# The effect of polyphenols on DNA methylation-assessed biological age attenuation:

### The DIRECT PLUS trial

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# Additional file 1

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	Fi	ish	Mea	ıt	F	Fruit	Vege	tables	Le	gumes
	$\mathbf{r}^1$	р	r <sup>1</sup>	р	$\mathbf{r}^{1}$	р	r <sup>1</sup>	р	$\mathbf{r}^{1}$	р
Age	0.158	0.01	0.003	0.96	0.284	4.50E-06	0.34	3.20E-08	0.264	2.10E-05
Li mAge	0.139	0.03	-7.80E-04	0.99	0.286	3.90E-06	0.32	1.70E-07	0.254	4.60E-05
Li AA	-0.012	0.84	0.019	0.76	0.093	0.137	0.01	0.852	0.04	0.52
Li IEAA	0.005	0.93	-0.089	0.16	0.043	0.492	0.07	0.205	0.046	0.46
Horvath mAge	0.167	0.007	0.019	0.76	0.293	2.30E-06	0.29	1.80E-06	0.262	2.50E-05
Horvath AA	0.055	0.37	0.041	0.52	0.092	0.14	-0.01	0.865	0.069	0.27
Horvath IEAA	0.056	0.37	0.009	0.88	0.077	0.220	-0.003	0.951	0.045	0.47
Hannum mAge	0.131	0.04	-0.002	0.96	0.279	6.80E-06	0.29	2.70E-06	0.237	1.40E-04
Hannum AA	0.004	0.95	-0.023	0.71	0.036	0.564	2.10E-05	0.999	0.037	0.55
Hannum IEAA	0.021	0.73	-0.061	0.33	0.009	0.880	0.04	0.539	0.045	0.47
Horvath skin mAge	0.164	0.008	0.0005	0.99	0.261	2.71e-05	0.325	1.34e-07	0.263	2.30e-05
Horvath skin AA	0.029	0.64	-0.019	0.75	-0.02	0.66	-0.005	0.93	0.031	0.61
Horvath skin IEAA	0.051	0.42	-0.053	0.39	-0.03	0.64	0.040	0.52	0.043	0.49
PhenoAge	0.136	0.03	0.003	0.96	0.251	5.51e-05	0.287	3.68e-06	0.217	0.0005
PhenoAge AA	0.005	0.93	0.007	0.90	-0.023	0.66	-0.022	0.71	-0.003	0.96
PhenoAge IEAA	0.016	0.79	-0.0002	0.99	-0.037	0.55	-0.007	0.91	-0.011	0.85
GrimAge	0.151	0.02	0.025	0.69	0.233	0.0001	0.273	1.09e-05	0.239	0.0001
Grim AA	0.053	0.40	0.099	0.11	-0.082	0.19	-0.109	0.08	-0.011	0.86
Grim IEAA	0.033	0.59	0.099	0.11	-0.09	0.12	-0.141	0.024	-0.019	0.75
DunedinPACE	0.021	0.73	0.017	0.78	-0.003	0.96	0.046	0.46	0.062	0.32

Table S1: Correlation of epigenetic clock measurements with the energy-adjusted fish, meat, fruit, legumes, and vegetable intakes.

<sup>1</sup>Spearman correlation coefficient. AA, age acceleration; IEAA, intrinsic epigenetics age acceleration; mAge, methylation age.

Table S2: Differences in mAge residu	als (age acceleration)	) with/out control	ling for cell type
(Intrinsic epigenetic age acceleration)			

