WEB APPENDIX

MATERIALS AND METHODS

Psychological measures

LOT is a validated instrument (1) made up of 12 items comprised of questions such as "If something can go wrong for me, it will." Respondents were asked to indicate their degree of agreement with each statement using a 5-point response scale ranging from 0 (*strongly disagree*) to 4 (*strongly agree*). While the LOT was designed to assess optimism as a bipolar dimension, and classifies optimists by high scores on the measure and pessimists by low scores, it can also be separated into two subscales that capture optimism and pessimism as more distinct constructs. Many studies have suggested these measures are distinct in relation to health and have recommended considering the subscales separately (2-5). Of the 12 items, 4 assess optimistic attitudes, 4 assess pessimistic attitudes and 4 are filler items. A mean of the items assessing pessimistic attitudes was calculated to yield a pessimism score with an observed range from 0 to 16 (higher scores indicate greater pessimism). A mean of the items assessing optimism was calculated to yield an optimism score with an observed range from 2 to 16 (higher scores indicate greater optimism). The LOT was administered during clinical exam visits to NAS subjects beginning in 1993 and continued through 2002. Because optimism and pessimism have been found to be stable personality traits (1, 6), we took the mean value from all available LOT measurements for an individual and assigned that mean value to all visits.

The BSI was administered during examination visits from 1991 to 2002, Respondents indicated their level of distress experienced over the last 30 days on a 5-point scale ranging from 0 (*not at all*) to 4 (*extremely*). As symptoms are measured over the last 30 days, these subscales have generally been found to identify different sources of current distress ranging from mild distress to significant psychopathology. Because items for the BSI were constructed to be clinically relevant, mean scores are generally skewed toward the low end in this non-clinically derived sample and thus may indicate poor emotional functioning rather than psychopathology per se. To characterize ongoing emotional functioning, we took the mean value of all available depression and anxiety measures over time.

DNA methylation analysis

For each assay, primer sets and conditions were evaluated using a two-step approach. In the first step, correct PCR amplification was verified on standard DNA by running the PCR products on an agarose gel. The standard DNA was comprised of a pool of human DNA from healthy volunteers. In the second step, five different samples were run starting from bisulfite treatment through PCR amplification and Pyrosequencing to test the entire analytic pipeline. Primer sets/conditions that did not produce successful pyrosequencing analyses, as indicated by the built-in Pyrosequencing software (Pyro Q-CpG Software), were modified and retested. In this second step, all samples were run in duplicates. Primer sets and conditions with low reproducibility were modified and retested.

The final elution was performed with 200 μL M-Elution Buffer. The assay for methylation was developed by locating the promoters using Genomatix Software

(Genomatix Software Inc, Ann Arbor, MI, USA) as described in Web Table 1. For each assay a 50-μL PCR was carried out in 25 μL GoTaq Green Master mix (Promega), 25 ng bisulfite-treated genomic DNA, and water. PCR cycling conditions and reagent amounts are described in Web Table 2. PCR products were purified and sequenced by pyrosequencing as previously described (7) using 0.3 μM sequencing primer. The primers and sequences analyzed for each assay are shown in Web Table 3.

Web Table 1 Localization of gene promoters and regions amplified

Gene	Chromosome	Promoter		Amplicon		CpGs (position)
		Start	*End*	*Start*	*End*	
iNOS	17	23149861	23150461	23149873	23149990	23149929 (pos1) 23149936 (pos2)
GCR	5	142760496	142761097	142760531	142760806	142760565

Web Table 2 PCR Conditions

Gene	Temperature	Time	# of cycles		Volume (µl)
iNOS	95°C	5 min.	1	H_2O	13
	95°C	1 min.		GoTaq Master mix	25
	60°C	1 min.	45	Forward primer	1
	72°C	1 min.		Reverse primer	1
	72°C	5 min.	1	Sample	10
	4°C	∞		Total Volume	50
GCR	95°C	5 min.	1	Water	13
	95°C	1 min.	50	GoTaq Master mix	25
	53.6°C	1 min.		Forward primer	1
	72°C	1 min.		Reverse primer	1
	72°C	5 min.	1	Sample	10
	4°C	∞		Total Volume	50

Web Table 3 Primers

Gene	Primer Forward	Primer Reverse	Primer Sequencing	Sequence to Analyze
iNOS	AATGAGAGTTGTTGGGAAGTGTTT	bio-CCACCAAACCCAACCAAACT	TAAAGGTATTTTTGTTTTAA	**C/T**GATTTT**C/T**GGGTTTTTTTTTATTTTG
GCR	bio-TTATATGTATTGGTTTTTAGAAAA	TACTCCCATTCAACATACCACATT	ATTCCTACCTCTTTTCAA	CAC**A/G**ACTATTT

