Association between metabolomics-based biomarker scores and 10-year cognitive decline in men and women. The Doetinchem Cohort Study

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RESEARCH PAPER

Association between metabolomics-based biomarker scores and 10-year cognitive decline in men and women. The Doetinchem Cohort Study

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Abstract

Background: Metabolomic scores based on age (MetaboAge) and mortality (MetaboHealth) are considered indicators of overall health, but their association with cognition in the general population is unknown. Therefore, the association between MetaboAge/MetaboHealth and level and decline in cognition was studied, as were differences between men and women.
Methods: Data of 2821 participants (50% women, age range 45–75) from the Doetinchem Cohort Study was used. MetaboAge and MetaboHealth were calculated from ^{1}H-NMR metabolomics data at baseline. Cognitive domain scores (memory, flexibility and processing speed) and global cognitive functioning were available over a 10-year period. The association between MetaboAge/MetaboHealth and level of cognitive functioning was studied using linear regressions while for the association between MetaboAge/MetaboHealth and cognitive decline longitudinal linear mixed models were used. Analyses were adjusted for demographics and lifestyle factors.
Results: Higher MetaboAge, indicating poorer metabolomic ageing, was only associated with lower levels of processing speed in men. Higher MetaboHealth, indicating poorer immune-metabolic health, was associated with lower levels of cognitive functioning for all three domains and global cognitive functioning in both men and women. Only in men, MetaboHealth was also associated with 10-year decline in flexibility, processing speed and global cognition. Metabolites that contributed to the observed associations were in men mainly markers of protein metabolism, and in women mainly markers of lipid metabolism and inflammatory metabolites.
Conclusions: MetaboHealth, not MetaboAge, was associated with cognitive functioning independent of conventional risk factors. Individual metabolites affect cognitive functioning differently in men and women, suggesting sex-specific pathophysiological pathways underlying cognitive functioning.

Keywords: metabolomics; cognitive function; longitudinal; sex differences; biomarker; older people

Key Points

- Associations between metabolomics-based biomarker scores such as MetaboHealth and cognition in the general population are unknown.

- Worse metabolomic health was associated with lower cognitive function in men and women.
- In men, worse metabolomic health was also associated with 10-year decline in flexibility, processing speed and global cognition.
- Individual metabolites that drove these associations differed between men (amino acids) and women (lipids and inflammation).
- Our results suggest sex-specific pathways involved in cognition and cognitive decline.

Introduction

The number of individuals with dementia rapidly increases [1, 2]. Dementia is characterised by a gradual decline in cognitive functioning impairing individuals' daily functioning [2], which can precede the diagnosis of dementia by several decades. There is large heterogeneity in cognitive functioning: some individuals preserve good cognitive functioning up to high ages, while others become cognitively impaired already relatively early in life [3]. Additionally, we know that this heterogeneity also differs between men and women [4]. With the current absence of curative interventions for dementia, gaining insight into modifiable factors that are associated with this heterogeneity is fundamental as potential targets for primary prevention of dementia [5].

Besides conventional risk factors, such as education and lifestyle factors [6], a variety of metabolites have been found to be associated with levels of cognitive functioning and rate of decline, with differences between men and women [7–10]. However, there is still a lack of consensus regarding the specific metabolites associated with cognitive decline, as each study included different metabolites/metabolite platforms [9]. A broader approach by using metabolomic scores might be useful to study in relation to cognitive functioning for risk stratification and guiding treatment options.

Metabolomic scores have been constructed from blood-based metabolomics data to either indicate the metabolomic age or immune-metabolic health of individuals, which indicates physiological vulnerability in middle and old age. Additionally, metabolomic scores have the potential to offer additional insights independent of conventional risk factors, identifying those individuals who are more susceptible to a lower level of cognitive functioning and/or faster decline. These specific scores were trained on either chronological age, i.e. MetaboAge [11], or prospective mortality, i.e. MetaboHealth [12]. Individuals that were metabolomically older, based on MetaboAge, were at increased risk for adverse health outcomes such as cardiovascular diseases and mortality [11]. For MetaboHealth, it was shown that it can be predictive, independent of classical markers, of all-cause and disease-specific mortality [12] and frailty [13]. MetaboHealth has also been shown to be associated with cognitive functioning in older adults with an increased risk of cardiovascular disease [14]. Nevertheless, it is unknown whether these metabolomic scores are associated with cognitive functioning in the general population.

