Unveiling the Dynamic Processes of Dietary Advanced Glycation End-Products (dAGEs) in Absorption, Accumulation, and Gut Microbiota Metabolism

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Supplementary Information

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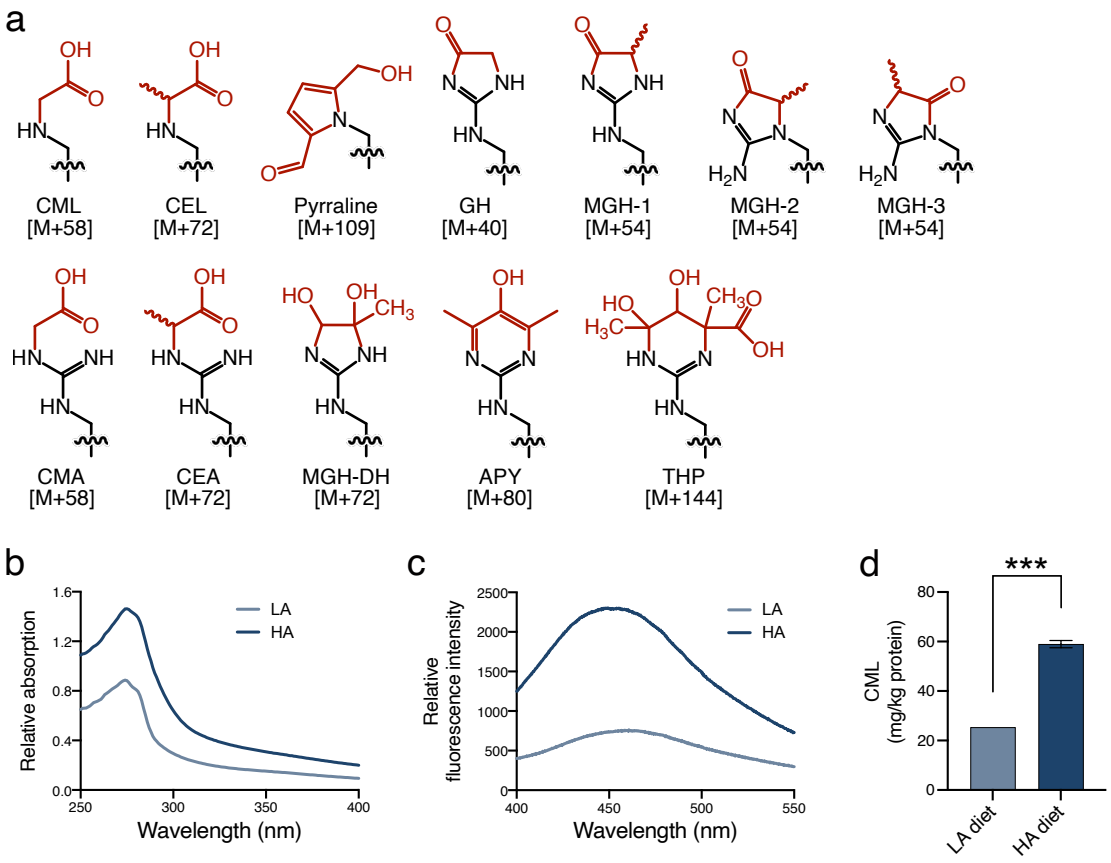
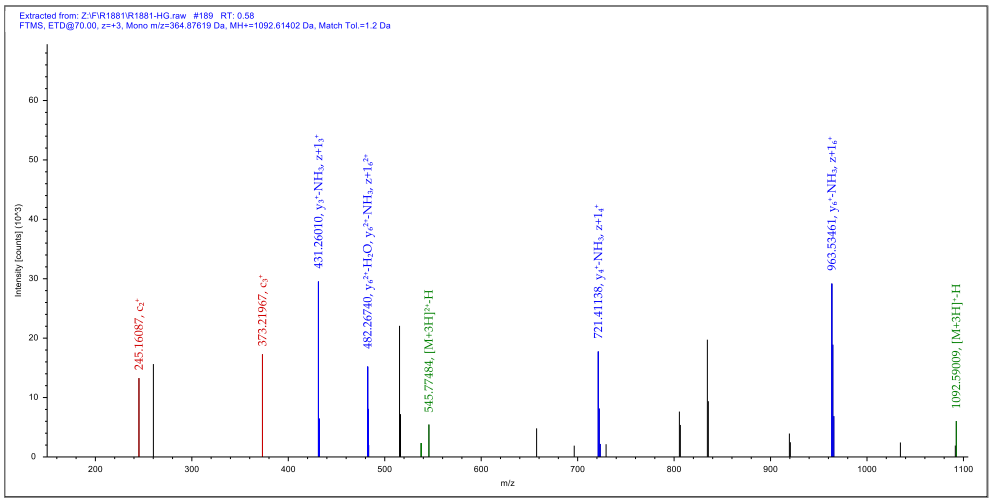
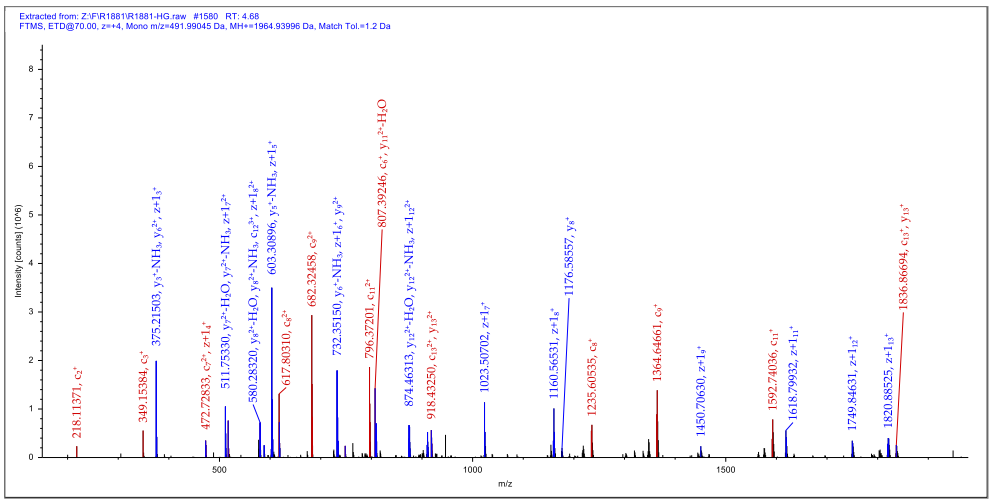


