	25.16
Sleeping less than 7 hours	0.01 (-0.02;0.04)	0.53	2.87	0.24	22.38	0.05 (0.02;0.09)	5.98×10^{-3}	2.94	0.23	35.35

B indicates beta coefficient; CI, 95 %-confidence interval; pFDR, p-value after false discovery rate correction; Q, Cochran's Q; Qp, Cochran's Q p-value.
Analyses were adjusted for sex, socioeconomic status, and season of blood collection, time between baseline and follow-up, and -in the RS- metabolomics measurement batch.

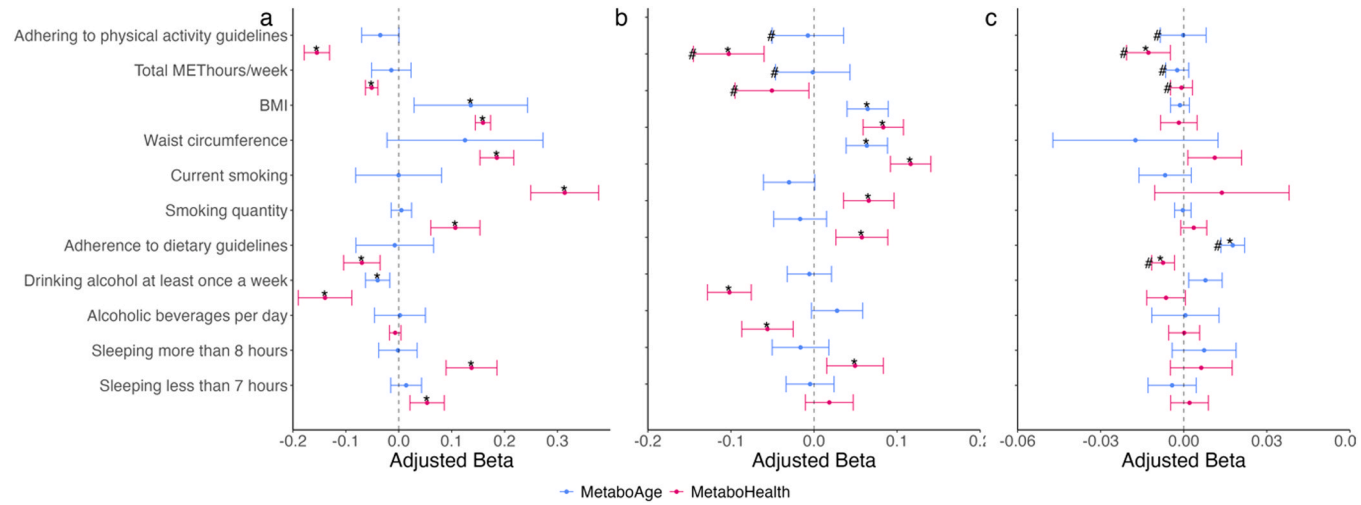

Fig. 2. Cross-sectional, retrospective and longitudinal associations of lifestyle with MetaboAge and MetaboHealth.

3.5. Retrospective: ten-year lifestyle changes and metabolomics-based aging biomarkers

In DCS and RS, but not in UKBB, information on lifestyle factors ten years prior to blood sampling was available (Fig. 1). We assessed the influence of lifestyle changes in the decade preceding blood collection on MetaboAge and MetaboHealth (Fig. 2b, Table 3, Appendix G). As results were similar across cohorts, we report the results of the meta-analysis. Results per cohort are detailed in Appendix G. Increases in BMI over a ten-year period were linked to higher MetaboAge and MetaboHealth, with effect sizes of 0.06[0.04;0.09] and 0.08[0.06;0.11], respectively. Similarly, increases in waist circumference over the same duration corresponded with higher MetaboAge (0.06[0.04;0.09]) and higher MetaboHealth (0.12[0.09;0.14]). Additionally, increase in smoking quantity (0.06[0.03;0.09]), smoking initiation (0.07 [0.04;0.10]), and starting to sleep long (0.05[0.02;0.08]) were related to higher MetaboHealth. Furthermore, initiation of regular alcohol consumption (-0.10[-0.13;-0.08]) or increase in alcohol consumption (-0.06 [-0.09;-0.03]) were associated with lower MetaboHealth. Starting to adhere to physical activity guidelines was linked to lower MetaboHealth (-0.10[-0.15;-0.06]) (DCS only, no data available in RS). All aforementioned associations remained significant after FDR-correction and all after adjustment for other lifestyle factors. We did not observe assay-differences nor notable alteration of the effect sizes after cell count adjustment (Appendix G).

