Supplementary Material

Methylglyoxal Reshapes Hepatic and Adipose Tissue Metabolism and Increases Viability of Lymphocytes

Naiara Cristina Lucredi[a] Lucas Paulo J. Saavedra[b] Silvano Piovan[b] Emanuele P. Lima[a] Mariane Aparecida F. Godoy[b] Rogério Marchiosi[a] Verônica Elisa P. Vicentini[b] Paulo Cezar F. Mathias[b] Anacharis B. Sá-Nakanishi[a] Lívia Bracht[a] Claudia C. S. Chini[c] Eduardo N. Chini[c] Adelar Bracht[a] Jurandir F. Comar[a]

[a]Department of Biochemistry, State University of Maringá, 87020900, PR, Brazil, [b]Department of Biotechnology, Genetics, and Cellular Biology, State University of Maringá, PR, Brazil, [c]Department of Anesthesiology and Perioperative Medicine, Mayo Clinic, Jacksonville, FL, USA

SUPPLEMENTARY MATERIAL

Table. S1. *Primer sequences used for real-time quantitative PCR (q-PCR) analysis*. FBPase-1, fructose 1,6-biphosphatase; G6Pase, glucose 6-phosphatase; GADPH, glyceraldehyde-3-phosphate dehydrogenase; GLO-I, glyoxalase I; GLO-II, glyoxalase II; IL-1β, interleukin 1 beta; IL-6, interleukin 6;PEPCK, phosphoenolpyruvate carboxykinase; RAGE, AGE receptor; TNF-α, tumoral necrosis factor alpha.

	Forward	Reverse	NM code
FBPase-1	TCGAAACGGATATCAGCACCC	GATGCCATAGAGCTGAGCGAT	NM_012558.3
G6ase	GATTCCGGTGCTTGAATGTCG	GCATTGTAGATGCCCCGGAT	NM_013098.2
GADPH	TTGTGCAGTGCCAGCCTC	GAGAAGGCAGCCCTGGTAAC	NM_017008.4
GLO-I	ACTGAAGATGACGAGACGCA	AATCCCAATGTGGCCAAATCCC	NM_207594.3
GLO-II	AGGGAACCGCAGACGAGAT	GAGGAAGCCGGCCTAAGACT	1
IL-1β	GCTTCCTTGTGCAAGTGTCT	TCTGGACAGCCCAAGTCAAG	NM_031512.2
IL-6	CATTCTGTCTCGAGCCCACC	GCTGGAAGTCTCTTGCGGAG	NM_012589.2
PEPCK	GGGGGTGTTTACTGGGAAGG	CGGTTCCTCATCCTGTGGTC	NM_198780.3
RAGE	GACAACTTTGGCATCGTGGA	ATGCAGGGATGATGTTCTGG	2
TNF-α	ATGGGCTCCCTCTCATCAGT	GCTTGGTGGTTTGCTACGAC	NM_012675.3

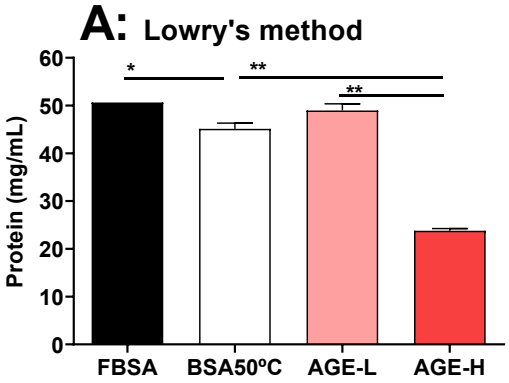
1:Masterjohn C, Park Y, Lee J, Noh SK, Koo SI, Bruno RS. Dietary fructose feeding increases adipose methylglyoxal accumulation in rats in association with low expression and activity of glyoxalase-2. Nutrients. 2013; 5:3311-3328. https://doi.org/10.3390/nu5083311

