

Supplementary Material

In-depth AGE and ALE profiling of human albumin in Heart Failure: *ex vivo* studies

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Table S1. Clinical and demographical characteristics of the study population.

* current smokers

DM II, Diabetes mellitus type 2; BMI, body mass index; FE, ejection fraction; BNP, brain natriuretic peptide;

Characteristics	control subjects		Heart failure patients		pvalue
	mean	SD	mean	SD	
Age	53,6	2,1	58,1	6,1	n.s.
Gender (F/M)	2/6		1/9		
Hypertension (n)	0		3		
Dyslipidemia (n)	0		3		
Smoke* (n)	0		1		
DM II (n)	0		3		
BMI (Kg/m ²)	23,5	2,0	26,6	4,0	n.s.
FE (%)	-		30,6	7,5	
Glycemia (mg/dL)	100,6	10,3	118,0	42,2	n.s.

Hb (g/dL)	14,8	0,9	13,5	1,8	n.s
Uric acid (mg/dL)	5,0	0,7	8,1	2,4	0,003
BNP (pg/ml)	-		635,0	462,6	
total cholesterol (mg/dL)	207,6	29,7	183,2	25,4	n.s

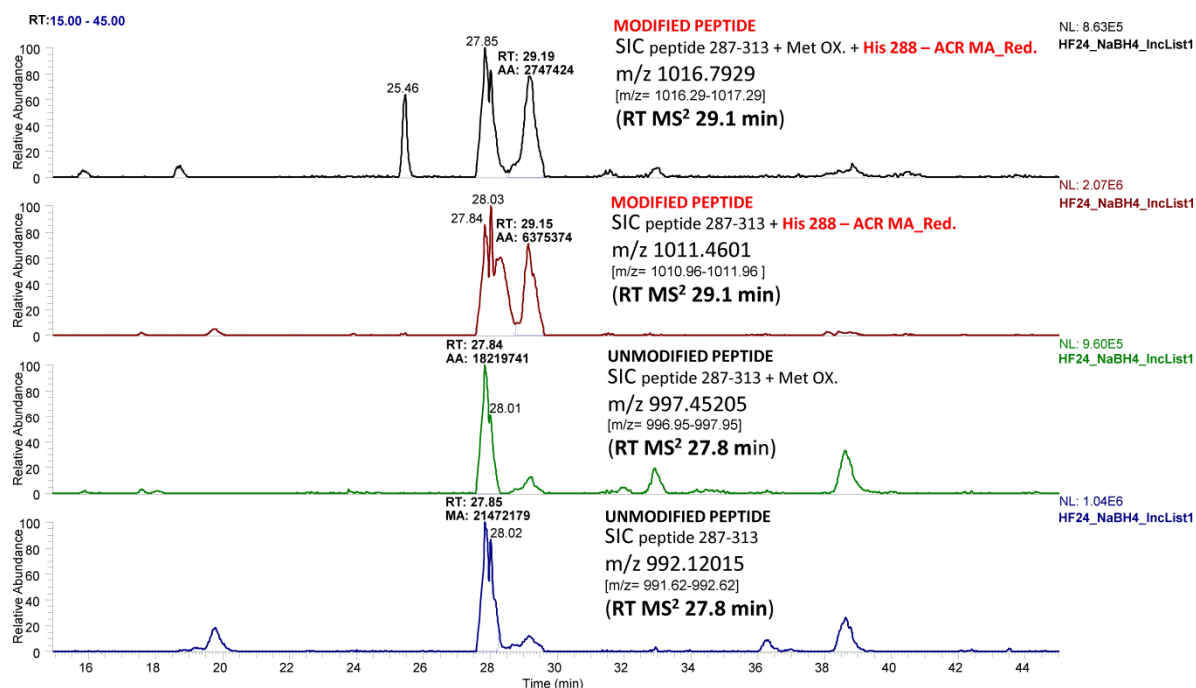
Table S2. Known (literature based) covalent adducts induced by HNE, ONE, MDA, ACR, GO, MGO, RED_Sugar derivatives and Cys-oxidation derivatives set as variable modifications within the PD parameters for the identification and localization of AGE/ALE-deriving adducts.

