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Supplementary Materials for

DNA methylation as a mediator of the association between prenatal adversity and risk factors for metabolic disease in adulthood

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Supplemental Materials

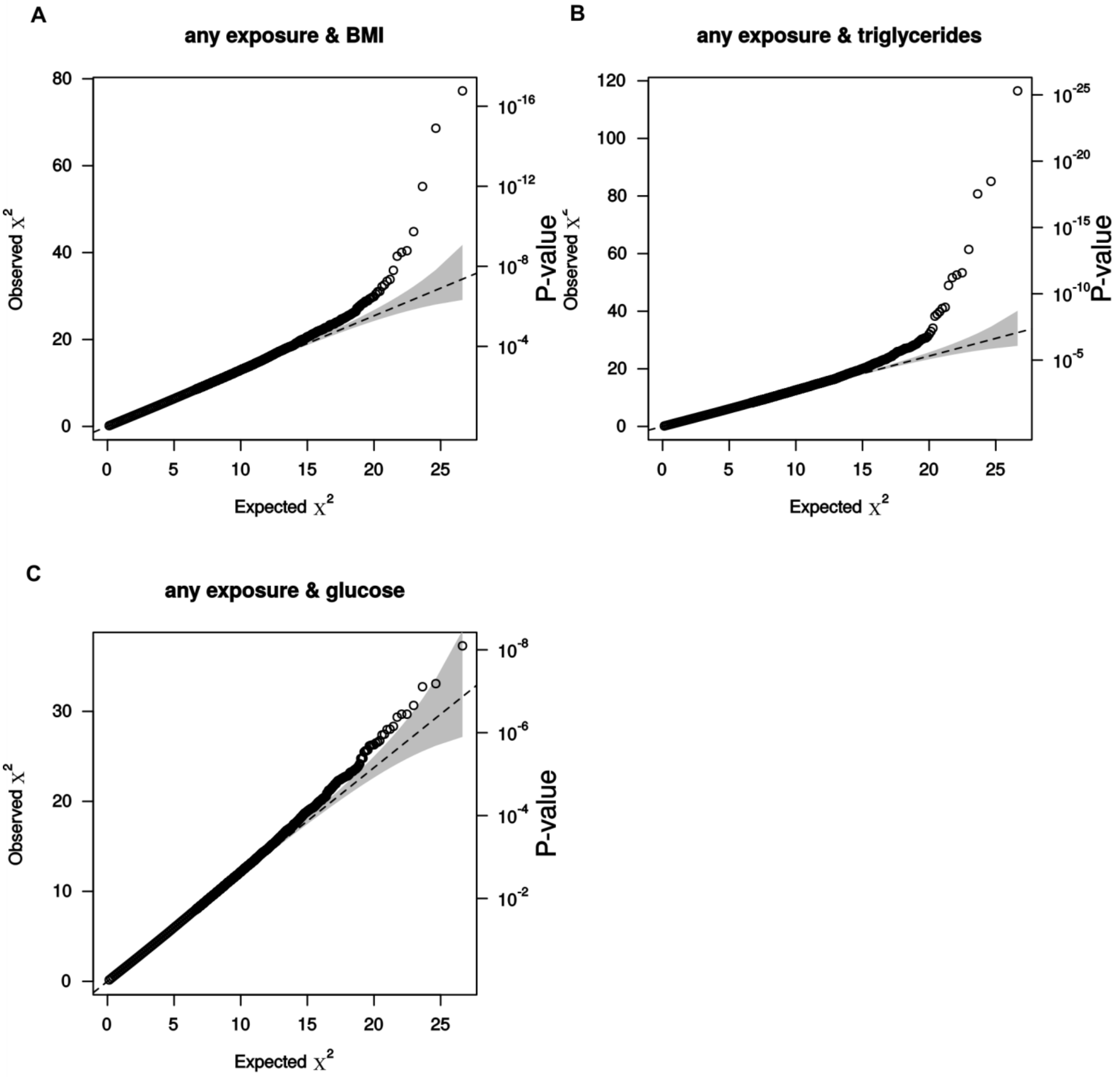

fig. S1. Quantile-quantile (QQ) plots for EWAS on famine exposure and phenotypes. Comparison of the expected versus observed statistic (χ2,df=2) for all 342K CpG dinucleotides tested. In gray the 95% C.I. is shown around the dotted line that represents the average trend between the expected and observed test statistic (λ, which ranged from 1.14-1.19).

