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How do metabolic processes age: Evidence from human metabolomic studies

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Abstract

Metabolomics, the global profiling of small molecules in the body, has emerged as a promising analytical approach for assessing molecular changes associated with ageing at the population level. Understanding root metabolic ageing pathways may have important implications for managing age-related disease risk. In this short review, relevant studies published in the last few years that have made valuable contributions to this field will be discussed. These include large-scale studies investigating metabolic changes with age, metabolomic clocks, and metabolic pathways associated with ageing phenotypes. Recent significant advances include the use of longitudinal study designs, populations spanning the whole life course, standardised analytical platforms of enhanced metabolome coverage and development of multivariate analyses. While many challenges remain, recent studies have demonstrated the considerable promise of this field.

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Introduction

Research interest in the ageing process has grown in recent years, partly due to the pressure on health systems due to a rapidly ageing global population [1], but also due to breakthroughs in the field of geroscience that suggest both health and lifespan can be extended through targeting of the ageing process itself as an underlying cause of disease [2]. Ageing can be considered hierarchical in nature incorporating functional ageing, phenotypic ageing and at its root, biological ageing [3]. The cellular and molecular changes that characterise biological ageing have been further delineated into nine "hallmarks of ageing" [4]. As biological ageing is believed to precede phenotypic and functional decline [3], identifying these molecular changes may have great implications for biomedical research.

Metabolomics, the global profiling of small molecules (typically <1500 Da) in the body, has emerged as a promising analytical approach for assessing molecular changes at the population level. Overall, the rate of metabolism declines with old age [5] and more specifically all ageing hallmarks are expected to have detectable effects on the metabolome, including hallmarks of ageing such as "nutrient sensing", "mitochondrial dysfunction", and "altered intracellular communication" which directly relate to metabolic alterations [6]. As the final product of metabolism, circulating metabolites, measured through liquid-chromatography mass spectrometry (LC-MS) or nuclear magnetic resonance spectroscopy (NMR) analysis of accessible biofluids such as blood, can provide a summary picture of biological processes associated with age in organs and tissues throughout the body.

Many recent reviews have summarised metabolomic studies of ageing published to date [7–11]. The most comprehensive review by Panyard et al. [12] identified 35 studies published since 2006, with sample sizes ranging from 60 to over 23,000 participants. They summarized their findings into major pathways of metabolomic aging and, despite the acknowledged difficulties in comparing results across metabolomic studies, some consistent findings emerged within these pathways. These include among amino acids measured in blood, a decrease in tryptophan and an increase in tyrosine with age; among lipids and lipoproteins there is a decrease in HDL, and increases in LDL, triglycerides, and cholesterol; and steroid hormones including DHEA-S, androgens, progestins and pregnenolones generally decrease. Among markers of renal excretion, urea and creatine in blood increased, while conversely urinary creatine decreased with age. Metabolites identified as indicative of oxidative stress included acylcarnitines, glutathione, ophthalmic acid and sphingomyelins; while metabolite changes grouped within the inflammation

pathway included increases in ornithine and kynurenic acid. In the following sections, we highlight the most interesting and relevant studies published in the last few years, that have made important contributions to this field.

Metabolic changes with age

Several recent studies have made important advances in characterising metabolic changes associated with age in large-scale population studies. Darst et al. [13] studied a large sample (N = 1212) of middle-aged participants (mean: 61 years) of the Wisconsin Registry for Alzheimer's Prevention (WRAP). The study is powerful due to the use of up to three longitudinal fasting plasma samples thereby reducing some of the uncertainties inherent in analysing age associations in cross-sectional data only and the wide metabolite assessment (1097 metabolites tested), covering most major metabolic pathways, provided through use of the Metabolon platform. Over half the metabolites tested were associated with age and these could be found across all major compound classes. One of the great advantages of studies using commercial platforms is facilitating comparison across studies, which is particularly important as metabolomic studies of age may be confounded by environmental cohort effects. An earlier analysis of the TwinsUK cohort (N = 6055, age range 17−85 years) also used an earlier version of the Metabolon platform covering 280 metabolites, of which 165 were associated with age [14], and the majority of these associations could be replicated in the WRAP study. Table 1 shows the top metabolites hits consistent across these studies, and among the most strongly associated metabolites are carbohydrate erythronate, amino acid related compounds including urea and C-glycosyltryptophan, steroid hormones, acylcarnitines, the fatty acid 3-carboxy-4-methyl-5-propyl-2-furanpropanoate and energy hub metabolite citrate, demonstrating the diversity of pathways associated with ageing. While this comparison is only between two studies that use the same metabolomic platform, it allows us to highlight a general consistency across studies and therefore the likelihood of these metabolites representing endogenous processes. However, a handful of metabolites showed inconsistent directions across studies, including several amino acids, indicating the need for these cross-cohort comparison to improve inference regarding causal relationships with age.

