Exploration of the gut-brain axis through metabolomics identifies serum propionic acid associated with higher cognitive decline in older persons

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Supplementary data

Supplementary table S1. List of the 206 food-related metabolites quantified in serum by the multi-metabolite metabolomics platform (with the 72 candidate food and gut microbiota-derived metabolites emphasized in bold)

Endogenous metabolites
glycine
L-alanine
L-valine
L-leucine
L-serine
L-threonine
L-proline
L-arginine
L-glutamate
L-aspartate
L-glutamine
L-asparagine
L-histidine
L-lysine
L-cysteine
L-methionine
L-phenylalanine
L-tyrosine
L-tryptophan
pyroglutamic acid
N6-trimethyl-lysine
phenyllactic acid
p-hydroxyphenyllactic acid
phenylacetylglutamine
epinephrine
p-cresol sulfate
p-cresol glucuronide
indoxyl sulfate
5-hydroxytryptophan
serotonin
indole-3-lactic acid
indole-3-acetic acid
5-hydroxyindole-3-acetic acid

indole-3-propionic acid
kynurenine
kynurenic acid
xanthurenic acid
anthranilic acid
picolinic acid
oxaloacetic acid
citric acid
lactic acid
pyruvic acid
propionic acid
butyric acid
valeric acid
octanoic acid
decanoic acid
lauric acid
myristic acid
pentadecanoic acid
palmitic acid
palmitoleic acid
margaric acid
stearic acid
oleic acid
linoleic acid
linolenic acid
eicosapentaenoic acid
arachidonic acid
docosatetraenoic acid
docosapentaenoic acid
docosahexaenoic acid
D-glucose
ornithine
citrulline
choline
trimethylamine N-oxide
betaine
taurine
urea
creatine
creatinine
guanosine
xanthine
uric acid
allantoin
hypoxanthine
adenine

adenosine
uracil
uridine
L-carnitine
Acetyl-L-carnitine
Propionyl-L-carnitine
Butyryl-L-carnitine
Hexanoyl-L-carnitine
Octanoyl-L-carnitine
Decanoyl-L-carnitine
Undecanoyl-L-carnitine
Dodecanoyl-L-carnitine
Myristoyl-L-carnitine
Palmitoyl-L-carnitine
Oleoyl-L-carnitine
Linoleyl-L-carnitine
Desmosterol
7-Ketocholesterol
Cholesterol sulfate
pregnenolone sulfate
dehydroepiandrosterone sulfate
testosterone
estrone 3-sulfate
17b-estradiol 3-sulfate
cortisol
corticosterone
11-deoxycortisol
Cholic acid
Glycocholic acid
Taurocholic acid
Chenodeoxycholic acid
Chenodeoxycholic acid 3-glucuronide
Glycochenodeoxycholic acid
Glycochenodeoxycholic acid 3-glucuronide
Taurochenodeoxycholic acid
Deoxycholic acid
Glycodeoxycholic acid
Glycodeoxycholic acid 3-sulfate
Taurodeoxycholic acid
Glycolitocholic acid 3-sulfate
Taurolitocholic acid 3-sulfate
Retinol
Thiamine
Riboflavin
Niacinamide
pantothenic acid