The aim of this study is to examine the association between both MetaboAge and MetaboHealth, and level of and decline in cognitive function (memory, flexibility, processing speed and global cognition) over a period of 10 years. The association between individual metabolites that constitute the metabolomic scores and cognitive functioning is studied to gain more insight into potential pathophysiological pathways. Given that metabolite levels and cognitive functioning differ between men and women, we hypothesise that our metabolomic scores are differently associated with cognitive functioning in men and women. Therefore, all our analyses were stratified by sex [4, 10].

Methods

Study population

Data of the Doetinchem Cohort Study (DCS), a long-running cohort study on the impact of lifestyle and biological risk factors on health in Dutch adults, were used [15, 16]. For the current analyses, participants from round 4 (R4) onwards ($n = 4517$) were included because metabolomics (Nightingale Health Ltd, Helsinki, Finland) were collected for R4. Cognitive functioning was assessed for all participants aged ≥45 years (age range 45–76) at R4, R5 and R6. Women who were pregnant at R4 were excluded ($N = 3$). Participants without missing values on metabolomics and covariates across rounds were included [R4 (2003–2007) $N = 2821$, R5 (2008–2012) $N = 2364$, R6 (2013–2017) $N = 1947$]. Visual impairments, such as colour blindness, which makes the Stroop Colour Word Test impossible, led to some variations in included number of participants across different cognitive domains (percentage of missing data ranged between 0.1% and 1.1%).

Written informed consent was received from all participants. The Medical Ethics Committees of the Netherlands Organization of Applied Scientific Research approved the DCS (approval number 07-233/O).

Cognition

Participants completed the 15-Word Verbal Learning Test [17], the Stroop Colour Word Test [18], the Letter Digit Substitution Test [19] and the Verbal Fluency Test [20]. Three domains of cognition were assessed (memory, flexibility and processing speed) as well as global cognitive functioning. More details about the assessment and calculation of the cognitive domains have been published before [21] and can be found in Appendix 1. As our cognitive domains and global cognitive functioning consisted of scores from various tests, sex-specific z-scores were calculated using the mean and

standard deviation of the baseline test (R4). A higher *z*-score represents a higher level of cognitive functioning.

MetaboAge and MetaboHealth

In 2020, 226 metabolomic markers were measured in stored EDTA plasma via high-throughput nuclear magnetic resonance (^{1}H NMR) for all participants at R4 with available blood samples ($N = 4464$) (Nightingale Health Ltd) [22].

MetaboAge [11] and MetaboHealth [12] were calculated using the scripts of the algorithms available on the GitHub of D. Bizzari (https://github.com/DanieleBizzarri/MiMIR). With elastic net, MetaboAge was trained based on the association with chronological age in 25 000 individuals. MetaboAge constitutes 63 metabolites [11] that were given a weight to calculate an individual's score (Appendix 2). A higher MetaboAge represents a higher metabolomic age. The MetaboHealth algorithm was trained based on the association with mortality in 44 000 persons and constitutes 14 metabolites. To calculate an individual's MetaboHealth, the metabolites [based on the results of this meta-analysis, i.e. ln(hazard ratio)] were multiplied by their weights and summed, generating a score ranging from −2 to +3 [12], reflecting metabolomic health (Appendix 2). A higher MetaboHealth represents higher mortality risk and poorer immune-metabolic health. As this algorithm is also scaled on the input sample, MetaboHealth was calculated separately for men and women. The 14 metabolites included in MetaboHealth are also included in MetaboAge.

