Supplemental Material 1 – Cognitive Tests:

The summary discusses various cognitive tests used to assess cognitive functioning, with more details provided elsewhere(Beydoun et al., 2023). These tests include the Mini-Mental State Examination (MMSE), California Verbal Learning Test (CVLT), Benton Visual Retention Test (BVRT), Wechsler Adult Intelligence Scale, Revised (DS-F and DS-B), Category Fluency, Brief Test of Attention (BTA), Trail Making Tests A and B (TRAILS A and B), Clock Drawing Test – Clock to Command (CDT), and Wide Range Achievement Test – 3rd Edition: Word and Letter Reading Subtest (WRAT).

The MMSE measures orientation, concentration, language, immediate and short-term memory, and constructional praxis. CVLT measures verbal learning and memory using a 16-item word list. BVRT assesses visuo-constructional skills and nonverbal memory. Wechsler Adult Intelligence Scale, Revised measures executive function, with the total score as the outcome variable. Category Fluency tests measure semantic verbal fluency, while BTA measures divided auditory attention. Trail Making Tests A and B focus on attention and executive functioning, while the Clock Drawing Test assesses visuospatial skills, executive function, and memory components. The WRAT Word and Letter Reading Subtest is used as a stand-in for literacy and educational quality.

The CES-D is a 20-item measure of depressive symptoms, with scores ranging from 0 to 60. Scores of >16 indicate significant depressive symptoms, and scores of >20 indicate a clinically significant level of depressive symptoms.

Supplementary Material 3: Directed Acyclic Graph

Fully adjusted model, Model 3: potential for incorrect adjustment

Exposure: Hcy
Outcome: cogn
Adjusted: BMI,COMORBID,DEP,HEI,SRH,age,drug,educ,literacy,pov_st,race,sex,smoking
Incorrectly adjusted.
No adjustment sets found.

The model implies the following conditional independences:

- BMI ⊥ DEP | Hcy, sex
- BMI ⊥ SRH | age, educ, literacy, pov_st, race, sex
- BMI ⊥ drug | age, educ, literacy, pov_st, race, sex
- COMORBID ⊥ DEP | Hcy, sex
- COMORBID ⊥ HEI | BMI, age, educ, pov_st, race, sex
- COMORBID ⊥ drug | age, educ, literacy, pov_st, race, sex
- COMORBID ⊥ drug | BMI, age, educ, pov_st, race, sex
- COMORBID ⊥ literacy | BMI, age, educ, pov_st, race, sex
- COMORBID ⊥ smoking | BMI, age, educ, pov_st, race, sex
- DEP ⊥ HEI | Hcy, sex
- DEP ⊥ age | Hcy, sex
- DEP ⊥ drug | Hcy, sex
- DEP ⊥ educ | Hcy, sex
- DEP ⊥ literacy | Hcy, sex
- DEP ⊥ pov_st | Hcy, sex
- DEP ⊥ race | Hcy, sex
- DEP ⊥ smoking | Hcy, sex
- HEI ⊥ SRH | age, educ, literacy, pov_st, race, sex
- HEI ⊥ drug | age, educ, literacy, pov_st, race, sex
- HEI ⊥ smoking | age, educ, literacy, pov_st, race, sex
- Hcy ⊥ SRH | age, educ, literacy, pov_st, race, sex
- SRH ⊥ drug | age, educ, literacy, pov_st, race, sex
- SRH ⊥ smoking | age, educ, literacy, pov_st, race, sex
- age ⊥ educ
- age ⊥ literacy
- age ⊥ pov_st
- age ⊥ race
- age ⊥ sex
- drug ⊥ smoking | age, educ, literacy, pov_st, race, sex
- pov_st ⊥ race | educ
- pov_st ⊥ sex | educ
- race ⊥ sex

Model R code:

```
dag {

bb="0,0,1,1"

BMI [adjusted,pos="0.669,0.083"]

COMORBID [adjusted,pos="0.771,0.027"]

DEP [adjusted,pos="0.877,0.122"]

HEI [adjusted,pos="0.465,0.046"]
```

Hcy [exposure,pos="0.112,0.260"]

SRH [adjusted,pos="0.930,0.275"]

age [adjusted,pos="0.111,0.634"]

cogn [outcome,pos="0.804,0.280"]

drug [adjusted,pos="0.324,0.024"]

educ [adjusted,pos="0.669,0.631"]

literacy [adjusted,pos="0.817,0.494"]

pov_st [adjusted,pos="0.517,0.574"]

race [adjusted,pos="0.328,0.572"]

sex [adjusted,pos="0.038,0.478"]

smoking [adjusted,pos="0.234,0.083"]