Bunning et al. [15] analysed age associations of 770 metabolites among 125 twin pairs measured using LC-MS. The study is important as it covered age range spanning almost the entire life course (6 months−82 years) enabling changes to age-metabolite relationships at different life periods to be detected. They were able to cluster metabolites into six different ageing trajectories and used pathway analysis to characterise the metabolites most representative of these clusters. For example, metabolites in cluster 1, enriched for xanthine and histidine metabolism, increased throughout the life course while cluster 6, enriched in monoacylglycerols and progestin sterols, increased during childhood and then decreased in late adult life. The study of Ahadi et al. [16] is unique as they took a longitudinal deep phenotyping approach in a cohort of 106 individuals, who were assessed quarterly over a period of four years. They applied integrative personal omics profiling including the same metabolomics platform as the study of Bunning et al. Pathway-enrichment analyses identified four major overlapping pathways - immunity, metabolic, liver and kidney dysregulation, to be associated with aging. Although general trends of individual changes in aging markers followed those of the cross-sectional markers, the authors identified distinct "ageotypes", with individuals falling in distinct patterns of ageing trajectories across these pathways highlighting the variation in ageing, which in some cases could be attributed to lifestyle changes such as weight loss. Insulin resistant individuals showed greater magnitude of ageing within inflammation pathways.

Verri Hernandes et al. [17] presented the largest single site study to date of 6872 study participants in an Italian Alpine region. They applied Biocrates' kit to quantify 175 metabolites with low technical variation. 148 metabolites were found associated with age and interestingly, all but 16 metabolites concentrations increased with age. 59 age associations replicated those reported in previous studies. They further analysed metabolite ratios in participants to find important nodes within metabolic pathways, identifying citrate/arginine and ornithine/arginine as amongst the most significant ratios. Metabolites citrate, symmetric dimethylarginine and ornithine were among the most significant single metabolite measures and along with the aforementioned ratios are all common to the urea cycle, highlighting the central importance of this pathway in ageing. The study of Chak et al. [18] also applied the same Biocrates' kit to examine metabolite changes over seven years using two longitudinal sampling points among participants aged 55−75 years at baseline in the German KORA study. Out of the 122 analysed metabolites, 72 and 81 metabolites were significantly associated with age in women and men, respectively, with 68 metabolites (about 80%) associated with age in both sexes. 60 metabolites were available in the similarly designed smaller CARLA study for replication, and arginine and ornithine were the only associations that could be replicated in both sexes.

Acylcarnitines are among the metabolite classes most consistently associated with age. They are required for import of long-chain fatty acids into mitochondria for β-oxidation and are viewed as markers of mitochondrial function, an aging hallmark, and are associated with activation of inflammation. Jarell et al. [19] examined the associations of 132 plasma acylcarnitines with age

Table 1

Top age-metabolites associations that were consistent across both the WRAP [13] and TwinUK studies [14], ordered by strength of association in the WRAP study. Only the top 28 metabolite associations are shown. P-values has not been adjusted for multiple-testing.