# a. Age acceleration

	Age acceleration pre intervention (baseline)	Age acceleration post intervention	p-value for paired t-test	p between groups baseline	p between group post intervention	
Li AA				0.577	0.563	
HDG	0.32 (3.7)	0.43 (3.7)	0.573			
MED	-0.46 (4.4)	-0.36 (4.4)	0.891			
Green-MED	0.104 (4.2)	-0.097 (3.9)	0.519			
Horvath AA				0.144	0.296	
HDG	0.65 (5.0)	0.49 (5.1)	0.752			
MED	-0.32 (4/2)	-0.31 (4.1)	0.973			
Green-MED	-0.37 (4.5)	-0.21 (4.1)	0.403			
Hannum AA				0.444	0.272	
HDG	0.49 (4.9)	0.63 (4.8)	0.671			
MED	-0.45 (4.6)	-0.51 (4.9)	0.865			
Green-MED	-0.08 (5.4)	-0.17 (4.9)	0.809			
Horvath skin and blood AA				0.16	0.068	
HDG	0.67 (3.5)	0.84 (3.7)	0.485			
MED	-0.57 (4.2)	-0.65 (4.3)	0.740			
Green-MED	-0.15 (3.9)	-0.25 (3.8)	0.725			
PhenoAge AA				0.677	0.217	
HDG	0.59 (4.4)	0.96 (5.2)	0.401			
MED	-0.92 (5.6)	-1.04 (5.7)	0.770			
Green-MED	0.25 (6.2)	0.0001 (4.5)	0.649			
PCGrimAge AA				0.373	0.416	
HDG	0.29 (2.9)	0.24 (3.0)	0.698			
MED	-0.25 (2.2)	-0.19 (2.2)	0.508			
Green-MED	-0.05 (2.4)	-0.07 (2.4)	0.911			
DunedinPACE				0.672	0.587	
HDG	1.12 (0.1)	1.11 (0.1)	0.022			
MED	1.10 (0.1)	1.08 (0.1)	0.058			
Green-MED	1.1 (0.1)	1.1 (0.1)	0.036			

Data presented as mean (SD). AA, age acceleration; HDG, healthy dietary guidelines; MED, Mediterranean.

# b. Intrinsic epigenetic age acceleration

	Age acceleration pre intervention (baseline)	Age acceleration post intervention	p-value for paired t-test	p between groups baseline	p between group post intervention
Li IEAA				0.461	0.247
HDG	0.41 (3.3)	0.46 (3.1)	0.793		
MED	-0.43 (4.1)	-0.32 (4.2)	0.613		

Green-MED	-0.007 (3.8)	-0.16 (3.6)	0.634		
Horvath IEAA				0.0392	0.102
HDG	0.85 (4.9)	0.63 (4.8)	0.673		
MED	-0.36 (4.0)	-0.26 (3.9)	0.754		
Green-MED	-0.51 (4.1)	-0.39 (3.7)	0.747		
Hannum IEAA				0.2	0.152
HDG	0.65 (4.6)	0.70 (4.1)	0.893		
MED	-0.49 (4.0)	-0.53 (4.3)	0.923		
Green-MED	-0.20 (4.6)	-0.21 (4.3)	0.970		
Horvath skin				0.076	0.031
and blood IEAA					
HDG	0.77 (3.4)	0.91 (3.1)	0.575		
MED	-0.63 (3.9)	-0.68 (4.0)	0.812		
Green-MED	-0.21 (3.6)	-0.28 (3.7)	0.781		
PhenoAge				0.58	0.16
IEAA					
HDG	0.66 (4.1)	1.06 (4.9)	0.323		
MED	-0.96 (5.6)	-1.16 (5.5)	0.632		
Green-MED	0.22 (5.9)	0.008 (4.3)	0.693		
PCGrimAge AA				0.382	0.355
HDG	0.25 (2.6)	0.27 (2.6)	0.725		
MED	-0.21 (2.0)	-0.22 (2.1)	0.727		
Green-MED	-0.05 (2.2)	-0.06 (2.2)	0.976		

Data presented as mean (SD). HDG, healthy dietary guidelines; IEAA, intrinsic epigenetic age acceleration; MED, Mediterranean.

cgID	r (Spearman)	p-value	FDR
Δcg16290275	0.24576	0.000217	0.047164
Δcg03172765	-0.17728	0.008108	0.853361
Δcg22454769	-0.16875	0.011798	0.853361
Δcg03032253	-0.15539	0.02054	0.992848
Δcg13187764	-0.15017	0.025251	0.992848
Δcg13576006	0.144667	0.031188	0.992848
Δcg07481335	0.142615	0.033688	0.992848
Δcg08202165	0.13959	0.037683	0.992848
Δcg09354241	-0.13708	0.04129	0.992848

Table S3: Specific CpGs associated with the GMD score. Top correlations (p<0.05).

FDR, false discovery rate.

