Supplementation with Ginseng, Lilii Bulbus, and Poria induces alterations in the serum metabolic profile of healthy adults

Fangzhi Xie[a], Liang Chen[b], Shuna Jin[c], Feng Qiu[a], Juntao Kan[b], Yujie Li[b], Hanjin Wang[a], Min Huang[a], Xiaojie Sun[a], Jun Du[b], Yuanyuan Li[a]

a. Key Laboratory of Environment and Health (HUST), Ministry of Education & Ministry of Environmental Protection, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China

b. Nutrilite Health Institute, Amway Innovation & Science, Shanghai, China

c. College of Basic Medicine, Hubei University of Chinese Medicine, 16 Huangjiahu West Road, Hongshan District, Wuhan, China

Contents

Supplementary Material 1. The inclusion and exclusion criteria of population study design

The inclusion criteria were: (1) healthy volunteers aged 18-35 years; (2) local residence in Wuhan within one year after the trial begins; (3) be able to participate in normal activities; (4) those who had not renovated their living rooms in the previous two years and would not do so in the coming year; (5) be willing to cooperate with the experimental study, provide biological specimens, give informed consent, and voluntarily participate in the study. Exclusion criteria: (1) psychopath; (2) inability to cooperate with the experimental study or the investigator due to dysgnosia or behavioral disorder; (3) suffering from systemic diseases (e.g., pneumonia, tuberculosis, atherosclerosis, and other immune system diseases that the investigators consider unsuitable for the study; (4) idiopathic sleep disorder >1 night/week; (5) alcohol consumption (>1 drink per week); (6) suffering from heart disease, chronic obstructive pulmonary disease, asthma, and malignancy; (7) inability to participate in normal activities.

Supplementary Material 2. Herbal preparation and dispensing

GLP was prepared as chewable tablets consisting of 200 mg of crude Ginseng powder containing 2% total ginsenosides, 120 mg of aqueous extract of Lilii bulbus containing ≥0.30% regaloside B and 50 mg of aqueous extract of Poria containing ≥10% crude polysaccharide. The detailed active ingredients of GLP were determined by UPLC mass spectrometry (Table S1). The placebo consisted of maltodextrin, sorbitol, caramel coloring and artificial ginseng flavoring, which were identical to GLP in color, shape, size and packaging. Subjects took two tablets/twice-daily of GLP or placebo as instructed. Both GLP and placebo were manufactured in a Good Manufacturing Practice pilot plant (Amway, Guangzhou, China) according to specific quality assurance instructions for the active compounds, microorganisms, heavy metals, and pesticide residues. The study staff and participants were not aware of the random assignment.

Supplementary Material 3. Details of metabolomics analysis method

Before analysis, all serum samples were thawed at 4 ℃. Then, each 100 mL of serum was mixed with 250 mL of acetonitrile (ACN) and 50 mL of internal standard (2 mg/mL fexofenadine). The extract was mixed vigorously for 5 min, followed by a 10 min centrifugation by 12000 rpm in 4 °C to remove precipitated proteins. The untargeted metabolomic analysis was conducted with Waters Xevo G2-XS ultra-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS/MS, Waters, Milford, Massachusetts) with ACQUITY UPLC BEH C18 column (2.1×100 mm, 1.8 mm, Waters Corporation). The temperature of the column stayed at 40 ℃ with a sample injection volume of 2 mL. The mobile phase A was water with 0.1% formic acid, and the mobile phase B was acetonitrile. The programmed gradient was: 1) 0 min, 5% (B); 2) 15 min, 95% (B); 3) 20 min, 95% (B); 4) 21 min, 5% (B); 5) 25 min, 5% (B). The mass spectrometer was performed and data was obtained with MSEmode. The parameters were as follows: capillary voltage, 3000 V; cone voltage, 30 V; source temperature, 200℃; desolvation gas flow, 800 L/h; scan interval, 100 ms; scan range, at 50-1500 m/z (mass-to-charge ratio). Besides, enkephalin was used to correct high-resolution molecular mass during acquisition, which cold ensure accurate mass measurement. All samples were assigned at random. QC samples were made by mixing equal volumes of serum from 20 random samples of participants in the present study. QC samples and blank samples were injected every ten samples in the analytical sequence to provide a set of data for repeatability assessment. The CV of peak intensity ranged from 2.88% to 14.4%. The accepted mass

difference was set as 10 ppm during the search.

