^{1}H-NMR-based metabolomics identifies disrupted betaine metabolism as distinct serum signature of pre-frailty

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Supplementary Material

Table S1. Biochemical pathways were identified through Pathway Enrichment. Hits is the actually matched number of metabolites from the user uploaded data. p value is calculated from the enriched analysis and adjusted using post-hoc Holm analysis. The false discovery rate (FDR) is the portion of false positives above the user-specified score threshold. Metabolites are the compounds corresponding to the Hits.

Pre-Frail vs Frail pathways	Hits	Raw p	Holm p	FDR	Metabolites
Betaine Metabolism	3	2,35E-04	1,64E-03	1,64E-03	Betaine,Choline,Methionine
Glycine and Serine Metabolism	11	1,40E-02	9,67E-01	4,91E-01	Arginine,Serine,Alanine,Ornithine,Betaine,Pyruvic,Threonine,Creatine,Methionine,Glycine,Glutamic acid
Methionine Metabolism	5	5,59E-02	0.00037983	0.00013033	Serine,Coline,Betaine,Methionine,Glycine
Arginine and Proline Metabolism	9	6,71E-01	0.0044956	0.0011742	Arginine,Aspartate,Succinate,Proline,Ornithine,Urea,Creatine,Glycine,Glutamate
Aspartate Metabolism	6	0.0001361	0.0089866	0.0019062	Aspartate,Acetic acid,Arginine,Asparagine,Glutamine,Glutamate
Urea Cycle	8	0.0002201	0.014308	0.0025681	Glutamate,Alanine,Aspartate,Urea,Ornithine,Glutamine, Pyruvate,Arginine
Pre-Frail vs Non-Frail pathways	Hits	Raw p	Holm p	FDR	
Methionine Metabolism	5	4,21E-09	2,94E-08	2,94E-08	Serine,Coline,Betaine,Methionine,Glycine
Glycine and Serine Metabolism	11	2,15E-08	1,48E-06	7,51E-07	Arginine,Serine,Alanine,Ornithine,Betaine,Pyruvic,Threonine,Creatine,Methionine,Glycine,Glutamic acid
Urea Cycle	8	1,91E-07	1,30E-06	4,46E-06	Glutamate,Alanine,Aspartate,Urea,Ornithine,Glutamine, Pyruvate,Arginine
Betaine Metabolism	3	3,62E-07	2,42E-05	5,63E-06	Betaine,Choline,Methionine
Nicotinate and Nicotinamide Metabolism	2	4,02E-07	2,65E-05	5,63E-06	Glutamine,Glutamate
Amino Sugar Metabolism	4	9,34E-07	6,07E-05	9,60E-06	Glutamine,Glutamate,Pyruvic,Acetic
Ammonia Recycling	8	9,60E-07	6,15E-05	9,60E-06	Aspartate,Serine,Glutamine,Glutamate,Histidine,Asparagine,Pyruvate,Glycine
Purine Metabolism	5	1,02E-05	6,23E-04	7,14E-05	Aspartate,Glutamine,Hypoxanthine,Glycine,Glutamic acid
Warburg Effect	7	2,73E-05	1,64E-03	1,74E-04	Glutamine,Succinic, Lactate, Citric, Pyruvic, Glucose,Glutamate
Aspartate Metabolism	6	3,41E-05	2,01E-03	1,93E-04	Aspartate,Acetic acid,Arginine,Asparagine,Glutamine,Glutamate
Glutamate Metabolism	7	3,58E-05	2,08E-03	1,93E-04	Glutamate,Glutamine,Succinic,Alanine,Pyruvate,Glycine,Aspartate
Tyrosine Metabolism	4	3,13E-03	1,79E-01	1,57E-02	Aspartate,Tyrosine,Acetoacetate,Glutamate

