ESM Methods

MRI Studies: MR imaging for determining abdominal fat deposits (subcutaneous and visceral fat) was performed as previously described [22, 23]. Briefly, MR imaging of the abdomen was performed with a 3 T whole body scanner (Philips Achieva) by using the 16-channel XL-Torso array coil for signal reception. Study subjects were imaged in a supine position with both arms parallel to their body. Whole-volume coverage of the abdomen is obtained by using cross sectional T1-weighted steady state free precession (SSFP) and strongly T1-weighted spoiled gradient echo sequences during breath holding. Image analysis was performed with software developed for volumetric measurement of muscle and fat in the abdomen using semi-automated image segmentation software implemented in a custom built software platform in Matlab (Natick, MA).

Carotid MRI was performed on a 3.0T whole body MR system (Philips Medical Systems, Cleveland, OH). A dedicated 8-element phased array coil was used for carotid imaging. After localization with fast gradient echo or TRUFI sequences, all images were obtained using 2D multi-slice double inversion recovery (DIR) turbo spin echo (TSE) technique. Carotid images were acquired with cardiac triggering, but free breathing. Proton density (PDW), T1 and T2 weighted images were acquired as described in previous studies [23]. Briefly, a total of 16 transverse images centered at the right carotid bifurcation were obtained. Imaging parameters were as follows: repetition time, 2RR/2RR/1RR(PDW/T2/T1 images); echo time 5.6/56/5.6ms (PDW/T2/T1 images); field of view 14 cm; slice thickness 2mm; 10% inter-slice gap; acquisition matrix 256 x 256; no phase wrap; number of signal averages 2/4/3 (PDW/T2/T1 images); turbo factor (echo train length), 15/15/3 (PDW/T2/T1 images); receiver bandwidth, 488 Hz/pixel; no

zero filling. A chemical shift suppression pulse is used to suppress signal from fat. Images were transferred to a dedicated workstation for off-line calculation of plaque burden (Vesselmass, Leiden, Netehrlands). Regions of interest encompassing the outer and inner wall contours were drawn by an experienced image analyst. Measures of wall area and wall thickness were automatically computed by the software program from these regions of interest tracings.

Materials: Plasma adiponectin and serum leptin were measured by commercial kits from Millipore Corp. Human plasma insulin was measured by an ELISA kit from ALPO Diagnostics. TNFα was measured in PMNC lysates by ELISA kit from Invitrogen. Plasma 8-isoprostanes ELISA kit was from Cayman. Anti-sirtuin 1 (SIRT1) antibody was from Millipore. Anti-advanced glycation endproduct receptor 1 (AGER1), anti-insulin receptor β, and anti-NF-κB p65 antibodies were from Santa Cruz Biotechnology. Anti- phosphor-Akt (Ser473) antibody and anti-p65 acetylated at lysine 310 position antibody were from Cell Signaling Technology. SIRT1inhibitor sirtinol was from Calbiochem. SIRT 1 activator SRT1720 was from Selleckchem.

RNA isolation and qRT-PCR: Total RNA was isolated from PMNC by Trizol according to the manufacturer's protocol (Sigma). First-strand cDNA synthesis was performed using Superscript III RT (Roche). AGER1, RAGE and SIRT1 mRNA expression were analyzed by quantitative SYBR Green real-time PCR. Primer sequences were: for AGER1, forward primer 5'-CTGGGGCTCTTCATCTTCAG-3', reverse primer 5'-GTTGCATCTCCCACAGAGGT-3', for RAGE, forward primer 5'-AGGAGCGTGCAGAACTGAAT-3', reverse primer 5'-TTGGCAAGGTGGGGTTATAC-3', and for SIRT1, forward primer 5'-

CGGAAACAATACCTCCACCT-3', reverse primer 5'-CACCCCAGCTCCAGTTAGAA-3'. Glyoxalase-1**,** forward primer 5"-GCAGACCATGCTACGAGTGA-3", reverse primer 5"-AGAGCGCCCAGGCTATTT-3'. 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 [17].