RESULTS

Web Table 4. Air pollution exposure by psychological category for men in the VA Normative Aging Study, Boston, MA, 1999 – 2009

Black Carbon, $\mu g/m^3$

Mean (SD)

Moving Average	High Pessimism	Low Pessimism	High Optimism	Low Optimism	High Depression	Low Depression	High Anxiety	Low Anxiety
4-hour	1.15 (0.69)	1.17 (0.75)	1.15 (0.72)	1.17 (0.73)	1.13 (0.62)	1.16 (0.74)	1.18 (0.69)	1.15 (0.73)
1-day	0.85 (0.42)	0.83 (0.43)	0.84 (0.42)	0.83 (0.43)	0.80 (0.39)	0.84 (0.43)	0.82 (0.40)	0.84 (0.43)
2-day	0.81 (0.37)	0.80 (0.38)	0.81 (0.36)	0.79 (0.38)	0.79 (0.38)	0.80 (0.37)	0.80 (0.37)	0.80 (0.37)
3-day	0.74 (0.31)	0.74 (0.32)	0.75 (0.31)	0.73 (0.33)	0.74 (0.33)	0.74 (0.31)	0.75 (0.31)	0.74 (0.32)
4-day	0.72 (0.27)	0.71 (0.28)	0.73 (0.27)	0.71 (0.28)	0.73 (0.29)	0.71 (0.27)	0.72 (0.27)	0.72 (0.27)
5-day	0.74 (0.25)	0.72 (0.25)	0.73 (0.25)	0.72 (0.26)	0.74 (0.27)	0.72 (0.25)	0.73 (0.25)	0.73 (0.25)
6-day	0.75 (0.24)	0.74 (0.24)	0.75 (0.24)	0.74 (0.24)	0.76 (0.26)	0.74 (0.23)	0.76 (0.24)	0.74 (0.24)
7-day	0.76 (0.23)	0.76 (0.23)	0.76 (0.23)	0.75 (0.23)	0.76 (0.25)	0.76 (0.23)	0.77 (0.23)	0.76 (0.23)
2-week	0.76 (0.20)	0.75 (0.20)	0.76 (0.19)	0.75 (0.20)	0.76 (0.21)	0.75 (0.20)	0.77 (0.20)	0.75 (0.20)
4-week	0.76 (0.18)	0.76 (0.18)	0.76 (0.17)	0.75 (0.19)	0.75 (0.19)	0.76 (0.18)	0.77 (0.17)	0.76 (0.18)

$PM_{2.5}$, $\mu g/m^3$

Mean (SD)

Moving Average	High Pessimism	Low Pessimism	High Optimism	Low Optimism	High Depression	Low Depression	High Anxiety	Low Anxiety
4-hour	11.6 (7.3)	11.2 (7.3)	11.2 (7.1)	11.6 (7.6)	11.7 (7.9)	11.3 (7.3)	12.2 (8.7)	11.3 (7.1)
1-day	10.8 (7.0)	10.3 (6.1)	10.6 (6.7)	10.4 (6.3)	10.4 (6.9)	10.6 (6.4)	11.1 (7.8)	10.5 (6.3)
2-day	10.5 (6.1)	10.3 (5.7)	10.5 (5.8)	10.3 (5.9)	10.2 (6.9)	10.4 (5.7)	10.9 (7.3)	10.3 (5.6)
3-day	10.1 (5.2)	10.2 (5.1)	10.2 (5.0)	10.1 (5.2)	10.1 (5.7)	10.1 (5.0)	10.6 (6.1)	10.1 (4.9)
4-day	10.1 (4.4)	10.1 (4.8)	10.2 (4.3)	10.0 (4.5)	10.2 (4.9)	10.1 (4.4)	10.5 (5.2)	10.0 (4.3)
5-day	10.2 (3.9)	10.1 (4.0)	10.2 (3.9)	10.0 (4.1)	10.3 (4.4)	10.1 (3.9)	10.5 (4.5)	10.1 (3.9)
6-day	10.1 (3.6)	10.1 (3.7)	10.2 (3.5)	10.0 (3.8)	10.3 (4.1)	10.1 (3.6)	10.5 (4.2)	10.1 (3.6)
7-day	10.0 (3.4)	10.2 (3.6)	10.1 (3.3)	10.1 (3.6)	10.2 (3.9)	10.1 (3.4)	10.4 (4.1)	10.1 (3.4)
2-week	10.1 (2.9)	10.2 (3.1)	10.2 (2.9)	10.1 (3.1)	10.1 (3.2)	10.2 (3.0)	10.3 (3.3)	10.1 (3.0)
4-week	10.1 (2.5)	10.2 (2.7)	10.2 (2.6)	10.2 (2.7)	10.0 (2.7)	10.2 (2.6)	10.3 (2.7)	10.2 (2.6)

