Supporting Information

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SI Materials and Methods

Reagents. Anti-survival factor sirtuin 1 (SIRT1), anti-a disintegrin and metalloproteinase binding protein 10 (ADAM10), and anti-specific nuclear cell protein (NeuN) antibodies were from Millipore, and anti-advanced glycation endproduct receptor 1 (AGER1) and anti-peroxisome proliferation-activated receptor γ (PPAR γ) antibodies were from Santa Cruz Biotechnology. Anti-nicotinamide phosphoribosyltransferase (NAMPT) antibody was from Bethyl Laboratories, and anti-amyloid precursor protein (APP) (6E10) and anti-soluble APPβ (sAPPβ) antibodies were from Covance. Anti-receptor for AGEs (RAGE) antibody was from Affinity BioReagents. Plasma 8-isoprostanes (Cayman), adiponectin (Invitrogen), and leptin (Invitrogen) were assessed by commercial kits as per manufacturer's procedures.

Animals. C57BL/6 mice [originally from National Institutes on Aging, calorie-restricted (CR) colony], which were bred for >10 generations on defined diets, were used in these studies (1). Mice (n = 18–20/group, 1:1 male/female,), after weaning, were assigned to two pair-fed groups, receiving either standard diet (NIH-31 open formula) prepared without heat exposure (low-AGE or MG^{-}) or the same nonheated formula but with synthetic MG-BSA added (1 mg/g food, MG^{+}) and air-dried at room temperature (1). These diets were identical in nutritional and caloric content, but the MG^{+} diet contained approximately twofold more MG-H1 and N^{ε}-carboxy-methyllysine (CML) than the MG^{-} diet. The standard (NIH-31 open formula) was exposed to the standard temperature during manufacturing (termed Reg; Table 1). An i.p. glucose tolerance test (IGTT) was performed at 6-mo intervals, as described previously (1). Impaired glucose tolerance was detected in MG^{+} mice at 18 mo, at which point both MG^{+} and age-matched MG^{-} mice were killed. Old C57B6 WT control mice, fed the Reg diet, previously shown to exhibit features of metabolic syndrome (MS; n = 8, 4:4 male/female), were killed at 24–26 mo after developing abnormal glucose tolerance (1). Blood and brain tissues from all mice were immediately isolated for further study. Mice were maintained in a pathogen-free environment (72 °F, 50% humidity, and 12:12-h light:dark cycles) at the Center for Laboratory Animal Science, Mount Sinai School of Medicine. All experimental procedures complied with the *Guide for the Care and Use of Laboratory Animals* (2).

Advanced Glycation End Product Determination. Fasting serum advanced glycation end products (AGEs) (monitored in all mice at 6-mo intervals) and brain AGEs were assessed at end of study. Two competitive ELISAs were used, based on non–cross-reactive mabs for MG derivatives, i.e., MG-H1 (3D11 mab), and for CML (4G9 mab) (1, 3, 4). Test sensitivity for CML and MG was 0.1 U/mL and 0.004 nmol/mL, respectively; the intraassay variation was ±2.6% (for CML) and ±2.8% (for MG), and the interassay variation is ±4.1% (CML) and ±5.2% (MG). Brain AGE lipids were measured by a direct ELISA using the above antibodies immediately after total brain tissue lipid extraction [2:1 chloroform-methanol (vol/vol); 20 mM butylated hydroxytoluene (BHT), Sigma] (5).

Cell Culture and Treatments. Primary mouse neurons were obtained from E14 mouse embryos (6, 7). Briefly, brain tissue sections, placed in cold DMEM [10% (vol/vol) FBS and 1% antibiotics], were mechanically dissociated by using a fire-polished Pasteur pipette, or a syringe and needle as needed (at room temperature). The dissociated tissues were filtered through a mesh (40 μM; BD Falcon) and seeded on six-well plates precoated with poly-L-lysine (0.1 mg/mL; Sigma). After 30 min, the medium was replaced with neurobasal medium (Invitrogen), 2% (vol/vol) B-27, 0.5% glutamine (2 mM), and 0.2% gentamicin (0.1 mg/mL), refreshed after 24 h (at 37 °C 5% CO_2), and maintained for up to 72 h.