			HDG				MED				Gre	en-MED
	N	Moon	N=10 SF	n velue <sup>2</sup>	N	Moon	N=9 SF	n valuo <sup>2</sup>	N	Moon	SE	$\frac{N=9}{n \text{ value}^2}$
	1	mAge <sup>1</sup>	512	p-value	14	mAge <sup>1</sup>	SE	p-value	1	mAge <sup>1</sup>	SE	p-value
Above 50y	3	-0.44	3.6	0.49	3	0.66	1.7	0.70	6	0.48	1.5	0.52
Below 50y	7	1.33	1.3		6	1.83	2.3		2	1.41	2.2	•
Baseline BMI < 30	5	-0.12	2.9	0.40	3	1.19	2.6	0.89	4	0.21	2.5	0.39
Baseline BMI >= 30	5	1.71	0.47		6	1.56	3.7		5	1.25	0.68	
No DM at	10	0.79	2.2	NA	7	1.42	2.4	0.88	9	0.79	1.7	NA
baseline DM (1)						1.40	0.2					
DM at baseline	-	-	-	214	2	1.49	0.2	0.15	-	-	-	0.11
No MS at baseline	9	0.74	2.3	NA	3	0.41	0.70	0.15	1	0.28	1.5	0.11
MS at baseline	1	-	_		6	1.95	2.4		2	2.55	1.2	
No Fatty liver at	7	0.51	2.6	1	4	0.36	1.3	0.19	6	0.65	1.6	0.62
baseline <sup>3</sup>												
Fatty liver at baseline <sup>3</sup>	3	1.46	0.3		4	2.45	2.7		2	2.03	2.0	
Successful weight	0	-	-	NA	2	0.98	1.5	1	2	1.89	2.5	0.19
lost												
Unsuccessful weight lost	10	0.79	2.2		7	1.57	2.3		7	0.47	1.8	
No Fatty liver	5	0.09	3.0	0.71	5	0.31	1.1	NA	6	0.16	1.5	NA
post intervention <sup>4</sup>												
Fatty liver post intervention <sup>4</sup>	4	1.7	0.5		1	-	-		1	-	-	
No DM post-	10	0.79	2.2	NA	7	1.42	2.4	0.88	9	0.79	1.7	NA
intervention						1.40	0.17					
DM post- intervention	-	-	-		2	1.49	0.17		-	-	-	
No MS post-	9	0.74	2.3	NA	5	0.49	1.4	0.39	7	0.28	1.5	0.11
intervention												
MS post- intervention	1	-	-		4	2.61	2.4		2	2.55	1.2	

Table S4: Biological aging across intervention groups in subgroups of health status, women only.

<sup>1</sup>mAge presented as absolute change. <sup>2</sup>Mann-Whiney test. <sup>3</sup> Liver MRI available for 26 participants at baseline. <sup>4</sup> Liver MRI available for 22 participants at T18. The presence of DM was defined for participants with baseline fasting plasma glucose levels  $\geq$ 126 mg/dL or haemoglobin-A1c levels  $\geq$ 6.5% or if regularly treated with oral antihyperglycemic medications or exogenous insulin. Liver status was based on MRI-measured live fat, as published before [35], with a cutoff >5% defining fatty liver. Interactions presented are between the health status and intervention group. BMI, body mass index; DM, diabetes mellitus; HDG, healthy dietary guidelines; MED, Mediterranean; MS, metabolic syndrome.

**Figure S1: The residuals from a regression model of mAge on actual age**. Positive residuals (red) represent mAge greater than age (biologically older). Negative residuals (green) represent mAge smaller than age (biologically younger).



Figure S2: Overlap between available CpGs for each clock



**Figure S3: 18-month absolute change in methylation age clocks across intervention groups for the entire cohort.** (a) Li mAge; (b) Horvath mAge; (c) Horvath skin and blood mAge; (d) Hannum mAge; (e) PhenoAge; (f) PCGrimAge. \* p<0.05 vs. baseline.



**Figure S4: 18-month absolute change in methylation age clocks across intervention groups, men only.** (a) Li mAge; (b) Horvath mAge; (c) Horvath skin and blood mAge; (d) Hannum mAge; (e) PhenoAge; (f) PCGrimAge.



**Figure S5: The distribution of the 9 components in the GMD score in each intervention group.** Preferred below median: Fruit, vegetables, legumes, fish, green tea, walnuts, and green Mankai. preferred above median: Meat and processed food. a. Healthy dietary guidelines; b. Mediterranean diet supplemented with walnuts; c. Green Mediterranean low in red and processed meat and supplemented with walnuts, green tea, and green Mankai.



**Figure S6: Correlation between changes in different mAge clocks and specific urine polyphenols.** a. The correlation between 18-month relative change in Li mAge and Hydroxytyrosol; b. The correlation between 18-month relative change in Li mAge and Tyrosol; c. The correlation between 18-month relative change in Li mAge and Urolithin C; d. The correlation between 18-month relative change in Horvath mAge and Hydroxytyrosol; e. The correlation between 18-month relative change in PCGrimAge mAge and Hydroxytyrosol. Blue line is a linear curve; red dashed line is a smooth curve.

