DHA-induced perturbation of human serum metabolome. Role of the food matrix and co-administration of oat β-glucan and anthocyanins

Supplementary Materials

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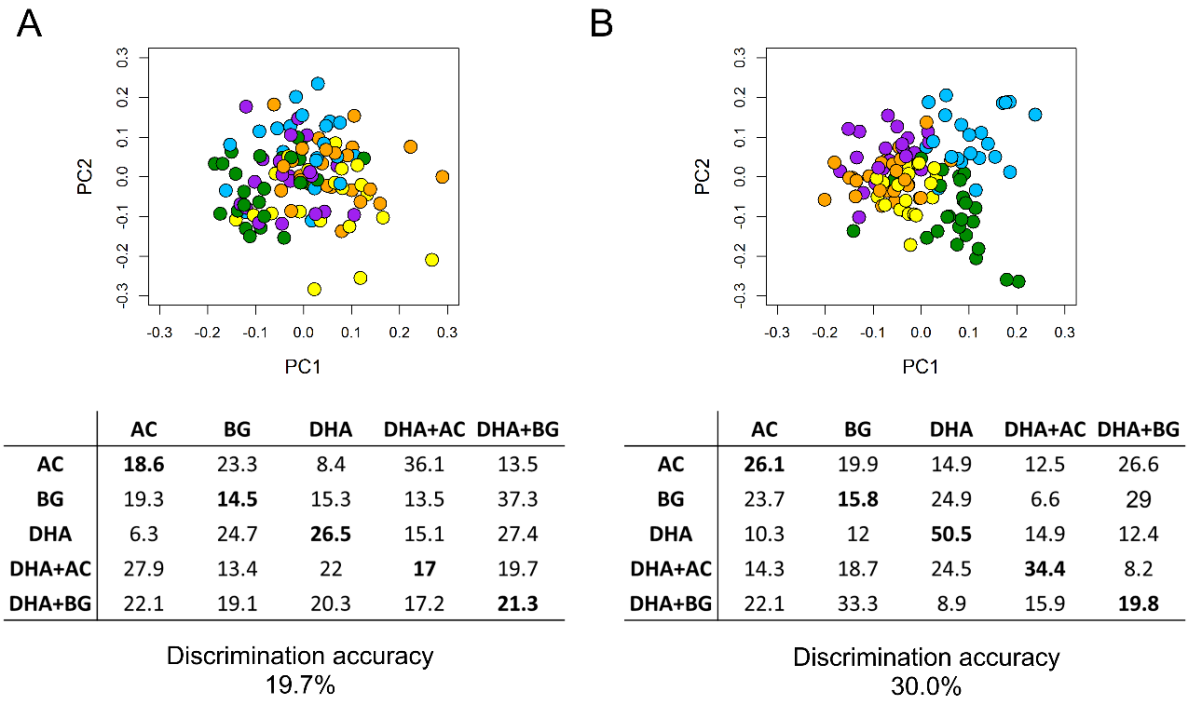

	AC	BG	DHA	DHA+AC	DHA+BG
AC	**18.6**	23.3	8.4	36.1	13.5
BG	19.3	**14.5**	15.3	13.5	37.3
DHA	6.3	24.7	**26.5**	15.1	27.4
DHA+AC	27.9	13.4	22	**17**	19.7
DHA+BG	22.1	19.1	20.3	17.2	**21.3**

Discrimination accuracy
19.7%

	AC	BG	DHA	DHA+AC	DHA+BG
AC	**26.1**	19.9	14.9	12.5	26.6
BG	23.7	**15.8**	24.9	6.6	29
DHA	10.3	12	**50.5**	14.9	12.4
DHA+AC	14.3	18.7	24.5	**34.4**	8.2
DHA+BG	22.1	33.3	8.9	15.9	**19.8**

Discrimination accuracy
30.0%

Figure S1. 5-Group discrimination. Score plot of PCA-CA analysis at T0 (a) and T1 (b). The values of the discrimination accuracy and the confusion matrices are also reported. In the score plot, each dot represents a different serum sample, and each colour represents a different group: dark green dots= AC; cyan dots= OBG; orange dots= DHA; yellow dots= DHA+AC; purple dots= DHA+OBG.