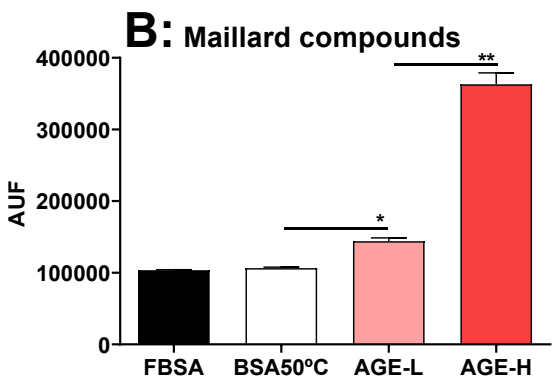
2: Xu L, Zang P, Feng B, Qian Q. Atorvastatin inhibits the expression of RAGE induced by advanced glycation end products on aortas in healthy Sprague-Dawley rats. Diabetol Metab Syndr. 2014; 6:102. https://doi.org/10.1186/1758-5996-6-102

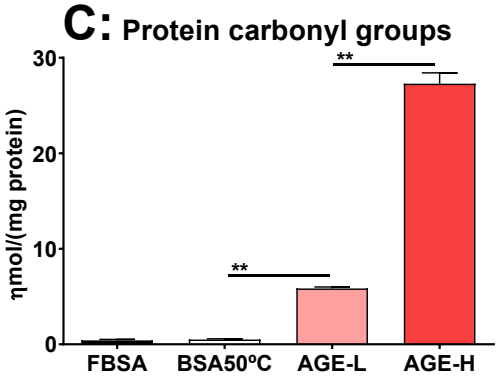
METHODS: AGE CHARACTERIZATION

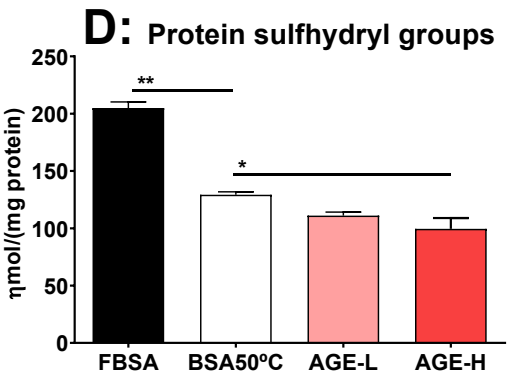
The AGE preparations were characterized in relation to non-oxidized amino acids, Maillard compounds, protein carbonyl groups, protein sulfhydryl groups (thiols), N-oxidation of protein amino acids, and contents of carboxymethyllysine (CML) and methylglyoxal-hydroimidazolone 1 (MGH1). Non-oxidized amino acids were quantified by their ability to react with the 2,4,6-trinitrobenzenesulfonic (TNBSA assay) [85]. Maillard compounds were quantified by spectrofluorimetry as described in the section 2.8. Protein carbonyl and sulfhydryl groups were determined by spectrophotometry as described in the section 2.6. N-oxidation of protein amino acids was determined with Folin reagent [46]. The contents of CML and MGH1 were determined by dot blot as described in the section 2.14.

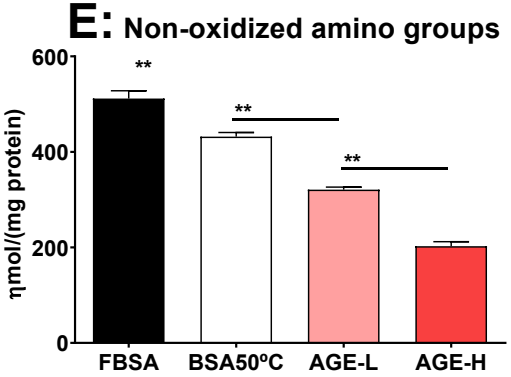
Fig. S1. *AGE characterization*. FBSA, fresh bovine serum albumin preparation; BSA 50 °C, BSA preparations heated at 50 °C in phosphate buffer; AGE-L and AGE-H, respectively, AGE prepared by incubating BSA with 50 and 250 mM methylglyoxal (MG) at 50 °C. **A:** Non-oxidized amino acids; **B:** Maillard compounds; **C:** Protein carbonyl groups; **D:** Protein sulfhydryl groups (thiols), **E:** N-oxidation of protein amino acids; **F:** representative dot blot quantifying the relative levels of carboxymethyllysine (CML), methylglyoxal-hydroimidazolone 1 (MGH1) and coomassie blue, with the sample for one preparation loaded into each lane (vertical column). The results of densitometric analysis of the respective dot blot are presented in Panels **G** for MGH1 and **H** for CML. Maillard compounds are presented as arbitrary units of fluorescence (AUF). Data are represented as mean ± SEM of 3-4 preparations. * $p < 0.05$; ** $p < 0.001$.