Arginine and Proline Metabolism	9	3,66E-04	2,05E-01	1,71E-03	Arginine,Aspartate,Succinate,Proline,Ornithine,Urea,Creatine,Glycine,Glutamate
Phenylalanine and Tyrosine Metabolism	4	7,74E-03	4,25E-01	3,38E-02	Phenyalanine,Tyrosine,Acetic a,Glutamate
Malate-Aspartate Shuttle	2	1,41E-02	7,34E-01	5,20E-02	Aspartate,Glutamate
Beta-Alanine Metabolism	3	1,96E-02	1,00E+00	6,86E-02	Aspartate,Histidine,Glutamate
Selenoamino Acid Metabolism	2	2,08E-02	0.00010404	6,94E-03	Serine,Alanine
Valine Leucine and Isoleucine Degradation	6	3,29E-02	0.00016128	9,06E-02	Valine,Succinic acid,Leucine, Acetoacetate,Isoleucine,Glutamic acid
Tryptophan Metabolism	4	3,31E-02	0.00016128	9,06E-02	Alanine,Formic,Tryptophan,Glutamate
Frail vs Non-Frail pathways	Hits	Raw p	Holm p	FDR	
Urea Cycle	8	9,30E-07	6,51E-05	6,51E-05	Glutamate,Alanine,Aspartate,Urea,Ornithine,Glutamine, Pyruvate,Arginine
Arginine and Proline Metabolism	9	2,97E-06	2,05E-04	1,04E-04	Arginine, Asparte,Succinate,Proline,Ornithine,Urea,Creatine,Glycine,Glutamate
Valine Leucine and Isoleucine Degradation	6	1,06E-05	7,21E-04	2,47E-04	Valine,Succinic acid,Leucine, Acetoacetate,Isoleucine,Glutamic acid
Selenoamino Acid Metabolism	2	1,90E-05	1,27E-04	3,33E-04	Serine,Alanine
Ketone Body Metabolism	3	2,17E-04	1,43E-03	3,04E-03	Acetone,Succinic,Acetoacetate
Aspartate Metabolism	6	3,81E-04	2,48E-02	4,45E-03	Aspartate,Acetic acid,Arginine,Asparagine,Glutamine,Glutamate
Oxidation of Branched Chain Fatty Acids	2	5,39E-04	3,45E-02	5,39E-03	Succinic, Carnitine
Warburg Effect	7	6,93E-04	4,37E-02	5,85E-03	Glutamine,Succinic, Lactate, Citric, Pyruvic, Glucose,Glutamate
Phosphatidylethanolamine Biosynthesis	2	7,52E-04	4,66E-02	5,85E-03	Serine,Choline
Ammonia Recycling	8	1,26E-03	7,67E-03	8,80E-04	Aspartate,Serine,Glutamine,Glutamate,Histidine,Asparagine,Pyruvate,Glycine
Glutamate Metabolism	7	4,97E-03	2,93E-02	2,90E-02	Glutamate,Glutamine,Succinic,Alanine,Pyruvate,Glycine,Aspartate
Sphingolipid Metabolism	2	8,52E-03	4,77E-01	3,98E-02	Serine,Glucose
Beta-Alanine Metabolism	3	9,73E-04	5,35E-01	4,26E-03	Aspartate,Histidine,Glutamate
Butyrate Metabolism	2	1,38E-02	7.45e-05	5,68E-02	Succinic,Acetoacetic acid
Glycine and Serine Metabolism	11	1,91E-02	0.00010105	6,81E-02	Arginine,Serine,Alanine,Ornithine,Betaine,Pyruvic,Threonine,Creatine,Methionine,Glycine,Glutamic acid
Carnitine Synthesis	4	5,01E-02	0.00025073	1,67E-01	Succinic,Carnitine,Lysine,Glycine