Fig. S1. AGEs content in HA and LA diet. a) Potential AGEs formatted in diet, including Nε-(carboxymethyl)-L-lysine (CML), Nε-(carboxyethyl)-L-lysine (CEL), formyline, pyrraline (Pyrr), maltosine, glyoxal hydroimidazolones (GH), methylglyoxal hydroimidazolones (MGH-1-3), carboxymethylarginine (CMA), carboxyethylarginine (CEA), dihydroxyimidazolidine (MGH-DH), argpyrimidine (APY), tetrahydropyrimidine (THP) and etc. b) The UV absorption spectrum of the β-casein and glycated β-casein in the LA and HA diet, respectively. c) The fluorescence spectroscopy of the β-casein and glycated β-casein in the LA and HA diet, respectively. d) Bar plot showing CML content in the LA and HA diet. Significance determined using one-way ANOVA with Tukey post hoc analysis and expressed as mean ± SEM. ***$P < 0.001$.

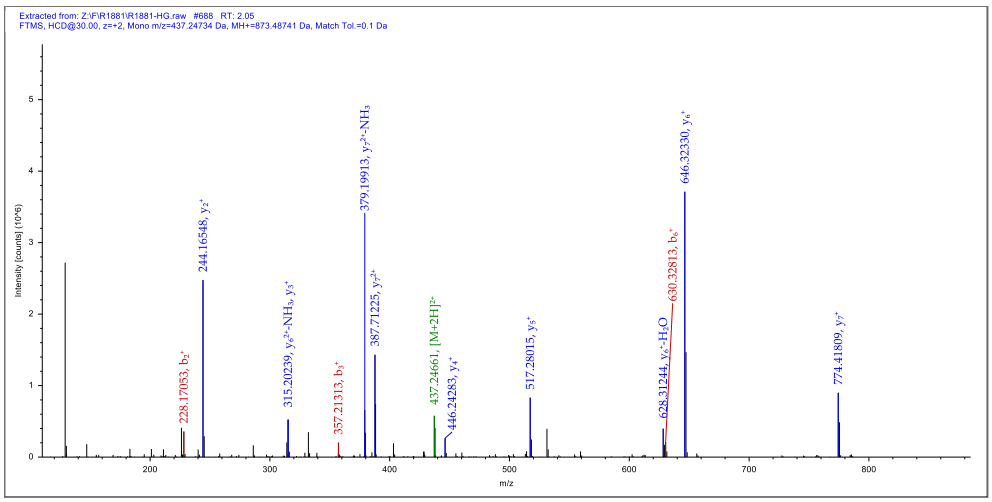
CM modification. E.g. Entry 2. K43 (C2O2H2)



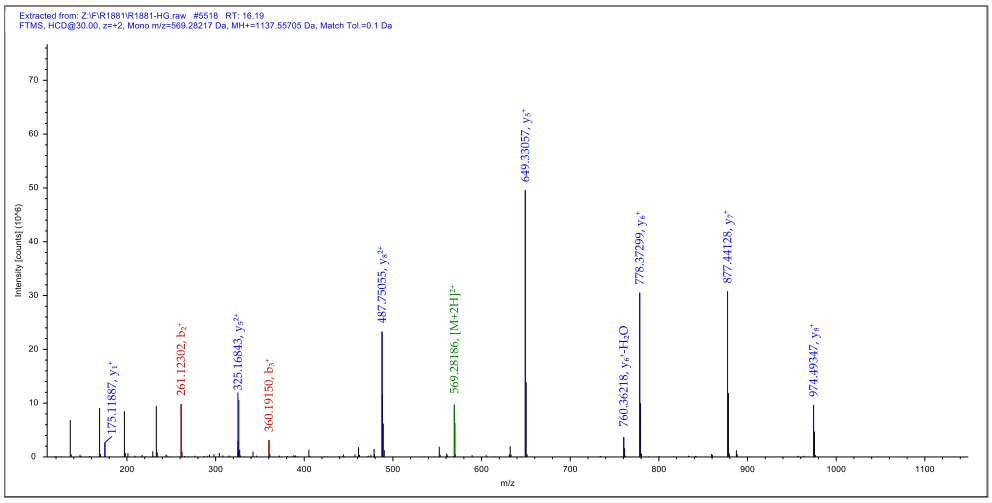
Pyrr modification. E.g. Entry 3. K44 (C6H4O2)