Web Table 5. Correlation matrix for psychological factors for men in the VA Normative Aging Study, Boston, MA, 1999 – 2009

	Optimism	Pessimism	Depression	Anxiety
Optimism	1.0	-0.44	-0.31	-0.26
		$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
Pessimism	-0.44	1.0	0.35	0.26
	$p < 0.0001$		$p < 0.0001$	$p < 0.0001$
Depression	-0.31	0.35	1.0	0.72
	$p < 0.0001$	$p < 0.0001$		$p < 0.0001$
Anxiety	-0.26	0.26	0.72	1.0
	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	

Web Table 6. Associations between acute and intermediate air pollution exposure and *iNOS* promoter methylation in the Normative Aging Study, 1999 – 2010[a]

	Change in % 5mC per BC			Change in % 5mC per 10 μg/m^3 $PM_{2.5}$		
Model with 4 hour + 1 week						
4 hour	-0.824	(-1.339	-0.309)	-0.478	(-1.042	0.087)
1 week	-1.048	(-3.127	1.031)	-0.872	(-2.060	0.315)
Model with 4 hour + 2 week						
4 hour	-0.824	(-1.329	-0.319)	-0.515	(-1.075	0.045)
2 week	-2.553	(-5.092	-0.013)	-0.743	(-2.101	0.615)
Model with 4 hour + 4 week						
4 hour	-0.843	(-1.347	-0.339)	-0.575	(-1.129	-0.021)
4 week	-2.461	(-5.731	0.809)	-0.044	(-1.620	1.533)

[a] Models adjusted for baseline age, season and time trend, apparent temperature, % lymphocytes, % neutrophils, BMI, diabetes mellitus, and hypertension medication

Web Table 7. Associations between air pollution and *iNOS* promoter methylation in the VA Normative Aging Study, Using the mean of two CpG sites 1999 – 2010

		Change in % 5mC per 1 mg/m^3 BC (95% Intervals)			Change in % 5mC per 10 mg/m^3 $PM_{2.5}$ (95% Intervals)		
iNOS[a]	4 hour	-0.893	(-1.450	-0.335)	-0.599	(-1.210	0.013)
	1 day	-0.478	(-1.501	0.545)	-0.374	(-1.155	0.407)
	2 day	-0.033	(-1.303	1.238)	-0.261	(-1.099	0.576)
	3 day	0.024	(-1.486	1.535)	-0.241	(-1.170	0.688)
	4 day	-0.543	(-2.281	1.195)	-0.510	(-1.555	0.534)
	5 day	-1.105	(-3.034	0.823)	-0.814	(-1.999	0.371)
	6 day	-1.135	(-3.247	0.977)	-1.012	(-2.313	0.288)
	7 day	-1.439	(-3.718	0.840)	-1.313	(-2.696	0.069)
	2 week	-3.363	(-6.341	-0.384)	-1.513	(-3.170	0.144)
	4 week	-4.571	(-8.508	-0.634)	-1.437	(-3.428	0.553)

[a] Models adjusted for baseline age, season and time trend, apparent temperature, % lymphocytes, % neutrophils, BMI, diabetes mellitus, and hypertension medication

Web Table 8. Associations between air pollution and gene promoter methylation in the VA Normative Aging Study, Using the full cohort, 1999 – 2010