Table 3
Random effect meta-analyzed associations between scaled ten-year-lifestyle-change and scaled MetaboAge and MetaboHealth.

	MetaboAge		MetaboHealth	
	B(CI)*	pFDR	B(CI)*	pFDR
Adhering to physical activity guidelines (Only available in DCS)	-0.01 (-0.05;0.04)	0.86	-0.10 (-0.15;-0.06)	<0.0001
Total METhours/week (Only available in DCS)	-0.00 (-0.05;0.04)	0.98	-0.05 (-0.10;-0.01)	0.07
BMI	0.06 (0.04;0.09)	<0.0001	0.08 (0.06;0.11)	<0.0001
Waist circumference	0.06 (0.04;0.09)	<0.0001	0.12 (0.09;0.14)	<0.0001
Current smoking	-0.03 (-0.06;0.00)	0.10	0.07 (0.04;0.10)	<0.0001
Smoking quantity	-0.02 (-0.05;0.02)	0.38	0.06 (0.03;0.09)	8.66×10^{-4}
Drinking alcohol at least once a week	-0.01 (-0.03;0.02)	0.74	-0.10 (-0.13;-0.08)	<0.0001
Alcoholic beverages per day	0.03 (-0.00;0.06)	0.12	-0.06 (-0.09;-0.03)	1.06×10^{-3}
Sleeping more than 8 hours	-0.02 (-0.05;0.02)	0.42	0.05 (0.02;0.08)	0.01
Sleeping less than 7 hours	-0.00 (-0.03;0.02)	0.81	0.02 (-0.01;0.05)	0.28

B indicates beta coefficient; CI, 95 %-confidence interval; pFDR, p-value after false discovery rate correction.
Analyses were adjusted for the baseline level of the lifestyle factor of interest, sex, socioeconomic status, season of blood collection, time between baseline and follow-up, and -in the RS- metabolomics measurement batch.
* Cochran's Q was 0.00 for all meta-analyzed results with a p-value of 1.00 and I^2 of 1.00

3.6. Retrospective: effects of sex

The Wald test did not reveal heterogeneity by sex in the retrospective analyses (Appendix H). Yet, we observed larger effect estimates in men for the association between increases in BMI and MetaboAge (0.11 [0.08;0.15] versus 0.03[0.00;0.07] in women). Additionally, we observed stronger inverse association in women for increase in alcohol consumption and initiation of regular alcohol consumption with MetaboHealth (daily alcoholic beverages:-0.10[-0.14;-0.05] versus −0.03[-0.07;0.02] in men, regular consumption:-0.14[-0.17;-0.10] versus −0.07[-0.11;-0.04] in men) (Appendix H).

3.7. Prospective: longitudinal changes in lifestyle and concomitant change in metabolomics-based aging biomarkers

Lifestyle data and metabolomics-based aging biomarkers were available at two distinct time points within both DCS and UKBB, respectively, ten years and five years apart (Fig. 1). In DCS, within-subject change accounted for 59.7 percent of the variation in MetaboAge and 54.9 percent of the variation in MetaboHealth. In UKB, these percentages were 49.1 for MetaboAge and 49.2 for MetaboHealth. MetaboAge changes exceeding the minimal detectable change threshold (MDC) were observed in 249 (8.2 %) DCS and 914 (6.1 %) UKBB participants. Additionally, in 172 (5.6 %) DCS and 711 (4.7 %) UKBB participants, we observed changes in MetaboHealth exceeding the MDC (Appendix I).

Using linear mixed-effect models with an interaction term of the lifestyle factors with observation time, we determined the longitudinal association between lifestyle factors and metabolomic aging in UKBB and DCS. Given the overall consistency of the results, we reported the results from the meta-analysis (Fig. 2c, Table 4, Appendix 9). Yet, in the

Table 4
Meta-analyzed interaction terms of age and lifestyle factors with MetaboAge and MetaboHealth.