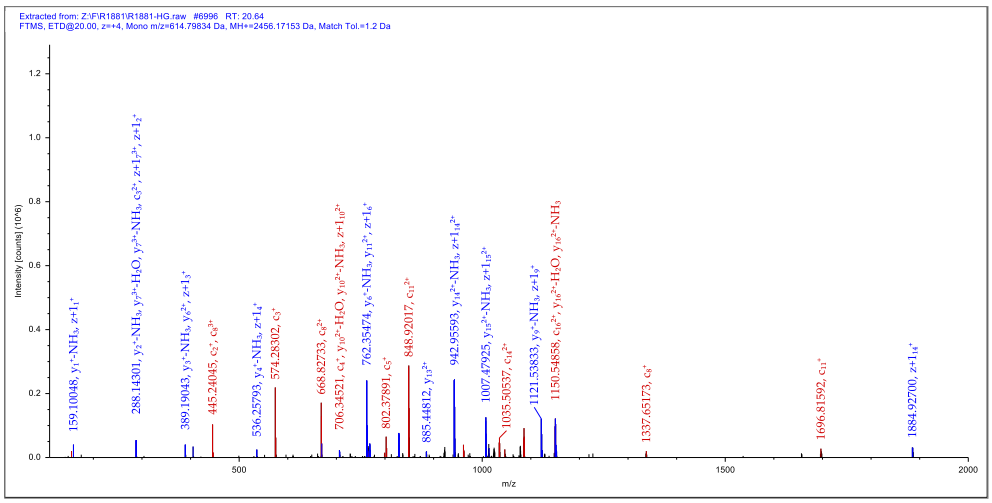
CE modification. E.g. Entry 5. K144 (C3H4O2)



AP modification. E.g. Entry 9. R137 (C5H4O)



GO modification. E.g. Entry 9. R137 (C2O)



MGO modification. E.g. Entry 11. R198 (C3H2O)

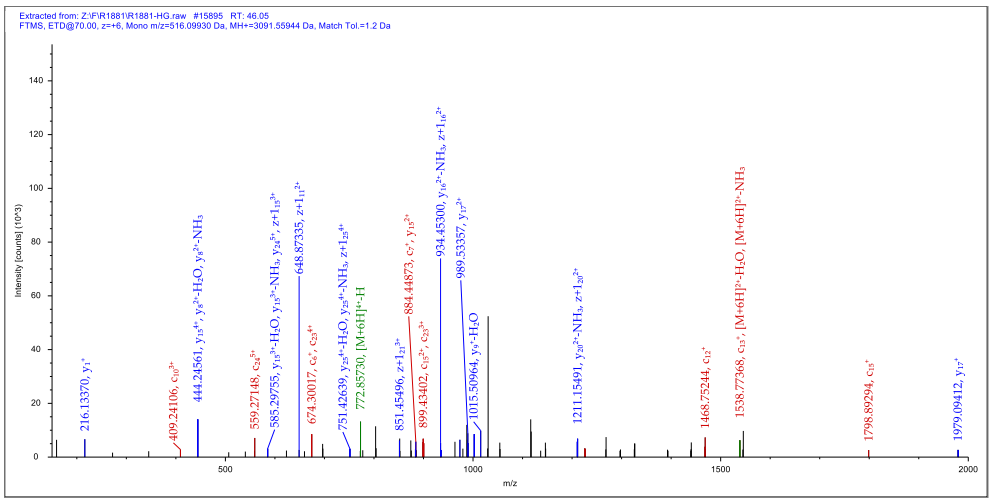
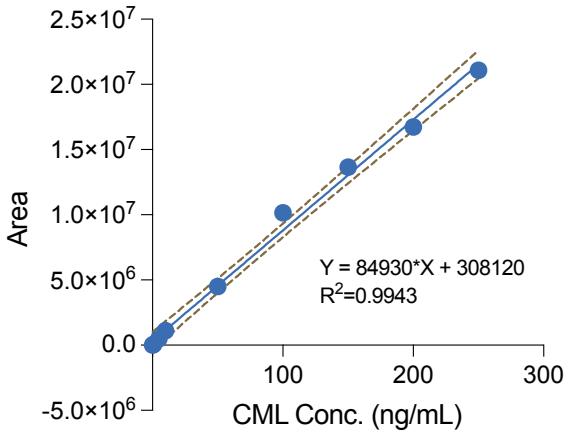