4-Pyridoxic acid

Biotin

ascorbic acid

25-hydroxyvitamin D3

α-Tocopherol

lynoleoyl-glycerophosphocholine

exogenous metabolites

2-hydroxybenzoic acid

3-hydroxybenzoic acid sulfate

4-hydroxybenzoic acid sulfate

2,6-dihydroxybenzoic acid

3,4-dihydroxybenzoic acid

dihydroxybenzoic acid glucuronide

hippuric acid

4-hydroxyhippuric acid

3-hydroxyhippuric acid

vanillic acid

isovanillic acid

2-hydroxyphenylacetic acid

4-hydroxyphenylacetic acid glucuronide

3-hydroxyphenylacetic acid sulfate

3,4-dihydroxyphenylacetic acid sulfate

o-coumaric acid sulfate

ferulic acid 4-sulfate

3-(3-hydroxyphenyl)propionic acid

hydroxyphenylpropionic acid sulfate

3-(3,5-dihydroxyphenyl)propionic acid sulfate

dihydrocaffeic acid 3-sulfate

dihydroferulic acid

dihydroferulic acid 4-sulfate

dihydroisoferulic acid 3-sulfate

3-(3-hydroxyphenyl)-3-hydroxypropionic acid

pyrogallol sulfate 1

methylpyrogallol sulfate 1

catechol sulfate

4-methylcatechol sulfate 1

vanillin

5-(4-hydroxy(3-4-dihydroxyphenyl)-valeric acid sulfate 1

3',4'-dihydroxyphenyl-γ-valerolactone sulfate

4'-hydroxy-3'-methoxyphenyl-γ-valerolactone sulfate

naringenin 7-glucuronide

naringenin glucuronide

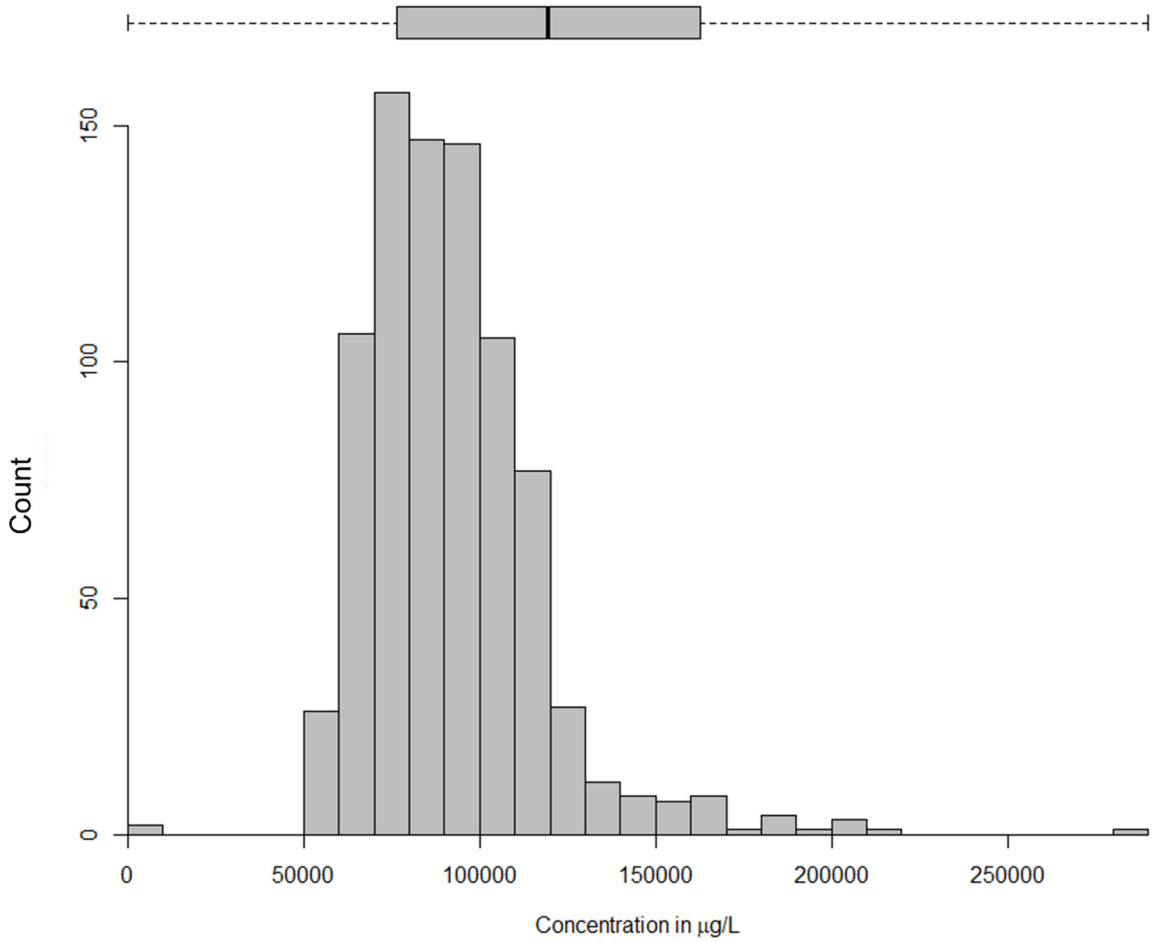
hesperetin 3'-glucuronide
hesperetin sulfate
bergaptol glucuronide
bergaptol sulfate
umbelliferone sulfate
4-methylumbelliferone sulfate
daidzein 4'-sulfate
genistein 7-sulfate
urolithin A glucuronide
urolithin A sulfate
urolithin B glucuronide
urolithin B sulfate
trans-resveratrol 3-sulfate
cis-resveratrol 3-sulfate
dihydroresveratrol sulfate 1
enterolactone
enterolactone sulfate
atractyligenin glucuronide
acesulfame K
saccharin
ethyl sulfate
tartaric acid
2-furoylglycine
1-methylxanthine
3-methylxanthine
paraxanthine +theofylline
theobromine
caffeine
1-methyluric acid
1,7-dimethyluric acid
5-acetamido-6-formylamino-3-methyluracil
cyclo(L-leucyl-L-prolyl)
cyclo(L-prolyl-L-valyl)
ergothioneine
proline betaine
4-hydroxyproline betaine
carnosine
1-methylhistidine
3-methylhistidine
arsenobetaine
N-methylpyridinium
α-chaconine