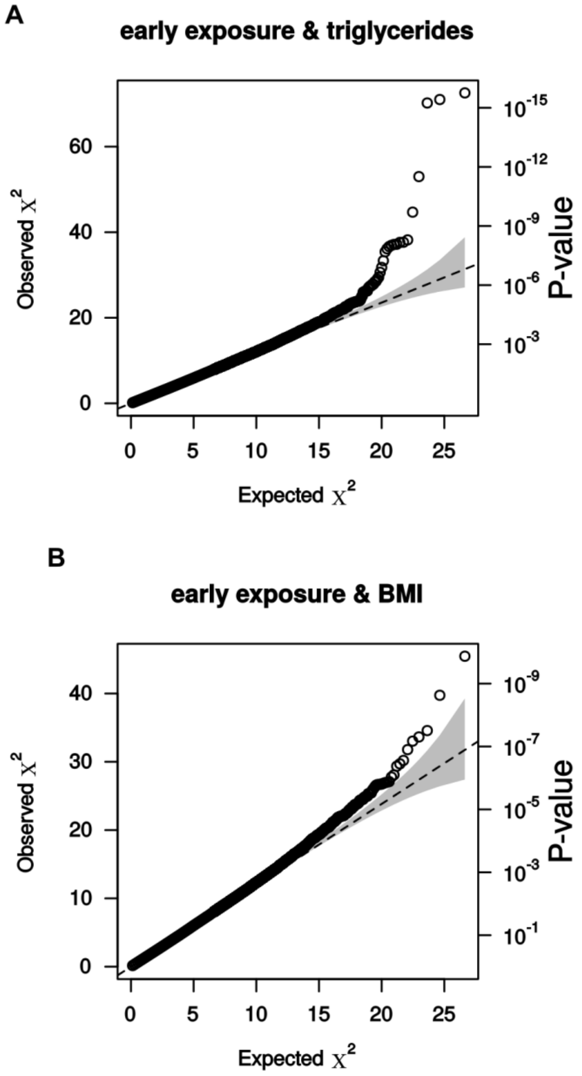

fig. S2. QQ plots for EWAS on early exposure and phenotypes. Comparison of the expected versus observed statistic (χ2,df=2) for all 342K CpG dinucleotides tested. In gray the 95% C.I. is shown around the dotted line that represents the average trend between the expected and observed test statistic (λ, which ranged from 1.12-1.19).