Western blotting. Mouse whole brain or cultured E14 neuronal cells were homogenized in RIPA buffer (150 mM NaCl, 1% Nonidet P-40, 0.5% deoxycholic acid, 0.1% SDS, 50 mM Tris, pH 8.0) including Complete Protease Inhibitor mixture (Roche). Lysates were then separated by SDS/PAGE and subjected to Western blot analysis with the indicated antibodies.

RNA isolation and real-time quantitative PCR. Total RNA from mouse brain, neocortical cells, or human mononuclear cells (MNCs) was extracted by using TRIzol (Sigma). For real-time quantitative PCR, first-strand cDNA was synthesized from total RNA, and PCR was performed in the presence of CYBR green (Bio-Rad). The murine ADAM10 specific primers were as follows: forward, TGGAACACGAGAAGCTGTGA; reverse, GGGAAACGGAAAGGATTTGT. Primer sequences for human AGER1, RAGE, and SIRT1 were as follows: for AGER1—forward, 5′-CTGGGGCTCTTCATCTTCAG-3′, reverse, 5′-GTTGCATCTCCCACAGAGGT-3′; for RAGE—forward, 5′-AGGAGCGTGCAGAACTGAAT-3′, reverse, 5′-TTGGCAAGGTGGGGTTATAC-3′; and for SIRT1—forward, 5′-CGGAAACAATACCTCCACCT-3′, reverse, 5′-CACCCCAGCTCCAGTTAGAA. β-actin and GAPDH housekeeping genes were used for internal normalization. The transcript copy number of target genes was determined based on their Ct values (4).

Aβ assay. $Aβ_{1–42}$ (Aβ) levels in the mouse brain were measured by ELISA for Aβ42 (Invitrogen), according to the manufacturer's directions. Briefly, brain tissue extracts were loaded onto plates precoated with a monoclonal antibody specific for the NH2 terminus of Aβ. After coincubating with a monoclonal antibody specific for the COOH terminus of Aβ, antibody binding was detected by an HRP-conjugated secondary antibody, and the color produced was assessed in proportion to the concentration of Aβ present (6).

H_2O_2 assay. Intracellular reactive oxygen species (iROS) in primary E14 brain neuronal cells, freshly isolated from each group of mice (n = 6/group) or after incubation of REG E14 cells with MG-BSA or BSA (30 and 60 μg/mL for 48 h), were measured by incubation with 2′,7′-dichlorofluorescein diacetate (DCFDA; Molecular Probes) (5 μM for 45 min), and dichlorofluorescein (DCF) was tested (excitation 485 nm and emission 530 nm) (1).

Immunohistochemistry. Anesthetized mice were transcardially perfused with 4% (vol/vol) paraformaldehyde (PF), and the brain was removed and fixed overnight in 4% PF followed by paraffin embedding. Then 20-μm-thick coronal sections were deparaffinized, rehydrated, and microwave-irradiated for antigen retrieval. The hippocampal (HC) area was identified with a neuron-specific nuclear protein marker (NeuN; Millipore) used at 1:100 dilution (6, 8). Sections were also incubated with anti-glial fibrillary acidic protein (GFAP) at 1:500 and anti-AGE (4G9) at 1:50 as primary antibodies overnight at 4 °C. Alexa Fluor-488 and Alexa Fluor 594 at 1:1,000 (Invitrogen) were used as secondary antibodies. All images were captured by a confocal microscope (Zeiss LSM 510 Meta) at the Mount Sinai School of Medicine-Microscopy Shared Resource Facility. The GFAP staining was quantified using the National Institutes of Health ImageJ program.

Brain Functional Tests. ***Rotarod test.*** Motor coordination, balance, and motor learning were tested on the accelerating-rotarod device with automatic timers and falling sensors (Series 8; IITC Life Science, Woodland Hills, CA) (9). The device speed accelerated between 4 and 40 rpm over 2 min. The latency to fall from the rotating rod was scored automatically. Mice were given three trials per day (15-min intertrial intervals) for 4 d, with 1 d of rest between the third and fourth days. The latency of falling from the rod was scored as an index of motor coordination, as was any improvement in latency between sessions that would indicate motor learning.