	MetaboAge					MetaboHealth				
	B (CI)	pFDR	Q	Qp	I^2	B(CI)	pFDR	Q	Qp	I^2
Adhering to physical activity guidelines (Only available in DCS)	0.000 (-0.008;0.008)	1.00				-0.013 (-0.021;-0.005)	0.01			
Total METhours/week (Only available in DCS)	-0.002 (-0.007;0.002)	0.60				-0.001 (-0.005;0.003)	0.87			
BMI*	-0.001 (-0.005;0.002)	0.78	1.61	0.20	38.02	-0.002 (-0.008;0.005)	0.80	6.80	9.09×10–3	85.30
Waist circumference	-0.017 (-0.047;0.012)	0.58	120.58	<0.0001	99.17	0.011 (0.001;0.021)	0.13	14.18	1.66×10–4	92.95
Current smoking	-0.007 (-0.016;0.003)	0.49	0.26	0.61	0.00	0.014 (-0.011;0.038)	0.58	6.01	0.01	83.36
Smoking quantity	0.000 (-0.003;0.003)	0.91	1.37×10–3	0.97	0.00	0.004 (-0.001;0.008)	0.45	2.72	0.10	63.28
Adherence to dietary guidelines (Only available in UKBB)	0.018 (0.013;0.022)	<0.0001				-0.007 (-0.012;-0.003)	3.09×10^{-3}			
Drinking alcohol at least once a week	0.008 (0.002;0.014)	0.08	0.32	0.57	0.00	-0.006 (-0.013;0.001)	0.29	1.46	0.23	31.65
Alcoholic beverages per day	0.001 (-0.012;0.013)	0.98	17.39	<0.0001	94.25	0.000 (-0.005;0.006)	0.99	4.08	0.04	75.49
Sleeping more than 8 hours	0.007 (-0.004;0.019)	0.56	0.56	0.45	0.00	0.006 (-0.005;0.017)	0.58	0.53	0.47	0.00
Sleeping less than 7 hours	-0.004 (-0.013;0.004)	0.66	1.49	0.22	32.85	0.002 (-0.005;0.009)	0.80	0.18	0.67	0.00

B indicates beta coefficient; CI, 95 %-confidence interval; DCS, Doetinchem Cohort Study; pFDR, p-value after false discovery rate correction; Q, Cochran's Q; Qp, Cochran's Q p-value; UKBB, UK Biobank.
Analyses were adjusted for time to first measurement, sex, socioeconomic status, and season of blood collection.
* using uncalibrated metabolomics-based aging biomarkers

UKBB we observed longitudinal associations between current smoking and smoking quantity with higher MetaboHealth (current smoking: 0.027[0.010;0.044], smoking quantity: 0.006[0.002;0.010]) and increased waist circumference with lower MetaboAge (-0.033[-0.037;-0.029]), which were not observed in DCS (current smoking:0.002 [-0.008;0.013], smoking quantity: 0.001[-0.003;0.005], waist circumference:-0.002[-0.007;0.002]). Additionally, in DCS but not in UKBB, we observed a longitudinal link between daily alcohol consumption and higher MetaboAge (DCS:0.007[0.003;0.011], UKBB:-0.006[-0.010;0.002]).

In both cohorts, we observed a relationship between larger waist circumference and increased MetaboHealth (0.011[0.001;0.021]). Regular alcohol consumption was longitudinally associated with higher MetaboAge (0.008[0.002;0.014]). However, both associations lost statistical significance in the meta-analysis after adjustment for multiple testing. Adherence to the physical activity guidelines was longitudinally linked to lower MetaboHealth (-0.013[-0.021;-0.005]) (DCS only, no data available in UKBB) and adherence to the dietary guidelines was longitudinally associated with increased MetaboAge (0.018 [0.013;0.022]) and decreased MetaboHealth (-0.007[-0.012;-0.003]) (UKBB only, no data available in DCS). Information on the associations with the different elements of the dietary indexes and cohort-specific results can be found in Appendix J. We did not observe heterogeneity by sex in the prospective associations (Appendix K).