Covariates

Socio-demographics (age, sex, education), lifestyle factors and chronic disease risk factors were collected at each round (R4, R5 and R6). Education was categorised according to highest attained educational level at R4; low; medium; high. Self-reported smoking status was categorised as never; former; current smoker. Self-reported physical activity per week was categorised as <0.5 h (inactive); 0.5–3.5 h (moderately inactive); ≥3.5 h, of which <2 h vigorous (moderately active); ≥3.5 h, of which ≥2 h vigorous (active) [23]. Self-reported alcohol use was categorised as [24] no; moderate [>0 and ≤1 glass/day (women) or >0 and ≤2 glasses/day (men)]; high [>1 glass/day (women) or >2 glasses/day (men)]. Trained staff measured waist circumference (centimetres) and systolic blood pressure (mmHg) according to standardised protocols. Use of antihypertensive medication (yes; no) was self-reported. ApoE4 status (non-carrier; carrier) was determined in DNA at round 4.

Statistical analyses

Baseline characteristics at R4 are displayed in Table 1 stratified by sex. In this table, participants without missing values on MetaboAge/MetaboHealth and covariates were included. Characteristics stratified by sex at R5 and R6 are displayed in Appendix 3 and Appendix 4. For descriptive purposes only, the 'MetaboHealth' score scaled on the input sample including both men and women is shown, as well as cognition scores at R4, which were standardised on the total sample (men and women combined).

The cross-sectional association between MetaboAge/MetaboHealth at baseline (R4) and level of cognitive functioning at baseline (R4) was analysed with multivariable-adjusted linear regression models. The longitudinal association between MetaboAge/MetaboHealth at baseline (R4) and decline in cognitive functioning during the subsequent rounds (R4, R5 and R6) was analysed using linear mixed models. Interaction effects between MetaboAge and MetaboHealth with chronological age (both age and age^2) were included to investigate cognitive change, i.e. cognitive decline. Chronological age was used to operationalise time due to the variability in calendar time between measurements. Age^2 was included for better age correction. The estimates and 95% confidence intervals (CI) each of MetaboAge and MetaboHealth reflect the level of cognitive functioning at R4. The estimates and 95% CI of the interaction effects of MetaboAge/MetaboHealth with chronological age and age^2 reflect the association of MetaboAge and MetaboHealth with cognitive decline per calendar year from R4 to R6.

Three models were fitted for each cognitive domain and global cognitive functioning in both the cross-sectional and longitudinal models. The first model included MetaboAge/MetaboHealth score, age, age^2, education, number of cognitive measurements (adjusting for learning effects) and ApoE4 status. In the longitudinal models, interaction effects between MetaboAge/MetaboHealth with chronological age and age^2 were also included. If these interaction effects were not statistically significant (*P*-values >.05), they were not included in the subsequent model. In the second model, lifestyle factors (i.e. smoking, alcohol and physical activity) were added. The third model additionally included cardiometabolic factors (i.e. waist circumference, systolic blood pressure and antihypertensive medication use) in order to investigate the possible mediating role of these cardiometabolic factors. We consider the second model (i.e. adjusted for demographics and lifestyle factors) as our main model. The aim of the third model is to investigate whether metabolomic age/health have added value beyond cardiometabolic factors that are often routinely collected.

For the longitudinal models, MetaboAge/MetaboHealth, education, number of cognitive measurements and ApoE4 status were taken into account as time-independent variables; the other covariates as time-dependent variables. Chronological age was centred around ∼65 years because this was the mean age of the population and to improve convergence of the model. For the cognitive domains and global cognitive functioning that were statistically significantly associated with MetaboAge or MetaboHealth after adjustment for demographic and lifestyle factors (model 2), the analyses were repeated for the individual metabolites that constitute the MetaboAge/MetaboHealth score. For single metabolites, we did not run model 3. In these analyses, for comparability the individual metabolites were standardised and a false discovery rate (FDR) was applied to account

for multiple comparisons [25]. All analyses were done in R.

Results

Table 1 shows baseline characteristics for participants without missing values on MetaboAge/MetaboHealth and the covariates stratified by sex at R4. At baseline, the mean age of the total study population was 60.3 years (SD 7.2, age range [45.0–75.9]) and 50% were men. Men seemed metabolically more healthy as they had a lower metabolomic age score and lower metabolomic health score than women. At baseline, men were more often highly educated, a former smoker, current drinker and had lower scores on all cognitive domains than women.