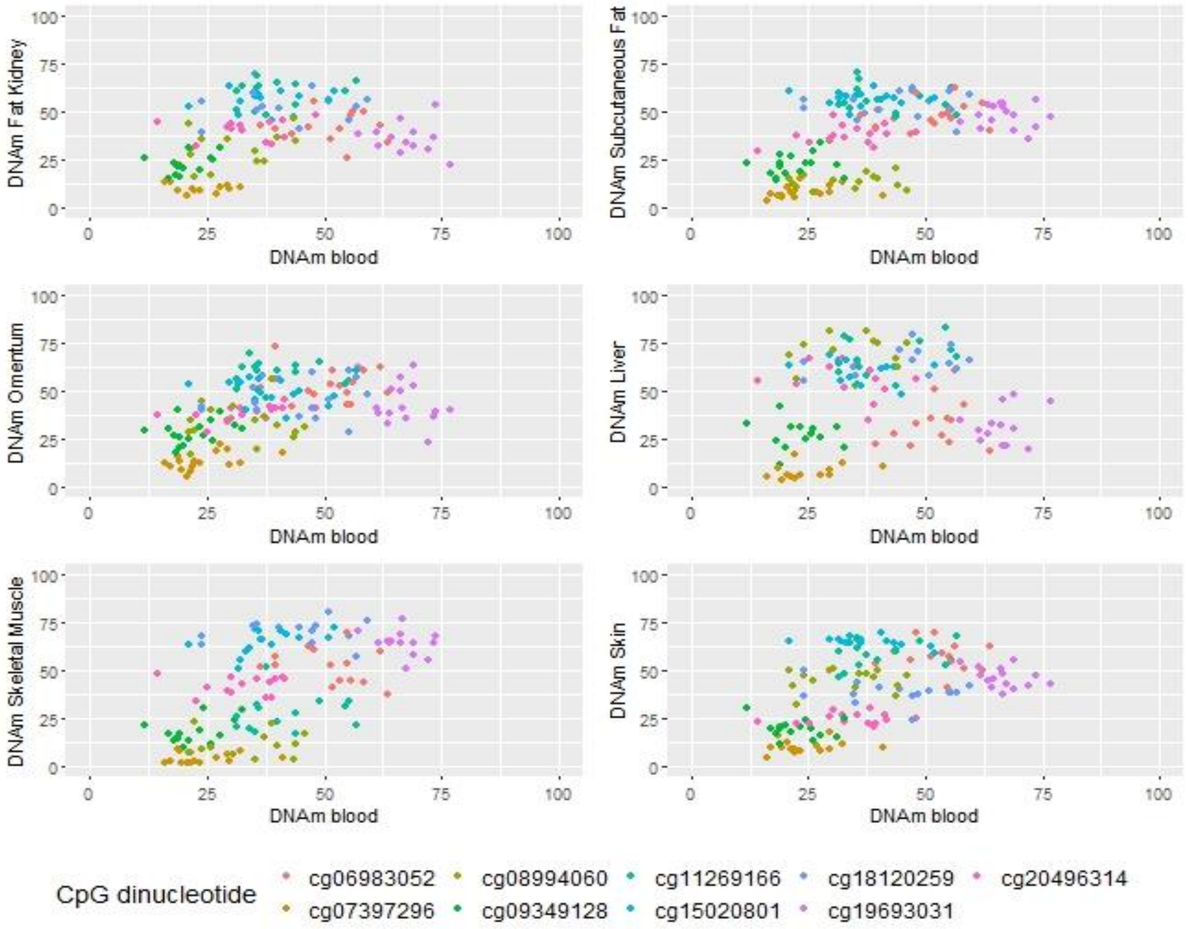

fig. S3. Correlation between tissues for the nine mediating CpG dinucleotides. Comparison of the percentage of methylation in whole blood (x-axis) with that in an (internal) tissue (y-axis) for the 9 CpG dinucleotides (each denoted in an specific colour).

table S1. Associations with gene expression and phenotypes.

CpG	BIOS DNAm-Expression N=2044 ($P_{FDR}<0.05$)	BIOS consortium data phenotype associations N=3296 (Dekker et al. Genome Biology 2016)		
CG ID	HGNC symbol	Phenotype	Estimate [S.E.]	P
cg09349128	*ZBED4, CRELD2, PIM3*	BMI	-8.7 [1.6]	$3.1\text{x}10^{-8}$
cg06983052	*LRRC8C,LRRC8D*	Triglycerides	-0.11 [0.06]	0.08
cg07397296	*ABCG1, AP001623.1*	Triglycerides	0.27 [0.07]	$1.1\text{x}10^{-4}$
cg20496314	*SYNGR1*	Triglycerides	0.18 [0.07]	0.015
cg15020801	*COPZ2, SP2*	Triglycerides	0.13 [0.07]	0.043
cg18120259	*none*	Triglycerides	-0.24 [0.07]	$6.9\text{x}10^{-4}$
cg19693031	*TXNIP, ANKRD34A*	Triglycerides	-0.64 [0.08]	$6.9\text{x}10^{-14}$
cg08994060	*PFKFB3*	Triglycerides	N.A.	N.A.
cg11269166	*METTL8*	Triglycerides	0.23 [0.06]	$3.6\ \text{x}10^{-4}$

table S2. Genome-wide screen for potential mediators: famine exposure and TG.