Citric Acid Cycle	3	8,69E-03	0.00042576	2,40E-01	Succinic,Citric,Pyruvic
Malate-Aspartate Shuttle	2	1,80E-01	0.00079314	4,67E-01	Aspartate,Glutamate
Nicotinate and Nicotinamide Metabolism	2	2,12E-01	0.00091015	5,29E-01	Glutamine,Glutamate
Histidine Metabolism	3	2,21E-01	0.00092991	5,34E-01	1-Methylhistidine,Glutamate,Histidine
Tyrosine Metabolism	4	4,25E-01	0.0017407	8,66E-01	Aspartate,Tyrosine,Acetoacetate,Glutamate
Amino Sugar Metabolism	4	4,33E-01	0.0017407	8,66E-01	Glutamine,Glutamate,Pyruvic,Acetic
Methionine Metabolism	5	4,68E-01	0.0017407	9,10E-01	Serine,Coline,Betaine,Methionine,Glycine
Purine Metabolism	5	0.00012261	0.0039236	0.00022007	Aspartate,Glutamine,Hypoxanthine,Glycine,Glutamic acid
Phenylalanine and Tyrosine Metabolism	4	0.00016232	0.0050318	0.00028405	Phenyalanine,Tyrosine,Acetic a,Glutamate
Propanoate Metabolism	3	0.00052029	0.015088	0.00086715	Valine,2-Hydroxybutyric a,Glutamate
Tryptophan Metabolism	4	0.001018	0.028503	0.0016572	Alanine,Formic,Tryptophan,Glutamate
Folate Metabolism	2	0.0017351	0.046849	0.0027604	Formic,Glutamate

Table S2. Studies investigating blood metabolomics profile in frailty not reported in Shekarchian et al[1].

Study	Study design	Fluid / Tissue	Study population	Frailty tool	Method	Main results
Corona et al 2014[2]	CS	Serum	Breast cancer females: 49 Fit 23 Unfit 17 Frail	CGA	Targeted LC-MS	↑ Beta-aminoisobutyric acid in unfit and frail compared to fit ↓ His and 3-methyl-His ↑ in unfit and frail ↑ Hydroxyproline in unfit and frail compared to fit ↓ Serine and Trp in frail and unfit compared to fit ↑ Cystine in frail and unfit ↑ Acylcarnitines in frail ↓ Sphingolipids and glicerophospholipids in frail
Rattray et al 2019[3]	L (4y)	Serum	1191 subjects (BL) 786 (L)	60-item FI	(U) LC-MS GC-MS	Discrete clusterization between the 4 FI-based groups 25 metabolites present within 4 pathways: carnitine shuttle, peroxisomal degradation, kynurenine pathway and vitamin E metabolism 12 metabolites significant in discriminating frailty classes Mendelian randomization: ↓ carnitine increases OR for frailty
Kameda et al 2020[4]	CS	Whole blood	9 frail 10 non-frail	EFS	(U) LC-MS	15 frailty markers: ↓ acetyl-carnosine, ergothioneine, S-methyl-ergothioneine, trimethylhistidine, ophthalmic acid, 2-ketobutyrate, urate, 1,5-anhydroglucitol, proline, isoleucine, leucine, tryptophan and methionine ↑ creatine and UDP-glucuronate
Brunelli et al 2021[5]	L (4y)	Plasma	Discovery: 65 fit, 65 frail Validation: 124 fit, 59 prefrail, 81 frail	32-item FI	(U) MS/MS	↓ Hippuric acid in frail group (driven by females) Hippuric acid – frailty relationship mediated by fruit-veg intake ↓ hippuric acid predicts high incident frailty risk
Gomez-Cabrero et al 2021[6]	CS	Plasma	552 robust 630 prefrail 340 frail	Fried	1H-NMR (+ genomic and proteomic)	Vitamin D3, lutein zeaxanthin and miRNA125b-5p associated with reduced OR of pre-frailty and frailty Troponin T increased OR for pre-frailty and frailty
Douzi et al 2022[7]	L (2y)	Urine	Hip fracture surgery: 5 non-frail 18 pre-frail 10 frail	Fried	1H-NMR	PLS-DA: good discrimination between non-frail and frail Hippuric acid ↑ in frail compared to non-frail Pathway analysis: dysregulated glycine, serine, and threonine metabolism; glyoxylate and dicarboxylate metabolism; citrate cycle; glutathione metabolism; pyruvate metabolism