BMI -> COMORBID

BMI -> Hcy

BMI -> cogn

COMORBID <-> Hcy

COMORBID <-> SRH

COMORBID <-> cogn

DEP <-> SRH

DEP <-> cogn

HEI -> BMI

HEI -> Hcy

HEI -> cogn

Hcy -> DEP

Hcy -> cogn

SRH <-> cogn

age -> BMI

age -> COMORBID

age -> HEI

age -> Hcy

age -> SRH

age -> cogn

age -> drug

age -> smoking

drug -> Hcy

drug -> cogn

educ -> BMI

educ -> COMORBID

educ -> HEI

educ -> Hcy

educ -> SRH

educ -> cogn

educ -> drug

educ -> pov_st

educ -> smoking

educ <-> literacy

literacy -> BMI

literacy -> HEI

literacy -> Hcy

literacy -> SRH

literacy -> cogn

literacy -> drug

literacy -> smoking

pov_st -> BMI

pov_st -> COMORBID

pov_st -> HEI

pov_st -> Hcy

pov_st -> SRH

pov_st -> cogn

pov_st -> drug

pov_st -> literacy

pov_st -> smoking

race -> BMI

race -> COMORBID

race -> HEI

race -> Hcy

race -> SRH

race -> cogn

race -> drug

race -> educ

race -> literacy

race -> smoking

sex -> BMI

sex -> COMORBID

sex -> DEP

sex -> HEI

sex -> Hcy

sex -> SRH

sex -> cogn

sex -> drug

sex -> educ

sex -> smoking

smoking -> BMI

smoking -> Hcy

smoking -> cogn

}

exposure(s) **Hcy**

outcome(s) **cogn**

covariates **13**

causal paths **1**
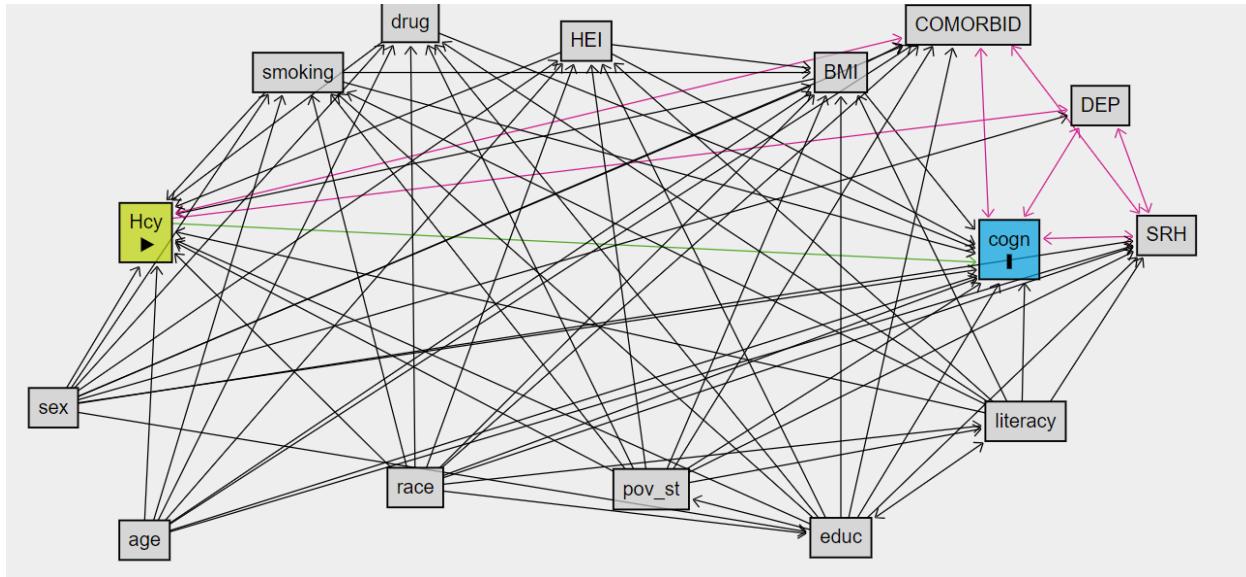
Fully adjusted Model 2: adjusted for socio-demographics, lifestyle factors and BMI

Exposure: Hcy
Outcome: cogn
Adjusted: BMI,HEI,age,drug,educ,literacy,pov_st,race,sex,smoking
Correctly adjusted.
Minimal sufficient adjustment sets containing BMI, HEI, age, drug, educ, literacy, pov_st, race, sex, smoking for estimating the total effect of Hcy on cogn:

- BMI, HEI, age, drug, educ, literacy, pov_st, race, sex, smoking

The model implies the following conditional independences:
- cogn ⊥ BMI | HEI, Hcy, age, drug, educ, literacy, pov_st, race, sex, smoking
- age ⊥ sex
- age ⊥ race

- age ⊥ pov_st
- age ⊥ educ
- age ⊥ literacy
- sex ⊥ race
- sex ⊥ pov_st | educ
- race ⊥ pov_st | educ
- smoking ⊥ HEI | age, educ, literacy, pov_st, race, sex
- smoking ⊥ drug | age, educ, literacy, pov_st, race, sex
- HEI ⊥ drug | age, educ, literacy, pov_st, race, sex
- drug ⊥ BMI | age, educ, literacy, pov_st, race, sex

Model R code:

```
dag {

bb="0,0,1,1"

BMI [adjusted,pos="0.669,0.083"]

HEI [adjusted,pos="0.465,0.046"]

Hcy [exposure,pos="0.112,0.260"]

age [adjusted,pos="0.111,0.634"]

cogn [outcome,pos="0.804,0.280"]

drug [adjusted,pos="0.324,0.024"]

educ [adjusted,pos="0.669,0.631"]

literacy [adjusted,pos="0.817,0.494"]

pov_st [adjusted,pos="0.517,0.574"]

race [adjusted,pos="0.328,0.572"]

sex [adjusted,pos="0.038,0.478"]

smoking [adjusted,pos="0.234,0.083"]

BMI -> Hcy
```

HEI -> BMI

HEI -> Hcy

HEI -> cogn

Hcy -> cogn

age -> BMI

age -> HEI

age -> Hcy

age -> cogn

age -> drug

age -> smoking

drug -> Hcy

drug -> cogn

educ -> BMI

educ -> HEI

educ -> Hcy

educ -> cogn

educ -> drug

educ -> pov_st

educ -> smoking

educ <-> literacy

literacy -> BMI

literacy -> HEI

literacy -> Hcy

literacy -> cogn

literacy -> drug

literacy -> smoking

pov_st -> BMI

pov_st -> HEI

pov_st -> Hcy

pov_st -> cogn

pov_st -> drug

pov_st -> literacy

pov_st -> smoking

race -> BMI

race -> HEI

race -> Hcy

race -> cogn

race -> drug

race -> educ

race -> literacy

race -> smoking

sex -> BMI

sex -> HEI

sex -> Hcy

sex -> cogn

sex -> drug

sex -> educ

sex -> smoking

smoking -> BMI

smoking -> Hcy

smoking -> cogn

}
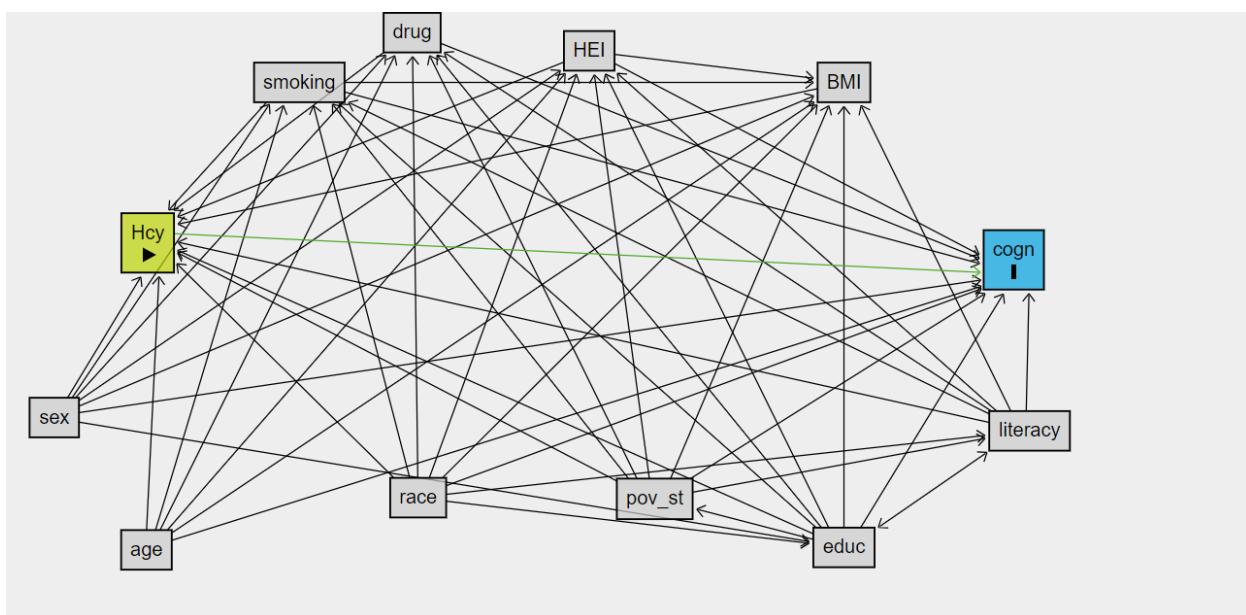
Supplemental Table 1. Relationship of baseline LnHcy (LnHcy$_{v1}$) and LnHcy trajectory (Hcy $_{traj}$) with 11 cognitive test scores (baseline and between-visit change) in fully-adjusted models: HANDLS 2004-2013 [a]

