

Impact of Biometric Patient Data, Probiotic Supplementation, and Selected Gut Microorganisms on Calprotectin, Zonulin, and sIgA Concentrations in the Stool of Adults Aged 18–74 Years

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Supplementary tables

Table S1. Characteristics of the participants.

Grouping variable		N	M	SD	Me	Q1	Q3	p-value
Age	-	160	40	12.7	39	31	47	-
Body mass [kg]		138	69	15.9	66	58	78	
BMI		138	23.41	4.65	22.53	20.38	25.86	
Sex								
Age	female	111	41	13.1	39	31	48	.3300 ^a
	male	49	38	11.7	38	31	44	
Body mass [kg]	female	97	65	15.4	63	54	78	<.0001 ^a
	male	41	79	12.8	75	70	90	
BMI	female	97	23.09	4.91	21.94	19.82	24.80	.0413 ^a
	male	41	24.17	3.91	23.89	21.22	26.86	
Probiotics usage								
Age	Non-supplementation	62	39	11.3	38	31	44	.6959 ^b
	Supplementation	45	40	13.3	37	31	47	
Body mass [kg]	Non-supplementation	59	71	17	70	58	86	.1548 ^b
	Supplementation	44	66	13	65	59	74	
BMI	Non-supplementation	59	24.18	5.60	23.53	20.31	26.88	.2627 ^a
	Supplementation	44	22.29	3.37	22.36	20.06	24.55	
	Present	86	23.10	4.149	22.44	20.38	25.61	

Legend: N – number of participants; M – Mean; SD – Standard deviation; Me – Median, Q1, Q3 – Lower and Upper Quartile; ^a – Mann-Whitney U test; ^b – t-Student test; p<0.05 are indicated in bold

Table S2. Biomarkers level characteristics in participants' stool.

Marker	Grouping variable	N	M	SD	Me	Q1	Q3	p-value
Calprotectin ug/g	-	74	85	180.1	30	30	42	-
slgA ug/ml		51	1702	1462	1399	549	2241	
Zonulin ng/ml		80	85.53	63.27	71.70	48.19	102.95	
Probiotics usage								
Calprotectin µg/g	Non-supplementation	29	100	232.6	30	30	35	<.9999 ^a
	Supplementation	21	69	91.5	30	30	36	
slgA µg/ml	Non-supplementation	18	1917	1567.5	1868	914	2290	.0464^b
	Supplementation	13	990	690.2	686	494	1653	
Zonulin ng/ml	Non-supplementation	31	96.34	56.341	83.26	57.70	119.50	.1819 ^a
	Supplementation	25	85.61	76.19	69.98	48.58	102.00	
Gastrointestinal disorders presence								
Calprotectin µg/g	Absent	18	109	197.4	32	30	107	.0712 ^a
	Present	49	65	148.1	30	30	35	
slgA µg/ml	Absent	20	1847	1486.3	1419	671	2274	.3762 ^a
	Present	29	1567	1439.9	1305	442	2121	
Zonulin ng/ml	Absent	35	81.05	52.853	68.49	45.39	86.90	.6379 ^a
	Present	42	89.21	72.718	73.50	48.70	104.10	

Legend: N – number of participants; M – Mean; SD – Standard deviation; Me – Median, Q1, Q3 – Lower and Upper Quartile; ^a – Mann-Whitney U test; ^b – t-Student test; $p < 0.05$ are indicated in bold

Table S3. Summary of analyzed microbiota characteristics regarding probiotic supplementation.