Object recognition and placement memory. For recognition memory, the mice were allowed to explore two different objects for 5 min up to 4 d (training phase), followed by a test phase on the fifth day with the replacement of one familiar object with a novel object. The exploration of the object was video recorded, and the time taken to explore each object was noted. The data were analyzed by measuring the time spent by the animal with the familiar vs. the novel object, a difference score (novel object interaction – familiar object interaction), and a discrimination ratio (novel object interaction/total interaction with both objects). Object recognition memory was reflected in longer interaction with the novel rather than the familiar object, with a positive difference score, and a discrimination ratio >0.5. A similar protocol was followed for object placement memory, only differing in the familiar object being placed in a new location on the test day (10).

Human Studies: Reagents/Methods. Serum AGEs (sCML and sMG) were quantified by ELISAs as above (3, 4). Plasma insulin was measured by an ELISA kit (ALPCO Diagnostics). Insulin resistance was estimated according to the homeostasis model assessment (HOMA) index as follows: FI × fasting glucose/22.5, where FI is insulin (μU/mL) and fasting glucose is expressed in millimoles per liter. Leptin and adiponectin were determined with ELISA kits (Millipore). TNFα was measured in MNC lysates by an ELISA kit (Biosource International). Human SIRT1, AGER1, RAGE, and PPARγ mRNA levels were assessed in isolated MNC by RT-PCR as described above (4).

Dietary AGE Assessment. Daily dietary AGE intake (dAGE) was estimated from a database, which lists food AGE content as AGE equivalents (Eq/d; 1 AGE equivalent = 1,000 kilounits) (11). A 3-d food record was obtained that emphasized preparation methods and amount of food and beverages consumed, based on established guidelines (11). In addition, subjects were administered a 7-d food frequency questionnaire on dietary habits and methods of food preparation, frequency, and portion size, providing data as an AGE score. Daily nutrient intake calculations were estimated from food records using a nutrient software program (Food Processor version 10.1; ESHA Research).

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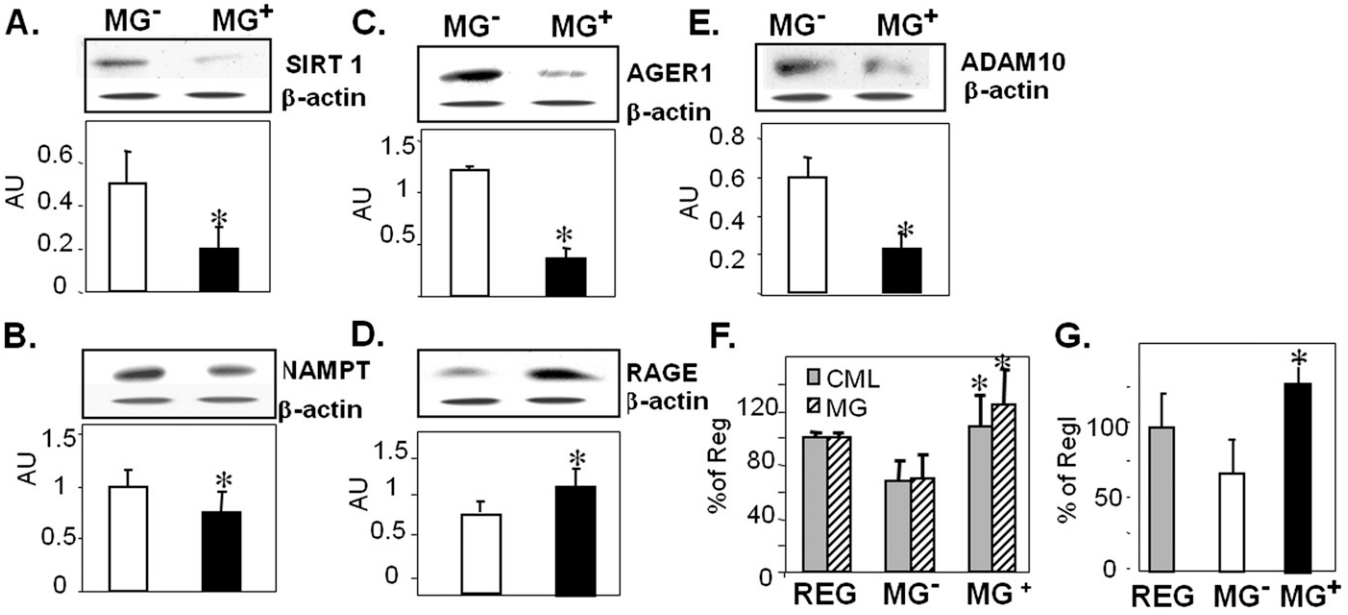