COGNITIVE TESTS [b]	LnHcy$_{v1}$ β (SE)	P value	Hcy $_{traj}$ β (SE)	P value
MMSE, normalized:	N= 1,430, K=1.9		N= 1,390, K=1.9	
[LnHcy$_{v1}$ | Hcy $_{traj}$]	+0.467 (1.084)	0.67	+0.061 (0.364)	0.86
[LnHcy$_{v1}$ × Time | Hcy $_{traj}$ × Time]	-0.007 (0.283)	0.98	+0.021 (0.087)	0.81
CVLT-List A:	N= 1,420, K=1.7		N=1,391, K=1.8	
[LnHcy$_{v1}$ | Hcy $_{traj}$]	**+1.231 (0.531)**	**0.020**	**+0.486 (0.171)**	**0.005**
[LnHcy$_{v1}$ × Time | Hcy $_{traj}$ × Time]	-0.077 (0.115)	0.51	-0.043 (0.036)	0.23
CVLT-DFR:	N=1,391, K=1.7		N=1,365, K=1.7	
[LnHcy$_{v1}$ | Hcy $_{traj}$]	**+0.601 (0.256)**	**0.019**	**+0.328 (0.083)**	**<0.001**
[LnHcy$_{v1}$ × Time | Hcy $_{traj}$ × Time]	-0.013 (0.057)	0.82	-0.034 (0.017)	0.055
BVRT:	N=1,443, K=1.9		N=1,412, K=1.9	
[LnHcy$_{v1}$ | Hcy $_{traj}$]	-0.117 (0.384)	0.76	-0.107 (0.123)	0.38
[LnHcy$_{v1}$ × Time | Hcy $_{traj}$ × Time]	+0.125 (0.082)	0.13	**-0.059 (0.026)**	**0.021**
BTA:	N=1,418, K=1.8		N=1,392, K=1.8	
[LnHcy$_{v1}$ | Hcy $_{traj}$]	-0.083 (0.188)	0.66	-0.006 (0.059)	0.92
[LnHcy$_{v1}$ × Time | Hcy $_{traj}$ × Time]	+0.006 (0.043)	0.89	-0.017 (0.013)	0.19
AF:	N= 1,446, K=1.9		N= 1,446, K=1.9	
[LnHcy$_{v1}$ | Hcy $_{traj}$]	-0.014 (0.429)	0.71	-0.053 (0.138)	0.70
[LnHcy$_{v1}$ × Time | Hcy $_{traj}$ × Time]	-0.036 (0.083)	0.62	0.036 (0.026)	0.16
DS-F:	N=1,443, K=1.9		N=1,412, K=1.9	
[LnHcy$_{v1}$ | Hcy $_{traj}$]	-0.044 (0.167)	0.80	-0.018 (0.053)	0.74
[LnHcy$_{v1}$ × Time | Hcy $_{traj}$ × Time]	-0.024 (0.034)	0.47	-0.014 (0.010)	0.17
DS-B:	N= 1,444, K=1.9		N= 1,413, K=1.9	
[LnHcy$_{v1}$ | Hcy $_{traj}$]	-0.028 (0.162)	0.86	-0.035 (0.052)	0.50
[LnHcy$_{v1}$ × Time | Hcy $_{traj}$ × Time]	0.011 (0.035)	0.76	+0.002 (0.011)	0.87
CDT:	N=1,445, K=1.9		N=1,414, K=1.9	
[LnHcy$_{v1}$ | Hcy $_{traj}$]	-0.059 (0.100)	0.56	-0.038 (0.032)	0.24
[LnHcy$_{v1}$ × Time | Hcy $_{traj}$ × Time]	-0.040 (0.026)	0.13	-0.007 (0.008)	0.43
Log$_e$ (TRAILS A):	N=1,428, K=1.9		N= 1,397, K=1.9	
[LnHcy$_{v1}$ | Hcy $_{traj}$]	**+0.081 (0.031)**	**0.008**	**+0.029 (0.010)**	**0.004**
[LnHcy$_{v1}$ × Time | Hcy $_{traj}$ × Time]	-0.011 (0.007)	0.14	-0.001 (0.002)	0.41
Log$_e$(TRAILS B):	N=1,414, K=1.8		N=1,383, K= 1.9	
[LnHcy$_{v1}$ | Hcy $_{traj}$]	+0.037 (0.051)	0.46	+0.012 (0.02)	0.46
[LnHcy$_{v1}$ × Time | Hcy $_{traj}$ × Time]	+0.011 (0.105)	0.31	**+0.006 (0.003)**	**0.049**

Abbreviations: Hcy = Homocysteine; Hcy $_{traj}$ = z-transformed probability of belonging to a group with increasing LnHcy over time according to group-based trajectory modeling; Ln or Log$_e$=Loge transformed; v1=visit 1 [a] Models are adjusted for age, sex, race, poverty status, education, literacy, smoking, drug use, 2010 healthy eating index, body mass index, hypertension, diabetes, dyslipidemia, cardiovascular disease, depressive symptoms score, self-rated health, inverse mills ratio as well as time on study in years between visits 1 and 2 and its interaction with LnHcyv1 or Hcy $_{traj}$ and covariates. [b] Cognitive tests include the Mini-Mental State Examination (MMSE), the California Verbal Learning Test (CVLT) Immediate (List A) and Delayed Free Recall (DFR), the Benton Visual Retention Test (BVRT, # of errors), Brief Test of Attention (BTA), Animal Fluency test (AF), the Digit Span Forward and Backwards tests (DS-F and DS-B), the Clock Drawing Test (CDT), the Trail making test Part A and B (TRAILS A and B, in seconds). K=mean observations/participant.