Cross-sectional associations of MetaboAge with level of cognitive functioning

In men, higher MetaboAge (R4) was only associated with a lower level of processing speed (R4) ($\beta = -0.005$, 95% CI = [−0.01;–0.001]) after adjustment for demographic and lifestyle factors (Table 2). This association was no longer statistically significant after further adjustment for cardiometabolic factors (Table 2). Exploring the individual metabolites that constitute MetaboAge, we did not observe any association with level of processing speed after applying an FDR correction (Appendix 5). In women, MetaboAge was not associated with any of the cognitive domains and global cognitive functioning (Table 2).

Cross-sectional association between MetaboHealth and level of cognitive functioning

Higher MetaboHealth (R4) was associated with lower level of memory, flexibility, processing speed and global cognitive functioning (R4) after adjustment for demographic and lifestyle factors in both men and women. In men, β estimates for the association between MetaboHealth and cognitive domains/global cognition ranged from $\beta = -0.15$ (95% CI = [−0.26;–0.04]) for memory to $\beta = -0.08$ (95%

Table 1. Baseline characteristics of round 4 stratified by sex

	Men (n = 1399)		Women (n = 1422)	
	Mean/%	SD/n	Mean/%	SD/n
Demographics				
Chronological age	60.5	7.1	60.2	7.2
Education				
Low	7.7	108	10.3	147
Medium	64.5	903	70.7	1006
High	27.7	388	18.9	269
Exposures				
MetaboAge [−17.7;123.7]	59.8	9.3	61.0	9.4
MetaboHealth[a] [−1.5;2.3]	−0.02	0.44	0.05	0.41
Smoking				
Never	26.9	376	39.7	565
Former	56.1	785	40.9	581
Current	17.0	238	19.4	276
Drinker				
0 glass/day	20.0	280	40.8	580
>0 and ≤2 (men) or >0 and ≤1 (women) glass/day	48.5	678	31.0	441
>2 (men) or >1 (women) glass/day	31.5	441	28.2	401
Physical activity[b]				
Inactive	10.4	146	9.4	133
Moderately inactive	30.4	425	32.7	465
Moderately active	42.0	587	46.9	667
Active	17.2	241	11.0	157
Waist circumference (cm)	101	9.7	92	11.5
Systolic pressure (mmHg)	136.0	17.5	132.0	18.5
Antihypertensive medication, yes (%)	19.5	273	21.7	309
ApoE4 carriers (%)	26.9	377	29.3	417
Cognitive function R4 [c]				
Memory	−0.23	0.9	0.23	0.9
Flexibility	−0.06	1.0	0.06	1.0
Processing speed	−0.08	0.9	0.09	0.9
Global cognition	−0.12	0.7	0.12	0.8

Percentage missing for the cognitive scores ranged between 0.1% and 1.1% [a]MetaboHealth calculated on input sample of men and women to facilitate the comparison of baseline characteristics [b]Physical activity: inactive <0.5 h; moderately inactive 0.5–3.5 h; moderately active ≥3.5 h, of which <2 h vigorous; active ≥3.5 h, of which ≥2 h or more vigorous [c]Z-scores were calculated in a sample including men and women to facilitate the comparison of baseline characteristics

Table 2. The association of MetaboAge/MetaboHealth and cognitive functioning. Beta estimates and 95% confidence intervals represent the effect of MetaboAge/MetaboHealth on *z*-scores of cognitive functioning stratified by sex