4-hydroxy-2-nonenal (HNE)			
PTM	AA Res. Involved	ΔM (no NaBH4)	ΔM (+ NaBH4)
<i>Michael adduct (HNE-MA)</i>	Cys	+ 156.110502 Da	+ 158.13067 Da
	His		
	Lys		
	Arg		
<i>Schiff Base (HNE-SB)</i>	Lys	+ 138.01446 Da	+ 140.12011 Da
	Arg		
<i>2-pentilpyrrole (PP)</i>	Lys	+ 120.0939 Da	-
	Arg		
<i>Dehydropentylfuran (DHPF)</i>	Cys	+ 138.10446 Da	-
	His		
	Lys		
	Arg		
4-oxo-2-nonenal (ONE)			
PTM	AA Res. Involved	ΔM (no NaBH4)	ΔM (+ NaBH4)
<i>Michael adduct (ONE-MA)</i>	Cys	+ 154.09937 Da	+ 158.13067 Da
	His		
	Lys		
	Arg		
<i>Schiff Base (ONE-SB)</i>	Lys	+ 136.08881 Da	+ 140.12011 Da
	Arg		
<i>ONE-pentylfuran (OPF)</i>	Cys	+ 136.08881 Da	-
	His		
	Lys		
	Arg		
Malondialdehyde (MDA)			
PTM	AA Res. Involved	ΔM (no NaBH4)	ΔM (+ NaBH4)

<i>N</i> -propenal-Lysine (NPK)	Lys	+ 54.01056 Da	+ 56.02621 Da
<i>Di</i> -dihydropyridine-lysine (DHPK)	Lys	+ 134.03678 Da	+ 138.06808 Da
Malondialdehyde argpyrimidine(MDA-RP)	Arg	+ 36.0000 Da	-
Acrolein (ACR)			
PTM	AA Res. Involved	ΔM (no NaBH₄)	ΔM (+ NaBH₄)
<i>Michael Adduct (propanal derivative) (ACR-MA)</i>	Cys		
	Lys	+ 56.02621 Da	+ 58.04186 Da
	His		
<i>Hydroxy-tetra-hydropyrimidine (Propane-arginine) (HTPR) (HTPR)</i>	Arg	+ 56.02621 Da	-
<i>Double Michael Adduct (2ACR-KMA)</i>	Lys	+ 112.05243 Da	+ 116.08373 Da
<i>Schiff Base (ACR-SB)</i>	Lys	+ 38.01565 Da	+ 40.0313 Da
<i>Formyl-dehydro-piperidyl-lysine (FDPK)</i>	Lys	+ 94.04186 Da	+ 96.05751 Da
<i>Methylpyridine-lysine (MPK)</i>	Lys	+ 77.03912 Da	-
Glyoxal (GO)			
PTM	AA Res. Involved	ΔM (no NaBH₄)	ΔM (+ NaBH₄)
<i>Carboxymethyl derivative (GO-CM)</i>	Lys	+ 58.005479 Da	-
	Arg		
<i>Glyoxal Imidazolone (GO_IR)</i>	Arg	+ 39.99492	-
Methyl Glyoxal (MGO)			
PTM	AA Res. Involved	ΔM (no NaBH₄)	ΔM (+ NaBH₄)
<i>Carboxyethyl derivative (MGO-CE)</i>	Cys		
	Lys	+ 72.02113 Da	-
	Arg		
<i>Methylglyoxal Methylimidazolone Derivative (MGO-RMI)</i>	Arg	+ 54.01056 Da	-
<i>Methylglyoxal Pirimidine Derivative (MGO-RP)</i>	Arg	+ 80.02622 Da	-
<i>Methylglyoxal Tetra Hydro Pirimidine Derivative (MGO-THPR)</i>	Arg	+ 144.04226 Da	-
Sugar Derivatives			
PTM	AA Res. Involved	ΔM (no NaBH₄)	ΔM (+ NaBH₄)
<i>Glucose Deoxy-fructosil Deirivative (DFK/DFR)</i>	Lys	+ 162.05282 Da	-
	Arg		
<i>3-Deoxyglucosone-Imidazolone Derivative (3DG-IR)</i>	Arg	+ 144.04226 Da	-
<i>Glucose Pirraline derivative (PK)</i>	Lys	+ 108.1723 Da	-
Cysteine-Oxo Derivatives			
PTM	AA Res. Involved	ΔM (no NaBH₄)	ΔM (+ NaBH₄)
<i>Sulfenic Acid-Cys (Sulfe_AC)</i>	Cys	+ 15.99492 Da	-