Metabolite	SUPER PATHWAY	SUB PATHWAY	HMDB ID	WRAP study		TWINS UK	
				Direction	p value	Direction	p value
erythronate	Carbohydrate	Aminosugar Metabolism	HMDB00613	+	6.25E-23	+	4.36E-73
Urea	Amino Acid	Urea cycle; Arginine and Proline Metabolism	HMDB00294	+	2.29E-19	+	4.78E-102
androsterone sulfate	Lipid	Steroid	HMDB02759	–	9.80E-16	–	2.63E-94
C-glycosyltryptophan	Amino Acid	Tryptophan Metabolism		+	2.44E-15	+	1.53E-150
CMPF[a]	Lipid	Fatty Acid, Dicarboxylate	HMDB61112	+	2.71E-15	+	3.21E-137
Citrate	Energy	TCA Cycle	HMDB00094	+	8.18E-15	+	9.97E-93
epiandrosterone sulfate	Lipid	Steroid	HMDB00365	–	2.51E-14	–	6.67E-85
4-acetamidobutanoate	Amino Acid	Polyamine Metabolism	HMDB03681	+	1.01E-12	+	1.11E-43
N-acetylalanine	Amino Acid	Alanine and Aspartate Metabolism	HMDB00766	+	7.05E-12	+	5.80E-68
myo-inositol	Lipid	Inositol Metabolism	HMDB00211	+	9.02E-12	+	3.87E-114
pseudouridine	Nucleotide	Pyrimidine Metabolism, Uracil containing	HMDB00767	+	1.54E-11	+	1.04E-27
phenylacetylglutamine	Peptide	Acetylated Peptides	HMDB06344	+	2.57E-11	+	8.13E-89
oleoylcarnitine	Lipid	Fatty Acid Metabolism (Acyl Carnitine)	HMDB05065	+	1.43E-10	+	7.22E-15
N-acetylthreonine	Amino Acid	Glycine, Serine and Threonine Metabolism		+	3.84E-10	+	1.00E-27
docosahexaenoate (DHA; 22:6n3)	Lipid	Polyunsaturated Fatty Acid (n3 and n6)	HMDB02183	+	4.47E-10	+	2.23E-55
gamma-glutamylphenylalanine	Peptide	Gamma-glutamyl Amino Acid	HMDB00594	+	7.67E-10	+	3.02E-73
erythritol	Xenobiotics	Food Component/Plant	HMDB02994	+	9.63E-09	+	2.35E-123
N1-methyladenosine	Nucleotide	Purine Metabolism, Adenine containing	HMDB03331	+	2.40E-08	+	1.05E-14
acetylcarnitine	Lipid	Fatty Acid Metabolism (Acyl Carnitine)	HMDB00201	+	4.62E-08	+	2.83E-63
gamma-glutamylvaline	Peptide	Gamma-glutamyl Amino Acid	HMDB11172	+	5.11E-08	+	3.13E-48
hexanoylcarnitine	Lipid	Fatty Acid Metabolism (Acyl Carnitine)	HMDB00705	+	5.50E-08	+	1.12E-78
succinylcarnitine	Energy	TCA Cycle		+	2.95E-07	+	9.04E-84
stearoylcarnitine	Lipid	Fatty Acid Metabolism (Acyl Carnitine)	HMDB00848	+	1.43E-06	+	9.96E-25
cis-4-decenoyl carnitine	Lipid	Fatty Acid Metabolism (Acyl Carnitine)		+	1.51E-06	+	5.34E-24
margarate (17:0)	Lipid	Long Chain Fatty Acid	HMDB02259	+	1.56E-06	+	2.85E-41
Malate	Energy	TCA Cycle	HMDB00156	+	1.70E-06	+	4.56E-45
glycerate	Carbohydrate	Glycolysis, Gluconeogenesis, Pyruvate Metabolism	HMDB00139	+	2.03E-06	+	6.42E-30
docosapentaenoate (n3 DPA; 22:5n3)	Lipid	Polyunsaturated Fatty Acid (n3 and n6)	HMDB01976	+	2.20E-06	+	3.02E-61

[a] 3-carboxy-4-methyl-5-propyl-2-furanpropanoate.

(range: 20–90 years) in 163 healthy, non-diseased Americans. Their work confirmed that increases in long-chain and very long-chain acylcarnitines are a part of normal ageing, while they observed many odd-chain acylcarnitines decreased with age possibly related to decreased dairy consumption. Beyene et al. [20] conducted a lipidomics study in over 10,000 people identifying 706 lipids across 36 classes/subclasses. Associations with age were found for 66.9% of lipid species, with remarkable positive associations with acylcarnitine and ceramide species, while ether-phospholipids, particularly alkylphosphatidylcholine, alkylphosphatidylethanolamine, and alkenylphosphatidylethanolamine species, were inversely associated with age. A specific set of triacylglycerol species, containing eicosapentaenoic acid (EPA) (20:5) fatty acids, were positively associated with age, even after adjusting for clinical measures of triglycerides.