	Memory		Flexibility		Processing speed		Global cognition	
	β	[95% CI]	*β*	[95% CI]	*β*	[95% CI]	*β*	[95% CI]
MetaboAge level*								
Men								
Model 1	−0.002	−0.007;0.003	0.001	−0.004;0.006	**−0.005**	**−0.01;–0.001**	−0.002	−0.006;0.002
Model 2	−0.002	−0.007;0.003	0.001	−0.004;0.006	**−0.005**	**−0.01;–0.001**	−0.002	−0.006;0.002
Model 3	−0.002	−0.007;0.004	0.001	−0.004;0.006	−0.004	−0.01;0.0003	−0.002	−0.01;0.002
Women								
Model 1	0.001	−0.004;0.01	−0.003	−0.01;0.002	0.00001	−0.004;0.004	−0.00001	−0.004;0.004
Model 2	0.001	−0.004;0.01	−0.003	−0.008;0.003	0.00001	−0.004;0.004	0.0001	−0.004;0.004
Model 3	0.001	−0.004;0.01	−0.002	−0.01;0.003	0.001	−0.003;0.005	0.001	−0.003;0.004
MetaboHealth level								
Men								
Model 1	**−0.17**	**−0.28;–0.06**	**−0.14**	**−0.25;–0.03**	**−0.15**	**−0.24;–0.06**	**−0.11**	**−0.19;–0.03**
Model 2	**−0.15**	**−0.26;–0.04**	**−0.12**	**−0.24;–0.01**	**−0.10**	**−0.19;–0.01**	**−0.08**	**−0.16;–0.002**
Model 3	**−0.11**	**−0.23;–0.0002**	−0.09	−0.20;0.02	−0.06	−0.16;0.03	−0.05	−0.13;0.03
*Women**								
Model 1	**−0.18**	**−0.29;–0.07**	**−0.22**	**−0.33;–0.11**	**−0.21**	**−0.30;–0.11**	**−0.16**	**−0.24;–0.08**
Model 2	**−0.17**	**−0.28;–0.06**	**−0.20**	**−0.31;–0.08**	**−0.18**	**−0.28;–0.09**	**−0.15**	**−0.23;–0.07**
Model 3	**−0.14**	**−0.25;–0.03**	**−0.17**	**−0.29;–0.06**	**−0.15**	**−0.25;–0.06**	**−0.12**	**−0.20;–0.04**
MetaboHealth decline								
Men								
Model 1	*		**−0.02**	**−0.03;−0.01**	**–0.01**	**−0.02;−0.01**	**−0.01**	**–0.01;–0.002**
Model 2	*		**−0.02**	**−0.03;−0.01**	**–0.01**	**−0.02;−0.01**	**−0.01**	**–0.01;–0.003**
Model 3	*		**−0.02**	**−0.03;−0.01**	**–0.01**	**−0.02;−0.01**	**−0.01**	**–0.01;–0.003**

Level: effect of MetaboAge/MetaboHealth at R4 on level of cognitive functioning at round 4 Decline: effect of MetaboAge/MetaboHealth at R4 on cognitive decline per calendar year (based on R4, R5 and R6) Model 1 adjusted for the following variables: age (centred around 65 years), age^2, education, number of cognitive measurements, ApoE4 Model 2 adjusted for the following variables: mod1 + lifestyle factors (i.e. smoking, alcohol and physical activity) Model 3 adjusted for the following variables: mod2 + cardiometabolic factors (i.e. waist circumference, systolic blood pressure and antihypertensive medication use) Bold values reflect statistically significant results ($P < .05$) *Decline was not included as the effect of MetaboAge/MetaboHealth at R4 on cognitive decline was not significant

CI = [−0.16;–0.002]) for global cognitive functioning. Except for memory, these associations did not remain statistically significant after adjustment for cardiometabolic factors (Table 2). Exploring the individual metabolites that constitute MetaboHealth, none of the individual metabolites was associated with memory and global cognitive functioning after FDR correction (Appendix 6 and Appendix 9). Lower levels of lactate were associated with lower level of flexibility and processing speed (Appendix 7 and Appendix 8). Lower levels of individual amino acids (i.e. leucine, isoleucine and valine) were associated with lower level of processing speed (Appendix 8).