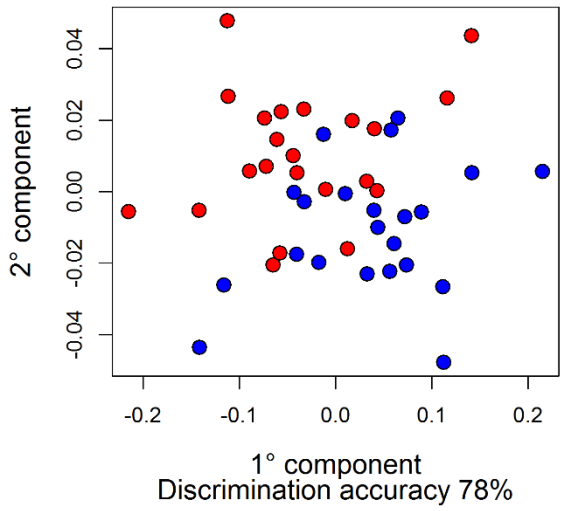

Figure S2. Score plot of M-PLS analysis (T0 vs. T1) of the spectral region (0.92 - 0.71 ppm) containing the signal of lipoprotein methyl group (-CH_3) resonances. The value of the discrimination accuracy is also reported. In the score plots, each dot represents a different serum sample of DHA+OBG group, and each colour represents a time point: blue dots= T0 samples; red dots= T1 samples.

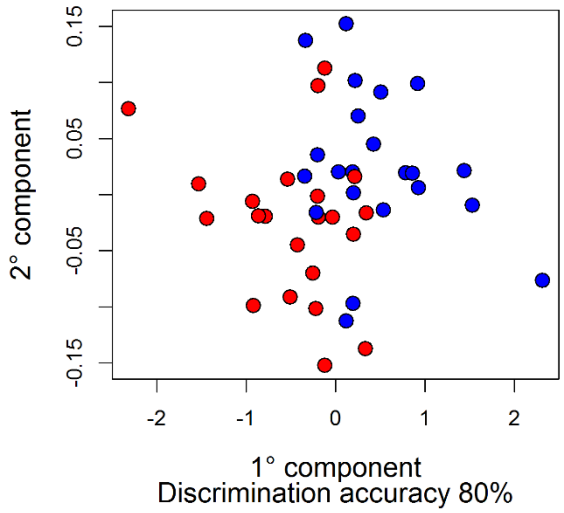

Figure S3. Score plot of M-PLS analysis (T0 vs. T1) of the spectral region (4.34 – 4.22 ppm) containing the signal of triglyceride resonances. The value of the discrimination accuracy is also reported. In the score plots, each dot represents a different serum sample of DHA+OBG group, and each colour represents a time point: blue dots= T0 samples; red dots= T1 samples.

Table S1. M-PLS discrimination accuracy values.

	Enriched Biscuits
DHA	85%
AC	48%
OBG	66%
DHA+AC	50%
DHA+OBG	100%

Table S2. Bruker IVdr Lipoprotein subclass analysis. Each significant comparison (T0 vs T1) is indicated with * for $p < 0.05$ and ** $p < 0.01$.