Mäkinen et al. [21] analysed blood measures from the NMR based Nightingale metabolomic platform in up to three repeat samples from ages 24–49 years in 4000 participants in two Finnish Cohorts. While this platform provides only limited metabolite coverage, including some lipids, fatty acids, glycolysis precursors, ketone bodies and amino acids, it provides the advantage of allowing profiling of lipoproteins and their constituents – structures that are destroyed during the the sample preparation process for LC-MS based platforms. This longitudinal analysis is notable as it avoids some of the biases inherent in cross sectional analysis, and the authors proposed a novel algorithm to normalise data across time-points to correct for batch effects and reduce pre-analytical and analytical technical variations. In addition to replicating many previously reported changes in this younger population such as decreases in creatinine and increases in LDL cholesterol, they isolated the influence of weight gain on some associations, with divergent patterns by weight gain observed for metabolites such as very-low-density-lipoprotein triglycerides, cholesterol, and branched-chain amino acids.

Metabolomic clocks

In recent years, various types of omic data have been combined to create "biological clocks", multi-variate models that are used to assess biological age. Most widely applied are the DNA methylation-based clocks, which can predict chronological age with extraordinary accuracy, but more recently transcriptomic, proteomic and metabolomic data have also been used, as described in a recent review [22]. Most are trained on chronological age itself and can be thought of as providing an average omic profile expected at a given age. The difference between predicted omic age and chronological age is then used to assess "accelerated biological age", and the associations shown with mortality and ageing phenotypes with age acceleration demonstrate the utility of the clock approach for the assessment of biological age. The use of metabolomics to develop biological clocks is supported by studies in model organisms under controlled conditions. These studies demonstrate that metabolic profiles change in a predictable manner with age [23–25], relate to age-related function [24] and metabolic aging may be slowed following experimental manipulation [25,26]. Two recent studies developed metabolomic clocks using quite different approaches. Van den Akker et al. [27] used Nightingale NMR based metabolomics, including 56 quantified metabolic variables in blood, to model age in a large Dutch Biobank sample of 25,000 people from 26 cohorts (age range 18–85), while our group [28] applied broad untargeted metabolomic profiling, covering nine LC-MS and NMR platforms in blood and urine, in the UK nationwide AIRWAVE study of over 2000 people (age range 20–65). Both studies applied internal subsampling to provide unbiased assessments of predictive ability within their respective study populations and assessed associations between age acceleration and ageing risk factors and phenotypes. Van den Akker et al. showed their metabolomic age acceleration measure was associated with BMI, T2D and C-reactive protein and predictive of cardiovascular events and mortality [27]. We found our measure of age acceleration to be associated with obesity, T2D, depression and heavy drinking [28]. The main advantage of the Van den Akker study is that their clock may be applied easily in other studies that use Nightingale data, while the untargeted metabolomics-based clock of the AIRWAVE study is generally not applicable to other studies. However, the limited sensitivity of the Nightingale platform does not capture many of the most important age-related metabolites, while pathway analysis of features included in the AIRWAVE study suggested enrichment for multiple aging pathways, including tyrosine and tryptophan metabolism, Vitamin D and E metabolism, urea cycle, carnitine shuffle and other pathways occurring in the mitochondria. Future developments in this area will need to combine the advantages of both: Broad, highly sensitive metabolomics to identify the most important clock predictors, combined with annotation and quantification to allow replication and validation across studies.

Interestingly, we found in AIRWAVE that metabolomic age acceleration was uncorrelated to DNA methylation age acceleration and the measures were sensitive to different ageing risk factors, suggesting that biological ageing is a multi-faceted process. This aspect has been explored further in recent studies which have systematically assessed and compared multiple biological clocks using different omics including metabolomics [29,30]. Jansen et al. [29] examined telomere length and four biological clocks in association with comorbidities, risk factors and functional aging measures in around 3000 Dutch subjects. All clocks, including a Nightingale metabolomics-based clock, were trained within the same population. Metabolomic age acceleration was associated with male sex, BMI (as observed for all clocks), smoking and cardiometabolic disease and showed moderate correlation with proteomic age acceleration. A composite clock, combining all molecular data types showed stronger associations generally, including with depression, childhood trauma and physical disability. Macdonald-Dunlop et al. [30] compared published and newly trained clocks from a variety of omic platforms in a Scottish cohort of around 1000 subjects. They found the metabolomic clock developed by Van den Akker et al. was only weakly correlated with chronological age in their data ($r = 0.2$), although newly trained clocks based on NMR, lipidomic and LC-MS based data, performed better in test portions of the data ($r > 0.7$), demonstrating the difficulty is developing metabolomic clocks that predict age well across separate populations. They found their NMR clock to associate after FDR correction with BMI, blood pressure and lung function, and their lipidomic clock to associate with cortisol. Overall, they found that clocks derived

from DNA methylation data or N-glycans attached to immunoglobulin G molecules to be more generalisable, tracking a broader range of age-associated disease risk than the metabolomic clocks they tested.