Guo et al 2022[8]	CS	Serum	5 middle-aged controls 5 older non-frail 5 pre-frail 5 frail	Fried	LC-MS	PLS-DA well separated non-frail from frail D-ribose, D-(-)-mannitol, creatine, indole, and dodecanedioic acid differentiated frail from non-frail Pathway analysis (frail vs nonfrail): Phe, Tyr, Trp; TCA cycle; fructose-mannose metabolism and others
Jaroch et al 2022[9]	L (pre and post-8 weeks nutritional intervention)	Serum	29 controls 16 prefrail	Fried	LC-MS	↓ Arachidonic acid, ↑ Oleoylethanolamide in prefrail
Meng et al 2022[10]	CS	Serum	Only men: 96 nonfrail 132 prefrail 18 frail (pre-frail and frail combined in one frail group)	Fried	Targeted HPLC-MS	↓ Trp, ↑ Gly, = Glu and Gln in frail compared to non-frail Frail vs non frail: ↑ Octanoyl-L-carnitine (C8), decanoyl-L-carnitine (C10), dodecanoyl-L-carnitine (C12) and tetradecanoyl-L-carnitine (C14); ↓ isovalerylcarnitine (C5); = Betaine Trp, C8, C12, LPC16:0, LPC18:2, and C5 were independently associated with frailty OPLS-DA did not discriminate frail from non-frail
Pan et al 2022[11]	CS	Serum	49 nonfrail 25 frail	Fried	LC-MS (U)	Pyruvic acid, dihydroxybutanoic acid, 1-methylguanine positively correlated with frailty 2-OH-glutarate, 3-pyridylacetic acid, 3-methylphenylacetic acid, pyruvic acid ↑ in frail; Hydroxyoctanoic acid, glyceric acid, DL-2-aminooctanoic acid, Ser-Phe-Val-Phe, 1-stearoylglycero-P-glycerol, glycoursodeoxycholic acid, taurodeoxycholic acid ↓ in frail Pathway analysis: glycolysis, pyruvaldehyde degradation, glycine-serine, glycerolipid enriched in frail subjects
Solvang et al 2022[12]	L	Serum	1691 subjects (not separated in classes)	38-item FI	LC-MS	CRP was the metabolite with strongest association with frailty Several kynurenines positively correlated with frailty
Tanaka et al 2022[13]	CS	Plasma	806 participants	44-item FI	LC-MS	The metabolomic Diet Score mediated 55% and 61% of the association between MIND and AHEI and FI, respectively. Glu modulated by diet
Westbrook et al 2022[14]	CS	Serum	45 young controls 29 frail 72 old non-frail	Fried (?)	HPLC-MS (U+T)	↑ glucose, pyruvate, cis-aconitate, L-citryl-L-Glu, ADP, Glutamate, GABA, succinic semialdehyde in frail compared to non-frail and young ↑ Aspartate in frail compared to non-frail G6P ↓ in frail compared to nonfrail Glycerol-3-P ↓ in frail compared to nonfrail and young

