

Supplementary Materials

Effect of a polyphenol-rich dietary pattern on subjects aged ≥ 60 years with higher levels of inflammatory markers: insights into microbiome and metabolome

Giorgio Gargari¹, Tomas Meroño^{2,3}, Gregorio Peron^{2,4}, Cristian Del Bo¹, Mirko Marino¹, Antonio Cherubini^{5,6}, Cristina Andres-Lacueva^{2,3}, Paul Antony Kroon⁷, Patrizia Riso¹, Simone Guglielmetti⁸

¹Department of Food, Environmental and Nutritional Sciences (DeFENS), Università Degli Studi di Milano, Milan 20133, Italy.

²Biomarkers and Nutrimetabolomics Laboratory, Department de Nutrició, Ciències de l'Alimentació i Gastronomia, Institut de Recerca en Nutrició i Seguretat Alimentària (INSA), Facultat de Farmàcia i Ciències de l'Alimentació, Universitat de Barcelona (UB), Barcelona 08028, Spain.

³Centro de Investigación Biomédica en Red de Fragilidad y Envejecimiento Saludable (CIBERFES), Instituto de Salud Carlos III, Madrid 28029, Spain.

⁴Department of Molecular and Translational Medicine, University of Brescia, Brescia 25121, Italy.

⁵Department of Geriatric Pathways of Frailty, Continuity of Care and Rehabilitation, Istituto di Ricovero e Cura a Carattere Scientifico - Istituto Nazionale di Ricovero e Cura per Anziani (IRCCS INRCA), Ancona 60127, Italy.

⁶Department of Clinical and Molecular Sciences, Università Politecnica delle Marche, Ancona 60131, Italy.

⁷Quadram Institute Bioscience, Norwich Research Park, Norwich NR4 7UQ, UK.

⁸µbEat lab, Department of Biotechnology and Biosciences (BtBs), University of Milano-Bicocca, Milan 20126, Italy.

Correspondence to: Dr. Giorgio Gargari, Department of Food, Environmental and Nutritional Sciences (DeFENS), Università Degli Studi di Milano, Milan 20133, Italy. E-mail: giorgio.gargari@unimi.it; Prof. Simone Guglielmetti, µbEat lab, Department of Biotechnology and Biosciences (BtBs), University of Milano-Bicocca, Milan 20126, Italy. E-mail: simone.guglielmetti@unimib.it

Supplementary Table 1. Comparative analysis of metabolite levels in fecal, serum, and urine samples between clusters cL and cH

Metabolites		p-value		Median/mean	
		Normality test	Mann-Whitney/Ttest	cH	cl
Feces	4-Aminobutyrate (gaba)	0.000	0.005	▼0.00	▲0.18
	Glycerol	0.000	0.030	▲0.66	▼0.33
	Nicotinate	0.000	0.008	▲0.14	▼0.11
Serum	2-hba	0.000	0.049	▼-0.23	▲-0.09
	3-hha	0.131	0.017	▲0.02	▼-0.37
	3-hphpa	0.442	0.013	▲0.09	▼-0.33
	3-methylindole	0.306	0.011	▲-0.01	▼-0.17
	3-methyl-2-oxovaleric	0.165	0.028	▼0.27	▲0.41
	4-imidazoleacrylic	0.360	0.044	▼-0.04	▲0.23
	asymmetric-dimethylarginine	0.028	0.015	▼-0.12	▲0.08
	betaine	0.122	0.030	▲0.13	▼-0.12
	dheas	0.814	0.020	▲0.24	▼-0.14
	dodecanedioic	0.006	0.006	▲-0.08	▼-0.27
	erg	0.462	0.002	▲0.27	▼-0.26
	hexanoic	0.010	0.036	▲-0.07	▼-0.20
	indolepropionic	0.000	0.039	▲0.07	▼-0.25
	n-acetylglucosamine-6s	0.003	0.031	▼-0.13	▲0.07
	pentadecanoic	0.000	0.002	▼-0.13	▲0.24
	phenylacetylglutamine	0.002	0.036	▼0.05	▲0.24
	pimelic	0.381	0.033	▲0.07	▼-0.21
	propionic	0.000	0.042	▲-0.05	▼-0.12
	riboflavin	0.023	0.025	▼-0.29	▲-0.05
	tryptophan	0.211	0.016	▲0.11	▼-0.04
	vanillylmandelic	0.689	0.007	▼-0.22	▲0.09
	xanthurenic	0.515	0.006	▲0.14	▼-0.06
Urine	4-HBAld	0.048	0.020	▼-0.39	▲-0.03
	DHRV-G2	0.000	0.041	▲-0.08	▼-0.28
	ED-S	0.569	0.010	▲0.14	▼-0.32
	FA	0.182	0.008	▼-0.14	▲0.44
	FOLIN	0.706	0.010	▼-0.19	▲0.10
	iVA	0.518	0.035	▼-0.23	▲0.16
	MeGA-S2	0.511	0.045	▲0.21	▼-0.26
	MePYR-S1	0.787	0.020	▲0.12	▼-0.38
	oCOU-G	0.102	0.037	▲0.14	▼-0.27
	TYR-S	0.000	0.019	▲-0.19	▼-0.26
	VAN-S	0.061	0.039	▼-0.33	▲0.19

Only metabolites with $P \leq 0.05$ are included. P -values were calculated using both parametric (t -test) and non-parametric (Mann-Whitney U) tests, according to the results of the Shapiro-Francia normality test. Mean or median values are reported depending on the applied test.

Supplementary Figure 1. Correlation between physiological parameters and bacteria assessed using the Kendall test. The τ index defines the color scale (blue for negative correlation, red for positive correlation). Asterisks indicate significance levels: * $0.1 < P < 0.05$; ** $0.001 < P < 0.01$; *** $P < 0.001$. Bacterial taxa that changed after treatment are shown in bold.