Supplementary table S2. List of the 72 candidate food and gut microbiota-derived metabolites measured in serum, with chemical class, endogenous versus exogenous origin and relation to the gut microbiota (derivative versus substrate)

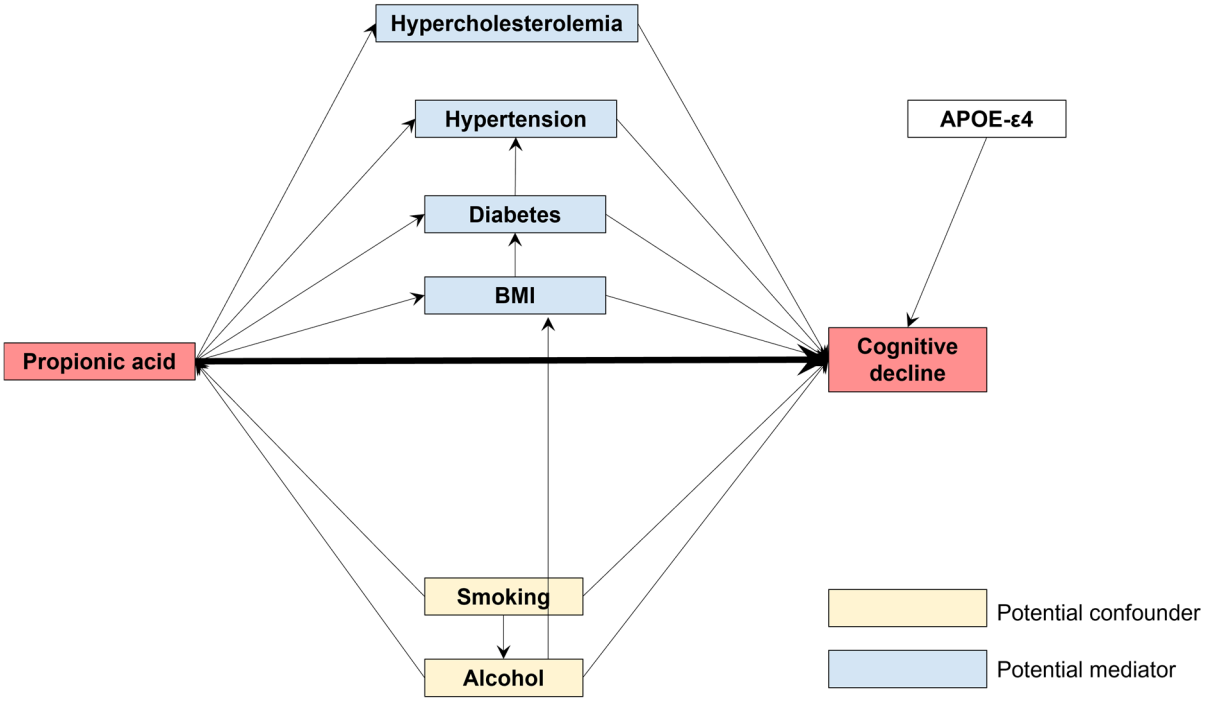
Class	Metabolite	Abbreviated name	Derivative (D)/ Substrate (S)
Amino-acids and derivatives	Phenylalanine	Phenylalanine	S
	Tryptophan	Tryptophan	S & D
	Tyrosine	Tyrosine	S & D
	Phenyl-lactic acid	Phenyl-lactic acid	D
	p-Hydroxyphenyl-lactic acid	p-HPLA	D
	Phenylacetylglutamine	Phenylacetylglutamine	D
	p-Cresol sulfate	p-cresol-S	D
	p-Cresol glucuronide	p-cresol-G	D
	Indole-3-propionic acid	IPA	D
	Indole-3-acetic acid	IAA	D
	Indoxyl sulfate	Indoxyl Sulfate	D
	Indole-3-lactic acid	Indolelactic acid	D
	5-Hydroxyindole-3-acetic acid	5-HIAA	D
	Kynurenine	Kynurenine	D
	Kynurenic acid	Kynurenic acid	D
	Xanthurenic acid	Xanthurenic acid	D
	Anthranillic acid	Anthranillic acid	S
	Picolinic acid	Picolinic acid	D
	Ergothioneine	Ergothioneine	D
	Epinephrine	Epinephrine	D
	Serotonin	Serotonin	D
Organic acids	Lactic acid	lactic acid	D
TMAO and substrates	Trimethylamine N-oxide	TMAO	D
	Betaine	betaine	S
	Choline	choline	S
	L-Carnitine	L-carnitine	S
Bile acids	Glycodeoxycholic acid	GDCA	D
B vitamins	Thiamine	Thiamine	D
	Riboflavin	Riboflavin	S&D
	Niacinamide	Niacinamide	D
	Pantothenic acid	Pantothenic acid	S&D
	4-Pyridoxic acid	4-pyridoxic acid	D
	Biotin	Biotin	D
Short chain fatty acids	Valeric acid	valeric acid	D
	Butyric acid	butyric acid	D
	Propionic acid	propionic acid	D
Phenolic compounds and derivatives	Urolithin A glucuronide	UroA-G	D
	Urolithin A sulfate	UroA-S	D
	Urolithin B glucuronide	UroB-G	D
	Urolithin B sulfate	UroB-S	D
	2-Hydroxybenzoic acid	2-HBA	D
	3-Hydroxybenzoic acid sulfate	3-HBA-S	D