Fig. S2. The typical MS² spectrum of the modifications in peptide mapping



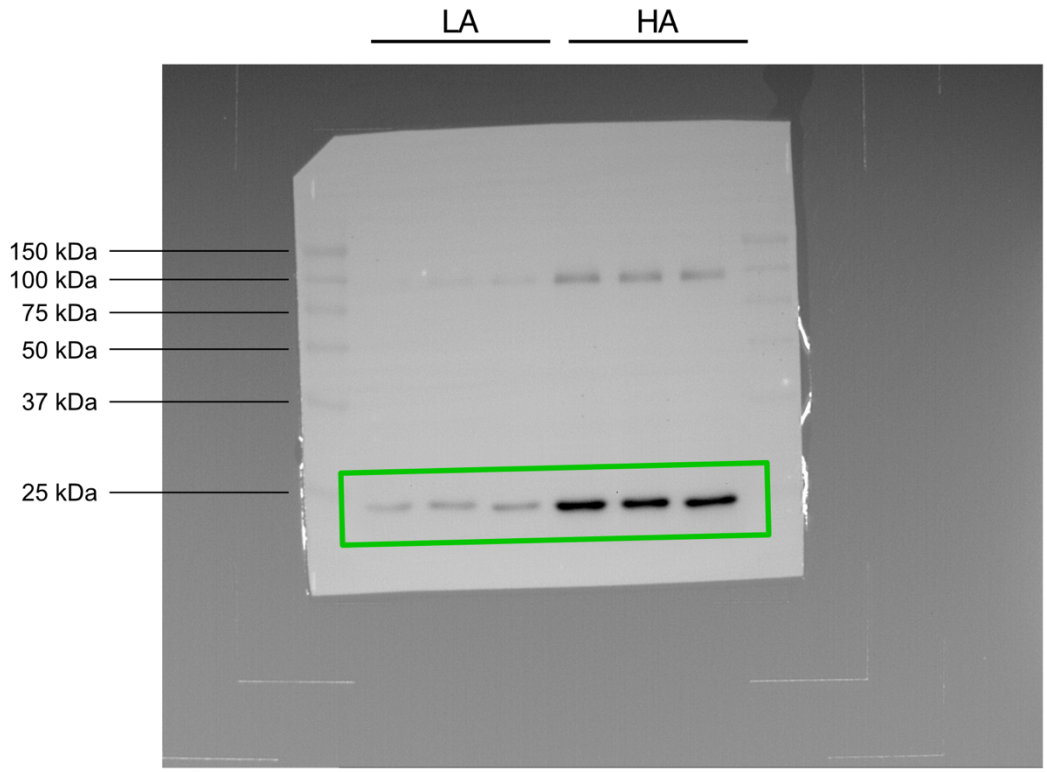
CML Conc. (ng/mL)	Area
0.1	6.24E+02
1	7.71E+04
5	5.07E+05
10	1.10E+06
50	4.50E+06
100	1.02E+07
150	1.37E+07
200	1.67E+07
250	2.11E+07

LOD: 0.1 ng/mL

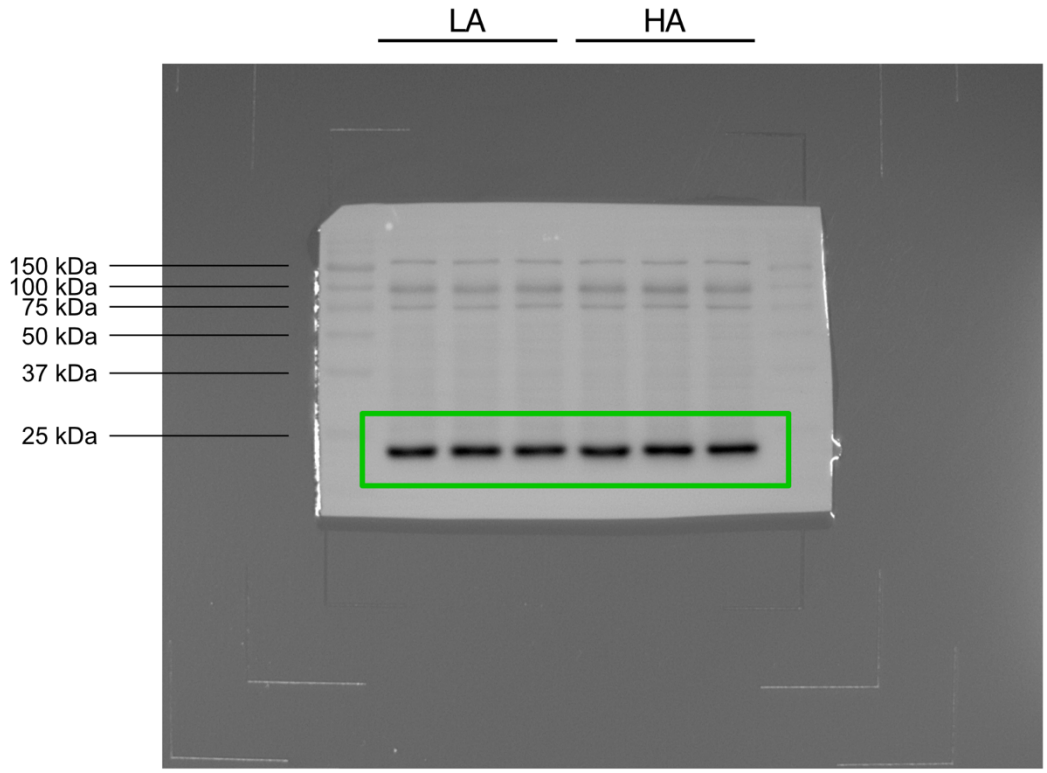
LOQ:250 ng/mL

Fig. S3. The standard curve of CML measured by LC-MS/MS.

