Geriatrics & Gerontology International

DOI: 10.1111/ggi.14684

REVIEW ARTICLE

Metabolites in aging and aging-relevant diseases: Frailty, sarcopenia and cognitive decline

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Received: 11 August 2023
Revised: 6 September 2023
Accepted: 11 September 2023

Aging shows biologically complex features with high individual variability, which reflects the exposure to several stimuli and the adaptation to them. Among them, metabolic changes are well observed as consequences or possible causes of aging. Calorie restriction extends organismal life span in experimental models. Several metabolites; for example, resveratrol or nicotinamide mononucleotide, are reported to mimic calorie restriction effects *in vivo*. Metabolomic research would be useful to evaluate metabolites as biomarkers in aging-relevant events and to identify metabolic regulation of aging. We recently developed the metabolomic approach for whole blood analysis, which functions as strong tool for this purpose. We review the update findings in aging-relevant metabolites detected by this method. **Geriatr Gerontol Int 2024; 24: 44–48**.

Keywords: aging, frailty, metabolites.

Abbreviations

CR	Calorie restriction
NMN	nicotinamide mononucleotide
LC–MS	liquid chromatography–mass spectrometer
AMPK	adenosine monophosphate-activated protein kinase
TOR	target of rapamycin
FOXO	forkhead box protein O
NAD^+	nicotinamide adenine dinucleotide$^+$
RBCs	red blood cells
OA	ophthalmic acid
1,5-AG	1,5-anhydroglucitol
$NADP^+$	nicotinamide adenine dinucleotide phosphate
IF	intermittent fasting
BCAAs	branched-chain amino acids
3-HB	3-hydroxybutyrate
TCA	tricarboxylic acid
ET	ergothioneine
PPP	pentose phosphate pathway
EFS	Edmonton Frailty Scale
MoCA-J	the Japanese version of the Montreal Cognitive Assessment
TUG	Timed Up and Go test
SMI	skeletal muscle mass index
S-methyl-ET	S-methyl-ergothioneine

A special issue entitled "Enhanced Wellbeing in Later Life through Innovation and Wisdom Sharing, from the IAGG Asia/Oceania Regional Congress 2023"

Introduction

Metabolites (small organic compounds), which are generated through various metabolic activities in living cells and tissues, are quantified by the metabolome approach; for example, using liquid chromatography–mass spectrometry. Metabolomics is utilized both for survey of clinical biomarkers and as important information on metabolic outlines in disease states and health conditions.[1,2]

It has been well accepted that organismal lifespan is prolonged significantly in several investigational models (*Drosophila*, *Caenorhabditis elegans* and mice) under calorie restriction (CR).[3] CR modifies distinct signal modules; for example, adenosine monophosphate-activated protein kinase, target of rapamycin kinase and sirtuin, followed by activation of transcription factor forkhead box protein O (Fig. 1).[4–6] During CR, forkhead box protein O upregulates a subset of radical scavenger genes, resulting in the decline of oxidative stress.[7] It is noteworthy that the mimetic metabolites for these CR pathways are also applicable for lifespan lengthening of experimental models; nicotinamide adenine dinucleotide (NAD^+) or nicotinamide mononucleotide (a precursor of NAD^+) as sirtuin activators,[8] rapamycin as an inhibitor against target of rapamycin kinase,[9] and metformin, which activates adenosine monophosphate-activated protein kinase.[10] These compounds are also applied for the treatment of several aging-relevant diseases, including diabetes, obesity and cancers in human.[11] Furthermore, trials for interventional approaches against human aging have been started by nicotinamide mononucleotide treatment.[12]

Several factors, including lifestyle, health, epigenetics and genetics, closely influence *in vivo* physiological states, which are reflected in human blood.[13] Blood comprises cellular and non-cellular components. Many reports on blood have been carried