Class	Metabolite	Abbreviated name	Derivative (D)/ Substrate (S)
	4-Hydroxybenzoic acid sulfate	4-HBA-S	D
	2,6-Dihydroxybenzoic acid	2,6-DHBA	D
	3,4-Dihydroxybenzoic acid	3,4-DHBA	D
	Hippuric acid	HA	D
	4-Hydroxyhippuric acid	4-HHA	D
	3-Hydroxyhippuric acid	3-HHA	D
	Isovanillic acid	iVA	D
	2-Hydroxyphenylacetic acid	2-HPAA	D
	4-Hydroxyphenylacetic acid glucuronide	4-HPAA-G	D
	3-Hydroxyphenylacetic acid sulfate	3-HPAA-S	D
	3,4-Dihydroxyphenylacetic acid sulfate	3,4-DHPAA-S	D
	Ferulic acid 4-sulfate	FA-4S	D
	3-(3-Hydroxyphenyl)propionic acid	3-HPPA	D
	Hydroxyphenylpropionic acid sulfate	HPPA-S	D
	3-(3,5-Dihydroxyphenyl)propionic acid sulfate	3,5-DHPPA-S	D
	Dihydrocaffeic acid 3-sulfate	DHCA-3S	D
	Dihydroferulic acid	DHFA	D
	Dihydroferulic acid 4-sulfate	DHFA-S	D
	Dihydroisoferulic acid 3-sulfate	DHiFA-S	D
	3-(3-Hydroxyphenyl)-3-hydroxypropionic acid	3-HPHPA	D
	Pyrogallol sulfate	PYR-S	D
	Methylpyrogallol sulfate	MePYR-S	D
	Catechol sulfate	CAT-S	D
	4-Methylcatechol sulfate	4-MeCAT-S	D
	Vanillin	VAN	D
	3’,4’-Dihydroxyphenyl-γ-valerolactone sulfate	3’,4’-DHPV-S	D
	4’-Hydroxy-3’-methoxyphenyl-γ-valerolactone sulfate	MHPV-S	D
	Dihydroresveratrol sulfate	DHRSV-S	D
	Enterolactone sulfate	EL-S	D
	Enterolactone	EL	D

Supplementary Figure S1. Distribution of plasmatic propionic acid concentration in the pooled sample (n=838)