	DHA		AC		OBG		DHA + AC		DHA + OBG	
	T0	T1	T0	T1	T0	T1	T0	T1	T0	T1
TG	156.48	138.83	168.67	176.2	166.39	166.96	160.29	162.52	193.56	153.63*
Chol	226.25	240.43*	228.49	218.60	239.18	241.74	237.95	230.67	252.82	250.90
LDL-Chol	122.91	129.37	123.37	114.94	131.35	129.25	133.44	134.43	136.43	147.09*
HDL-Chol	52.04	53.515	48.89	49.17	49.77	51.13	49.47	47.85	50.41	51.50
Apo A1	147.1	148.06	147.03	143.32	147.58	149.97	140.9	137.12	146.04	142.07
Apo A2	32.71	32.84	32.66	33.52	35.77	34.15	32.36	30.83	35.42	34.98*
Apo B100	100.41	108.63*	100.49	104.33	105.43	115.31	111.06	106.76	114.74	113.23
Calculated Figures										
LDL-Chol/HDL-Chol	2.40	2.52	2.35	2.325	2.66	2.58	2.64	2.6	2.745	2.53
Apo B100/ Apo A1	1.42	1.26*	1.43	1.33	1.43	1.50	1.26	1.30	1.27	1.29
Total Apo B100 Particle Number	1825.71	1975.17*	1827.19	1896.97	1916.98	2096.64	2019.42	1941.34	2086.31	2058.91
VLDL Particle Number	192.87	175.21	226.40	229.415	203.68	210.67	210.08	198.28	236.63	208.71*
IDL Particle Number	114.72	125.52	111.30	110.66	112.87	121.54	133.99	120.06	140.01	113.54
LDL Particle Number	1496.1	1589.47*	1498.29	1485.65	1574.71	1641.95	1660.61	1576.62	1703.64	1726.34
LDL 1 Particle Number	232.84	261.34	197.92	192.17	229.24	224.15	222.97	211.18	236.15	234.76
LDL 2 Particle Number	135.96	146.20	127.89	107.465	144.12	144.61	156.2	149.43	128.89	136.96
LDL 3 Particle Number	168.93	175.43	150.52	112.33	172.15	154.01	186.58	188.54	162.15	168.93
LDL 4 Particle Number	236.97	250.86	202.88	177.61	233.12	219.68	263.76	259.80	218.57	257.18
LDL 5 Particle Number	282.27	340.76	295.64	335.28	294.8	362.03	334.17	327.82	324.57	356.65
LDL 6 Particle Number	417.67	476.1	478.34	531.35	458.74	513.02	486.81	441.21	538.21	505.63
Lipoprotein Main Fractions										
TG-VLDL	105.3	92.49	113.92	126.08	121.77	115.56	104.23	108.85	130.67	112.03*
TG-IDL	17.61	14.07	19.86	19.62	19.45	19.15	17.51	16.67	23.67	16.52*
TG-LDL	22.05	25.26*	20.805	22.585	23.32	23.16	24.9	23.22	24.55	22.80
TG-HDL	10.69	10.97	10.85	11.52	10.46	9.71	10.84	10.21	11.69	10.10*
Chol-VLDL	27.59	22.44	33.35	34.38	29.47	28.26	29.04	27.21	34.54	28.24*
Chol-IDL	15.97	16.93	16.4	16.78	16.02	17.52	18.51	16.27	19.76	15.57*
Chol-LDL	122.91	129.37	123.37	114.94	131.35	129.25	133.44	134.43	136.43	147.09*
Chol-HDL	52.04	53.51	48.895	49.175	49.77	51.13	49.47	47.85	50.41	51.50
Free Chol-VLDL	12.65	11.27	14.71	14.13	13.13	13.59	12.56	12.93	15.23	12.54*
Free Chol-IDL	4.48	4.95	4.615	4.71	4.59	5	5.33	4.595	5.48	4.47*
Free Chol-LDL	36.23	36.81	34.575	32.25	38.09	34.93	37.02	37.78	39.24	41.04
Free Chol-HDL	12.46	13.29	11.69	10.95	12.15	11.33	11.68	11.2	12.66	11.89
Phospholipids-VLDL	28.25	24.50	31.355	32.84	30.71	31.17	29.44	29.69	33.71	28.06*
Phospholipids-IDL	8.67	8.925	9.945	10.335	10.36	9.51	10.75	9.01	12.03	10.23*
Phospholipids-LDL	69.93	72.97*	67.985	64.355	75.3	72.86	75.47	73.96	75.48	81.04
Phospholipids-HDL	71.10	73.07	69.76	68.11	69.69	74.35	68.24	66.52	70.88	70.82
Apo A1-HDL	142.37	144.01	142.035	141.075	145.98	145.79	135.37	130.66	137.75	138.77
Apo A2-HDL	33.53	33.99	33.575	34.265	36.38	35.12	33.05	31.83	36.26	35.63*