Calvani et al 2023[15]	CS	Serum	94 frail+sarcopenic (PF&S) 66 frail/prefrail + T2DM (F-T2DM) 40 robust	Index BIOSPHERE	UPLC-MS	F-T2DM: ↑ 3-methylhistidine, alanine, arginine, ethanolamine and glutamate. PF&S and control participants discriminated based on aminoadipic acid, aspartate, citrulline, cystine, taurine and Trp PLS-DA: scarce accuracy in discriminating PF&S from controls; F-T2DM had a distinct profile.
Kuiper et al 2023[16]	CS	Plasma	Rotterdam cohort: 1347 subjects Leiden cohort: 1875 subjects	5 frailty measures	NMR + epigenetics	Weak to moderate correlations between DNA methylation and metabolomics biological aging biomarkers (MetaboAge and MetaboHealth)
Mak et al 2023[17]	CS	Plasma	Discovery: 90,573 subjects Validation cohorts: 11,025 subjects 6,073 subjects	FI Fried	NMR (T)	Glycoprotein acetyls had the strongest positive association with frailty
Marron et al 2023[18]	CS	Plasma	643 vigorous 774 average 772 frail	SAVE	LC-MS (U)	Frailty-metabolites association not dependent by race or sex ↑ acyl carnitines, fatty acids, amino acid derivatives, carboximidic acids, dicarboxylic acids, keto acid derivatives, carbohydrates, metabolites involved in the TCA, trimethylamine *N*-oxide associated with frailer scores TCA: most significant pathway
Zhou et al 2024[19]	CS	Serum	95 nonfrail 67 prefrail 31 frail	Fried FRAIL	UPLC-MS/MS	PLS-DA discriminated well between frail and nonfrail (pre-frail not reported!) Fried: 9 amino acids with VIP > 1: Trp, Asp, Ser, Val, Gly, Gln, Phe, Ala, Cys FRAIL scale: ↑ Cys, L-glutamine, Citrulline, Tyr, Kyn, Phe, and Glu different between the 2 groups; Pathway analysis: serotonergic synapses, kynurenine, melatonin, and serotonin, urea cycle, Asp metabolism, ammonia recycling, Glu metabolism, Warburg effect
Kim et al 2024[20]	CS	Plasma (female patients)	Older females: 8 robust 31 pre-frail 9 frail	Fried 50-item FI	LC-MS/MS (T+U)	↑ α-ketoglutarate associated with increased frailty risk
Pu et al 2024[21]	L (2y)	Plasma	253 non-frailty 962 mild-frailty 413 moderate-frailty 193 severe-frailty	33-item FI	NMR + Gut metagenome	PLS-DA moderately separated the 4 groups HDL, VLDL, amino acids (leucine, lysine), acetoacetic acid, acetic acid, glycerol, glucose, trimethylamine-N-oxide associated with frailty
Yao et al 2024[22]	CS	Plasma	UK Biobank	Fried 49-item FI	NMR	Four dietary patterns (DASH, aMED, RFS, MIND) independently associated with frailty

						Metabolites associated with dietary patterns included lipids, amino acids, inflammatory markers, short-chain fatty acids Metabolites partially mediated the diet-frailty association
Zhao et al 2024[23]	CS	Serum	102 non-frail 156 frail	Not reported	(T) LC-MS/MS	Tyr, Trp, Phe and total aromatic amino acids negatively associated with malnutrition in non-frail subjects In frail group no correlation between malnutrition, MNA-SF and aromatic amino acids levels.

Abbreviations: ↑, increased; ↓, decreased; =, equal; aMED, alternate mediterranean diet; AHEI, Alternate Healthy Eating Index-2010; CRP, C reactive protein; CGA, comprehensive geriatric assessment; CS, cross-sectional design; Cys, cysteine; DASH, dietary approaches to stop hypertension; EFS, Edmonton Frail Scale; FI, frailty index; Glu, glutamate; HDL, high density lipoprotein; His, histidine; HPLC, High-Performance Liquid Chromatography; L, longitudinal; LC-MS, liquid chromatography-mass spectrometry; LPC, lysophosphatidylcholine; MIND, Mediterranean–DASH Diet Intervention for Neurodegenerative Delay; MNA-SF, Mini Nutritional Assessment – Short Form; OR, odds ratio; OVX, ovariectomized; Phe, phenylalanine; PLS-DA, Partial Least Squares Discriminant Analysis; RFS, recommended food score; SAVE, Scale of Aging Vigor in Epidemiology; T2DM, type 2 diabetes mellitus; TCA, tricarboxylic acid cycle; Trp, Tryptophan; Tyr, Tyrosine; U, untargeted; UPLC, Ultra High-Performance Liquid Chromatography; Val, valine; VIP, variable importance in projection; VLDL, very low density lipoprotein.

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