Supplemental Table 2. Interaction effects by serum folate and vitamin B-12 levels (Log_e transformed, z-scored) for the effects of baseline blood homocysteine level (Log_e transformed), $LnHcy_{v1}$ on 11 cognitive test scores (baseline and between-visit change): HANDLS 2004-2013 [c]

	Model 1 [a]		Model 2 [b]	
	β (SE)	P value	β (SE)	P value
FOLATE				
MMSE, normalized [FOL_{v1}]	**+0.137 (0.061)**	**0.024**	.070 (.055)	0.203
MMSE, normalized [$FOL_{v1} \times$ Time]	-0.0046 (0.015)	0.755	-.0137 (.0151)	0.364
MMSE, normalized [$FOL_{v1} \times LnHcy_{v1}$]	**-0.424 (0.152)**	**0.005**	-.220 (.130)	0.092
MMSE, normalized [$FOL_{v1} \times LnHcy_{v1} \times$ Time]	0.036 (0.038)	0.342	.029 (.037)	0.431
CVLT-List A [FOL_{v1}]	-0.0017 (0.028)	0.950	-.0402 (.027)	0.145
CVLT-List A [$FOL_{v1} \times$ Time]	*0.0095 (0.0054)*	*0.081*	**.0115 (.0057)**	**0.044**
CVLT-List A [$FOL_{v1} \times LnHcy_{v1j}$]	-0.153 (.068)	0.024	-.092 (.063)	0.143
CVLT-List A [$FOL_{v1} \times LnHcy_{v1} \times$ Time]	-0.019 (0.014)	0.151	-.020 (.014)	0.148
CVLT-DFR [FOL_{v1}]	.0098 (0.013)	0.447	-.000097 (.013)	0.994
CVLT-DFR [$FOL_{v1} \times$ Time]	0.0018 (0.0026)	0.499	.0017 (.0027)	0.533
CVLT-DFR [$FOL_{v1} \times LnHcy_{v1}$]	**-0.065 (0.032)**	**0.043**	-.0455 (.031)	0.143
CVLT-DFR [$FOL_{v1} \times LnHcy_{v1} \times$ Time]	-0.00018 (0.0067)	0.977	.00093 (.0067)	0.889
BVRT [FOL_{v1}]	-0.033 (0.021)	0.116	-.012 (.021)	0.584
BVRT [$FOL_{v1} \times$ Time]	.0016 (.0038)	0.665	.0013 (.0039)	0.749
BVRT [$FOL_{v1} \times LnHcy_{v1}$]	0.032 (0.048)	0.495	-.0096 (.045)	0.833

13

BVRT [FOL$_{v1}$ × LnHcy$_{v1}$ × Time]	**0.023 (0.0096)**	**0.017**	**.022 (.0097)**	**0.020**
BTA [FOLv1]	0.0038 (0.0095)	0.683	-.0037 (.0094)	0.692
BTA [FOL$_{v1}$ × Time]	0.00090 (0.0019)	0.645	.00055 (.0020)	0.787
BTA [FOL$_{v1}$ × LnHcy$_{v1}$]	0.012 (0.023)	0.611	.031 (0.023)	0.173
BTA [FOL$_{v1}$ × LnHcy$_{v1}$ × Time]	-0.0052 (0.0051)	0.305	-.0049 (.0051)	0.338
AF [FOL$_{v1}$]	**0.062 (0.021)**	**0.004**	.0320 (.0214)	0.135
AF [FOL$_{v1}$ × Time]	-0.0025 (0.0039)	0.525	-.00016 (.00417)	0.969
AF [FOL$_{v1}$ × LnHcy$_{v1}$]	-0.074 (0.054)	0.166	-.0373 (.0513)	0.467
AF [FOL$_{v1}$ × LnHcy$_{v1}$ × Time]	0.014 (0.0099)	0.140	.0140 (.0100)	0.161
DS-F [FOL$_{v1}$]	.0154 (.0093)	0.097	.0067 (.0085)	0.435
DS-F [FOL$_{v1}$ × Time]	-.00076 (.0015)	0.628	-.00096 (.0017)	0.565
DS-F [FOL$_{v1}$ × LnHcy$_{v1}$]	-.0376 (.022)	0.089	-.0124 (.020)	0.541
DS-F [FOL$_{v1}$ × LnHcy$_{v1}$ × Time]	.00248 (.0040)	0.535	.0026 (.0040)	0.509
DS-B [FOL$_{v1}$]	**.0196 (.0088)**	**0.027**	.0089 (.0082)	0.280
DS-B [FOL$_{v1}$ × Time]	-.00098 (.0016)	0.550	-.00124 (.00171)	0.467
DS-B [FOL$_{v1}$ × LnHcy$_{v1}$]	-.0258 (.0215)	0.231	.000099 (.019)	0.996
DS-B [FOL$_{v1}$ × LnHcy$_{v1}$ × Time]	-.0053 (.0041)	0.199	-.0055 (.0042)	0.182
CDT [FOL$_{v1}$]	**0.0122 (0.0050)**	**0.015**	.0077 (.0052)	0.137
CDT [FOL$_{v1}$ × Time]	-0.00087 (0.0013)	0.491	-.00076 (.0013)	0.569
CDT [FOL$_{v1}$ × LnHcy$_{v1}$]	0.0188 (0.0123)	0.128	**.0261 (.012)**	**0.032**
CDT [FOL$_{v1}$ × LnHcy$_{v1}$ × Time]	-0.00618 (0.00319)	0.053	-.0057 (.0032)	0.077
Log$_e$ (TRAILS A) [FOL$_{v1}$]	-0.0017 (0.0016)	0.256	-.00126 (.0015)	0.424
Log$_e$ (TRAILS A) [FOL$_{v1}$ × Time]	0.000056 (0.00034)	0.869	.000036 (.00035)	0.919
Log$_e$ (TRAILS A) [FOL$_{v1}$ × LnHcy$_{v1}$]	-0.00435 (0.00402)	0.279	*-.0068 (.0039)*	*0.088*
Log$_e$ (TRAILS A) [FOL$_{v1}$ × LnHcy$_{v1}$ × Time]	**0.00297 (0.00092)**	**0.001**	**.0031 (.00092)**	**0.001**
Log$_e$ (TRAILS B) [FOL$_{v1}$]	0.00050 (0.0026)	0.850	.0026 (.0026)	0.313
Log$_e$ (TRAILS B) [FOL$_{v1}$ × Time]	.000088 (0.00051)	0.863	.00015 (.00053)	0.766
Log$_e$ (TRAILS B) [FOL$_{v1}$ × LnHcy$_{v1}$]	0.011 (0.0069)	0.111	.0040 (.0064)	0.532
Log$_e$ (TRAILS B) [FOL$_{v1}$ × LnHcy$_{v1}$ × Time]	-0.00040 (0.0013)	0.762	-.00016 (.0013)	0.901