AGE determination: AGEs in serum, urine and PMNC lysates were determined by well validated standard competitive (for AGE-proteins) [24,25] enzyme-linked immunosorbent assays (ELISA). For CML, anti-AGE-KLH monoclonal antibody (4G9 mab) (Alteon, Inc., NJ) was used, which was shown to be strongly reactive with CML-modified BSA [12, 24, 25]. For MG, anti-MG monoclonal antibody (3D11 mab) was developed in our laboratory based on HPLC-characterized MG-modified ovalbumin as immunogen [12, 14]. 3D11 was found to be highly reactive with MG-derivatives, i.e., arginine-MG-H1 and MG-modified BSA [12, 14, 26]. In brief, for sample preparation, serum and urine samples were diluted to 1/5 and 1/3 respectively. AGE-immunoreactivity was based on ligand inhibition. AGE measurements were performed in 96-well microtiter plates (COSTAR, Corning, NY) coated with AGE-BSA (3ug/ml). After washing (x3 with PBS-Tween-20) and blocking with SuperBlock blocking buffer in PBS (Pierce, Rockford, IL), competing antigen was added, followed by the antisera and incubated for 2h at room temperature. Wells were then washed with PBS-Tween-20 and developed with an alkaline phosphatase-linked anti-mouse IgG [24, 25, 37]. Optical density (OD) at 405 nm was determined by an ELISA reader [25]. For sera and urine, AGE values were expressed as CML units/ml or MG nmol/ml. For PMNC lysates, prior to ELISA, protein concentration was determined by Bio-Rad and intracellular CML (*iCML*) was expressed as units/mg and *iMG* as nmol/mg cell protein. Test sensitivity for CML and MG: 0.1 u/ml and 0.04 nmol/ml, respectively; intra-assay variation: ±2.6 % (CML) and ±2.8 % (MG); inter-assay variation: ±4.1 % (CML) and ±5.2 % (MG).

MG-BSA preparation. Low-endotoxin BSA (Sigma) was incubated in sodium phosphate buffer with pure MG (1 mmol/L) for 3 days, dialyzed against ammonium bicarbonate buffer (pH 8, 4 °C), lyophilized and stored at −80 °C. Based on HPLC, the

thus-derived MG-BSA contained 19.2 MG-Arg/mol BSA. Based on ELISA using anti-MG mab (3D11) it contained 9.2 nmol MG/mg, and by an anti-CML mab (4G9), 48 CML U/mg [12,14].

ESM Table 1. Changes from baseline to 12 months by intervention group and between intervention groups