Supplementary Material 4. Serum biomarker measurements

As mentioned in the text, we tested a total of 10 serum biomarkers of oxidative stress and inflammation. Interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor (TNF-α), and Paraoxonase1 (PON1) were measured by enzyme-linked immunosorbent assay; total antioxidant capacity (TAC), glutathione peroxidase (GSH-PX) and γ-glutamyl transpeptidase (γ-GT) were measured by colorimetric method; Superoxide dismutase (SOD) was measured by hydroxylamine method; Triglyceride (TG), and Triglyceride (TC) were performed using enzymatic methods.

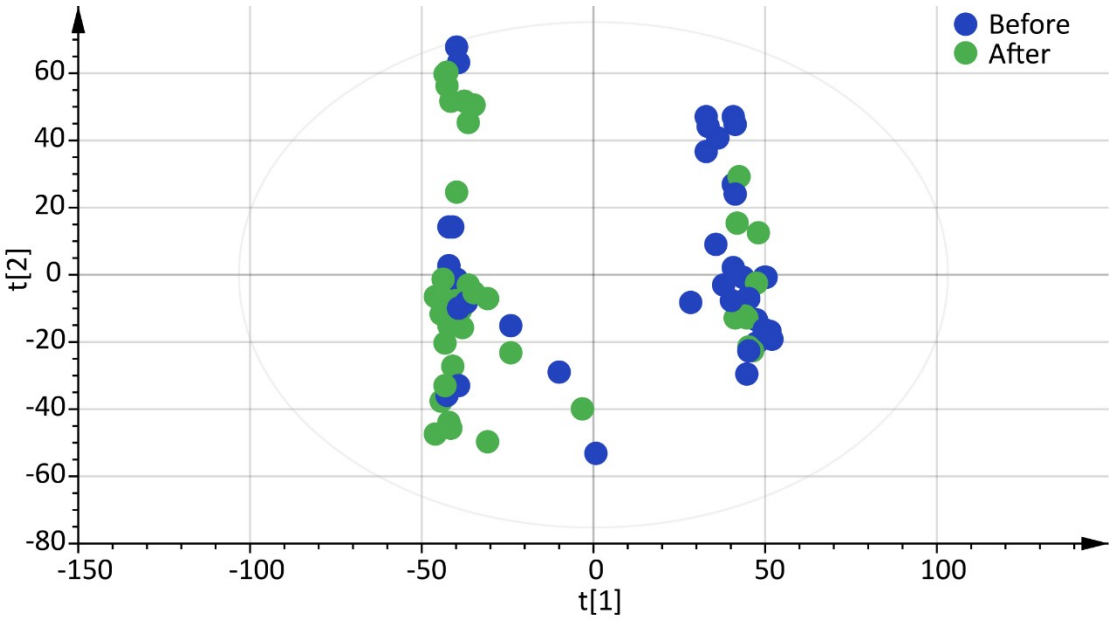

Figure S1. Scoring plots for principal component analysis (PCA) model of before- and after- placebo.