CpG dinucleotide characteristics				Mediation EWAS			Associations famine exposure or TG^4				Literature
CpG	*Location (hg19)*	*Nearest gene^1*	*Methylation (SD)^2*	*Rank*	*EWAS P^3*	*EWAS P_{FDR}*	β_{famine}	P_{famine}	β_{TG}	P_{TG}	*prior studies*
cg00574958	chr11:68607622	*CPT1A*	13.6 (2.5)	1	$2.1x10^{-21}$	$7.0x10^{-16}$	-0.3	0.11	-1.6	$7.9x10^{-27}$	(*33, 72, 73*)
cg06500161	chr21:43656587	*ABCG1*	65.9 (3.0)	2	$7.8x10^{-16}$	$1.3x10^{-10}$	-0.2	0.44	1.6	$2.2x10^{-18}$	(*33, 43, 73*)
cg17058475	chr11:68607737	*CPT1A*	16.1 (4.1)	3	$4.6x10^{-15}$	$5.2x10^{-10}$	-0.1	0.74	-1.9	$4.0x10^{-19}$	(*30, 72-74*)
cg26262157	chr10:6214079	*PFKFB3*	42.9 (5.5)	4	$1.2x10^{-11}$	$1.0x10^{-6}$	-0.3	0.31	-1.9	$5.4x10^{-15}$	
cg11024682	chr17:17730094	*SREBF1*	53.1 (3.2)	5	$3.4x10^{-10}$	$2.3x10^{-5}$	-0.1	0.46	1.1	$3.9x10^{-12}$	(*30, 33, 43, 49*)
cg19693031	chr1:145441552	*TXNIP*	77.5 (4.3)	6	$4.6x10^{-10}$	$2.6x10^{-5}$	-0.9	$4.8\ x10^{-3}$	-2	$2.3x10^{-11}$	(*41-45*)
cg08994060	chr10:6214026	*PFKFB3*	38.5 (6.5)	7	$6.7x10^{-10}$	$3.3x10^{-5}$	-0.4	0.2	-2.2	$8.8x10^{-13}$	
cg09737197	chr11:68607675	*CPT1A*	20.5 (4.6)	8	$2.0x10^{-9}$	$8.7x10^{-5}$	-0.4	0.078	-1.6	$5.7x10^{-12}$	(*33, 73*)
cg17901584	chr1:55353706	*DHCR24*	58.7 (6.0)	9	$4.5x10^{-8}$	$1.7x10^{-3}$	-0.4	0.24	-1.8	$2.5x10^{-10}$	(*30, 35, 44*)
cg18120259	chr6:43894639	*LOC100132354*	60.4 (4.7)	10	$5.3x10^{-8}$	$1.8x10^{-3}$	-0.8	$6.6\ x10^{-4}$	-1.1	$6.4x10^{-8}$	(*44*)
cg05014727	chr10:6214016	*PFKFB3*	34.4 (6.4)	11	$8.6x10^{-8}$	$2.7x10^{-3}$	-0.2	0.58	-1.6	$3.7x10^{-10}$	
cg15020801	chr17:46022809	*PNPO*	36.1 (3.4)	12	$1.2x10^{-7}$	$3.5x10^{-3}$	0.7	$7.1\ x10^{-4}$	1.1	$6.0x10^{-8}$	(*30*)
cg06983052	chr1:90288099	*LRRC8D*	64.8 (3.8)	13	$1.6x10^{-7}$	$4.2x10^{-3}$	-0.8	$1.0\ x10^{-5}$	-0.7	$5.3x10^{-6}$	
cg07397296	chr21:43655316	*ABCG1*	26.9 (3.8)	14	$8.7x10^{-7}$	0.021	0.7	$5.1\ x10^{-3}$	1.2	$1.9x10^{-7}$	(*49*)
cg20496314	chr22:39759864	*SYNGR1*	40.2 (4.3)	15	$1.4x10^{-6}$	0.032	0.6	$3.9\ x10^{-3}$	1.1	$1.5x10^{-7}$	(*45, 46*)
cg07504977	chr10:102131012	*LINC00263*	47.2 (4.9)	16	$2.0x10^{-6}$	0.043	-0.1	0.64	1.4	$2.1x10^{-8}$	(*30, 49*)
cg26199857	chr12:54764265	*ZNF385A*	68.9 (5.7)	21	$4.2x10^{-6}$	0.068	2	$3.3\ x10^{-}$	0.2	0.67	(*28*)