In women, β estimates for the association between MetaboHealth and cognitive domains/global cognition ranged from $\beta = -0.20$ (95% CI = [−0.31;–0.08]) for flexibility to $\beta = -0.15$ (95% CI = [−0.23;–0.07]) for global cognitive functioning after adjustment for demographic and lifestyle factors (Table 2). After further adjustment for cardiometabolic factors, these associations remained statistically significant (Table 2). Exploring the individual metabolites that constitute MetaboHealth, no associations were found for memory and flexibility after FDR correction (Appendix 6 and Appendix 7). Higher levels of total lipids in chylomicrons and extremely large very-low-density lipoprotein (VLDL) were associated with lower levels of processing speed (Appendix 8), while higher levels of glycoprotein acetyls were associated with a lower level of processing speed and global cognitive functioning after FDR correction (Appendix 8 and Appendix 9).

MetaboAge and MetaboHealth and cognitive decline

There was no interaction effect of MetaboAge with age and age^2 in neither men nor women, indicating that MetaboAge was not associated with cognitive decline. Studying the interaction effects between MetaboHealth with age and age^2 showed that an interaction effect between age and MetaboHealth was observed only in men, for all domains except for memory. This means that men with higher MetaboHealth scores at baseline had a faster decline in flexibility, processing speed and global cognition scores than men with a lower score (Table 2). The effect sizes of the interaction effects with age were similar for all domains, with a β estimate of $\beta = -0.02$ (95% CI = [−0.03;–0.01]) for the interaction between MetaboHealth score and flexibility, which means an extra decline of 0.02 (*z*-score) in flexibility per year per MetaboHealth unit. After further adjustment for

cardiometabolic factors, these associations remained nearly identical. Exploring the individual metabolites that constitute MetaboHealth, higher baseline levels of glucose were associated with faster decline in flexibility and processing speed in men (Appendix 7 and Appendix 8).

Discussion

The association of MetaboAge and MetaboHealth with level of and decline in cognitive functioning with 10-year follow-up was investigated in both men and women. A higher MetaboAge was only associated with a lower level of processing speed in men. A higher MetaboHealth was associated with lower levels of cognitive functioning across all domains and global cognitive functioning in both men and women after adjustment for demographic and lifestyle factors. Only in men, a higher MetaboHealth was associated with faster decline in flexibility, processing speed and global cognitive functioning over 10 years of observation. Some of these associations remained significant after adjustment for other cardiometabolic factors. This was the case for all domains of cognitive functioning in women and for cognitive decline in most domains in men, indicating that the vulnerability detected by the MetaboHealth adds to markers of cardiometabolic health.

MetaboAge was not associated with any of the cognitive domains, except for level of processing speed in men. This result might indicate that an indicator based on the relation of metabolites with chronological age is not informative on cognitive function. However, as this study is the first population-based study, this research needs to be replicated to substantiate this conclusion. Higher MetaboHealth, however, based on time-to-event analysis (mortality), was associated with lower levels of cognitive functioning in all cognitive domains and global cognitive functioning in both men and women. Our results are consistent with studies that showed that second-generation biomarkers, such as MetaboHealth, that are trained to predict health-related outcomes outperform first-generation biomarkers, such as MetaboAge, that are trained to predict chronological age [13, 26]. In our study, the effect sizes for the association of MetaboHealth at baseline with each domain of level of cognitive functioning (i.e. memory, flexibility and processing speed) and global cognitive functioning were comparable between men and women. These findings suggest that this score similarly represents the level of cognitive functioning in men and women. In addition, in women MetaboHealth adds additional information to markers of cardiometabolic health. Our results highlight that the importance of factors and pathways differs between men and women. However, previous research rarely stratified their analyses by sex and thus research into sex differences in metabolomic age/health is still scarce. Fortunately, this area of research is gaining more attention, and we recommend that future studies stratify analyses by sex to better understand these differences.

MetaboAge was not associated with cognitive decline in any domain, neither in men nor women. Only in men, a higher MetaboHealth at baseline was associated with faster decline in flexibility, processing speed and global cognitive functioning. Previous studies on this topic are absent. A systematic review on metabolites that appear to be linked to memory decline (not stratified by sex) identified metabolites that are not included in the MetaboHealth score [27], like sphingolipids. This finding implies that other metabolites could further contribute to the metabolomic score used in this study with respect to the association with memory decline. Further investigation into this area is warranted. Our results highlight that MetaboHealth is differently associated with decline in cognitive functioning in men and women because only in men a high MetaboHealth score is associated with faster cognitive decline.