<i>Sulfinic Acid-Cys (Sulfi_AC)</i>	Cys	+ 31.98983 Da	
<i>Sulfonic Acid-Cys (Sulfo_AC)</i>	Cys	+ 47.98474 Da	
<i>Glutathione Disulfide Adduct (GSS-)</i>	Cys	+ 305.681596 Da	-
<i>S-Nitrosilation (S-Nicro_Cys)</i>	Cys	+ 28.99016 Da	-

Figure S1. Example of the SIC extraction from the total chromatogram (HF 24 sample used for the semi-quantitative analysis of selected adducts. Peptide 287_SH*CIAEVENDEMPADLPSLAADFVESK_313 bearing an ACR-MA on the His 288 residue has been detected in the peptide mixture in two forms: one with oxidized methionine (m/z 1016.7929) and one without methionine oxidation (m/z 1011.4601), that were present also for the native peptide (Met_Ox m/z 997.45205; No_Ox m/z 992.12015); for the semi-quantitative analysis, all the species mentioned were considered. Other aspecific peaks were detected in the SIC chromatograms of the two modified species: however, only the peaks at 29.17 ± 0.028 min were integrated to calculate the area values since the corresponding MS2 spectra confirmed the peptide sequence; the other peaks probably refer to isobaric species.



$$Add. Rel. Ab. (\%) = \left[\frac{\frac{Mod.Peptide}{Peak Area}}{\frac{Mod.Peptide}{Peak Area} + \frac{UnMod.Peptide}{Peak Area}} \right] * 100 = \left[\frac{(2747424+6375374)}{(2747424+6375374+18219741+2142179)} \right] * 100 = 18.688\%$$

Table S3. Inclusion List. For each listed adduct are reported: (i) the peptide sequence; (ii) the modification bearing by the peptide; (iii) the aminoacidic site; (iv) the predominant charge state, and the corresponding (v) experimental m/z of the precursor and (vi) the theoretical one.

Peptide Sequence	AGE/ALE Adduct	AA residue involved	Charge	m/z Sp.	m/z th. (Inclusion list)
337_RHPDYSVLLLR_348	1xGO_Carboxymethyl [R1]	Arg 337	3	509,6208	509,2861
337_RHPDYSVLLLR_348	-	-	3	489,9513	489,95249
485_RPCFSALEVDETYVPK_500	1xCarbamidomethyl [C3] 1xGO_Carboxymethyl [R1]	Arg 485	3	657,3184	657,3184
485_RPCFSALEVDETYVPK_500	1xCarbamidomethyl [C3]	-	3	637,982	637,982
11_FKDLGEENFK_20	1xGO_Carboxymethyl [K2]	Lys 12	3	428,87495	428,87495
11_FKDLGEENFK_20	-	-	3	409,5388	409,5388
373_VFDEFKPLVEEPQNLK_389	1xGO_Carboxymethyl [K6]	Lys 378	3	702,0385	702,0385
373_VFDEFKPLVEEPQNLKQNCLEQLGEYK_402	1xCarbamidomethyl [C20]; 1xGO_Carboxymethyl [K6]	Lys 378	3	1247,5939	1247,95628
373_VFDEFKPLVEEPQNLKQNCLEQLGEYK_402	1xCarbamidomethyl [C20]; 1xGlu_deoxy-fructosil [K17]	Lys 389	4	962,4763	962,4763
373_VFDEFKPLVEEPQNLK_389	-	-	3	682,7026	682,37068
390_QNcELFEQLGEYkFQNALLVR_410	C3(Carbamidomethyl); 1xGO_Carboxymethyl [K13]	Lys 402	3	886,7759	886,44583
390_QNcELFEQLGEYkFQNALLVR_410	C3(Carbamidomethyl);	-	3	867,4366	867,10416
414_KVPQVSTPTLVEVSR_428	1xGO_Carboxymethyl [K1]	Lys 414	3	566,9925	566,65944
414_KVPQVSTPTLVEVSR_428	-	-	3	547,3155	547,31749

Figure S2. SDS-PAGE analysis of *in vitro* produced AGEs/ALEs adducts by incubating HSA with glyoxal (GO) at increasing molar ratios between protein and GO (1:10, 1:100, 1:1000); the time-dependence effect was also evaluated stopping the reaction at two time points: after 48 h (*panel a*) or 72 h (*panel b*). HSA modified by GO is characterized by a different migration pattern on the gel, with the appearance of oligomeric bands proportional to the increase of the HSA-GO molar ratio.