B-12

MMSE, normalized [B12$_{v1}$]	.00125 (.0018)	0.490	.00022 (.0016)	0.890
MMSE, normalized [B12$_{v1}$ × Time]	-.000469 (.00041)	0.255	-.00052 (.00041)	0.212
MMSE, normalized [B12$_{v1}$ × LnHcy$_{v1}$]	-.00099 (.0049)	0.840	-.0024 (.0042)	0.556
MMSE, normalized [B12$_{v1}$ × LnHcy$_{v1}$ × Time]	-.00033 (.0011)	0.772	-.00022 (.0011)	0.847
CVLT-List A [B12$_{v1}$]	.0013 (.00088)	0.120	.00077 (.00083)	0.350
CVLT-List A [B12$_{v1}$ × Time]	-.00014 (.00017)	0.381	-.00013 (.00017)	0.428
CVLT-List A [B12$_{v1}$ × LnHcy$_{v1}$]	-.0010 (.0023)	0.657	-.0015 (.0021)	0.464
CVLT-List A [B12$_{v1}$ × LnHcy$_{v1}$ × Time]	-.00026 (.00047)	0.570	-.00017 (.00047)	0.707
CVLT-DFR [B12$_{v1}$]	.00012 (.00039)	0.763	-.000010 (.00038)	0.978
CVLT-DFR [B12$_{v1}$ × Time]	.000092 (.000083)	0.264	.000086 (.000084)	0.301
CVLT-DFR [B12$_{v1}$ × LnHcy$_{v1}$]	-.00094 (.0010)	0.368	-.0011 (.00099)	0.282
CVLT-DFR [B12$_{v1}$ × LnHcy$_{v1}$ × Time]	-.000034 (.00023)	0.882	0.000 (.00023)	0.966
BVRT [B12$_{v1}$]	-.00067 (.00062)	0.284	-.00030 (.00059)	0.608
BVRT [B12$_{v1}$ × Time]	.000024 (.00012)	0.834	.000035 (.00012)	0.773

	Model 1[a]		Model 2[b]	
BVRT [$B12_{v1} \times LnHcy_{v1}$]	.0011 (.0017)	0.520	.0012 (.0016)	0.443
BVRT [$B12_{v1} \times LnHcy_{v1} \times Time$]	**-.00074 (.00032)**	**0.019**	**-.00073 (.00031)**	**0.021**
BTA [$B12_{v1}$]	.00043 (.00028)	0.129	.00032 (.00027)	0.246
BTA [$B12_{v1} \times Time$]	.000049 (.000061)	0.420	.000044 (.000062)	0.471
BTA [$B12_{v1} \times LnHcy_{v1}$]	.00062 (.00077)	0.422	.00050 (.00073)	0.491
BTA [$B12_{v1} \times LnHcy_{v1} \times Time$]	-.00014 (.00017)	0.428	-.00013 (.00017)	0.449
AF [$B12_{v1}$]	.00106 (.00065)	0.104	.00058 (.00063)	0.347
AF [$B12_{v1} \times Time$]	.000015 (.00012)	0.902	.000052 (.00012)	0.675
AF [$B12_{v1} \times LnHcy_{v1}$]	-.0027 (.0017)	0.119	-.0027 (.0016)	0.091
AF [$B12_{v1} \times LnHcy_{v1} \times Time$]	.00026 (.00032)	0.419	.00022 (.00033)	0.502
DS-F [$B12_{v1}$]	-.000024 (.000048)	0.621	.00024 (.00026)	0.336
DS-F [$B12_{v1} \times Time$]	.00061 (.00070)	0.386	-.000024 (.000049)	0.622
DS-F [$B12_{v1} \times LnHcy_{v1}$]	0.000 (.00013)	0.993	.00034 (.00065)	0.603
DS-F [$B12_{v1} \times LnHcy_{v1} \times Time$]	-.000024 (.000048)	0.621	.000 (0.000)	0.958
DS-B [$B12_{v1}$]	**.00070 (.00026)**	**0.008**	**.00058 (.00024)**	**0.015**
DS-B [$B12_{v1} \times Time$]	-.000044 (.000051)	0.394	-.000056 (.000051)	0.270
DS-B [$B12_{v1} \times LnHcy_{v1}$]	*.0012 (.00072)*	*0.087*	.00094 (.00064)	0.138
DS-B [$B12_{v1} \times LnHcy_{v1} \times Time$]	-.00016 (.00014)	0.239	-.00016 (.00014)	0.245
CDT [$B12_{v1}$]	.000052 (.00015)	0.730	-.000047 (.00015)	0.749
CDT [$B12_{v1} \times Time$]	0.000 (.000038)	0.864	0.000 (.000039)	0.887
CDT [$B12_{v1} \times LnHcy_{v1}$]	.00036 (.00040)	0.363	.00030 (.00039)	0.443
CDT [$B12_{v1} \times LnHcy_{v1} \times Time$]	.000055 (.00010)	0.592	.000058 (.00010)	0.573
Log_e (TRAILS A) [$B12_{v1}$]	0.000 (.00004)	0.920	.000014 (.000046)	0.759
Log_e (TRAILS A) [$B12_{V1} \times Time$]	.000012 (.000011)	0.259	.000012 (.000011)	0.231
Log_e (TRAILS A) [$B12_{V1} \times LnHcy_{v1}$]	-.000043 (.00012)	0.722	-.000036 (.000122)	0.765
Log_e (TRAILS A) [$B12_{V1} \times LnHcy_{v1} \times Time$]	.000038 (.000028)	0.183	.000039 (.000027)	0.151
Log_e (TRAILS B) [$B12_{v1}$]	.000018 (.000079)	0.815	.000061 (.000074)	0.410
Log_e (TRAILS B) [$B12_{V1} \times Time$]	0.000 (.000015)	0.669	0.000 (.000016)	0.665
Log_e (TRAILS B) [$B12_{V1} \times LnHcy_{v1}$]	.000042 (.00021)	0.837	.00011 (.00019)	0.566
Log_e (TRAILS B) [$B12_{V1} \times LnHcy_{v1} \times Time$]	**-.000085 (.000042)**	**0.041**	**-.000086 (.000041)**	**0.036**