Fig. S1. Chronic exposure to high maternal serum MG levels alters fetal brain SIRT and related gene products. (*A–E*) Western blots and densitometric analysis of SIRT1, AGER1, NAMPT, RAGE, and ADAM10 protein expression in primary E14 brain neuronal cell lysates from MG^+ and MG^- mice ($n = 6$/group). Data show the ratio (mean ± SEM) of target protein to β-actin. *$P < 0.05$ vs. MG^- mice. (*F*) Intracellular CML and MG, by ELISA (*G*) Intracellular ROS by DCF. Data for *F* and *G* are shown as percent (mean ± SEM) of Reg ($n = 3–5$ experiments, each in triplicate), *$P < 0.05$ vs. MG^- mice. The data suggest that physiologic levels of SIRT1 may be influenced by external (including maternal) AGEs/OS. Thus, during fetal life, high maternal AGE levels can down-regulate levels of brain SIRT1, rendering the brain more susceptible to OS injury. Because in addition to SIRT1, NAMPT and AGER1 levels were also suppressed, chronic excess of AGEs/OS could negatively impact several host defenses that are critical in brain development/function. These findings may also be of relevance to the increasing incidence of cognitive decline in younger adults with the MS or diabetes. Neurons from MG^- brain, however, exhibited low AGEs/OS and markedly elevated SIRT1, as well as AGER1, NAMPT, and ADAM10 (but low RAGE) levels, a pattern likely to afford these neurons significant protection against OS injury. Extracellular AGEs may thus represent a modifiable risk factor active throughout life, and the timely reduction of this factor may prove brain protective.