4. Discussion

This study is the first to measure MetaboAge and MetaboHealth multiple times, allowing for a comprehensive assessment of their responsiveness to lifestyle factors. Cross-sectionally and longitudinally, smoking, sleeping long, higher BMI, and larger waist circumference were associated with higher MetaboHealth, with BMI and waist also with higher MetaboAge. Higher scores on the metabolomics-based aging biomarkers indicate being biologically "older" than the chronological age's population average. Furthermore, we observed inverse links between alcohol consumption, adhering to dietary guidelines, and physical activity with MetaboHealth. The observed relationships among anthropometric measures, diet, and physical activity suggest the potential application of MetaboHealth as an early response outcome in dietary or physical activity trials.

Results were generally consistent across cohorts. I^2 and Cronchan's Q were larger in analyses including the UK Biobank. This is probably caused by the more in-depth phenotyping and distinct nature of DCS and RS as population-based cohorts from the UKBB, which suffers from healthy volunteer bias (Fry et al., 2017). Yet, the general consistency of the results strengthened our confidence in the described associations.

Fewer lifestyle factors were associated with MetaboAge than MetaboHealth, supporting existing evidence that MetaboHealth is a better predictor of adverse outcomes (Kuiper et al., 2023). This disparity may be due to MetaboAge being trained on chronological age, naturally increasing with time, while MetaboHealth is trained on mortality, influenced by lifestyle changes, which may better reflect biological age. MetaboHealth's higher responsiveness to longitudinal lifestyle modifications reinforces its effectiveness in capturing biological age over MetaboAge and the effects of lifestyle on biological aging.

The metabolomics-based aging biomarkers seemed to be relatively stable over time. Only a small number of individuals showed changes exceeding the MDC. This finding suggests that metabolomics-based aging biomarkers are better suited for capturing population-level changes than individual-level changes, which aligns with their original design as both MetaboAge and MetaboHealth metabolites are scaled per biobank (Van Den Akker et al., 2020; Deelen et al., 2019; Bizzarri et al., 2023a). Lifestyle intervention studies are needed to gain insight into the ability of metabolomics-based aging markers to capture individual changes in intervention settings, which means changes should preferably be measurable over a relatively short period of time (months).

Our results align with previous cross-sectional research linking higher BMI with higher MetaboAge and MetaboHealth, as well as larger waist circumference and higher MetaboHealth (Van Den Akker et al., 2020; Smit et al., 2023) Furthermore, we observed cross-sectional, retrospective, and prospective inverse associations between adhering to the physical activity guidelines and lower MetaboHealth. Additionally, adherence to dietary guidelines, both cross-sectionally and longitudinally, was linked to lower MetaboHealth. Importantly, most

associations between lifestyle factors and MetaboHealth and MetaboAge were independent of other lifestyle factors, suggesting that targeting a single lifestyle factor could already reduce aging disparities.

We also observed the earlier described cross-sectional inverse relationship between alcohol use and MetaboAge (Van Den Akker et al., 2020) Moreover, we observed cross-sectional associations between regular alcohol consumption and increases in alcohol consumption and starting regular alcohol consumption with lower MetaboHealth. HDL, one of the metabolites used to build the MetaboHealth and MetaboAge biomarkers, has a well-established inverse relationship with alcohol intake, as well as physical activity (Couillard et al., 2001; Kodama et al., 2007; De Oliveira e Silva et al., 2000; van de Luitgaarden et al., 2022) Conversely, we observed longitudinal associations between regular alcohol consumption and higher MetaboAge. Previous research on alcohol use and epigenetic aging biomarkers suggests a potential non-linear relationship, with both low and high alcohol consumption associated with higher epigenetic aging scores (Oblak et al., 2021) Studies on alcohol use and telomere length indicate that alcohol abuse accelerates aging, while no effect was found with moderate alcohol intake (Oblak et al., 2021) Yet, interpreting the effect of moderate alcohol intake on metabolomic aging as potentially beneficial is cautioned due to adverse outcomes associated with alcohol intake and potential biases in observational studies comparing no to low/moderate alcohol intake (Naimi et al., 2017; Room et al., 2005)