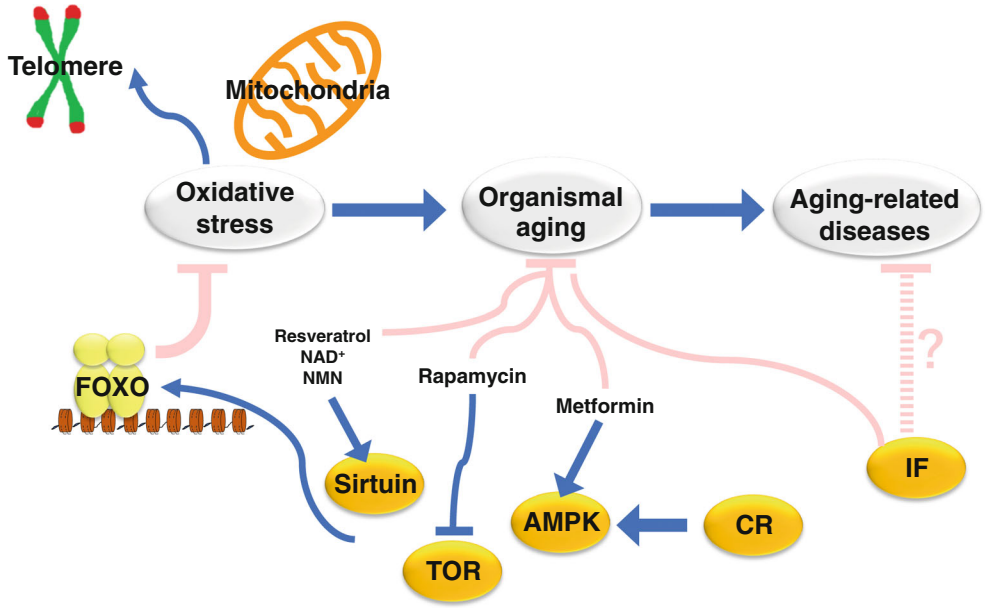

Figure 1 Impact of metabolism on aging. In experimental models, the lifespans are extended by calorie restriction (CR), which activates or inactivates several signaling pathways: adenosine monophosphate-activated protein kinase, target of rapamycin (TOR) kinase and sirtuin. The mimetic metabolites for the CR pathways are also applicable for lifespan lengthening; nicotinamide adenine dinucleotide phosphate (NAD^+) or nicotinamide mononucleotide (NMN; a precursor of NAD^+), rapamycin and metformin. The downstream target of CR signals is forkhead box protein O (FOXO) transcriptional factor, which activates radical scavengers. Recently, intermittent fasting (IF) was also reported to extend organismal lifespan. AMPK, adenosine monophosphate-activated protein kinase.

out on plasma or serum, not on cellular components.[14] This is partly due to the instability of cell-derived metabolites, which makes it difficult to analyze.[15]

Recently, we established "the whole blood metabolome" for examining both cellular and non-cellular components in whole blood. Approximately 130 metabolites are listed, including >14 subgroups.[16] Approximately half of these ~130 metabolites are enriched in red blood cells.[16] Thus, as our "the whole blood metabolome" comprehensively captures multiplex alterations with individualistic variations, it would be applicable for research of human aging. Indeed, "the whole blood metabolome" has identified several compounds that function as biomarkers relevant to aging, fasting and frailty/sarcopenia, as reviewed here.

Biomarker for aging and fasting

We carried out "the whole blood metabolome" for comparison between healthy young and older people, whose average ages were 29 ± 4 and 81 ± 7 years, respectively.[17] Among approximately 130 metabolites, 14 compounds were identified as aging markers. Nine significantly-decreased metabolites in older adults includes carnosine, acetyl-carnosine, ophthalmic acid (OA), 1,5-anhydroglucitol, isoleucine, leucine, nicotinamide adenine dinucleotide phosphate, NAD^+ and UDP-acetyl-glucosamine, suggesting the decline of anti-oxidants, nitrogen and muscle-related metabolites. Five significantly increased metabolites are pantothenate, citrulline, N-acetyl-arginine, dimethyl-guanosine and N6-acetyl-lysine, some of which are known as indicators of kidney function (Fig. 2). Thus, these 14 aging markers reflect impairment in relevant physiological functions in older adults.

In addition to CR, intermittent fasting, cyclic feeding and fasting expand the lifespan of *C. elegans* by approximately 50% compared with the control on a normal diet.[18] The majority of research on human fasting has reported its physiological aspects on energy substitution.[19] During fasting, non-carbohydrate nutrients, such as branched-chain amino acids and lipids, are utilized as energy sources in the human body.[19] Most prominently, the level of 3-hydroxybutyrate, a butyrate, is upregulated by >30-fold, followed by its conversion into acetyl-CoA, which functions as an alternative energy source.[19] Acylcarnitines, transporters for lipid into mitochondria, are also elevated.[20] Third, fasting muscle release branched-chain amino acids, which are utilized in the liver lipogenesis or tricarboxylic acid cycle.[21] Collectively, in the blood of fasting participants, the levels of these three compounds were greatly elevated.