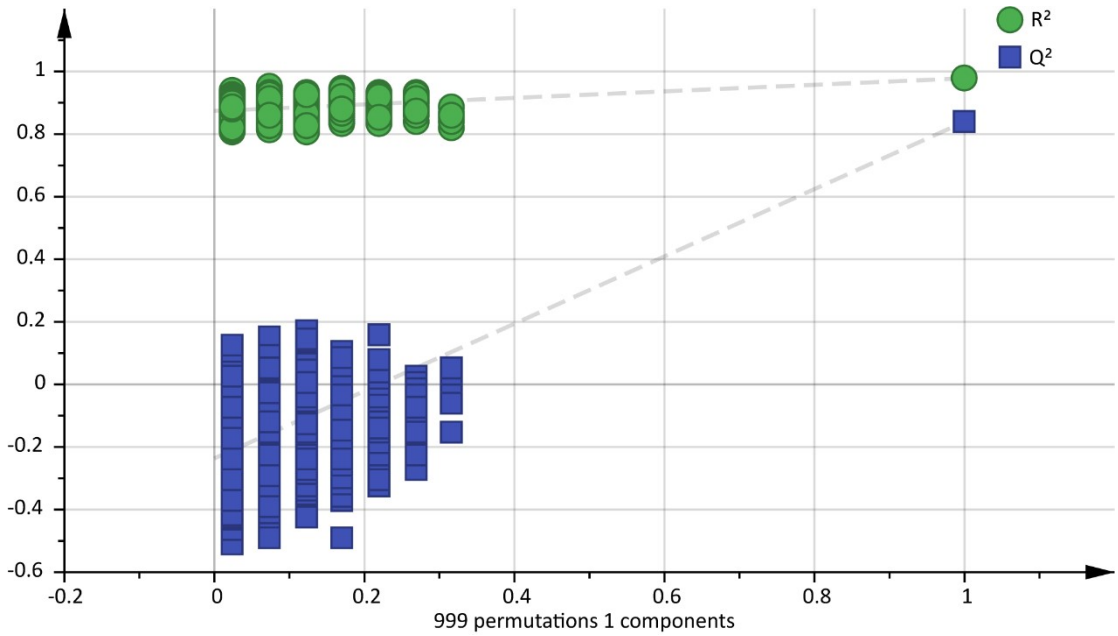

Figure S2. Scoring plots for 999-time permutation validation test for OPLS-DA models of GLP.

Abbreviations: OPLS-DA, orthogonal partial least squares discrimination analysis.

Table S1. Identification result of chemical composition of GLP.