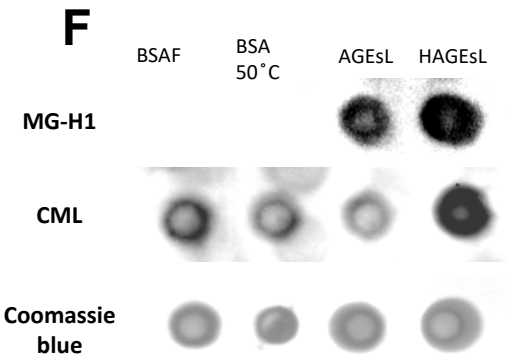

A: Lowry's method
Protein (mg/mL)
60
50
40
30
20
10
0
*
**
**
FBSA
BSA50ºC
AGE-L
AGE-H

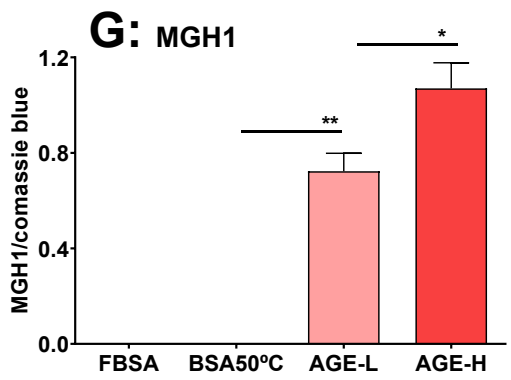

B: Maillard compounds
AUF
400000
300000
200000
100000
0
**
*
FBSA
BSA50ºC
AGE-L
AGE-H

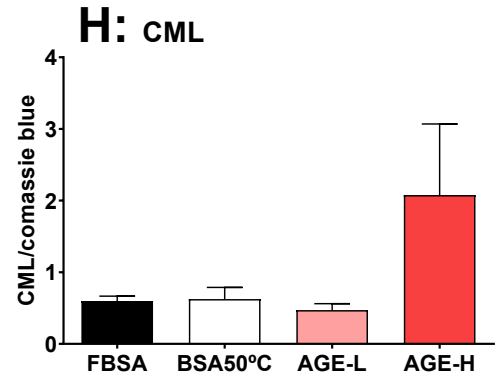

C: Protein carbonyl groups
ηmol/(mg protein)
30
20
10
0
**
**
FBSA
BSA50ºC
AGE-L
AGE-H


D: Protein sulfhydryl groups
ηmol/(mg protein)
250
200
150
100
50
0
**
*
FBSA
BSA50ºC
AGE-L
AGE-H


E: Non-oxidized amino groups
ηmol/(mg protein)
600
400
200
0
**
**
**
FBSA
BSA50ºC
AGE-L
AGE-H


F
BSAF
BSA 50˚C
AGEsL
HAGEsL
MG-H1
CML
Coomassie blue


G: MGH1
MGH1/comassie blue
1.2
0.8
0.4
0.0
**
*
FBSA
BSA50ºC
AGE-L
AGE-H


H: CML
CML/comassie blue
4
3
2
1
0
FBSA
BSA50ºC
AGE-L
AGE-H