Abbreviations: Hcy=Homocysteine; Ln or Log_e=Log_e transformed; v1=visit 1 [a] Model 1 is adjusted for age, sex, race, poverty status, inverse mills ratio as well as time on study in years between visits 1 and 2 and its interaction with $LnHcy_{v1}$ and covariates. [b] Model 2 is adjusted for age, sex, race, poverty status, education, literacy, smoking, drug use, 2010 healthy eating index, body mass index, inverse mills ratio as well as time on study in years between visits 1 and 2 and its interaction with $LnHcy_{v1}$ and covariates. [c] Cognitive tests include the Mini-Mental State Examination (MMSE), the California Verbal Learning Test (CVLT) Immediate (List A) and Delayed Free Recall (DFR), the Benton Visual Retention Test (BVRT, # of errors), Brief Test of Attention (BTA), Animal Fluency test (AF), the Digit Span Forward and Backwards tests (DS-F and DS-B), the Clock Drawing Test (CDT), the Trail making test Part A and B (TRAILS A and B, in seconds).

Supplemental Table 3. Interaction effects serum folate and vitamin B-12 levels (Log$_e$ transformed, z-scored) for the effects of homocysteine trajectory on 11 cognitive test scores (baseline and between-visit change):HANDLS 2004-2013 [c]

	Model 1 [a]		Model 2 [b]	
	β (SE)	P value	β (SE)	P value
FOLATE				
MMSE, normalized [FOL$_{v1}$]	**.153 (.058)**	**0.009**	.068 (.054)	0.205
MMSE, normalized [FOL$_{v1}$ × Time]	-.0059 (.0139)	0.670	-.013 (.014)	0.355
MMSE, normalized [FOL$_{v1}$×Hcy $_{traj}$]	**-.142 (.051)**	**0.006**	-.071 (.044)	0.105
MMSE, normalized [FOL$_{v1}$ ×Hcy $_{traj}$ × Time]	.013 (.013)	0.327	.0094 (.012)	0.465
CVLT-List A [FOL$_{v1}$]	-.00531 (.0270)	0.844	***-.048 (.026)***	***0.069***
CVLT-List A [FOL$_{v1}$ × Time]	.00916 (.0052)	0.081	**.011 (.005)**	**0.044**
CVLT-List A [FOL$_{v1}$ × Hcy $_{traj}$]	**-.0478 (.0229)**	**0.037**	-.028 (.021)	0.175
CVLT-List A [FOL$_{v1}$ × Hcy $_{traj}$ × Time]	**-.00371 (.0045)**	**.0045**	-.0035 (.0045)	0.436
CVLT-DFR [FOL$_{v1}$]	.0100 (.012)	0.424	-.0019 (.0127)	0.878
CVLT-DFR [FOL$_{v1}$ × Time]	.0011 (.0025)	0.664	.0010 (.0026)	0.689
CVLT-DFR [FOL$_{v1}$ × Hcy $_{traj}$]	***-.020 (.012)***	***0.086***	-.0118 (.0107)	0.271
CVLT-DFR [FOL$_{v1}$ × Hcy $_{traj}$ × Time]	.00013 (.0022)	0.953	.00026 (.0022)	0.906
BVRT [FOL$_{v1}$]	-.033 (.020)	0.103	-.0079 (.0206)	0.699
BVRT [FOL$_{v1}$ × Time]	.00062 (.0037)	0.867	.00041 (.0038)	0.915
BVRT [FOL$_{v1}$ × Hcy $_{traj}$]	.0165 (.0164)	0.315	.0028 (.0155)	0.857

BVRT [FOL$_{v1}$ × Hcy $_{traj}$ × Time]	.0040 (.0031)	0.199	.00384 (.0031)	0.217
BTA [FOLv1]	.0043 (.0092)	0.639	-.0048 (.0092)	0.596
BTA [FOL$_{v1}$ × Time]	.00077 (.0019)	0.684	.00042 (.0019)	0.830
BTA [FOL$_{v1}$ × Hcy $_{traj}$]	-.0011 (.0077)	0.887	.0041 (.0073)	0.575
BTA [FOL$_{v1}$ × Hcy $_{traj}$ × Time]	-.00088 (.0016)	0.577	-.00072 (.0015)	0.649
AF [FOL$_{v1}$]	**.064 (.021)**	**0.002**	.0317 (.021)	0.129
AF [FOL$_{v1}$ × Time]	-.0025 (.0038)	0.508	-.00023 (.0040)	0.954
AF [FOL$_{v1}$ × Hcy $_{traj}$]	-.0248 (.018)	0.175	-.0118 (.017)	0.496
AF [FOL$_{v1}$ × Hcy $_{traj}$ × Time]	.000094 (.0032)	0.976	-.00013 (.0032)	0.967
DS-F [FOL$_{v1}$]	*.017 (.0088)*	*0.051*	.0074 (.0083)	0.376
DS-F [FOL$_{v1}$ × Time]	-.00061 (.0015)	0.687	-.00083 (.0016)	0.603
DS-F [FOL$_{v1}$ × Hcy $_{traj}$]	-.0040 (.0074)	0.590	.0038 (.0068)	0.575
DS-F [FOL$_{v1}$ × Hcy $_{traj}$ × Time]	-.00087 (.0013)	0.497	-.00095 (.0013)	0.460
DS-B [FOL$_{v1}$]	**.019 (.0085)**	**0.020**	.0074 (.0080)	0.355
DS-B [FOL$_{v1}$ × Time]	-.00090 (.0016)	0.571	-.0012 (.0016)	0.471
DS-B [FOL$_{v1}$ × Hcy $_{traj}$]	-.0103 (.0073)	0.161	-.0016 (.0065)	0.804
DS-B [FOL$_{v1}$ × Hcy $_{traj}$ × Time]	-.0019 (.0013)	0.158	-.0020 (.0013)	0.119
CDT [FOL$_{v1}$]	**.0127 (.0048)**	**0.009**	.0078 (.0049)	0.115
CDT [FOL$_{v1}$ × Time]	-.00042 (.0012)	0.728	-.00032 (.0013)	0.805
CDT [FOL$_{v1}$ × Hcy $_{traj}$]	.0057 (.0042)	0.169	.0081 (.0041)	0.046
CDT [FOL$_{v1}$ × Hcy $_{traj}$ × Time]	**-.00275 (.0010)**	**0.007**	**-.0027 (.0010)**	**0.008**
Log$_e$ (TRAILS A) [FOL$_{v1}$]	-.0022 (.0015)	0.138	-.0015 (.0015)	0.317
Log$_e$ (TRAILS A) [FOL$_{v1}$ × Time]	.00014 (.00033)	0.662	.00014 (.00034)	0.673
Log$_e$ (TRAILS A) [FOL$_{v1}$ × Hcy $_{traj}$]	.000022 (.0014)	0.988	-.00068 (.0014)	0.635
Log$_e$ (TRAILS A) [FOL$_{v1}$ × Hcy $_{traj}$ × Time]	.00010 (.00031)	0.740	.0001 (.00031)	0.748
Log$_e$ (TRAILS B) [FOL$_{v1}$]	-.00057 (.0026)	0.822	.0021 (.0026)	0.408
Log$_e$ (TRAILS B) [FOL$_{v1}$ × Time]	.000083 (.00048)	0.864	.00012 (.00050)	0.804
Log$_e$ (TRAILS B) [FOL$_{v1}$ × Hcy $_{traj}$]	.00453 (.0025)	0.066	.0022 (.0023)	0.341
Log$_e$ (TRAILS B) [FOL$_{v1}$ × Hcy $_{traj}$ × Time]	-.000032 (.00046)	0.946	.000030 (.00046)	0.948