Biological aging may also be measured through extent of physiological dysregulation, which often underlie the increase in disease risk with age. Flores-Guerrero et al. [31] applied Mahalanobis distance (MD), a statistical technique for measuring multivariate distance to population norms, on 32 circulating biomarkers including metabolites, to quantify the degree of loss of homeostasis in 6000 Dutch participants. Interestingly, they found MD scores to be associated with T2D incidence, even after adjustment for multiple T2D risk factors.

Metabolic changes with ageing phenotypes

A key question for the field is how metabolic changes associated with age relate to phenotypic measures of ageing and age-related disease risk. The study of Pietzner et al. [32] examined over 1000 metabolites, using the Metabolon platform, in over 11,000 people in relation to 27 incident diseases. Many metabolites were found to have pleiotropic effects across multiple diseases, and notably both the modified nucleotide pseudouridine and C-glycosyltryptophan were associated with nine different diseases. These metabolites were among those most strongly associated with age [13,14] (Table 1), suggesting some age-related metabolic changes may underlie risk of multiple diseases. Similarly, two studies constructed multivariate models of 24 incident diseases [33] and all-cause mortality [34] in very large populations of over 100,000 and 44,000 people respectively, facilitated by the use of the relatively cost-effective Nightingale NMR platform. The metabolic state models added predictive information over comprehensive clinical variables for eight common diseases [33], while the mortality model had greater predictive accuracy than a model containing conventional risk factors [34]. Other studies have examined metabolic profiles of frailty, a key indicator of functional decline [35–38]. The largest of these studies, in over 1000 older individuals, identified enrichment of carnitine shuffle, mono-saccharide pathway and vitamin E pathways and increases in citrate associated with frailty. The authors suggested these pathways may be linked through cyclic AMP as a hub metabolite [38]. Again, these pathways have been consistently reported in association with age itself [12,28].

Another approach is profiling metabolomic changes associated with established markers of biological age. Telomere length, which shortens with age and is often used as a proxy of biological age, was associated with alterations to lipid metabolism by two studies [39,40], including a replicated positive association in the proportion of omega-6 fatty acids to total fatty acids with telomere length [40].

The study of Johnson et al. is of note [41] as they examined a biological age score constructed from clinical measurements, reporting alterations to metabolites including carnitines and citrate, although the study sample is too small to draw firm conclusions. Similarly, Polonis et al. [42] reported decreases in four lysophosphaditylcholines among hypertensives with early vascular ageing defined by arterial stiffness.

Future perspectives

Since the first large-scale metabolomic studies on ageing in humans around 15 years ago, many important advances have been made, including the use of longitudinal study designs, populations covering the whole life course, and large-scale population-based studies with increasing coverage of the metabolome. Greater comparison between studies is being facilitated by the availability of commercial metabolomic service providers such as Metabolon, Biocrates and Nightingale and also through publication of analytical protocols by academic metabolomic research centres [43]. The metabolomic clock approach appears promising for assessing the contribution of overall metabolic ageing for disease risk, although greater validation across studies is required, particularly as many metabolites are impacted by short-term environmental effects such as diet. The causal relationship of age-related metabolic changes to functional ageing measures and disease risk is also an important consideration for the utility of metabolomics clocks: although some consistently reported age related changes may directly influence disease risk [32], others such as increases in plasma docosahexaenoic acid levels may potentially be beneficial to health [44]. Indeed, the epigenetic clock has recently been partitioned to assess both damaging and adaptive age-related changes, through incorporation of causal inference under a Mendelian Randomisation framework [45]. The availability of databases of genetic instruments for metabolites [46] is an important development in this regard. Other important recent advances include: the increased use of cerebrospinal fluid for metabolomic analysis [47–49], with may be more directly relevant for neurodegenerative disease [50]; analytical methods development to provide greater precision in assessment of age related pathways [51,52]; and continued development of statistical and bioinformatic tools [53,54], including a novel approach investigating how correlations and ratios between metabolites change with age [54]. While many challenges remain, including incomplete metabolome coverage, relatively poor cross-study comparability, and lack of longitudinal studies, recent studies have demonstrated the great promise of this important research field.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as

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Data availability

No data was used for the research described in the article.

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