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cg15659713	chr8:38586183	*TACC1*	24.6 (3.9)	22	4.9x10^{-6}	0.077	1.2	5.3x10^{-8}	-0.1	0.61	(*28*)

1. Nearest gene within 100kb
2. The Illumina 450k array beta value (ranging from 0-1) multiplied by 100 for easy interpretation.
3. The p-value belonging to an anova test (χ^2,2 df) between a GEE model with and without both famine exposure and BMI.
4. The estimate and (nominal) p-value belonging to the EWAS for famine exposure (β=exposed-unexposed) or BMI (Beta-value*100/log(TG))

table S3. Early exposure.

	Controls	Preconception exposure	Early exposure
N	463	94	73
Age (y) [SD]	58.0[5.4]	58.4[0.5]	58.6 [0.4]*
Male (%)	43.0	52.1	46.6
BMI	27.0 [4.2]	27.7 [4.6]	28.7 [4.9]**
Triglycerides	1.48 [0.86]	1.64 [0.92]	1.68 [0.86]**

*P<0.05; **P<0.01; ***P<0.001: from a linear mixed effects model with as dependent the denoted variable and family identifier as random effect. In the model with age as outcome the difference in variance between exposed and unexposed for age was taken into account. Models for BMI, serum measurements and depression score were corrected for age and gender and the model for cholesterol measures for the use of statins.

1. Only individuals who were fasted at examination included: excluding 2 individuals designated as controls, 5 individuals with any and 1 individual with preconception exposure.
2. Only individuals who were fasted at examination and did not have prior diagnosed diabetes (thus receiving diabetes treatment) before the clinical examination were included: excluding an additional 17 individuals designated as controls, 27 individuals with any, 5 individuals with early and 6 individuals with preconception exposure in addition to those non-fasted individuals removed for the cholesterol analyses.

table S4. Genome-wide screen for potential mediators: early exposure and triglycerides.