Then, we carried out "the whole blood metabolome" for the samples from four healthy young people after 58 h of fasting.[22] Unexpectedly, this study showed that 44 metabolites, including the aforementioned well-known fasting markers, significantly increased compared with the basal levels of those before fasting, indicating excessive metabolic activation during fasting (Fig. 2). This study discovered several novel aspects of fasting. First of all, tricarboxylic acid cycle metabolites significantly increase during starvation due to the activation of mitochondrial function. Next, anti-oxidant compounds, urate, xanthine (the precursor of urate), carnosine, OA and ergothioneine, increased significantly. Ergothioneine is generated by mushrooms and fungi. Four metabolites generated through the pentose phosphate pathway significantly increased in plasma during fasting: 6-phosphogluconate, pentose phosphate, glucose-6-phosphate and sedoheptulose-7-phosphate. The pentose phosphate pathway supports the intracellular redox control, by production of nicotinamide adenine dinucleotide phosphate.[23] Thus, anti-oxidative ability during starvation is much enhanced by these elevated anti-oxidant metabolites. The present study also noticed the fasting-dependent significant increase of

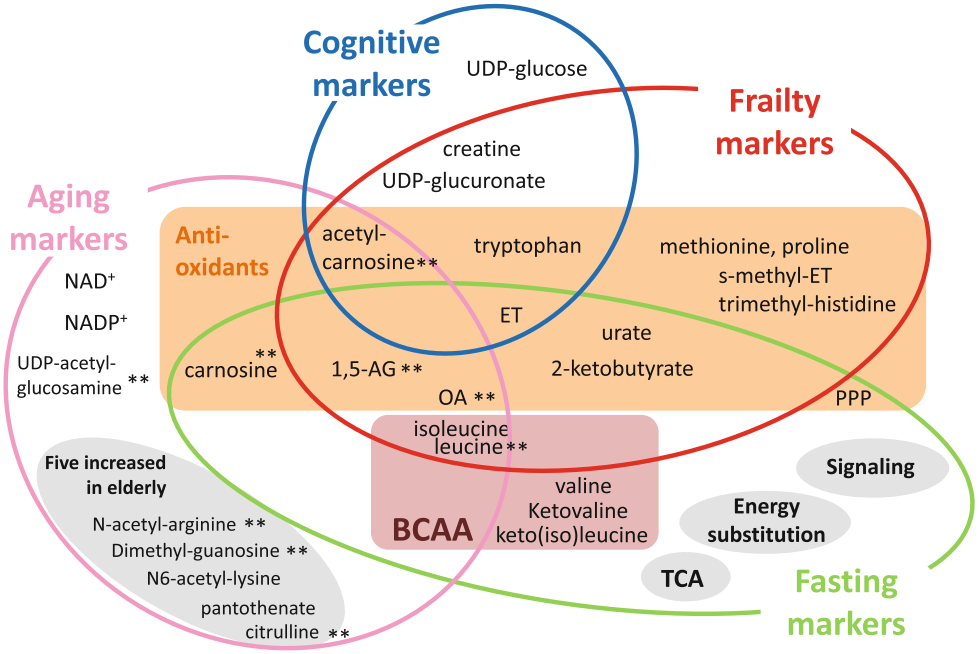

Figure 2 Metabolite markers identified by the whole blood metabolomics. Whole blood metabolomics identified markers for fasting, frailty, cognition and aging. Several anti-oxidative metabolites are included in the lists. The majority of results on the listed markers showed statistical significance, with $P < 0.05$, whereas nine aging markers are detected with statistical significance of $P < 0.01$, which are marked by **. 1,5-AG, 1,5-anhydroglucitol; BCAA, branched chain amino acid; ET, ergothioneine; s-methyl-ET, S-methyl-ergothioneine; NAD^+, nicotinamide adenine dinucleotide; NADP, nicotinamide adenine dinucleotide phosphate; OA, ophthalmic acid; PPP, pentose phosphate pathway; TCA, tricarboxylic acid.

purines/pyrimidines and some signaling metabolites, consistent with the notion that fasting genetically or epigenetically provokes global reprogramming of transcriptional networks. 3-Hydroxybutyrate is also known as a histone deacetylase inhibitor.[24]

Noteworthy, four fasting markers are identical as aging markers – carnosine, OA, leucine and isoleucine (Fig. 2) – suggesting the possibility that upregulation of these aging metabolites by fasting might exert an anti-aging effect.