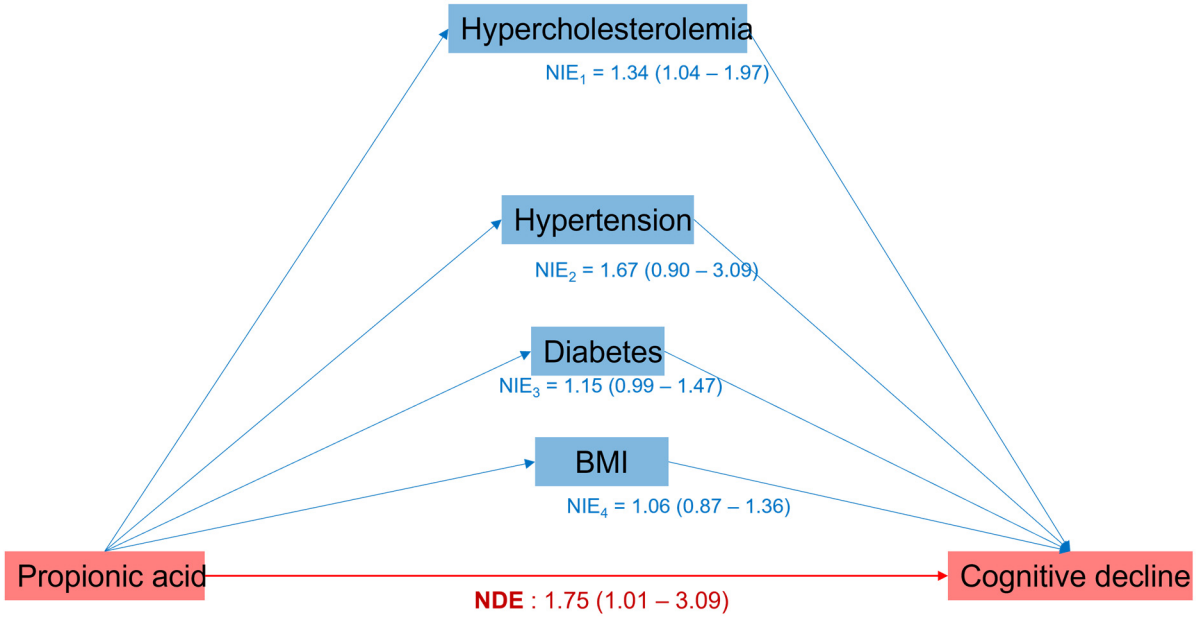


Supplementary Figure S2. Directed Acyclic Graph to identify potential confounders and mediators in the relation of propionic acid in serum to cognitive decline



Footnote: A directed acyclic graph (DAG) was implemented using DAGitty software (version 3.0) to identify the factors of adjustment in the model estimating the association between propionic acid in serum and cognitive decline. Covariates and their potential role in the relation of propionic acid to cognitive decline (beyond the matching variables age, sex and education) were selected from the literature.

Supplementary Figure S3. Counterfactual mediation analysis for matched data to estimate the mediating effect of hypertension, hypercholesterolemia and diabetes in the relation of propionic acid in serum to the odds of cognitive decline over 12 years in the pooled sample (n=838)



Footnote: A counterfactual mediation analysis for matched data[1,2] was run independently for each of the four potential mediators using conditional logistic regression models adjusted for confounders (alcohol consumption and smoking) and other potential mediators (BMI, hypercholesterolemia, hypertension or diabetes). Propionic acid and mediators were modeled as a binary variables (concentration ≥75th vs <25th percentiles for propionic acid; ≥30 kg/m² vs < 30 kg/m² for BMI, yes/no for hypertension, hypercholesterolemia and diabetes).
Note that there is no method available yet, to our knowledge, to assess in a unique analysis multiple mediation through potentially correlated mediators in matched case-control studies; here, we adapted the method by Kim et al[2] to several mediators, modeled non-concomitantly. In counterfactual approaches, two assumptions about unmeasured confounders must hold: (i) There is no mediator – outcome confounder affected by exposure, and (ii) no exposure-mediator interaction. There was no statistically significant interaction between propionic acid (the exposure) and any of the 4 mediators on the odds of cognitive decline. In each model, the total effect of propionic acid on cognitive decline was decomposed into a natural direct effect (NDE, red arrow) and a natural indirect effect (NIE_1, NIE_2, NIE_3 and NIE_4, blue arrows) through the mediator. NDE is interpreted as the effect of propionic acid on cognitive decline when the path between each mediator and cognitive decline is blocked. NIE represents the impact of propionic acid on cognitive decline when the value of the mediator changes.

References

1. VanderWeele, T. J. Mediation Analysis: A Practitioner's Guide. *Annu. Rev. Public Health* **2016**, *37***,** 17–32
2. Kim, Y. M. *et al.* Causal mediation analysis in nested case-control studies using conditional logistic regression. *Biom. J.* **2020**, *62***,** 1939–1959