NO	RT（min）	M/Z Actual value	M/Z Theoretical value	Formula	Active ingredient	MS^2 data	Medicinal herbs
1	0.91	341.1095	341.1089	$C_{12}H_{22}O_{11}$	Sucrose	179.0567；161.0471；143.0356；119.0357	Poria
2	1.51	191.0202	191.0197	$C_6H_8O_7$	Citric acid	129.0189；111.0089；87.0087；85.0294；67.0186；57.0344	Poria
3	1.94	243.0623	243.0623	$C_9H_{12}N_2O_6$	L-uridine	200.0526；127.8685；110.0243；82.0288	Poria
4	3.61	142.0516	142.051	$C_6H_9NO_3$	/	142.0511	Poria
5	5.84	232.119	232.119	$C_{10}H_{19}NO_5$	/	146.0819；102.0562；86.0605；84.0454；71.0498	Poria
6	8.19	415.1243	415.1246	$C_{18}H_{24}O_{11}$	Regaloside C	179.0354；161.0244；135.0451	*Lilii Bulbus*
7	8.57	399.1298	399.1297	$C_{18}H_{24}O_{10}$	Regaloside A	163.0401；145.0302；119.0507；117.0346；59.0138	*Lilii Bulbus*
8	10.09	399.1302	399.1297	$C_{18}H_{24}O_{10}$	Regaloside D	163.0399；145.0291；119.0501	*Lilii Bulbus*
9	11.23	429.1412	429.1402	$C_{19}H_{26}O_{11}$	Regaloside F	429.1403；193.0506；175.0400	*Lilii Bulbus*
10	13.22	457.1359	457.1352	$C_{20}H_{26}O_{12}$	Regaloside E	415.1249；397.1144；179.0354；161.0251；135.0460；59.0144	*Lilii Bulbus*
11	16.09	441.1412	441.1402	$C_{20}H_{26}O_{11}$	Regaloside B	399.1324；381.1232；163.0518；145.0308；119.0508；117.0355；59.0138	*Lilii Bulbus*
12	16.76	441.1408	441.1402	$C_{20}H_{26}O_{11}$	4-Acetyl Regaloside D	399.1302；381.1198；261.0781；163.0393；145.0286	*Lilii Bulbus*
13	19.94	1007.5478	1007.5432	$C_{48}H_{82}O_{19}$	20-gluco-ginsenoside Rf	1007.5540；961.5434；799.5075；637.4490；475.342	Ginseng
14	20.65	977.5366	977.5327	$C_{47}H_{80}O_{18}$	Notoginsenoside R1	977.5509；931.5279；799.4854	Ginseng
15	21.89	845.4924	845.4904	$C_{42}H_{72}O_{14}$	Ginsenoside Rg1	799.4898；637.4347；619.4236；475.3807；179.0565；161.0445	Ginseng
16	22.04	991.5522	991.5483	$C_{48}H_{82}O_{18}$	Ginsenoside Re	945.5561；799.4943；783.5009；637.4403；475.3876	Ginseng
17	24.76	947.4857	947.4857	$C_{45}H_{74}O_{18}$	Protobioside	755.4265；739.4282	*Lilii Bulbus*
18	24.92	949.5026	949.5014	$C_{45}H_{76}O_{18}$	/	903.4967；757.4385；595.3824	
19	26.55	845.4926	845.4904	$C_{42}H_{72}O_{14}$	Ginsenoside Rf	845.4915,799.4852,637.4312,475.3778	Ginseng
20	27.36	815.4803	815.4798	$C_{41}H_{70}O_{13}$	Isomer of notoginsenoside R2	769.4818；637.4337；475.3806；161.0464	Ginseng
21	28.36	1153.6049	1153.6011	$C_{54}H_{92}O_{23}$	Ginsenoside Rb1	1107.6036；945.5470；783.4812；621.4299；459.3779	Ginseng
22	28.5	683.4379	683.4376	$C_{36}H_{62}O_9$	Ginsenoside Rh1	683.4397；637.4343；475.2761；391.2934	Ginseng
23	29.17	1123.5937	1123.5906	$C_{53}H_{90}O_{22}$	Ginsenoside Rc	1077.5951；945.5486；783.5000	Ginseng
24	29.61	955.4931	955.4908	$C_{48}H_{76}O_{19}$	Ginsenoside Ro	793.4421；731.4407；569.3839；523.3804	Ginseng
25	29.89	1165.6052	1165.6011	$C_{55}H_{92}O_{23}$	Ginsenoside Rs1 or isomer	1119.5945；1077.5769；1059.5705	Ginseng
26	30.06	1123.5947	1123.5906	$C_{53}H_{90}O_{22}$	Ginsenoside Rb2	1077.5965；945.5510；915.5387；783.5034；149.0459	Ginseng
27	30.81	1165.6025	1165.6011	$C_{55}H_{92}O_{23}$	Ginsenoside Rs1 or isomer	1119.6023；1077.5999；1059.5777	Ginseng
28	31.31	1195.6147	1195.6117	$C_{56}H_{94}O_{24}$	Quinquenoside R1 or isomer	1149.6114；1107.5923；1089.5809；945.5369	Ginseng
29	32.08	991.5527	991.5483	$C_{48}H_{82}O_{18}$	Ginsenoside Rd	945.5557；783.4979；621.4441；161.0457；459.3830	Ginseng
30	32.4	1165.6058	1165.6011	$C_{55}H_{92}O_{23}$	Ginsenoside Rs1 or isomer	1119.5968；1077.5796；1059.5753	Ginseng
31	32.64	793.4377	793.438	$C_{42}H_{66}O_{14}$	Chikusetsusaponin Iva	793.4398；673.3938；631.3876；569.3860	Ginseng
32	33.43	1165.6055	1165.6011	$C_{55}H_{92}O_{23}$	Ginsenoside Rs1 or isomer	1119.5978；1077.5848；1059.5715；945.5447	Ginseng
33	35.24	1033.5607	1033.5589	$C_{50}H_{84}O_{19}$	Quinquenoside III or isomer	987.5667；945.5516；927.5426；765.4833	Ginseng
34	36.63	665.4301	665.427	$C_{36}H_{60}O_8$	Ginsenoside Rh4 or isomer	619.426；161.0460	Ginseng
35	36.81	793.4413	793.438	$C_{42}H_{66}O_{14}$	Zingibroside R1	793.4362；613.3902；569.3817；455.3568	Ginseng

36	37.13	829.499	829.4955	$C_{42}H_{72}O_{13}$	Ginsenoside Rg3	829.4944；783.4881；621.4381；459.3887	Ginseng
37	38.53	811.489	811.4849	$C_{42}H_{70}O_{12}$	Ginsenoside Rg5 or isomer	765.4850；603.4311	Ginseng
38	38.66	811.4875	811.4849	$C_{42}H_{70}O_{12}$	Ginsenoside Rg5 or isomer	765.4901；603.4334；161.0463	Ginseng

Table S2. Difference in serum biomarkers before and after GLP administration group at baseline.