		Change in % 5mC per 1 μg/m^3 BC (95% Intervals)				Change in % 5mC per 10 μg/m^3 $PM_{2.5}$ (95% Intervals)		
iNOS[a]	4 hour	-0.812	(-1.352	-0.272)	4 hour	-0.431	(-0.977	0.115)
	1 day	-0.188	(-1.098	0.722)	1 day	-0.341	(-1.037	0.354)
	2 day	0.290	(-0.842	1.422)	2 day	-0.257	(-1.013	0.499)
	3 day	0.237	(-1.110	1.584)	3 day	-0.342	(-1.175	0.491)
	4 day	-0.450	(-2.005	1.105)	4 day	-0.623	(-1.556	0.310)
	5 day	-1.085	(-2.808	0.638)	5 day	-0.937	(-1.990	0.115)
	6 day	-1.149	(-3.024	0.727)	6 day	-1.094	(-2.245	0.058)
	7 day	-1.621	(-3.639	0.398)	7 day	-1.349	(-2.569	-0.128)
	2 week	-3.818	(-6.451	-1.185)	2 week	-1.636	(-3.087	-0.185)
	4 week	-5.903	(-9.371	-2.435)	4 week	-1.710	(-3.451	0.030)
GCR[b]	4 hour	-0.395	(-0.858	0.067)	4 hour	-0.076	(-0.551	0.399)
	1 day	-0.588	(-1.423	0.247)	1 day	-0.201	(-0.802	0.400)
	2 day	-0.645	(-1.654	0.363)	2 day	-0.345	(-1.005	0.315)
	3 day	-0.711	(-1.919	0.498)	3 day	-0.146	(-0.865	0.573)
	4 day	-1.066	(-2.459	0.326)	4 day	-0.207	(-1.026	0.611)
	5 day	-0.606	(-2.139	0.928)	5 day	-0.076	(-1.001	0.850)
	6 day	-1.329	(-2.991	0.333)	6 day	-0.256	(-1.275	0.762)
	7 day	-1.354	(-3.122	0.413)	7 day	-0.375	(-1.449	0.699)
	2 week	-0.904	(-3.208	1.400)	2 week	-0.289	(-1.570	0.993)
	4 week	0.005	(-3.042	3.052)	4 week	-0.418	(-1.924	1.088)

[a] Models adjusted for baseline age, season and time trend, apparent temperature, % lymphocytes, % neutrophils, BMI, diabetes mellitus, and hypertension medication

[b] Models adjusted for baseline age, season and time trend, apparent temperature, % lymphocytes, % neutrophils, diabetes mellitus, and race

Web Table 9. Associations between air pollution and gene promoter methylation in the VA Normative Aging Study, Restricted to residents living within 40 km of central site air pollution monitor, 1999 – 2010

		Change in % 5mC per 1 μg/m^3 BC (95% Intervals)				Change in % 5mC per 10 μg/m^3 $PM_{2.5}$ (95% Intervals)		
iNOS[a]	4 hour	-1.166	(-1.778	-0.554)	4 hour	-0.482	(-1.157	0.192)
	1 day	-0.626	(-1.653	0.400)	1 day	-0.055	(-0.896	0.787)
	2 day	-0.113	(-1.399	1.172)	2 day	0.421	(-0.516	1.359)
	3 day	-0.013	(-1.539	1.513)	3 day	0.390	(-0.611	1.391)
	4 day	-0.673	(-2.455	1.110)	4 day	0.168	(-0.943	1.278)
	5 day	-1.332	(-3.302	0.638)	5 day	-0.092	(-1.337	1.153)
	6 day	-1.213	(-3.381	0.956)	6 day	-0.079	(-1.440	1.282)
	7 day	-1.425	(-3.759	0.908)	7 day	-0.137	(-1.592	1.319)
	2 week	-2.415	(-5.431	0.600)	2 week	-0.300	(-1.993	1.393)
	4 week	-4.297	(-8.261	-0.332)	4 week	-0.751	(-2.749	1.247)
GCR[b]	4 hour	-0.303	(-0.835	0.229)	4 hour	0.076	(-0.516	0.668)
	1 day	-0.596	(-1.550	0.358)	1 day	-0.110	(-0.873	0.652)
	2 day	-0.652	(-1.825	0.520)	2 day	-0.163	(-1.006	0.680)
	3 day	-0.735	(-2.133	0.662)	3 day	-0.130	(-1.025	0.766)
	4 day	-1.082	(-2.695	0.531)	4 day	-0.207	(-1.225	0.810)
	5 day	-0.461	(-2.233	1.311)	5 day	-0.157	(-1.299	0.985)
	6 day	-1.106	(-3.040	0.828)	6 day	-0.361	(-1.608	0.885)
	7 day	-1.219	(-3.274	0.837)	7 day	-0.567	(-1.884	0.750)
	2 week	-0.843	(-3.528	1.842)	2 week	-0.551	(-2.104	1.001)
	4 week	0.482	(-3.044	4.008)	4 week	-0.296	(-2.082	1.491)

[a] Models adjusted for baseline age, season and time trend, apparent temperature, % lymphocytes, % neutrophils, BMI, diabetes mellitus, and hypertension medication