Microbiota	Probiotics												p-value ^a
	Non-supplementation						Supplementation						
	N	M	SD	Me	Q1	Q3	N	M	SD	Me	Q1	Q3	
<i>Escherichia coli</i>	61	2.16E7	4.03E7	4.00E6	8.00E5	3.00E7	45	2.14E7	4.46E7	4.00E6	1.00E6	1.00E7	.8746
<i>Escherichia coli</i> (NLF)	62	1.15E5	4.74E5	2.00E3	2.00E3	2.00E3	45	1.43E6	4.38E6	2.00E3	2.00E3	2.00E3	.0291
<i>Pseudomonas</i> spp.	62	5.19E3	2.51E4	2.00E3	2.00E3	2.00E3	44	2.01E4	8.66E4	2.00E3	2.00E3	2.00E3	.4009
<i>Klebsiella</i> spp.	61	3.41E5	1.47E6	2.00E3	2.00E3	2.00E3	45	2.73E5	1.32E6	2.00E3	2.00E3	2.00E3	.2627
<i>Enterobacter</i> spp.	62	6.33E5	1.76E6	2.00E3	2.00E3	2.00E3	45	1.08E5	4.22E5	2.00E3	2.00E3	2.00E3	.9135
<i>Citrobacter</i> spp.	62	1.70E5	1.27E6	2.00E3	2.00E3	2.00E3	45	1.56E6	6.64E6	2.00E3	2.00E3	2.00E3	.1650
<i>Enterococcus</i> spp.	62	3.36E7	1.18E8	2.00E3	2.00E3	8.00E5	45	4.18E7	1.15E8	2.00E3	2.00E3	6.00E6	.7951
<i>Bifidobacterium</i> spp.	62	1.04E9	9.37E8	8.00E8	4.00E8	1.00E9	45	8.77E8	9.67E8	6.00E8	2.00E8	1.00E9	.6872
<i>Bacteroides</i> spp.	62	2.39E9	2.02E9	2.00E9	1.00E9	3.00E9	45	2.24E9	1.64E9	2.00E9	1.00E9	3.00E9	.2819
<i>Lactobacillus</i> spp.	62	3.86E5	9.69E5	3.00E4	2.00E3	3.00E5	45	8.93E5	2.13E6	6.00E4	2.00E3	7.00E5	.7050
H ₂ O ₂ <i>Lactobacillus</i>	62	9.70E4	3.04E5	2.00E3	2.00E3	4.00E4	44	2.09E5	5.66E5	2.00E3	2.00E3	7.50E4	.9246
<i>Clostridium</i> spp.	61	1.77E4	4.91E4	2.00E3	2.00E3	2.00E3	45	7.36E4	2.26E5	2.00E3	2.00E3	2.00E3	.1315
<i>Faecalibacterium prausnitzii</i>	62	4.57E9	3.69E9	3.00E9	2.00E9	7.00E9	45	5.24E9	4.45E9	4.00E9	2.00E9	8.00E9	<.9999
<i>Akkermansia muciniphila</i>	62	8.06E7	1.75E8	6.00E6	8.00E3	8.00E7	45	9.40E7	2.01E8	7.00E6	1.00E2	7.00E7	.3422
TBC	62	1.38E10	1.42E10	1.00E10	5.00E9	2.00E10	45	1.03E10	1.06E10	7.00E9	4.00E9	1.00E10	.7926
<i>Candida</i> spp.	62	1.74E3	5.43E3	1.00E2	1.00E2	5.00E2	44	4.71E3	1.52E4	1.00E2	1.00E2	1.00E3	.7037

Legend: N – number of participants; M – Mean; SD – Standard deviation; Me – Median, Q1, Q3 – Lower and Upper Quartile; ^a – Mann-Whitney U test; *p*<0.05 are indicated in bold

Table S4. Significant correlations between analyzed biomarkers and selected microorganisms in all studied cases.

From	To	R	p-value
Calprotectin µg/g	H ₂ O ₂ <i>Lactobacillus</i>	0.25	.0343
Calprotectin µg/g	<i>Bifidobacterium</i> spp.	0.25	.0030
slgA µg/ml	<i>Enterococcus</i> spp.	0.28	.0447
slgA µg/ml	<i>Citrobacter</i> spp.	-0.39	.0042
slgA µg/ml	Calprotectin µg/g	-0.60	.0085
Zonulin ng/ml	<i>Lactobacillus</i> spp.	-0.27	.0145
<i>Bacteroides</i> spp.	TBC	0.58	<.0001
<i>Bacteroides</i> spp.	<i>Pseudomonas</i> spp.	0.19	.0158
<i>Bacteroides</i> spp.	<i>Bifidobacterium</i> spp.	0.36	<.0001
<i>Bifidobacterium</i> spp.	TBC	0.50	<.0001
<i>Candida</i> spp.	<i>Faecalibacterium prausnitzii</i>	0.18	.0232
<i>Candida</i> spp.	<i>Lactobacillus</i> spp.	0.19	.0197
<i>Candida</i> spp.	H ₂ O ₂ <i>Lactobacillus</i>	0.21	.0067
<i>Citrobacter</i> spp.	<i>Escherichia coli</i> (NLF)	0.16	.0465
<i>Enterobacter</i> spp.	<i>Escherichia coli</i>	0.16	.0391
<i>Enterococcus</i> spp.	<i>Citrobacter</i> spp.	0.16	.0471
<i>Enterococcus</i> spp.	<i>Escherichia coli</i>	0.16	.0454
<i>Escherichia coli</i>	<i>Escherichia coli</i> (NLF)	-0.17	.0319
<i>Faecalibacterium prausnitzii</i>	<i>Bifidobacterium</i> spp.	0.16	.0378
<i>Faecalibacterium prausnitzii</i>	TBC	0.30	<.0001
H ₂ O ₂ <i>Lactobacillus</i>	<i>Escherichia coli</i> (NLF)	0.22	.0052
H ₂ O ₂ <i>Lactobacillus</i>	<i>Bifidobacterium</i> spp.	0.24	.0021
H ₂ O ₂ <i>Lactobacillus</i>	<i>Lactobacillus</i> spp.	0.61	<.0001
<i>Lactobacillus</i> spp.	<i>Escherichia coli</i>	0.18	.0249
<i>Lactobacillus</i> spp.	<i>Bifidobacterium</i> spp.	0.22	.0048
<i>Lactobacillus</i> spp.	<i>Escherichia coli</i> (NLF)	0.23	.0031
<i>Lactobacillus</i> spp.	<i>Enterococcus</i> spp.	0.31	<.0001
<i>Lactobacillus</i> spp.	TBC	0.18	.0204