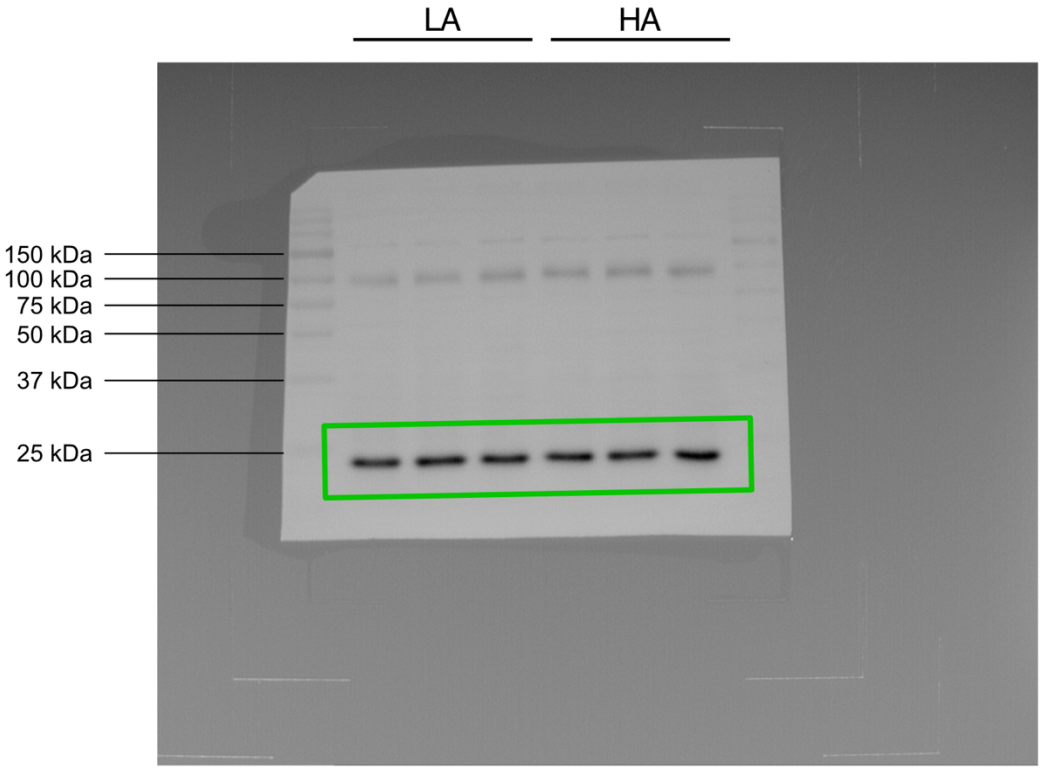
CML of small intestine



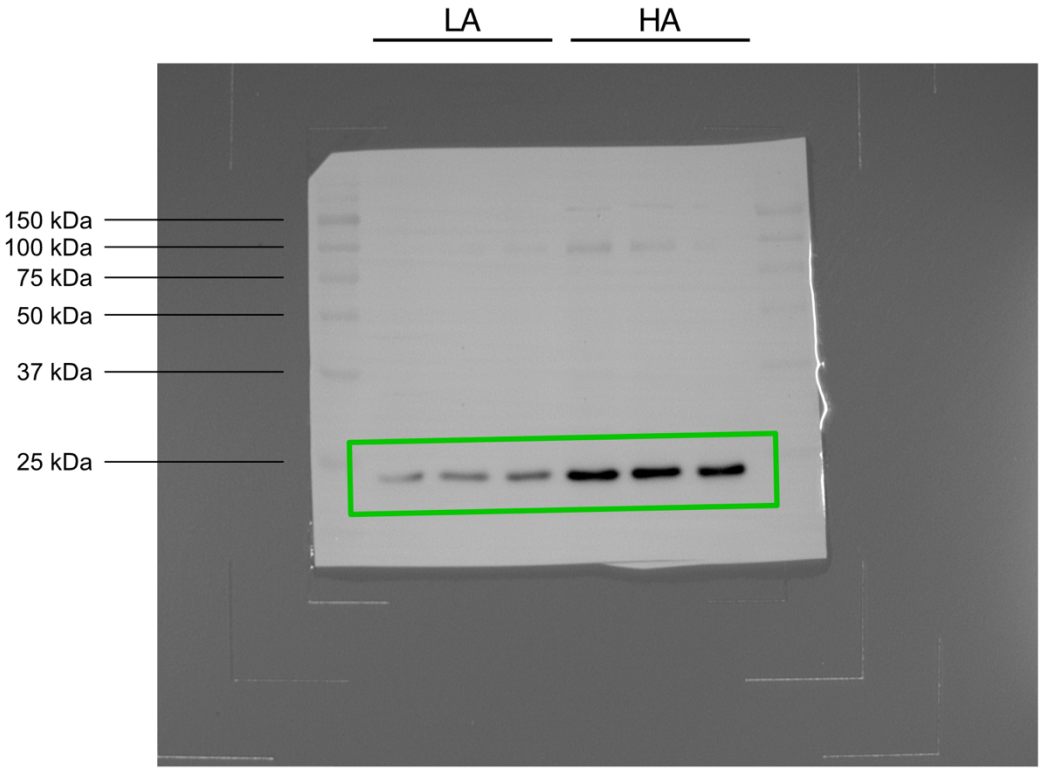
CML of colon



CML of liver



CML of kidney



B-actin of kidney

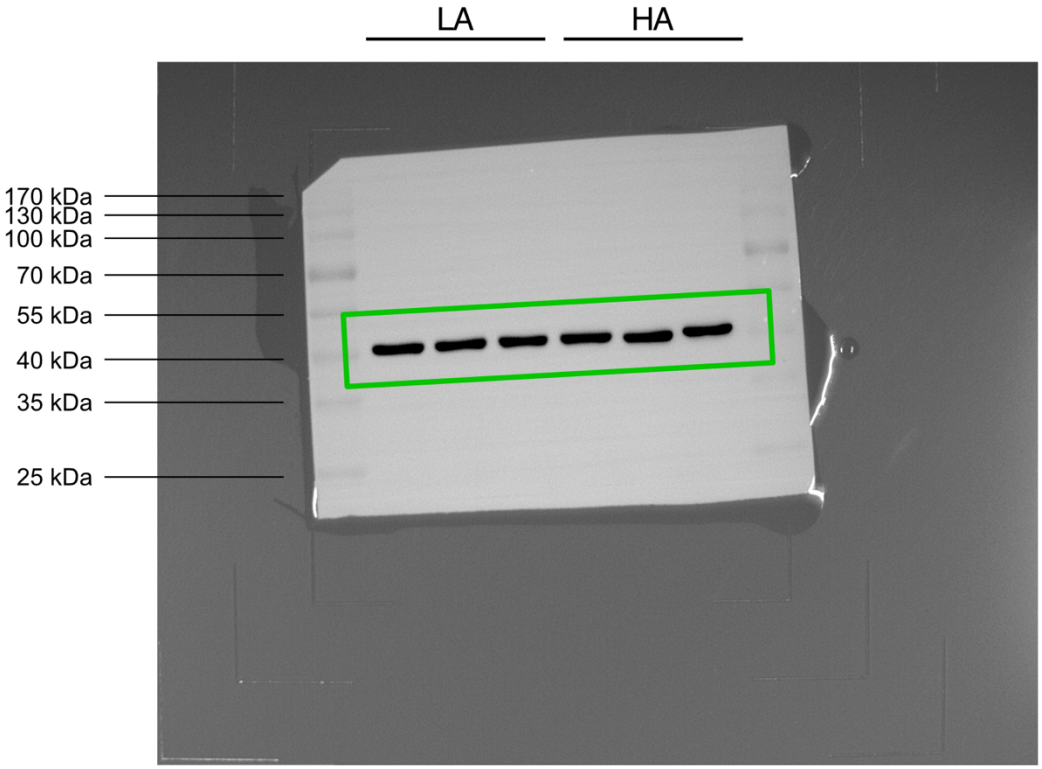