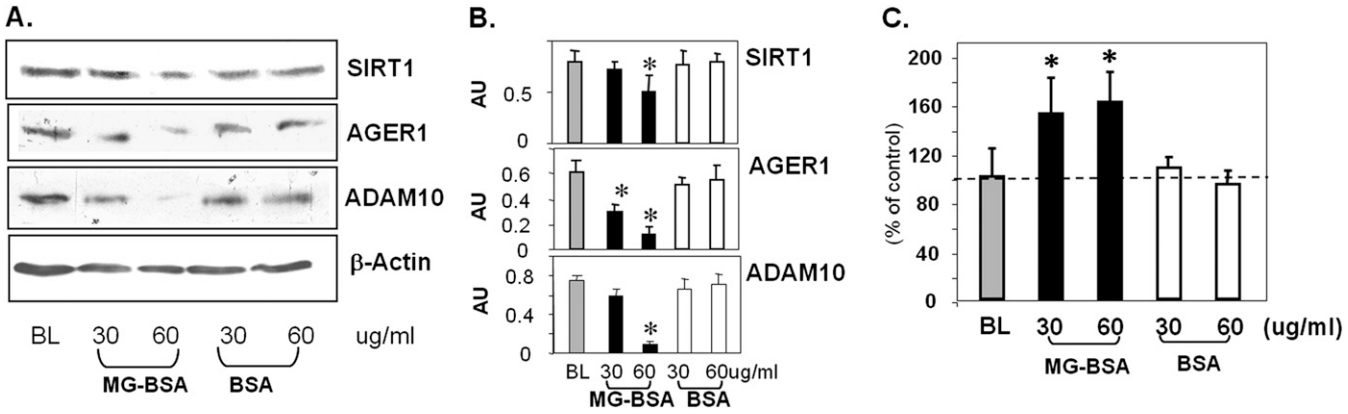

Fig. S2. Prolonged in vitro exposure of primary brain neurons to MG-BSA suppresses neuronal SIRT1, AGER1, and ADAM10 protein and raises ROS generation. (*A*) Western blots and (*B*) densitometric analysis of SIRT1, AGER1, and ADAM10 levels in brain primary neuronal cell lysates from Reg mice, cultured with MG-BSA or BSA (30 or 60 μg/mL, 72 h) ($n = 6$). Data are shown as the ratio (mean ± SEM) of target protein to β-Actin. *$P < 0.05$ vs. nonstimulated cells. (C) Intracellular ROS levels (by DCF). Nonstimulated neuronal cells served as baseline controls. Data are expressed as percent of baseline (mean ± SEM), $n = 5$, each in triplicate. *$P <$ 0.05 vs. baseline. The data indicate that chronic (72 h) in vitro MG stimulation of neuronal cells from control mice also suppresses SIRT1, ADAM10, and AGER1, replicating the pattern seen in vivo with MG^+ neurons. The data support the role of exogenous AGEs in the genesis of lifelong brain toxicity.

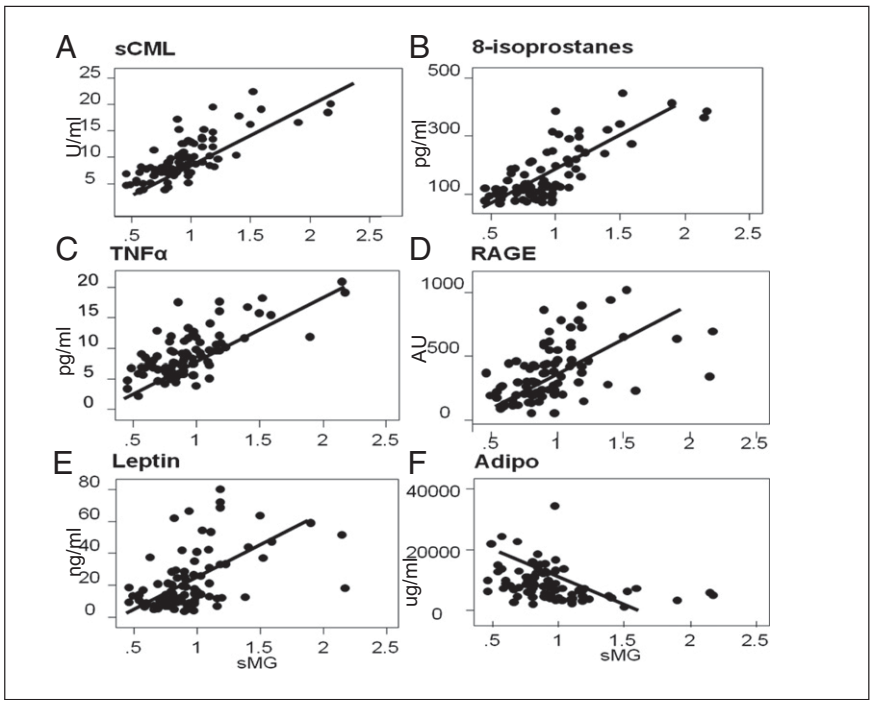

Fig. S3. Cross-sectional correlations between baseline levels of serum MG and markers of inflammation and OS in older humans: (*A*) serum CML (sCML), (*B*) plasma 8-isoprostanes, (*C*) MNC TNFα protein, (*D*) MNC RAGE mRNA, (*E*) plasma leptin, and (*F*) plasma adiponectin. All values are obtained from fasting blood samples; fitted linear regression lines are as shown. Serum MG and CML, plasma 8-isoprostanes, leptin, MNC TNFα protein, and adiponectin were measured by ELISAs, and MNC SIRT1 and RAGE mRNA levels were measured by RT-PCR.