Looking into the individual metabolites that constitute MetaboHealth, in men specifically amino acid–related metabolites were associated with processing speed, while in women specifically the inflammatory marker glycoprotein acetyls and lipid-related metabolites were associated cognitive functioning. In past studies, metabolites, among others high-density lipoprotein subfractions, docosahexaenoic acid and glycoprotein acetyls, were separately studied in relation to cognitive functioning and were found to be associated with cognitive functioning [7, 9, 10]. Only in the study of Proitsi *et al*. [10] analyses were stratified by sex, and they also observed that metabolites associated with cognitive functioning differ between men and women. These results support our findings suggesting the presence of distinct pathophysiological pathways for men and women concerning cognitive functioning.

The strengths of our study include the 10-year follow-up period with a broad range of cognitive tests. Furthermore, the large sample size enabled us to stratify for sex. A limitation is that our population consists mainly of white participants; therefore, the results cannot be generalised to other ethnic groups. Future studies are thus needed to replicate this research with a more diverse ethnic population sample. Moreover, as in each cohort study, there is a potential for attrition bias. However, as those who dropped out are in general more unhealthy than those included, the association of MetaboHealth with cognition and cognitive decline might be even stronger.

In conclusion, a higher MetaboHealth score was consistently inversely associated with cognitive functioning in both men and women, and also with 10-year cognitive decline in flexibility, processing speed and global cognitive functioning in men. MetaboAge was not associated with cognitive functioning, except for processing speed in men. In men, markers of amino acids were contributing most to the association of MetaboHealth with cognitive functioning, while in women, markers of lipid metabolism and inflammatory metabolites contributed most. This suggest the existence of sex-specific pathophysiological pathways in cognitive functioning. MetaboHealth, considered to be an indicator of overall health in older individuals, may enrich scores

based on dementia-specific markers to predict outcomes. However, as this study is one of the first studies exploring the relationship between MetaboAge and MetaboHealth and (decline in) cognitive functioning in the general population, it is essential for our findings to be replicated.

Research data transparency and availability

Data and code(books) that support this manuscript are available from the corresponding author upon reasonable request. Before the data can be shared, a data access agreement needs to be signed.

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Supplementary Data: Supplementary Data is available at *Age and Ageing* online.

Declaration of Conflicts of Interest: None declared.