B-12

MMSE, normalized [B12$_{v1}$]	.0019 (.0017)	0.258	.00053 (.0015)	0.730
MMSE, normalized [B12$_{v1}$ × Time]	-.00042 (.00039)	0.285	-.00047 (.00040)	0.237
MMSE, normalized [B12$_{v1}$ ×Hcy $_{traj}$]	.00038 (.0024)	0.879	.00028 (.0019)	0.886
MMSE, normalized [B12$_{v1}$ ×Hcy $_{traj}$ × Time]	.00014 (.00053)	0.786	.000 (.00051)	0.992
CVLT-List A [B12$_{v1}$]	*.0015 (.00084)*	*0.075*	.0007 (.00079)	0.359
CVLT-List A [B12$_{v1}$ × Time]	-.00013 (.00016)	0.424	-.00010 (.00017)	0.520
CVLT-List A [B12$_{v1}$ × Hcy $_{traj}$]	.00055 (.0011)	0.605	.00026 (.00098)	0.793
CVLT-List A [B12$_{v1}$ × Hcy $_{traj}$ × Time]	-.000078 (.00021)	0.712	-.000013 (.00021)	0.952
CVLT-DFR [B12$_{v1}$]	.00034 (.00039)	0.381	.00014 (.00038)	0.707
CVLT-DFR [B12$_{v1}$ × Time]	.000069 (.000081)	0.396	.000069 (.000082)	0.400
CVLT-DFR [B12$_{v1}$ × Hcy $_{traj}$]	.00042 (.00055)	0.446	.00036 (.00050)	0.465
CVLT-DFR [B12$_{v1}$ × Hcy $_{traj}$ × Time]	-.000016 (.00011)	0.882	.000012 (.00010)	0.910
BVRT [B12$_{v1}$]	-.0006 (.00059)	0.249	-.00020 (.00057)	0.719
BVRT [B12$_{v1}$ × Time]	.000051 (.00011)	0.653	.000058 (.00011)	0.614
BVRT [B12$_{v1}$ × Hcy $_{traj}$]	.00053 (.00083)	0.529	.00060 (.00077)	0.439

BVRT [B12$_{v1}$ × Hcy $_{traj}$ × Time]	-.00014 (.00013)	0.301	-.00015 (.00014)	0.287
BTA [B12$_{v1}$]	***.00045 (.00027)***	***0.089***	.00032 (.00026)	0.232
BTA [B12$_{v1}$ × Time]	.000040 (.000059)	0.491	.000033 (.000059)	0.576
BTA [B12$_{v1}$ × Hcy $_{traj}$]	.00011 (.00035)	0.756	.000078 (.00033)	0.811
BTA [B12$_{v1}$ × Hcy $_{traj}$ × Time]	-.00002 (.000071)	0.739	-.000024 (.000070)	0.735
AF [B12$_{v1}$]	**.00150 (.00063)**	**0.016**	.00092 (.00061)	0.127
AF [B12$_{v1}$ × Time]	-.000044 (.00012)	0.706	.000 (.00011)	0.980
AF [B12$_{v1}$ × Hcy $_{traj}$]	.00017 (.00083)	0.837	.00012 (.00077)	0.873
AF [B12$_{v1}$ × Hcy $_{traj}$ × Time]	-.00006 (.00014)	0.635	-.000078 (.00014)	0.582
DS-F [B12$_{v1}$]	.00042 (.00026)	0.110	.00022 (.00024)	0.366
DS-F [B12$_{v1}$ × Time]	-.000027 (.000046)	0.546	-.000029 (.000048)	0.532
DS-F [B12$_{v1}$ × Hcy $_{traj}$]	.00013 (.00032)	0.688	.000014 (.00029)	0.964
DS-F [B12$_{v1}$ × Hcy $_{traj}$ × Time]	-.000014 (.000056)	0.809	-.000019 (.000057)	0.743
DS-B [B12$_{v1}$]	**.00059 (.00025)**	**0.018**	.00041 (.00023)	0.072
DS-B [B12$_{v1}$ × Time]	-.000032 (.000049)	0.513	-.000044 (.000049)	0.367
DS-B [B12$_{v1}$ × Hcy $_{traj}$]	.00023 (.00032)	0.467	.00016 (.00028)	0.567
DS-B [B12$_{v1}$ × Hcy $_{traj}$ × Time]	-.000043 (.000059)	0.465	-.000052 (.000058)	.372
CDT [B12$_{v1}$]	.000040 (.00014)	0.779	-.000075 (.0001)	0.598
CDT [B12$_{v1}$ × Time]	.000 (.000037)	0.897	.000 (.000)	0.930
CDT [B12$_{v1}$ × Hcy $_{traj}$]	.00022 (.00019)	0.248	.00018 (.0002)	0.324
CDT [B12$_{v1}$ × Hcy $_{traj}$ × Time]	-.000036 (.000045)	0.421	-.00003 (.00004)	0.487
Log$_e$ (TRAILS A) [B12$_{v1}$]	.000 (.000044)	0.958	.00001 (.00004)	0.769
Log$_e$ (TRAILS A) [B12$_{V1}$ × Time]	.000010 (.000)	0.306	.00001 (.00001)	0.291
Log$_e$ (TRAILS A) [B12$_{V1}$ × Hcy $_{traj}$]	-.000017 (.000058)	0.763	-.00002 (.00006)	0.706
Log$_e$ (TRAILS A) [B12$_{V1}$ × Hcy $_{traj}$ × Time]	.000 (.000012)	0.609	.000 (.000)	0.600
Log$_e$ (TRAILS B) [B12$_{v1}$]	-.000013 (.000076)	0.862	.00004 (.00007)	0.536
Log$_e$ (TRAILS B) [B12$_{V1}$ × Time]	.000 (.000015)	0.486	.000 (.00001)	0.709
Log$_e$ (TRAILS B) [B12$_{V1}$ × Hcy $_{traj}$]	-.00006 (.000096)	0.486	-.00004 (.00009)	0.604
Log$_e$ (TRAILS B) [B12$_{V1}$ × Hcy $_{traj}$ × Time]	.000018 (.000018)	0.332	.000018 (.000018)	0.312