	Regular AGE Diet: last - first value			Low Age Diet: last - first value			Regular minus Low Age Diet		
Variable	**n**	**Mean**	**95% CI**	**n**	**Mean**	**95% CI**	**Mean**	**95% CI**	**p value**
BP systolic, kPa	49	0.174	(-2.5, 3.2)	51	0.234	(-3.2, 3.7)	-0.150	(-0.99, 0.69)	0.73
BP diastolic, kPa	49	0.245	(-1.7, 2.4)	51	0.037	(-2.1, 2.0)	0.208	(-0.3, 0.7)	0.42
Weight, Kg	49	-0.410	(-5.2, 5.2)	51	-1.799	(-7.6, 3.7)	1.389	(0.1, 2.7)	**0.03**
BMI, kg/m^2	49	-0.139	(-2.7, 2.0)	51	-0.514	(-2.7, 1.7)	0.375	(-0.2, 0.9)	0.17
Waist circumference, cm	49	-2.931	(-11.6, 4.9)	51	-2.616	(-13.5, 6.4)	-0.315	(-2.6, 1.9)	0.78
Body fat (BIA), %	49	0.233	(-4.5, 6.5)	51	-0.082	(-6.2, 5.1)	0.315	(-1.0, 1.6)	0.64
Abdominal SAT, cm^2	39	0.628	(-4.9, 32.0)	43	-0.987	(-9.2, 5.6)	1.615	(4.3, 26.4)	0.41
Abdominal VAT, cm^2	39	-1.080	(-1.07, 2.98)	43	-1.599	(-11.5, 5.4)	0.594	(-3.6, 4.8)	0.78
Fasting plasma glucose, mmol/l	49	0.060	(-0.83, 1.4)	51	-0.020	(-1.3, 1.4)	0.070	(-0.2, 0.3)	0.61
Glucose AUC (OGTT)	49	2.939	(-83.0, 60.5)	51	-6.843	(-95.0, 87.0)	9.782	(-8.8, 28.4)	0.31
Fasting plasma insulin, pmol/l	49	15.3	(-63.9, 87.5)	50	-33.900	(-179.2, 39.6)	49.20	(26.4, 72.2)	**<0.0001**
Insulin AUC (OGTT)	49	-0.377	(-145.5, 104.0)	50	-1.486	(-143.0, 104.7)	1.109	(-31.6, 33.8)	0.95
HbA_{1c}, %	49	-0.010	(-0.50, 0.50)	51	-0.061	(-0.40, 0.50)	0.051	(-0.07, 0.17)	0.40
HOMA-IR	49	0.527	(-1.9, 3.1)	50	-1.106	(-6.2, 1.6)	1.633	(.9, 2.4)	**<0.0001**
Triacylglycerol, mmol/l	49	-0.160	(-0.77, 0.40)	51	0.080	(-1.48, 1.01)	-0.240	(-0.55, 0.07)	0.13
HDL cholesterol, mmol/l	49	0.030	(-0.39, 0.41)	51	0.060	(-0.36, 0.47)	-0.030	(-0.13, 0.07)	0.57
Serum CML, U/ml	49	4.639	(-7.4, 18.2)	51	-4.316	(-18.1, 7.3)	8.955	(5.4, 12.5)	**<0.0001**
Serum MG, nmol/ml	49	0.602	(-0.8, 1.9)	51	-0.593	(-1.9, 0.6)	1.195	(0.9, 1.5)	**<0.0001**
iCML, U/mg protein	33	1.714	(-6.0, 9.2)	29	-1.792	(-5.4, 1.3)	3.506	(2.0, 5.0)	**<0.0001**
iMG, nmol/mg protein	33	0.197	(-0.8, 1.4)	29	-0.281	(-0.9, 0.3)	0.478	(0.2, 0.7)	**0.0002**
Plasma 8-isoprostanes, pg/ml	49	72.355	(-251, 229)	51	-75.161	(-301, 81)	147.516	(93, 202)	**<0.0001**
Serum VCAM1, ng/ml	13	255.031	(-106, 608)	11	-120.455	(-456, 135)	375.486	(206, 545)	**<0.0001**
Serum leptin, ng/ml	49	8.590	(-14.8, 26.0)	51	-10.961	(-45.3, 11.8)	19.551	(13.7, 25.4)	**<0.0001**
Plasma adiponectin, μg/ml	49	-1.059	(-8.7, 6.8)	51	7.205	(-1.8, 20.1)	-8.264	(-10.6, -5.9)	**<0.0001**
AGER1, mRNA	47	-16.851	(-169, 136)	51	158.020	(-47, 346)	-174.871	(-221, -129)	**<0.0001**
RAGE, mRNA	47	106.851	(-393, 408)	51	-157.784	(-582, 246)	264.635	(172, 357)	**<0.0001**