Our observations align with research investigating the associations of lifestyle factors with epigenetic aging biomarkers and telomere length. A meta-analysis of 156 studies examining the epigenetic aging biomarkers DNAm Horvath, DNAm Hannum, DNAm GrimAge, and DNAm PhenoAge found that BMI was associated with acceleration in all four biomarkers (Oblak et al., 2021) Additionally, smoking was linked to accelerated aging as measured by DNAm GrimAge, DNAm PhenoAge and telomere length, while physical activity was associated with deceleration in these three aging biomarkers (Oblak et al., 2021; Buttet et al., 2022) Waist circumference associations have been reported with accelerated epigenetic aging (Li et al., 2024; Kresovich et al., 2021) although contrary to the current study, results attenuated when adjusted for physical activity (Kresovich et al., 2021) While lifestyle associations with telomere length, epigenetic, and metabolomics-based aging biomarkers are largely consistent, these biomarkers are known to have a poor to moderate correlation (Kuiper et al., 2023; Vetter et al., 2019; Hastings et al., 2019; Belsky et al., 2018; Li et al., 2020) Intervention studies with combined measurements of these aging biomarkers are needed to more precisely identify which aspects of accelerated aging due to lifestyle are captured by these different biomarkers.

The study reveals cross-sectional variation by sex in the associations between lifestyle factors and metabolomics-based aging biomarkers. We observed stronger inverse associations of increases in and initiation of alcohol consumption over a ten-year period, albeit borderline statistically significant, with MetaboHealth in women compared to men. These findings suggest potential sex-related differences in the impact of alcohol on metabolomic aging, in line with previous research (Parker et al., 1996; Weidner et al., 1991) However, sex differences were longitudinally not observed with either MetaboAge or MetaboHealth. Yet, the point estimate in women (-0.016[-0.023;-0.008]) compared to men (-0.009[-0.020;0.002]) suggests also longitudinally stronger inverse effects of alcohol on MetaboHealth. Further research into the sex-specific impact of alcohol on metabolomic aging is necessary to gain a more comprehensive understanding.

Furthermore, we observed cross-sectional sex differences in the association between smoking and sleeping long with MetaboHealth. Additionally, we observed, although not statistically significant, sex differences in the association between ten-year BMI increase with MetaboAge. Cross-sectionally, modification by sex of the associations between sleep and BMI with metabolic syndrome and the association between smoking with MetaboHealth have been reported (Smit et al., 2023; Smiley et al., 2019; Slagter et al., 2017) The absence of observed longitudinal sex differences suggests that these differences may not be causal. However, our results lack sufficient conclusiveness to definitively support this inference. Further research on the longitudinal sex-specific association between smoking, sleep, and BMI with metabolomic aging is warranted.

Strengths of our study include the large study population originating from three prospective cohorts, the use of longitudinal lifestyle and metabolomics measures, and robustness across Nightingale assays. Furthermore, the inclusion of middle-aged participants enabled us to identify early determinants of accelerated aging. Limitations were the predominantly white study population, limiting our ability to investigate race differences and the generalizability of the results. Additionally, the varying time between measurements between DCS (ten years) and UKBB (five years) introduces a source of heterogeneity. This heterogeneity may potentially pose challenges to the direct comparison of the results and could impact the robustness of our findings.

In conclusion, in the first combined cross-sectional and longitudinal study on lifestyle factors associated with MetaboAge and MetaboHealth, we discovered robust associations between waist circumference, smoking, and alcohol status with metabolomics-based biomarkers of biological age. Dietary or physical activity trials are needed to further establish the performance of MetaboHealth in the assessment of accelerated aging on an individual level. Moreover, our findings demonstrated variations in these associations based on sex. Collectively, these results contribute to our understanding of the role of lifestyle in biological aging and offer valuable insights for future studies using metabolomics-based aging biomarkers as early response outcomes of accelerated aging.

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Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) used ChatGPT and Grammarly in order to identify redundant words and refine the English language in this work's preparation. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Declaration of Competing Interest

The authors declare that they have no competing interests.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mad.2024.111958.

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