Serum biomarkers	Before-GLP group	After-GLP group	P[a]
IL-1β, pg/mL	2.89±0.83	3.24±1.08	0.0859
IL-6, pg/mL	3.43±1.30	2.96±1.47	0.0749
TNF-α, pg/mL	1.12±0.62	0.83±0.52	0.0034
SOD, U/mL	325.15±141.88	434.05±150.36	0.0005
GSH-PX, mU/mL	1178.99±333.64	1063.06±335.80	0.0894
TAC, U/mL	15.62±4.48	15.68±4.96	0.9513
PON1, ng/mL	623.83±295.30	1209.62±322.77	<.0001
TC, mmol/L	4.24±0.66	4.39±0.72	0.0287
TG, mmol/L	0.78±0.29	0.71±0.21	0.0731
γ-GT, U/L	15.44±5.06	15.02±5.47	0.0987

Abbreviations: γ-GT, γ-glutamyl transpeptidase; GSH-PX, Glutathione Peroxidase; IL-1β, Interleukin1β; IL-6, Interleukin-6; PON1, paraoxonase1; SOD, Superoxide Dismutase; Tumor necrosis factor-α, TNF-α; T-AOC, total antioxidant capacity; TC, Total cholesterol; TG, Total triglycerides

[a]compared with the after-GLP group and obtained from independent Student's t test for continuous normal distribution data or independent Wilcoxon signed rank test for continuous non-normal distribution data.

Table S3. OPLS-DA Model Parameters Table

Model Type	R2X(cum)	R2Y(cum)	Q2(cum)
OPLS-DA	0.219	0.978	0.839

Table S4. The correlation coefficients of differential metabolites with TNF-α, SOD, and TC.

Metabolites	TNF-α	SOD	TC
Tryptophan	-0.07	0.23	0.27
5-hydroxytryptophol	0.30	-0.31	-0.15
Palmitoylcarnitine	0.24	-0.25	-0.16
Hydroxyhexadecanoylcarnitine	-0.21	0.30	0.08
12-oxo-leukotriene B4	-0.25	0.32	0.08
Prostaglandin E2 ethanolamide	0.22	-0.31	-0.04
20-dihydroxyleukotriene B4	0.25	-0.21	-0.05
Leukotriene E4	0.18	-0.25	-0.19
PC (12:0/14:0)	-0.16	0.37	0.22
LysoPA(16:0)	-0.15	0.36	0.18
cPA(18:0)	0.17	-0.18	-0.09
Phytosphingosine	0.27	-0.35	-0.10
Pantetheine 4'-phosphate	0.29	-0.32	-0.13
Dihydroceramide	0.23	-0.21	-0.16

Table S5. Levels of 14 serum metabolites in placebo and intervention group [mean ± SD]. (n=82)

Metabolites	No.	before-GLP group	after-GLP group	before-placebo	after-placebo
Tryptophan	C1	31198.64±17295.70	50823.74±13987.10	27080.02±13400.56	37536.71±20373.05
5-hydroxytryptophol	C2	1634.66±375.10	450.24±127.77	1540.35±435.25	1719.62±244.55
Prostaglandin E2 ethanolamide	C3	3837.35±1770.55	415.05±238.54	2998.02±1212.11	3228.48±947.55
Phytosphingosine	C4	827.49±132.28	1680.27±408.35	838.53±373.12	842.99±364.74
Pantetheine 4'-phosphate	C5	692.91±89.83	1820.78±242.80	854.09±390.96	879.03±319.67
Dihydroceramide	C6	8021.79±2437.47	3664.02±1908.46	5880.03±1558.81	6314.08±1541.94
12-oxo-leukotriene B4	C7	9265.75±4567.13	3637.84±784.31	11368.59±5985.03	9633.35±4898.85
PC (12:0/14:0)	C8	31228.99±9356.34	13060.52±6557.68	29662.92±14100.85	25357.59±10769.92
Palmitoylcarnitine	C9	2088.36±1435.99	11145.37±5687.36	2407.84±679.79	2330.33±575.07
Hydroxyhexadecanoylcarnitine	C10	3058.20±2746.56	11528.03±5746.28	2799.20±463.07	2686.34±605.57
LysoPA(16:0)	C11	16137.70±13671.66	2348.95±1354.04	13261.12±4594.66	11771.495029.66
cPA(18:0)	C12	12606.62±2597.63	3660.82±996.38	11230.35±2417.82	10611.05±2715.50
20-dihydroxyleukotriene B4	C13	11141.29±2827.04	1651.10±336.55	13513.22±2634.93	12164.70±1648.00
Leukotriene E4	C14	2326.06±1074.71	599.86±249.04	1732.20±572.94	1553.93±392.62