Abbreviations: Hcy=Homocysteine; Hcy $_{traj}$ = z-transformed probability of belonging to a group with increasing LnHcy over time according to group-based trajectory modeling; Ln or Log$_e$=Log$_e$ transformed [a] Model 1 is adjusted for age, sex, race, poverty status, inverse mills ratio as well as time on study in years between visits 1 and 2 and its interaction with trajectory in LnHcy and covariates. [b] Model 2 is adjusted for age, sex, race, poverty status, education, literacy, smoking, drug use, 2010 healthy eating index, body mass index, inverse mills ratio as well as time on study in years between visits 1 and 2 and its interaction with trajectory in LnHcy and covariates. [c] Cognitive tests include the Mini-Mental State Examination (MMSE), the California Verbal Learning Test (CVLT) Immediate (List A) and Delayed Free Recall (DFR), the Benton Visual Retention Test (BVRT, # of errors), Brief Test of Attention (BTA), Animal Fluency test (AF), the Digit Span Forward and Backwards tests (DS-F and DS-B), the Clock Drawing Test (CDT), the Trail making test Part A and B (TRAILS A and B, in seconds).

Supplemental Material 4 – Mixed-effects linear regression models:

The main multiple mixed-effects regression models can be summarized as follows, detailed further elsewhere(Beydoun et al., 2023):

| **Multi-level models** | vs. **Composite models** |

Eq. 1.1-1.4

$$Y_{ij} = \pi_{0i} + \pi_{1i}Time_{ij} + \varepsilon_{ij}$$

$$\pi_{0i} = \gamma_{00} + \gamma_{0a}X_{aij} + \sum_{k=1}^{l}\gamma_{0k}Z_{ik} + \zeta_{0i}$$

$$\pi_{1i} = \gamma_{10} + \gamma_{1a}X_{aij} + \sum_{m=1}^{n}\gamma_{1m}Z_{im} + \zeta_{1i}$$

$$Y_{ij} = \gamma_{00} + \gamma_{0a}X_{aij} + \sum_{k=1}^{l}\gamma_{0k}Z_{ik}$$
$$+ \gamma_{10}Time_{ij} + \gamma_{1a}X_{aij}Time_{ij}$$
$$+ \sum_{m=1}^{n}\gamma_{1m}Z_{im}Time_{ij}$$
$$+ (\zeta_{0i} + \zeta_{1i}Time_{ij} + \varepsilon_{ij})$$

Where Y_{ij} is the outcome (11 cognitive test scores measured at v_1 and/or v_2) for each individual "i" and visit "j"; π_{0i} is the level-1 intercept for individual i; π_{1i} is the level-1 slope for individual i; γ_{00} is the level-2 intercept of the random intercept π_{0i}; γ_{10} is the level-2 intercept of the slope π_{1i}; Z_{ik} is a vector of fixed covariates for each individual *i* that are used to predict level-1 intercepts and slopes, which can include socio-demographic variables among others. In this analysis, mixed-effects regression models included Hcy exposure measured at v_1 or as a trajectory exposure (Probability of belonging to "High increasing" group, z-scored) (X_{ij}), along with covariates (Z_{ik} and Z_{im}). ζ_{0i} and ζ_{1i} are level-2 disturbances; ε_{ij} is the within-person level-1 disturbance (Blackwell et al., 2006).

Supplementary Material 5 –Homocysteine

Aeon Technologies, LLC tested Hcy (Frostburg, MD). First, the Alinity I analyzer was used to test the serum quality by looking for icteria, lipemia, and hemolysis. Every serum sample met the Aeon

Technologies-established quality testing level. The Alinity i Homocysteine assay on the Alinity i analyzer was used to quantify Hcy. Chemiluminescent microparticle immunoassay (CMIA) technology is used in this one-step immunoassay. The Alinity i Homocysteine assay has an analytical measuring range (AMR) of 1.00 to 50.00 mmol/L (0.14 to 6.76 mg/mL). Twelve batches of samples were processed, and as a control, a serum sample (Cat # 200-0162; Stem Cell Technologies) was processed in every batch. This serum control's Hcy levels ranged from 4.55 to 6.18, with an 8.38% inter-assay coefficient of variation. The main exposure variable of interest was Hcy measured at v1 of the HANDLS study, Loge transformed (LnHcy$_{v1}$).

Additionally, groups of individuals with comparable developmental trajectories throughout time were identified by the use of a STATA plugin (*traj* and *trajplot*) developed from a well-established SAS approach (Jones, 2001; Jones, 2007), for group-based trajectory modeling for LnHcy. This group-based method uses maximum likelihood and a multinomial modeling strategy to estimate model parameters. The quasi-Newton procedure is used to optimize the results. We presented group-based trajectories over time with 95% confidence intervals (CI) and specified a zero-inflated Poisson (zip) distribution for the chosen outcomes, with intercept (0), linear (1) or quadratic (2) orders for each group trajectory, as appropriate.

The One Carbon Metabolism (OCM) is a metabolic pathway that involves the transfer of methyl groups from folate's active form, tetrahydrofolate (THF), to specific enzymes(Troesch et al., 2016). It consists of methionine, thymidylate, and purine cycles(Troesch et al., 2016). The neuro-toxic substance Hcy is metabolized through the methionine or thymidylate cycles, with a negative feedback loop where Hcy is remethylated into methionine under low methionine levels(Shane, 2008; Troesch et al., 2016). Methionine is further metabolized into S-adenosylmethionine (SAM), which is the principal methyl-donor in DNA methylation and the synthesis of phospholipids, myelin, and neurotransmitters(Shane, 2008; Troesch et al., 2016). The cycle ends with the reduction of 5,10-methylene-THF to 5-methylenetetrahydrofolate (MTHF), catalyzed by methylenetetrahydrofolate reductase (MTHFR) (Shane,

2008; Troesch et al., 2016). Many OCM enzymes depend on vitamins B-2, B-6, folate (B-9), and B-

12(Shane, 2008; Troesch et al., 2016).

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