Fig. S4. Row data of western blot.

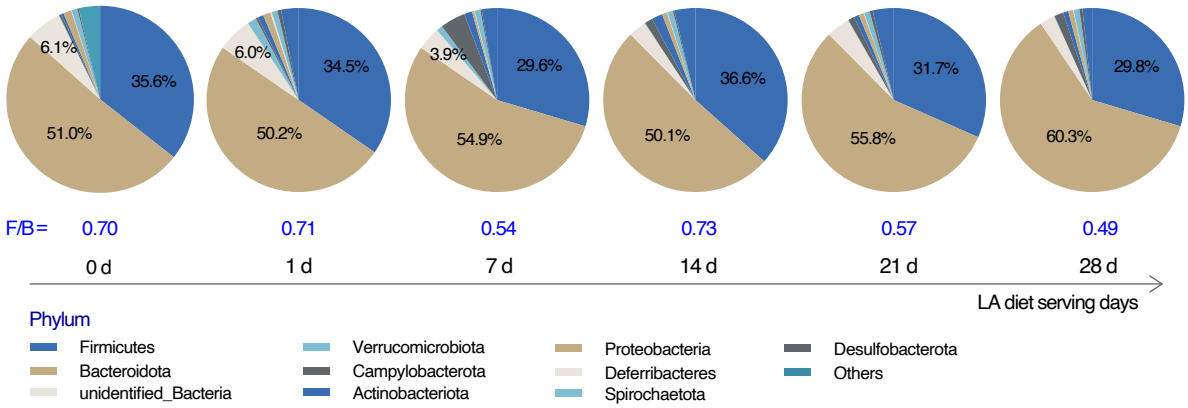


Fig. S5. Dynamic changes in the relative abundance of microbial taxa at the phylum level of LA group.