Legend: R – Spearman's rank coefficient of correlation; TBC – total bacteria count; NLF – non-lactose fermenting; *p*<0.05 are indicated in bold

R values ranges:	-1	-0.8	-0.79	-0.6	-0.59	-0.4	-0.39	-0.2	-0.19	0	0.19	0.2	0.39	0.4	0.59	0.6	0.79	0.8	1
Proportionality	Negative/inversely proportional									No correlation	Positive/directly proportional								
Strength Interpretation	very strong		strong		moderate		weak			very weak		weak		moderate		strong		very strong	

Table S5. Significant correlations between analyzed biomarkers and selected microorganisms groups distinguished probiotics usage accordingly.

Probiotics non-supplementation				Probiotics supplementation			
From	To	R	p-value	From	To	R	p-value
Calprotectin µg/g	<i>Klebsiella</i> spp.	0.44	.0203	Calprotectin µg/g	<i>Escherichia coli</i>	0.43	.0496
Zonulin ng/ml	<i>Lactobacillus</i> spp.	-0.43	.0164	Calprotectin µg/g	<i>Lactobacillus</i> spp.	0.47	.0304
Zonulin ng/ml	<i>Clostridium</i> spp.	0.36	.0468	Calprotectin µg/g	H ₂ O ₂ <i>Lactobacillus</i>	0.64	.0024
<i>Bacteroides</i> spp.	<i>Bifidobacterium</i> spp.	0.51	<.0001	Zonulin ng/ml	<i>Escherichia coli</i> (NLF)	0.40	.0468
<i>Bacteroides</i> spp.	TBC	0.67	<.0001	<i>Akkermansia muciniphila</i>	<i>Enterobacter</i> spp.	0.37	.0114
<i>Candida</i> spp.	<i>Bacteroides</i> spp.	0.33	.0084	<i>Bacteroides</i> spp.	<i>Bifidobacterium</i> spp.	0.37	.0127
<i>Candida</i> spp.	TBC	0.28	.0294	<i>Bacteroides</i> spp.	TBC	0.62	<.0001
<i>Bifidobacterium</i> spp.	TBC	0.57	<.0001	<i>Bifidobacterium</i> spp.	TBC	0.52	.0003
<i>Faecalibacterium prausnitzii</i>	<i>Candida</i> spp.	0.34	.0066	<i>Candida</i> spp.	<i>Lactobacillus</i> spp.	0.41	.0052
<i>Faecalibacterium prausnitzii</i>	TBC	0.39	.0019	<i>Candida</i> spp.	H ₂ O ₂ <i>Lactobacillus</i>	0.45	.0027
H ₂ O ₂ <i>Lactobacillus</i>	<i>Lactobacillus</i> spp.	0.62	<.0001	<i>Escherichia coli</i> (NLF)	<i>Escherichia coli</i>	-0.40	.0068
<i>Lactobacillus</i> spp.	<i>Enterococcus</i> spp.	0.29	.0238	<i>Faecalibacterium prausnitzii</i>	<i>Bifidobacterium</i> spp.	0.34	.0234
				<i>Faecalibacterium prausnitzii</i>	TBC	0.43	.0031
				H ₂ O ₂ <i>Lactobacillus</i>	<i>Lactobacillus</i> spp.	0.62	<.0001
				<i>Lactobacillus</i> spp.	<i>Enterococcus</i> spp.	0.33	.0262
				<i>Lactobacillus</i> spp.	<i>Bifidobacterium</i> spp.	0.34	.0242
				<i>Lactobacillus</i> spp.	TBC	0.36	.0155

Legend: R – Spearman's rank coefficient of correlation; TBC – total bacteria count; NLF – non-lactose fermenting; $p < 0.05$ are indicated in bold

R values ranges:	-1	-0.8	-0.79	-0.6	-0.59	-0.4	-0.39	-0.2	-0.19	0	0.19	0.2	0.39	0.4	0.59	0.6	0.79	0.8	1
Proportionality	Negative/inversely proportional									No correlation	Positive/directly proportional								
Strength Interpretation	very strong	strong	moderate	weak						very weak	weak	moderate	strong	very strong					