Apo B-VLDL	10.61	9.63	12.45	12.62	11.2	11.59	11.55	10.90	13.01	11.48*
Apo B-IDL	6.31	6.90	6.125	6.085	6.21	6.68	7.37	6.60	7.7	6.245
Apo B-LDL	82.28	87.42*	82.4	81.71	86.61	90.3	91.33	86.71	93.7	94.94
VLDL Subfractions										
TG-VLDL 1	49.54	42.19	53.345	60.545	54.47	50.86	46.315	46.12	62.56	55.9*
TG-VLDL 2	19.73	13.31	19.99	22.74	19.33	19.7	19.805	18.32	23.62	19.14*
TG-VLDL 3	15.46	12.54	17.975	18	17.3	16.04	16.29	14.33	18.71	16.97*
TG-VLDL 4	10.82	9.63	12.345	13.975	11.74	11.53	12.81	11.53	13.48	12.31*
TG-VLDL 5	3.50	3.4	3.425	3.13	3.35	3.53	3.17	3.32	3.59	3.31*
Chol-VLDL 1	9.93	8.89	10.37	9.765	10.78	10.22	9.37	9.86	11.59	9.13*
Chol-VLDL 2	4.68	3.73	5.115	5.425	5.06	4.76	4.65	4.34	5.67	4.87*
Chol-VLDL 3	4.45	4.04	5.215	6	5.15	4.62	5.52	4.67	6.10	5.62
Chol-VLDL 4	6.21	6.9	7.57	7.26	6.42	6.68	7.75	6.52	8.27	6.48
Chol-VLDL 5	1.76	1.59	1.64	1.69	1.46	1.88	1.61	1.64	1.68	1.62
Free Chol-VLDL 1	3.51	3.32	3.73	3.92	3.54	3.99	3.71	3.86	4.11	3.98*
Free Chol-VLDL 2	1.93	1.67	2.11	2.225	2.15	1.9	1.95	1.74	2.31	1.93*
Free Chol-VLDL 3	2.07	1.69	2.485	2.64	2.39	2.17	2.32	1.91	2.55	2.42*
Free Chol-VLDL 4	2.51	2.87	2.735	2.72	2.39	2.45	2.98	2.5	3.09	2.37
Free Chol-VLDL 5	0.83	0.75	0.84	0.95	0.85	0.94	0.66	0.73	0.88	0.63
Phospholipids-VLDL 1	8.43	6.92	8.765	9.88	9.1	8.7	8.11	8.44	10.17	9.39*
Phospholipids-VLDL 2	4.81	3.71	5.1	5.515	5.09	4.88	5.08	4.65	5.86	4.905*
Phospholipids-VLDL 3	4.83	4.09	5.86	6.195	5.6	5.5	5.60	5.19	5.91	5.74*
Phospholipids-VLDL 4	5.58	5.57	6.305	6.89	5.76	5.72	6.75	5.76	6.8	6.04
Phospholipids-VLDL 5	2.30	2.06	2.16	2.14	2.04	2.23	2.13	2.05	2.305	1.94
LDL Subfractions										
TG-LDL 1	6.57	6.42	5.85	5.91	5.55	6.39	6.63	6.01	6.84	6.1*
TG-LDL 2	2.16	2.47	1.96	2.03	2.16	2	2.72	2.51	2.3	2.14
TG-LDL 3	2.54	2.735	2.55	2.53	2.92	2.85	3.02	2.96	2.98	2.95
TG-LDL 4	2.76	2.88*	2.625	2.665	2.93	2.81	3.08	2.99	3.00	3.03
TG-LDL 5	3.13	3.79*	3.69	4.105	3.69	4.21	4.23	3.97	3.94	4.01
TG-LDL 6	4.16	4.87	4.865	5.605	4.95	4.84	5.17	5.04	5.39	5.42
Chol-LDL 1	24.47	27.52	20.345	19.71	23.52	22.86	24.48	21.78	24.05	24.05
Chol-LDL 2	12.69	14.44	12.04	9.26	13.49	13.12	14.89	14.15	12.38	12.83
Chol-LDL 3	15.71	16.03	13.43	9.46	15.14	14.22	17.16	16.4	13.43	14.5
Chol-LDL 4	20.24	21.23	15.67	14.68	20.13	19.35	22.45	22.54	18.11	21.15
Chol-LDL 5	22.34	25.72	23.79	25.87	23.5	27.32	26.53	25.70	24.98	27.01
Chol-LDL 6	27.9	32.68	32.14	34.89	32.19	34.64	32.36	29.87	35.63	34.76
Free Chol-LDL 1	7.87	8.60	6.64	6.23	7.44	7.04	7.60	6.91	7.845	7.36
Free Chol-LDL 2	4.34	4.68	4.22	3.35	4.56	3.96	4.53	4.26	4.18	4.61
Free Chol-LDL 3	5.19	5.18	4.49	3.63	5.11	4.52	5.36	4.95	5.35	5.16
Free Chol-LDL 4	5.88	6.05	4.53	4.24	5.93	5.46	6.24	6.14	5.50	6.21
Free Chol-LDL 5	6.08	6.66	6.22	6.64	5.99	7.4	6.99	6.64	6.63	7.06
Free Chol-LDL 6	6.51	7.32	8.16	8.03	7.86	8.28	7.99	7.18	8.83	8.42
Phospholipids-LDL 1	13.54	15.39	11.63	11.19	13.36	13.12	13.52	12.46	13.85	13.48
Phospholipids-LDL 2	7.30	8.05	7.05	5.34	7.59	7.49	8.27	8.16	7.08	7.57