SIRT1, mRNA	47	-31.255	(-228, 145)	51	136.529	(-62, 405)	-167.784	(-221, -115)	**<0.0001**
Glyoxalase 1, mRNA	47	-1.851	(-18.0, 22.0)	51	11.843	(-13.0, 36.0)	-13.694	(-19.3, -8.1)	**<0.0001**
TNFα, pg/mg protein	41	1.110	(-6.2, 8.6)	46	-4.423	(-12.0, 2.4)	5.533	(3.4, 7.7)	**<0.0001**
Dietary calories, kJ/day	49	-862.643	(-5342, 3023)	51	-1691.467	(-5024, 1482)	828.986	(-71, 1729)	0.07
Dietary AGEs, AGE Eq/day	49	0.949	(-18.7, 20.1)	51	-10.676	(-25.5, 2.1)	11.625	(7.3, 15.9)	**<0.0001**
eGFR, ml/min/1.72m^2	49	-0.038	(-5.54, 2.70)	51	1.375	(-5.55, 2.71)	1.413		0.498
Urine protein excretion, mg/day	49	-0.375	(-70.0, 83.2)	50	1.741	(-49.8, 71.4)	-2.116	(-17.8, 13.6)	0.79
Urine albumin excretion, mg/day	48	-0.228	(-2.10, 0.83)	50	-0.056	(-1.02, 0.81)	-0.172	(-0.51, 0.16)	0.32
Urine CML excretion, U/day	49	24897	(-90325, 162780)	51	-32773	(-147870, 30096)	57670	(27892, 87448)	**<0.0001**
Urine MG excretion, nmol/day	49	666.689	(-1259, 3267)	51	-386.405	(-1497, 1099)	1053.094	(564, 1542)	**<0.0001**
Average Carotid Wall Area, mm^2	36	23.729	(15.6, 40.7)	42	23.927	(16.7, 33.4)	-0.198	(-2.7, 2.3)	0.88
Average Carotid Wall Thickness, mm	36	0.991	(0.79, 1.51)	42	0.993	(0.83, 1.20)	-0.002	(-0.07, 0.07)	0.95

P value = statistical significant differences of changes between both groups; CI = confidence intervals; BIA = Bioelectrical impedance; Abdominal SAT = Abdominal subcutaneous fat; Abdominal VAT = Abdominal visceral fat; AUC=Area under the curve; OGTT = Oral glucose tolerance test; iCML = intracellular CML per mg protein in PMNC; iMG = intracellular MG per mg protein in PMNC; eGFR = Estimated GFR by MDRD equation.

ESM Table 2. Changes in selected parameters in selected subgroups of patients during intervention

	Low AGE diet without weight loss			Reg-AGE diet with weight loss		
Variable	**Month 0**	**Month 12**	**P[1]**	**Month 0**	**Month 12**	**P[2]**
N	12	12		25	25	
dAGE, AGE Eq/day	16±14	9±8	.007	19±11	19±10	.840
dCal, kJ/day	7432±2269	6033±1838	.033	8759±3061	8068±2680	.219
Weight, kg	87±13	89±13	.003	93±22	90±21	.001
HOMA-IR	2.90±1.23	1.82±1.05	.001	2.76±0.97	3.7±1.45	.008
FBG*, mmol/l	5.4±0.8	5.3±0.6	.612	4.9±0.6	4.8±0.4	.618
FPI*, pmol/l	83.3±31.9	53.5±31.3	.001	88.9±29.2	116.0±45.1	.007
sCML, U/ml	17±9	12±6	.073	18±7	24±7	.007
sMG, mmol/ml	2.16±0.74	1.76±0.70	.098	2.33±0.50	3.14±0.80	.001
Leptin, ng/ml	25±15	17±13	.138	28±10	39±13	.003
Adiponectin, μg/ml	10±3	16±7	.017	9±3	7±2	.207
TNFα, pg/mg protein	15±6	10±3	.017	15±5	17±4	.252
Glyoxalase, mRNA	25±15	37±17	.024	28±11	26±10	.518
Isoprostane, pg/ml	203±137	113±44	.034	210±100	318±118	.002
AGER1, mRNA	180±85	328±162	.006	178±70	152±65	.181
SIRT1, mRNA	225±76	378±130	.009	239±102	209±91	.239
RAGE, mRNA	515±288	287±128	.017	490±219	641±208	.005

All data presented as mean ± SD

Weight loss was defined by any change in weight ≥0.01kg between baseline and end of study.

FBG* = Fasting blood glucose; FPI* = fasting plasma insulin.

P^1 = Difference between baseline and end of study for the low AGE diet without weight loss

P^2 = Difference between baseline and end of study for the Reg- AGE diet with weight loss.

Both p values obtained by paired t-test analyses.