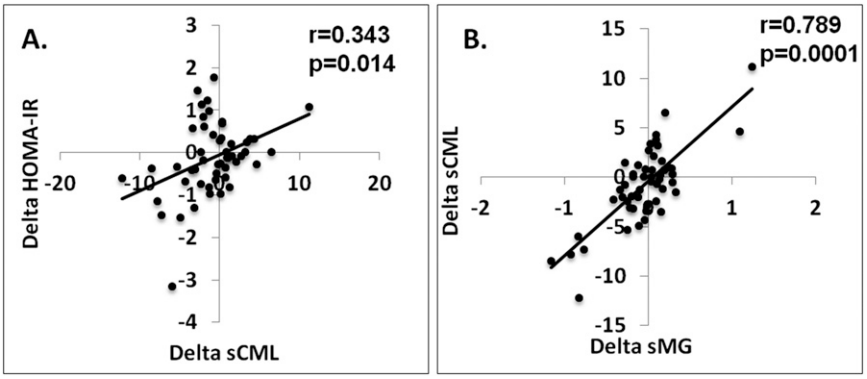

Fig. S4. Temporal relationships between changes in serum AGEs and HOMA-IR over 9 mo. (*A*) Correlation between changes (Δ) in sCML and changes (Δ) in sMG. (*B*) Correlation between changes in sCML and HOMA-IR. All tests were performed in fasting sera; fitted regression lines are as shown.

Table S1. Clinical cohort baseline summary statistics

Variable	Mean	SD	Median	First quartile	Third quartile
Age (y)	70.2	7.9	69.0	63.3	75.3
Body mass index (kg/m^2)	26.1	6.5	26.1	22.9	29.9
Dietary AGE (AGE Eq/d)	13.0	7.6	11.7	7.2	16.2
Dietary calories (kcal/d)	1,873	645	1,746	1,451	2,134
Education (y)	15.6	3.4	16.0	14.0	18.0
MMSE	28.7	1.7	29.0	28	30
Serum CML (U/mL)	9.8	4.1	8.7	7.2	12.0
Serum MG (mmol/mL)	0.96	0.32	0.90	0.77	1.1
8-isoprost. (pg/mL)	167	93	129	100	214
VCAM1 (ng/mL)	818	272	801	619	986
Insulin (μU/mL)	7.5	4.5	6.5	4.0	9.2
HOMA-IR	1.6	1.2	1.4	0.8	1.8
Adiponectin (μg/mL)	8.8	5.6	7.4	4.6	11.9
Leptin (ng/mL)	22.4	18.5	14.6	9.0	30.3
TNFα (pg/mg protein)	9.2	3.9	8.3	6.5	11.5
RAGE (mRNA)	379	217	325	211	471
AGER1 (mRNA)	196	113	176	113	261
SIRT1 (mRNA)	336	93	307	201	426

Transcript copy number of target genes is shown, based on Ct values (3). MMSE, Mini Mental State Examination; mRNA, RNA extracted from MNC.

Table S2. Clinical study: Correlations between selected parameters at baseline

Baseline	Baseline	*r* value	*P* value	*N*
dAGE*	sCML	0.71	<0.0001	80
dAGE	sMG	0.64	<0.0001	80
dAGE	TNFα	0.54	<0.0001	80
dAGE	RAGE	0.58	<0.0001	79
dAGE	Leptin	0.38	0.0006	80
dAGE	8-isoprostane	0.49	<0.0001	81
dAGE	AGER1	0.42	0.0001	79
dAGE	SIRT1	−0.55	<0.0001	84
dAGE	Adiponectin	−0.36	0.0013	80
sMG	sCML	0.74	<0.0001	85
sMG	TNFα	0.59	<0.0001	85
sMG	RAGE	0.53	<0.0001	84
sMG	AGER1	0.35	0.0013	84
sMG	8-isoprostane	0.62	<0.0001	85
sMG	Adiponectin	−0.47	<0.0001	85
sMG	Leptin	0.48	<0.0001	85
sMG	dAGE	0.64	<0.0001	80
sMG	SIRT1	−0.61	<0.0001	84

r values are Spearman correlations adjusted for age and sex. MG, serum methylglyoxal-derivatives; sCML, serum N$^{\varepsilon}$carboxy-methyl-lysine.
*Dietary AGE intake (dAGE), estimated by 3-d food records, is shown as AGE Eq/d (10).