## Long-term Consumption of 2-*O*-β-D-Glucopyranosyl-L-ascorbic Acid from the Fruits of *Lycium barbarum* Modulates Gut Microbiota in C57BL/6 Mice

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**Preparation of AA-2\betaG.** The dried fruits of *L. barbarum* were ground into powder by using a grinder and extracted 2 times with 30% (v/v) aqueous ethanol solution in a ratio of material/solvent of 1:10 (w/v) at 70 °C for 1 h. The resulting extracts were concentrated, the residues were dissolved with distilled water and loaded onto a column (5 × 30 cm) of Dowex 1-X8 anion exchange resin (Sigma Co., Ltd., USA). The column was washed with distilled water, 0.1 M acetic acid solution and subsequently eluted with 0.4 M acetic acid solution. The eluent was monitored at 280 nm and collected (5.0 mL/tube) with an automatic fraction collector (Baixian BS-100A, Shanghai, China). The fractions eluted with 0.4 M acetic acid were checked by HPLC, and fractions containing AA-2 $\beta$ G were combined and concentrated. The resulting residues were dissolved with distilled water and concentrated to dry repeatedly, and the final concentrated residues were dissolved with distilled water for use of animal experiments.

Target gene	Primer Sequence (5'-3')	Annealing T (°C)	NCBI gene ID	
ZO-1	FW: TGAGTGCGTTTCTCTCCCTT	60	ID: 21872	
	RV: CCCTCTGTGTTCCTCATGGT	00		
Occludin	FW: AGCACTTAACCTGCCTGGAT	50	ID: 18260	
	RV: AGCCTGTGGAAGCAAGAGAT	59		
Claudin-1	FW: AGCTGCCTGTTCCATGTACT	(0	ID: 12737	
	RV: CTCCCATTTGTCTGCTGCTC	60		
FFAR2	FW: CGACTAGAGATGGCTGTGGT	(0)	ID: 233080	
	RV: AGAAGATGAGCAGTGTGGCT	60		
FFAR3	FW: TCCAGCCTGGCTTTCCAATA	50	ID: 233079	
	RV: GCCTGCAGGAGACATTTCAG	59		

 Table S1. Primer Sequences Used in RT-qPCR Assay in Colonic Tissue

Table S2 Concentration (nn	nol/mg) of Short-chai	n Fatty Acids	s (SCFAs) in	<b>Contents of Caecum</b>	(nmol/mg) and Ser	um (nmol/mL) of
Mice						

		SCFAs							
		Acetic	Propio	<i>i</i> -buty	<i>n</i> -butyri	<i>i</i> -valeric	<i>n</i> -valeri	lactic	Tatal
		acid	nic	ric	c acid	acid	c acid	acid	Total
Conte	Ν	18.44±3.	3.84±0.	ND	3.74±1.	0.37±0.0	0.28±0.	4.55±1.5	31.21±4.
	С	96	98	ND	49	7	06	9	51
nts of	А	14.82±3.	3.10±0.	ND	1.72±0.	0.37±0.1	0.23±0.	6.69±1.3	26.94±5.
caecu m	А	71*	76	ND	7**	0	04	2**	06
Serum	N	1.49±0.2		ND	0.12±0.	0.27±0.0	0.05±0.	23.71±4.	25.63±4.
	С	0	ND	ND	05	5	01	28	16
	А	0.86±0.2		ND	0.13±0.	0.49±0.0	0.05±0.	24.72±12	26.25±12
	А	7**	ND	ND	02	7**	01	.13	.24

\*p < 0.05 and \*\*p < 0.01 vs the NC group tested by independent sample T test, and ND not detected.



**Figure S1.** The apparent indexes of mice in the NC and AA groups (A, body weight; B, mass of cecum contents; C, pH of mice feces). Data are presented as means  $\pm$  SD (n = 10). #p < 0.05 and ##p < 0.01 vs the NC group tested by independent sample T test.