Biomarker for frailty and sarcopenia

Frailty patients are defined as vulnerable to stressors, due to gradual impairment of organs during aging.[25,26] Frailty covers various aspects of aging, including impairment in cognition, mobility and social activity.[27] In contrast, older adults with sarcopenia – muscle aging – suffer from a loss of muscle mass and strength.[28] Frail patients clinically overlap with sarcopenic populations,[29] as both frailty and sarcopenia significantly impact the daily life of older people.[25] Indeed, the symptomatic standards to diagnose sarcopenia are similar to that for physical frailty, a subtype of frailty.[25,28]

Several metabolomic studies on frailty have been reported.[30–34] However, no unified conclusion was observed among these reports (Table 1). Such gaps are probably due to a large difference in study designs (Table 2). In addition to experimental size, patient profiles and experimental protocols are also important in metabolome research. First, in some studies, the participants also included bed-bound patients,[30] mainly prefrailty[31] or younger generations (aged <60 years).[33,34] The frailty group with bed-bound populations in a study by Adachi *et al.* showed significantly lower body mass index, compared with non-frail participants, suggesting the possibility that malnutrition affects patient status.[30] Second, the reports by Pujos-Guillot *et al.* and Marron *et al.* diagnosed frailty with Fried CHS, which does not include cognitive evaluation, whereas other studies by Rattray *et al.* and Livshits *et al.* utilized the Rockwood Frailty index, which covers not only hypomobility, but also cognitive, social function, medical evaluation and so on (Table 2). In this way, the large difference in patient backgrounds might affect the metabolomic findings. Furthermore, metabolomic measurement protocols are greatly influenced by dietary intake[22] and sample preparation. As previous studies were carried out under different fasting conditions and cryopreservation protocols, we applied our whole blood metabolome.

Thus, "the whole blood metabolome" was achieved in relation to frailty and sarcopenia,[35,36] under the design to capture multiple aspects of frailty. For this purpose, we applied the Edmonton Frailty Scale as an analytical device for frailty evaluation, as it evaluates cognition, walking, social status and multimorbidity. At the same time, the Japanese version of the Montreal Cognitive Assessment,[37] the Timed Up and Go Test,[38] skeletal muscle mass index and muscle strength were evaluated. A total of 19 older people with average age of 84.2 years participated in the study. They comprised nine frailty patients and 10 non-frailty patients, and six sarcopenic and 13 non-sarcopenic patients were included.

The combined findings from these studies identified distinct, but overlapping, markers in aging-relevant diseases: 15 frailty (blue box), 22 sarcopenia (dark green box) and six cognitive markers (orange box) are identified (Fig. 3).[35,36] These 15 frailty markers include seven anti-oxidant metabolites: urate, OA, acetyl-carnosine, trimethyl-histidine, ergothioneine, S-methyl-ergothioneine and 2-ketobutyrate. Cognitive markers largely overlap with frailty markers (Fig. 3). In frailty, diverse anti-oxidants are decreased. In contrast, among 22 sarcopenia markers, 10 compounds were associated with mitochondria; carnitines, metabolites

Table 1 The summary of metabolomic study for frailty

	Anti-oxidant, anti-inflammation	Amino acids	Carnitine	Purine metabolite	Urea cycle, kidney markers	TCA	Monosaccharides	Glucuronate
Adachi *et al.*[30]		↓						
Pujos-Guillot *et al.*[31]		↑	↑					
Marron *et al.*[32]		↓	↑	↑	↑	↑		↑
Rattray *et al.*[33]	↓	↑	↓	↓	↑	↑	↑	
Livshits *et al.*[34]		↑	↑	↑			↑	
Kameda *et al.*[35]	↓	↓	↓	↓				↑

Note: Several metabolomic studies for blood samples from frailty have been reported. ↑, Upregulated in frailty; ↓, downregulated in frailty; TCA, tricarboxylic acid.