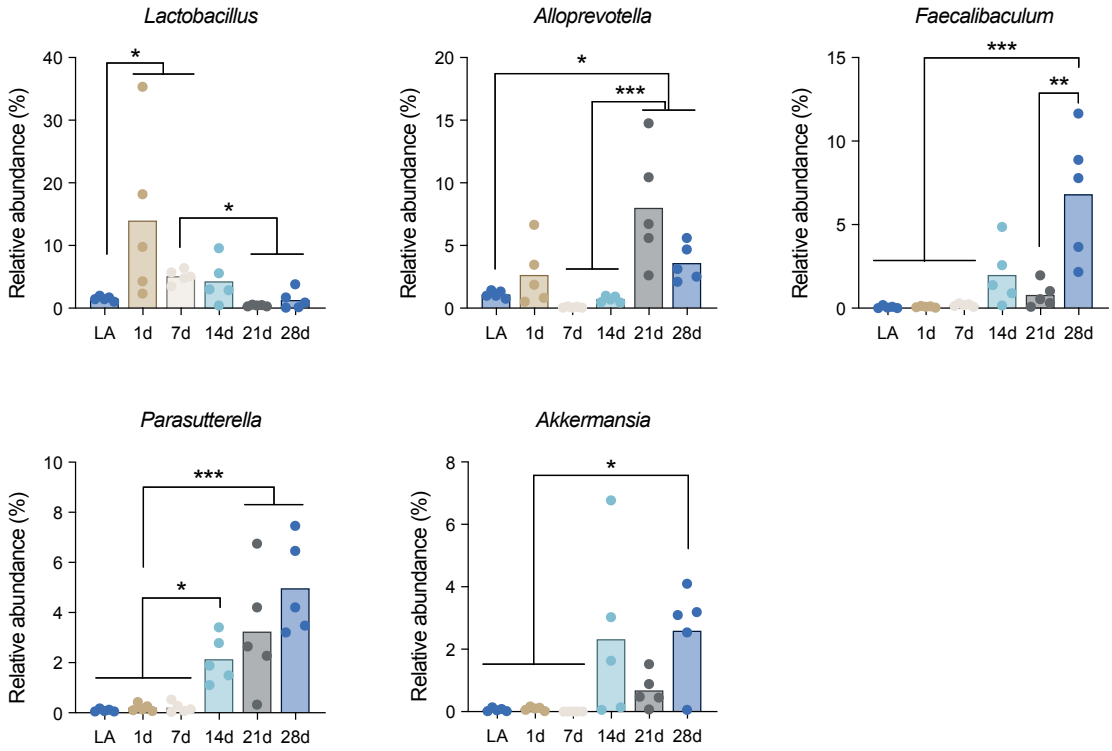


Fig. S6. Relative abundance (%) of the genera *Lactobacillus*, *Alloprevotella*, *Faecalibaculum*, *Parasutterella*, and *Akkermansia*. Significance determined using one-way ANOVA with Tukey post hoc analysis and expressed as mean ± SEM. *P < 0.05, ***P < 0.001.

Fig. S7. Predicting potential host functional changes resulting from differential microbiota-metabolite interactions induced by HA diet.

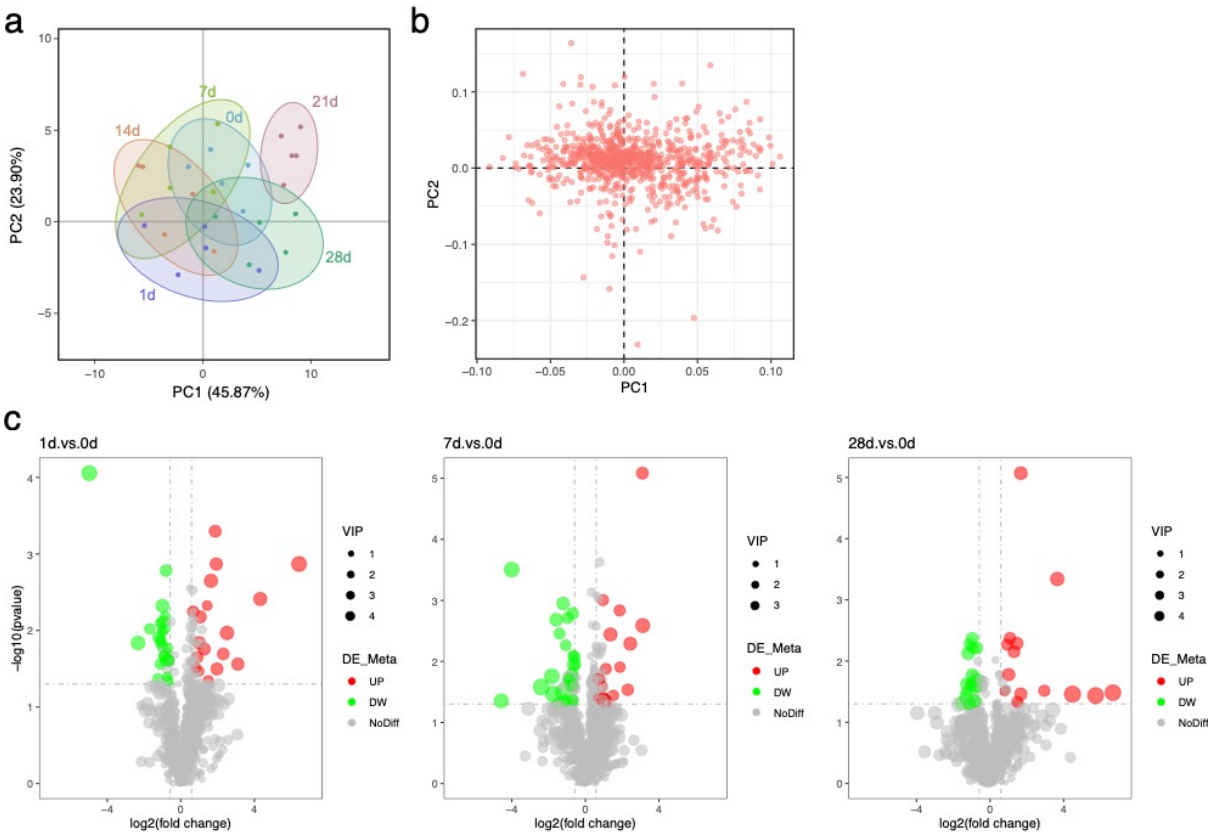


Fig. S8. Fecal Metabolomics Changes with Varied LA Diet Intake Periods. a) Principal component analysis (PCA) plot comparing the effects of the LA diet intake periods on fecal metabolite distribution. b) Metabolites that significantly change in the same direction (blue) or opposite direction (red) at LA diet intake days 7 and 28 compared to 0 d are plotted as loading coefficients, as they contribute to PLSR scores of the fecal metabolome. Each metabolite is represented by a dot. c) Volcano plot depicting the significance of differences between fecal metabolomes of LA diet intake days 1, 7, and 28 compared to 0 d.