Phospholipids-LDL 3	9.01	8.97	7.88	6.03	8.6	8.09	9.43	9.38	7.78	8.2
Phospholipids-LDL 4	11.45	12.20	9.09	8.51	11.03	10.64	12.2	12.35	10.08	11.69
Phospholipids-LDL 5	11.90	13.72	12.84	13.78	12.58	14.88	14.33	13.66	13.23	14.41
Phospholipids-LDL 6	15.04	16.93	16.89	18.39	17.63	18.59	17.31	16.1	19.04	18.39
Apo B-LDL 1	12.80	14.37	10.88	10.57	12.61	12.33	12.26	11.61	12.98	12.91
Apo B-LDL 2	7.48	8.04	7.03	5.91	7.93	7.95	8.59	8.21	7.09	7.53
Apo B-LDL 3	9.29	9.65	8.28	6.17	9.47	8.47	10.26	10.37	8.91	9.29
Apo B-LDL 4	13.03	13.8	11.15	9.77	12.82	12.08	14.51	14.29	12.02	14.14
Apo B-LDL 5	15.52	18.74	16.26	18.44	16.21	19.91	18.38	18.03	17.85	19.61
Apo B-LDL 6	22.97	26.18	26.31	29.22	25.23	28.21	26.77	24.26	29.6	27.81
HDL Subfractions										
TG-HDL 1	2.58	2.93	2.86	2.62	2.44	1.95	2.575	2.61	2.46	2.695
TG-HDL 2	1.69	1.79	1.67	1.85	1.49	1.48	1.765	1.66	1.67	1.415*
TG-HDL 3	2.33	2.5	2.455	2.6	2.33	2.34	2.42	2.41	2.7	2.11*
TG-HDL 4	4.24	4.105	4.655	4.56	4.43	4.45	4.11	4.13	4.61	4.01*
Chol-HDL 1	13.02	13.39	11.66	12.2	12.16	11.22	15.5	12.56	13.99	14.35
Chol-HDL 2	7.025	7.035	6.32	6.5	6.06	6.01	6.89	6.59	6.97	6.63
Chol-HDL 3	9.975	10.31	9.855	9.015	10.11	10.07	9.29	9.04	9.67	10.20
Chol-HDL 4	21.77	21.55	23.45	22.535	21.37	21.18	18.94	18.97	22.83	21.55
Free Chol-HDL 1	3.39	3.24	3.01	3.025	3.9	3.2	3.65	3.37	3.74	3.79
Free Chol-HDL 2	1.73	1.68	1.445	1.435	1.76	1.58	1.58	1.49	1.8	1.69*
Free Chol-HDL 3	2.33	2.47	2.235	2.15	2.63	2.41	2.18	1.95	2.70	2.29*
Free Chol-HDL 4	4.57	4.475	4.42	4.125	4.42	4.12	3.99	3.63	4.97	4.41*
Phospholipids-HDL 1	14.3	15.81	13.175	12.78	14	12.5	16.92	14.62	15.17	15.3
Phospholipids-HDL 2	10.64	10.51	10.16	10.275	10.88	11.22	11.42	10.96	11.32	10.75
Phospholipids-HDL 3	15.59	15.15	15.72	14.98	16.84	17.06	15.06	14.85	15.94	16.25*
Phospholipids-HDL 4	29.9	29.39	30.865	30.44	29.54	29.34	27.36	26.74	31.34	29.25
Apo A1-HDL 1	16.82	16.82	15.95	15.48	13.65	13.99	17.88	16.69	13.33	15.95
Apo A1-HDL 2	16.43	17.87*	16.205	16.635	18.6	19.18	17.11	16	18.35	17.33
Apo A1-HDL 3	25.77	26.76	25.995	25.135	27.4	28.76	24.69	24.03	26.60	26.46*
Apo A1-HDL 4	81.00	80.94	86.72	85.84	84.15	81.3	75.14	74.75	83.71	82.01
Apo A2-HDL 1	1.71	1.97	1.66	1.695	1.81	2.01	2.10	2.13	2.04	2.065
Apo A2-HDL 2	2.98	3.19	2.955	3	3.56	3.61	3.76	3.5	3.74	3.57*
Apo A2-HDL 3	6.49	6.68	6.395	6.47	7.56	7.09	6.65	6.59	7.43	6.95*
Apo A2-HDL 4	22.02	22.15	23.18	23.08	22	21.77	19.59	20.16	23.46	23.05