Table 2 Design of the metabolomic study for frailty

	Size	Sample	Diagnosis	Average age	State	BMI	Fasting condition
Adachi *et al.*[30]	$n = 59$	Plasma	CSHA-CFS	83.0	Including bed-bound	↓	Overnight
Pujos-Guillot *et al.*[31]	$n = 60$	Serum	CHS	71.3	Including prefrail	→	ND
Marron *et al*[32]	$n = 287$	Plasma	SAVE (modified CHS)	70–79		→	Over 8 h
Rattray *et al.*[33]	$n = 1191$	Serum	Rockwood	67.7 (56–84)		ND	ND
Livshits *et al.*[34]		Plasma	Rockwood	60.5 (23–85)		ND	>6 h
Kameda *et al.*[35]	$n = 19$	Whole blood	EFS	84.2 (74–96)		→	>12 h

Note: Each study was based on different design of study. Except for Kameda *et al.*,[35] most studies utilized serum or plasma samples. Frailty diagnosis also differs among studies. Some studies included prefrail or non-frail (bed-bound or younger generations) as their target. ↓, Downregulated in frailty; →, not changed in frailty; CSHA-CFS, the Chinese-Canadian study of health and aging clinical frailty scale; EFS, Edmonton Frailty Scale; ND, not described; SAVE; the Scale of Aging Vigor in Epidemiology.

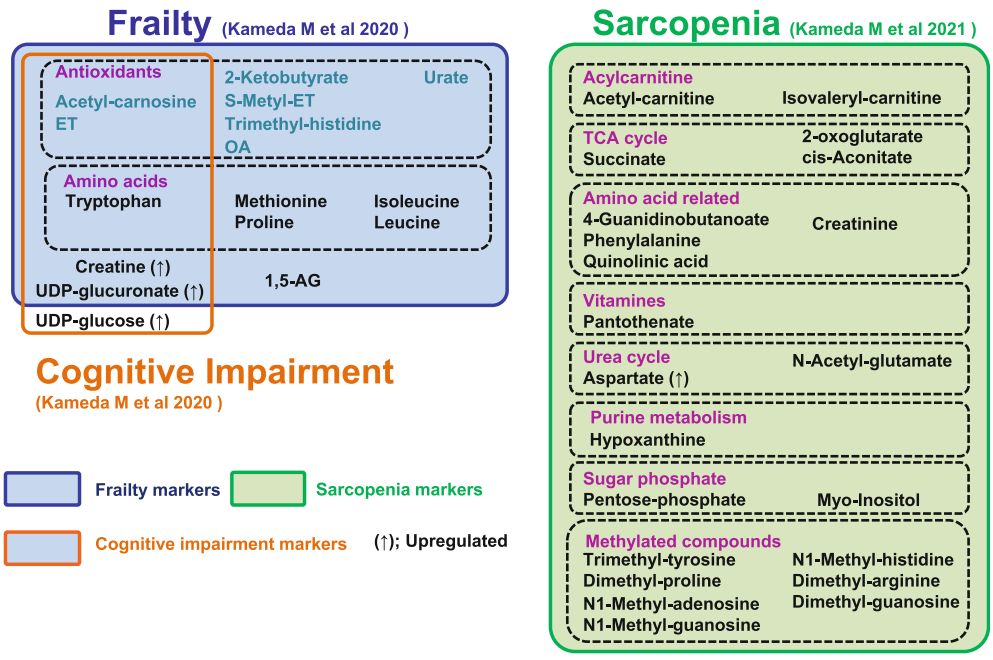

Figure 3 The difference in metabolites between of frailty and sarcopenia. A total of 15 frailty markers (blue box) did not overlap with 22 sarcopenia markers (dark green box). 1,5-AG, 1,5-anhydroglucitol; ET, ergothioneine; s-methyl-ET, S-methyl-ergothioneine; OA, ophthalmic acid.

relevant to the tricarboxylic acid and urea cycle. There is no overlay between sarcopenia and frailty markers, despite clinical commonality, which is shared both in frailty and sarcopenia. Noteworthy, 21 sarcopenia-related markers are identical to markers that are increased in kidney failure.[39]

Finally, the similarities between frailty and sarcopenia indicators are shown (Fig. 3). Half of frailty markers consist of antioxidants, whereas one-third of sarcopenia markers are related to mitochondria. These metabolite differences might reflect different pathology in frailty and sarcopenia.

Summary

Collectively, "the whole blood metabolome" detects the similarity and differences in markers for sarcopenia, frailty and cognition.

Notably, several markers for frailty overlap with aging and fasting markers (Figure 2), implying that frailty possibly shares the metabolic properties of physiological aging.

Disclosure statement

The authors declare no conflict of interest.

Data availability statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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How to cite this article: Kondoh H, Kameda M. Metabolites in aging and aging-relevant diseases: Frailty, sarcopenia and cognitive decline. Geriatr. Gerontol. Int. 2024;24:44–48. https://doi.org/10.1111/ggi.14684