Table S1. Composition of the experimental diets.

Component	Unit calorific value (kcal/g)	LA (g/kg)	Calories (kcal)	^{13}C-HA (g/kg)	Calories (kcal)	HA (g/kg)	Calories (kcal)
Soyabean oil	9	100	900	100	900	100	900
Casein	4	200	800				
^{13}C-labeled glycated casein	4			200	800		
Glycated casein	4					200	800
Methionine	4	3	12	3	12	3	12
Maize starch	4	630	2520	630	2520	630	2520
Salt mixture*	1.6	35	56	35	56	35	56
Vitamin mixture†	3.9	10	39	10	39	10	39
Methyl cellulose	0	31	0	31	0	31	0
Choline chloride	0	1	0	1	0	1	0
Total		1010	4327	1010	4327	1010	4327

LA, low-AGEs diet; HA, high-AGEs diet.

* The salt mixture contained the following (mg/g): calcium phosphate diabasic, 500; sodium chloride, 74; potassium sulfate, 52; potassium citrate monohydrate, 20; magnesium oxide, 24; manganese carbonate, 3.5; ferric citrate, 6; zinc carbonate, 1.6; curpric carbonate, 0.3; potassium iodate, 0.01; sodium selenite, 0.01; chromium potassium sulfate, 0.55.

† The vitamin mixture contained the following (mg/g): thiamin hydrochloride, 0.6; riboflavin, 0.6; pyridoxine hydrochloride, 0.7; nicotinic acid, 3; calcium pantothenate, 1.6; D-biotin, 0.05; cyanocobalamin, 0.001; retinyl palmitate, 1.6; DL-a-tocopheryl acetate, 20; cholecalciferol, 0.25; menaquinone, 0.005.

Table S2. AGE modifications in β-casein measured by peptide mapping.

Site	Entry	11-mer Sequence	LA	(^{13}C-) HA
R40	1	EESIT**R**INKKI		CM; MG-H
K43	2	ITRIN**K**KIEKF	**CM**	**CM**; CE
K44	3	TRINK**K**IEKFQ	CE	**CM**; Pyrr
K47	4	NKKIE**K**FQSEE	CE	CE; Pyrr
K114	5	GVSKV**K**EAMAP		**CM**; CE
K120	6	EAMAP**K**HKEMP	**CM**; CE	**CM**; CE
K122	7	MAPKH**K**EMPFP	**CM**; CE	**CM**; CE
K128	8	EMPFP**K**YPVEP	**CM**; CE	CE
R137	9	EPFTE**R**QSLTL	CE; GO-H; MG-H; AP	CE; GO-H; AP
K191	10	LPVPQ**K**AVPYP	**CM**; CE	**CM**
R198	11	VPYPQ**R**DMPIQ	CM; CE; GO-H; AP	CM; CE; GO-H; MG-H

Carboxymethylation (CM), carboxyethylation (CE), pyrrolization (Pyrr), arginine derived pyrimidine (AP), glyoxal derived hydroimidazolone (GO-H), and methylglyoxal derived hydroimidazolone (MG-H). Modifications that form CML are shown in bold.

Supplementary Methods

Orbitrap-MS/MS-Based Peptide Mapping.

Glycation sites of Two types of glycated casein were analyzed using a proteomics method described by Sjoblom et al. [1] with some modifications. Briefly, protein samples were treated by DTT, followed by hydrolyzed by sequencing grade modified trypsin (Sigma-Aldrich, USA). The Ultimate 3000 RSLC nanosystem (Thermo Scientific, USA) was connected to an AdvanceBio Peptide column (2.7 μm, 2.1 x 150 mm, Agilent, USA). Orbitrap Fusion (Thermo Scientific, USA) was used to detect the MS/MS of the peptide at the mode of positive ion. Fragmentation energy was applied at a slope of 3.6 V/100 Da and -4.8 V offset, and at a slope of 3.0 V/100 Da and 2 V offset. Analysis was completed using Proteome Discover 1.4 software as follows: carboxymethylation (Lys and Arg, $C_2H_2O_2$), carboxyethylation (Lys and Arg, $C_3H_4O_2$), pyrrolization (Lys, $C_6H_4O_2$), furanization (Lys and Arg, $C_6H_4O_2$), arginine derived pyrimidine (Arg, C_5H_4O), glyoxal derived hydroimidazolone (Arg, C_2O), and methylglyoxal derived hydroimidazolone (Arg, C_3H_2O) were set as variable modification.

Reference

1. N. M. Sjoblom, M. M. G. Kelsey and R. A. Scheck, A Systematic Study of Selective Protein Glycation, *Angew Chem Int Ed Engl*, 2018, **57**, 16077-16082.