CpG dinucleotide characteristics				Mediation EWAS			Associations with either any famine exposure or TG				Literature
cpg	*Location (hg19)*	*nearest gene*[1]	*Methylation (SD)*[2]	*Rank*	*EWAS P*[3]	*EWAS* P_{FDR}	β_{famine}	P_{famine}	β_{TG}	P_{TG}	*prior studies*
cg00574958	chr11:68607622	*CPT1A*	13.8 (2.6)	1	$4.1\text{x}10^{-14}$	$1.3\text{x}10^{-8}$	-0.4	0.31	-1.6	2.6x10-16	(*33, 72, 73*)
cg17058475	chr11:68607737	*CPT1A*	16.2 (4.2)	2	$8.0\text{x}10^{-14}$	$1.3\text{x}10^{-8}$	-0.4	0.41	-2.2	4.1x10-16	(*30, 72-74*)
cg06500161	chr21:43656587	*ABCG1*	66.1 (3)	3	$1.1\text{x}10^{-13}$	$1.3\text{x}10^{-8}$	-0.1	0.80	1.7	4.0x10-15	(*33, 43, 73*)
cg06649410	chr6:154360483	*OPRM1*	29.6 (5.2)	4	$1.7\text{x}10^{-10}$	$1.4\text{x}10^{-5}$	-0.2	0.87	-0.6	0.65	
cg26262157	chr10:6214079	*PFKFB3*	42.7 (5.6)	5	$5.7\text{x}10^{-9}$	$3.9\text{x}10^{-4}$	-0.7	0.21	-2.0	1.2x10-10	
cg11024682	chr17:17730094	*SREBF1*	53.2 (3.2)	6	$9.0\text{x}10^{-8}$	$4.8\text{x}10^{-3}$	-0.4	0.22	1.2	5.4x10-8	(*33, 43, 73*)
cg27370573	chr19:292167	*PPAP2C*	84.4 (4)	7	$1.1\text{x}10^{-7}$	$4.8\text{x}10^{-3}$	2.9	$8.9\text{x}10^{-8}$	-0.3	0.34	(*28*)
cg08994060	chr10:6214026	*PFKFB3*	38.3 (6.5)	8	$1.2\text{x}10^{-7}$	$4.8\text{x}10^{-3}$	-1.4	0.037	-2.6	4.3x10-9	
cg11269166	chr2:172203847	*METTL8*	48.2 (3.1)	9	$1.4\text{x}10^{-7}$	$4.8\text{x}10^{-3}$	0.8	0.025	1.2	4.1x10-8	(*75*)
cg05014727	chr10:6214016	*PFKFB3*	34.1 (6.5)	10	$1.4\text{x}10^{-7}$	$4.8\text{x}10^{-3}$	-0.8	0.13	-1.9	1.1x10-8	
cg10354880	chr12:46737123	*SLC38A2*)	87.7 (1.2)	11	$1.5\text{x}10^{-7}$	$4.8\text{x}10^{-3}$	0.7	$1.3\text{x}10^{-8}$	0.1	0.24	(*28*)
cg09737197	chr11:68607675	*CPT1A*	20.6 (4.7)	12	$1.7\text{x}10^{-7}$	$4.9\text{x}10^{-3}$	-0.2	0.72	-1.8	5.0x10-9	(*33, 73*)
cg17901584	chr1:55353706	*DHCR24*)	59 (6.2)	13	$2.2\text{x}10^{-7}$	$5.7\text{x}10^{-3}$	-0.1	0.91	-2.1	6.5x10-9	
cg19693031	chr1:145441552	*TXNIP*	77.8 (4.2)	14	$2.9\text{x}10^{-7}$	$7.2\text{x}10^{-3}$	-0.9	0.17	-1.7	2.8x10-8	(*41-45*)
cg22307029	chr19:49891270	*CCDC155*	53.2 (5.7)	15	$7.1\text{x}10^{-7}$	0.016	3.8	$3.4\text{x}10^{-6}$	-0.3	0.64	
cg11496778	chr11:3225076	*OSBPL5/MRGPRG*	26.3 (4.6)	16	$1.4\text{x}10^{-6}$	0.03	-2.4	$1.5\text{x}10^{-7}$	0.1	0.7	(*28*)
cg24324837	chr19:49891574	*CCDC155*	61.9 (11.1)	17	$2.2\text{x}10^{-6}$	0.045	7.5	$3.6\text{x}10^{-6}$	-0.9	0.32	
cg20823026	chr2:366113	*FAM150B*	85.1 (4.6)	24	$8.6\text{x}10^{-6}$	0.12	2.1	$9.4\text{x}10^{-7}$	-0.2	0.63	(*28*)

1. Nearest gene within 100kb
2. The Illumina 450k array beta value (ranging from 0-1) multiplied by 100 for easy interpretation.
3. The p-value belonging to an anova test (χ^2,2 df) between a GEE model with and without both famine exposure and BMI.
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