Table S3. List of metabolites whose signals were assigned and integrated in the NMR spectra. DHA+OBG discrimination between T0 and T1 in all samples and only in milkshake; each significant comparison (T0 vs T1) is indicated with * for $p < 0.05$ and ** $p < 0.01$.

	All BEF		**Milkshake only**	
Metabolites	median T0	median T1	median T0	median T1
valine	0.2802	0.2971	0.2775	0.3329
isoleucine	0.0362	0.0395**	0.0405	0.0429
leucine	0.1123	0.1254*	0.112	0.1377
3-hydroxybutyrate	0.022	0.0208	0.0305	0.0222**
alanine	0.3994	0.4509*	0.3574	0.4676
acetate	0.0108	0.0147	0.0105	0.0136
glucose	0.8177	0.8947**	0.8368	0.968*
lactate	0.268	0.3168*	0.2501	0.3049
phenylalanine	0.0396	0.0474	0.04	0.0508
tyrosine	0.041	0.0482	0.0445	0.0545
creatine	0.0283	0.0361	0.0303	0.0344
creatinine	0.0613	0.063	0.0609	0.0598
glycine	0.1441	0.1693**	0.1407	0.1659**
glycerol	0.0159	0.0151	0.015	0.0136
histidine	0.0349	0.0348	0.038	0.037
formate	0.0025	0.0029*	0.0016	0.0026*
pyruvate	0.05	0.0558	0.0495	0.0554*
succinate	0.0141	0.0161	0.011	0.014
acetoacetate	0.0148	0.0286	0.0148	0.029*
dimethylamine	0.0086	0.012**	0.0084	0.013*
methionine	0.013	0.0207	0.0087	0.0308*
methanol	0.0524	0.0513	0.0478	0.0478
glutamine	0.0037	0.0068	0.0037	0.0077
lysine	0.0184	0.019	0.019	0.0192
proline	0.0135	0.0158	0.0115	0.0148
histidine	0.037	0.036	0.0376	0.0365

citrate	0.0216	0.0289**	0.0214	0.0328*
dimethylglycine	0.0128	0.0161	0.0114	0.0168