[b] Models adjusted for baseline age, season and time trend, apparent temperature, % lymphocytes, % neutrophils, diabetes mellitus, and race

Web Table 10. Associations between air pollution and gene promoter methylation in the VA Normative Aging Study, Using a quadratic term for apparent temperature, 1999 – 2010

		Change in % 5mC per 1 μg/m^3 BC (95% Intervals)				Change in % 5mC per 10 μg/m^3 $PM_{2.5}$ (95% Intervals)		
iNOS[a]	4 hour	-0.891	(-1.438	-0.344)	4 hour	-0.616	(-1.200	-0.031)
	1 day	-0.368	(-1.283	0.548)	1 day	-0.365	(-1.109	0.380)
	2 day	0.089	(-1.056	1.234)	2 day	-0.168	(-0.977	0.642)
	3 day	0.041	(-1.326	1.407)	3 day	-0.271	(-1.168	0.626)
	4 day	-0.662	(-2.247	0.923)	4 day	-0.693	(-1.708	0.323)
	5 day	-1.301	(-3.057	0.455)	5 day	-1.129	(-2.286	0.028)
	6 day	-1.338	(-3.253	0.578)	6 day	-1.354	(-2.631	-0.076)
	7 day	-1.694	(-3.752	0.365)	7 day	-1.608	(-2.976	-0.239)
	2 week	-3.712	(-6.398	-1.025)	2 week	-1.787	(-3.463	-0.111)
	4 week	-5.512	(-9.124	-1.900)	4 week	-1.689	(-3.713	0.335)
GCR[b]	4 hour	-0.381	(-0.853	0.091)	4 hour	-0.182	(-0.697	0.332)
	1 day	-0.662	(-1.515	0.190)	1 day	-0.372	(-1.025	0.282)
	2 day	-0.762	(-1.802	0.277)	2 day	-0.502	(-1.221	0.216)
	3 day	-0.963	(-2.212	0.286)	3 day	-0.478	(-1.280	0.324)
	4 day	-1.303	(-2.744	0.137)	4 day	-0.537	(-1.459	0.385)
	5 day	-0.822	(-2.407	0.763)	5 day	-0.372	(-1.420	0.675)
	6 day	-1.522	(-3.240	0.197)	6 day	-0.630	(-1.784	0.524)
	7 day	-1.629	(-3.453	0.196)	7 day	-0.845	(-2.063	0.372)
	2 week	-1.182	(-3.557	1.194)	2 week	-0.848	(-2.339	0.643)
	4 week	-0.068	(-3.258	1.194)	4 week	-0.607	(-2.385	1.170)

[a] Models adjusted for baseline age, season and time trend, apparent temperature, % lymphocytes, % neutrophils, BMI, diabetes mellitus, and hypertension medication

[b] Models adjusted for baseline age, season and time trend, apparent temperature, % lymphocytes, % neutrophils, diabetes mellitus, and race

References

1. Scheier MF, Carver CS. Optimism, coping, and health: assessment and implications of generalized outcome expectancies. *Health Psychol*. 1985;4(3):219-247.

2. Mroczek DK, Spiro A,3rd, Aldwin CM, et al. Construct validation of optimism and pessimism in older men: findings from the normative aging study. *Health Psychol*. 1993;12(5):406-409.

3. Robb KA, Simon AE, Wardle J. Socioeconomic disparities in optimism and pessimism. *Int J Behav Med*. 2009;16(4):331-338. (doi: 10.1007/s12529-008-9018-0).

4. Robinson-Whelen S, Kim C, MacCallum RC, et al. Distinguishing optimism from pessimism in older adults: is it more important to be optimistic or not to be pessimistic? J *Pers Soc Psychol*. 1997;73(6):1345-1353.

5. Kubzansky LD, Kubzansky PE, Maselko J. Optimism and pessimism in the context of health: bipolar opposites or separate constructs? *Pers Soc Psychol Bull*. 2004;30(8):943-956. (doi: 10.1177/0146167203262086).

6. Schou I, Ekeberg O, Sandvik L, et al. Stability in optimism-pessimism in relation to bad news: a study of women with breast cancer. *J Pers Assess*. 2005;84(2):148-154. (doi: 10.1207/s15327752jpa8402_04).

7. Bollati V, Baccarelli A, Hou L, et al. Changes in DNA methylation patterns in subjects exposed to low-dose benzene. *Cancer Res*. 2007;67(3):876-880.