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References

1. World Health Organization. *World Report on Ageing and Health*. Geneva: World Health Organization, 2015.
2. 2022 Alzheimer's disease facts and figures. *Alzheimers Dement* 2022; **18**: 700–89.
3. Tucker-Drob EM, Brandmaier AM, Lindenberger U. Coupled cognitive changes in adulthood: a meta-analysis. *Psychol Bull* 2019; **145**: 273–301.
4. Nooyens ACJ, Wijnhoven HAH, Schaap LS *et al.* Sex differences in cognitive functioning with aging in the Netherlands. *Gerontology* 2022; **68**: 999–1009.
5. Gonzales MM, Garbarino VR, Pollet E *et al.* Biological aging processes underlying cognitive decline and neurodegenerative disease. *J Clin Invest* 2022; **132**: e158453.
6. Baumgart M, Snyder HM, Carrillo MC *et al.* Summary of the evidence on modifiable risk factors for cognitive decline and dementia: a population-based perspective. *Alzheimers Dement* 2015; **11**: 718–26.
7. van der Lee SJ, Teunissen CE, Pool R *et al.* Circulating metabolites and general cognitive ability and dementia: evidence from 11 cohort studies. *Alzheimers Dement* 2018; **14**: 707–22.
8. Huo Z, Rana BK, Elman JA *et al.* Metabolic profiling of cognitive aging in midlife. *Front Aging Neurosci* 2020; **12**: 555850.
9. Jiang Y, Zhu Z, Shi J *et al.* Metabolomics in the development and progression of dementia: a systematic review. *Front Neurosci* 2019; **13**: 343.
10. Proitsi P, Kuh D, Wong A *et al.* Lifetime cognition and late midlife blood metabolites: findings from a British birth cohort. *Transl Psychiatry* 2018; **8**: 203.
11. van den Akker EB, Trompet S, Barkey Wolf JJH *et al.* Metabolic age based on the BBMRI-NL (1)H-NMR metabolomics repository as biomarker of age-related disease. *Circ Genom Precis Med* 2020; **13**: 541–7.
12. Deelen J, Kettunen J, Fischer K *et al.* A metabolic profile of all-cause mortality risk identified in an observational study of 44,168 individuals. *Nat Commun* 2019; **10**: 3346.
13. Kuiper LM, Polinder-Bos HA, Bizzarri D *et al.* Epigenetic and metabolomic biomarkers for biological age: a comparative analysis of mortality and frailty risk. *J Gerontol A* 2023; **78**: 1753–62.
14. Zonneveld MH, Kuhaili NA, Mooijaart SP *et al.* Increased ^{1}H-NMR metabolomics-based health score associates with declined cognitive performance and functional independence in older adults at risk of cardiovascular disease. *medRxiv* 2023.
15. Verschuren WM, Blokstra A, Picavet HS *et al.* Cohort profile: The Doetinchem Cohort Study. *Int J Epidemiol* 2008; **37**: 1236–41.
16. Picavet HSJ, Blokstra A, Spijkerman AM *et al.* Cohort profile update: The Doetinchem Cohort Study 1987–2017: lifestyle, health and chronic diseases in a life course and ageing perspective. *Int J Epidemiol* 2017; **46**: 1751–1751g.
17. Van Der Elst W, Van Boxtel MP, Van Breukelen GJ *et al.* Rey's verbal learning test: normative data for 1855 healthy participants aged 24–81 years and the influence of age, sex, education, and mode of presentation. *J Int Neuropsychol Soc* 2005; **11**: 290–302.
18. Van der Elst W, Van Boxtel MP, Van Breukelen GJ *et al.* The Stroop color-word test: influence of age, sex, and education; and normative data for a large sample across the adult age range. *Assessment* 2006; **13**: 62–79.
19. Van der Elst W, van Boxtel MP, van Breukelen GJ *et al.* The Letter Digit Substitution Test: normative data for 1,858 healthy participants aged 24–81 from the Maastricht Aging Study (MAAS): influence of age, education, and sex. *J Clin Exp Neuropsychol* 2006; **28**: 998–1009.
20. Van Der Elst W, Van Boxtel MP, Van Breukelen GJ *et al.* Normative data for the Animal, Profession and Letter M Naming verbal fluency tests for Dutch speaking participants and the effects of age, education, and sex. *J Int Neuropsychol Soc* 2006; **12**: 80–9.
21. Nooyens AC, Bueno-de-Mesquita HB, van Boxtel MP *et al.* Fruit and vegetable intake and cognitive decline in middle-aged men and women: The Doetinchem Cohort Study. *Br J Nutr* 2011; **106**: 752–61.
22. Würtz P, Kangas AJ, Soininen P *et al.* Quantitative serum nuclear magnetic resonance metabolomics in large-scale epidemiology: a primer on -omic technologies. *Am J Epidemiol* 2017; **186**: 1084–96.
23. Pols MA, Peeters PH, Ocké MC *et al.* Estimation of reproducibility and relative validity of the questions included in the

EPIC Physical Activity Questionnaire. *Int J Epidemiol* 1997; **26**: S181–9.
24. U.S. Department of Agriculture and U.S. Department of Health and Human Services. *Dietary Guidelines for Americans, 2020–2025*9th Edition. December 2020. Available at DietaryGuidelines.gov.
25. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc B Methodol* 1995; **57**: 289–300.
26. Maddock J, Castillo-Fernandez J, Wong A *et al.* DNA methylation age and physical and cognitive aging. *J Gerontol A* 2019; **75**: 504–11.
27. Tian Q, Mitchell BA, Corkum AE *et al.* Metabolites associated with memory and gait: a systematic review. *Metabolites* 2022; **12**: 356.

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