Electronic Supplementary Material (ESI) for Food & Function.
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Supplemental Information

Supplementary Materials and Methods

Materials

Assay kits of total cholesterol (TC), total triglyceride (TG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), high density lipoprotein (HDL) and low density lipoprotein (LDL) were obtained from Huili Biotechnology Co., Ltd. (Changchun, China). Additionally, detection kits of glutathione peroxidase (GSH-P*x*), superoxide dismutase (SOD), alkaline phosphatase (ALP) and malonaldehyde (MDA) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Moreover, enzyme-linked immuno sorbent assay kits of apoprotein A1 (Apo-A1), apoprotein B (Apo-B), fatty acid synthetase (FAS), tumor necrosis factor-α (TNF-α), interleukin 1 (IL-1) and interleukin 6 (IL-6) were also purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Histopathological observation

A portion of each liver tissue (cut into several portions) and abdominal fat pad were preserved into 4% paraformaldehyde solution, and then embedded in paraffin. The paraffin blocks were sliced into 4-6 μm sections, and stained with hematoxylin-eosin (H & E) for the fat pad and hepatic tissue. Sections of liver were also stained with Oil Red O before histopathological evaluation. Finally, the samples were analyzed by observing the morphological changes with an Olympus light microscope[1].

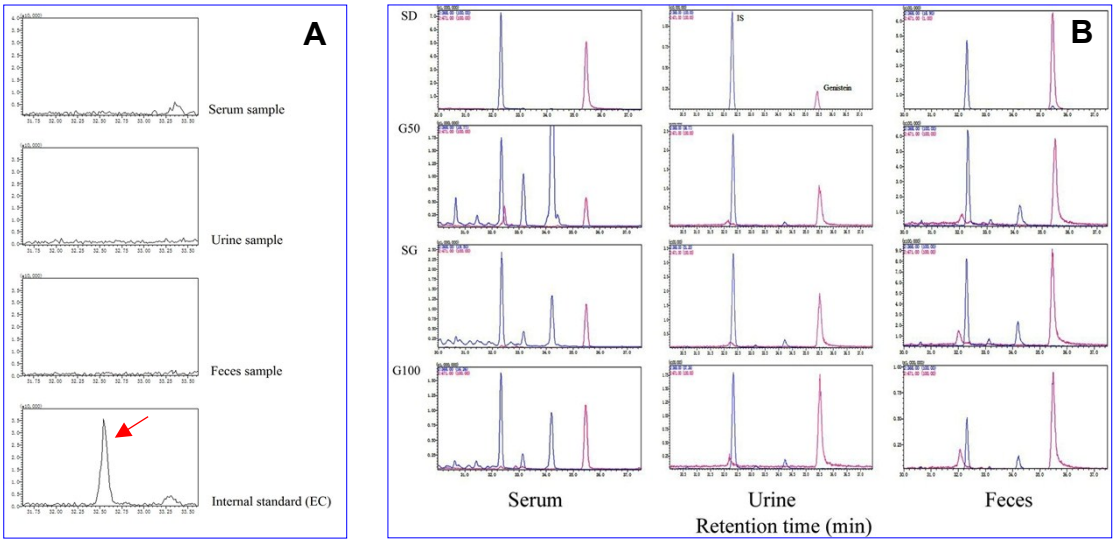
Western blot analysis

Liver tissue of 100 mg was homogenized in 1mL lysis buffer (200 mM of pH 6.8 Tris-HCl, 0.32 M SDS, 125 ng/mL bromophenol blue, 15.6% glycerinum, 21% Tris-Base, 6.25% β-mercaptoethanol). After 10 min, the centrifugation was performed at 15000 rad/min for 10 min and the supernatant was collected. Protein concentration of lysate was determined with a commercial kit (BestBio, Shanghai, China). Equal amounts of lysate proteins were heated at 95ºC for 5 min with one-fourth volume of 5× loading buffer. Then, electrophoresis was performed by SDS-PAGE for 30 min at 80 V in the first stage, and 60 min at 120 V in the 2nd stage, and the separated proteins was electro-blotted to

PVDF membranes. The nonspecific binding sites were blocked with 5% defatted milk in TBST (Tris 100mM, NaCl 166mM, 0.5% Tween 20) for 2h, and the transferred membrane was incubated at 4ºC for 12h with primary anti-CDP antibody (1:1000, Proteintech Group, Inc., Chicago, USA), followed by incubation at room temperature for 1h with horseradish peroxidase conjugated goat anti-rabbit secondary antibody (1:5000, Proteintech Group, Inc., Chicago, USA). The immunoreactive bands were detected using a Chemiluminescence Imaging Systems (Ultra-Violet Products, USA).

References

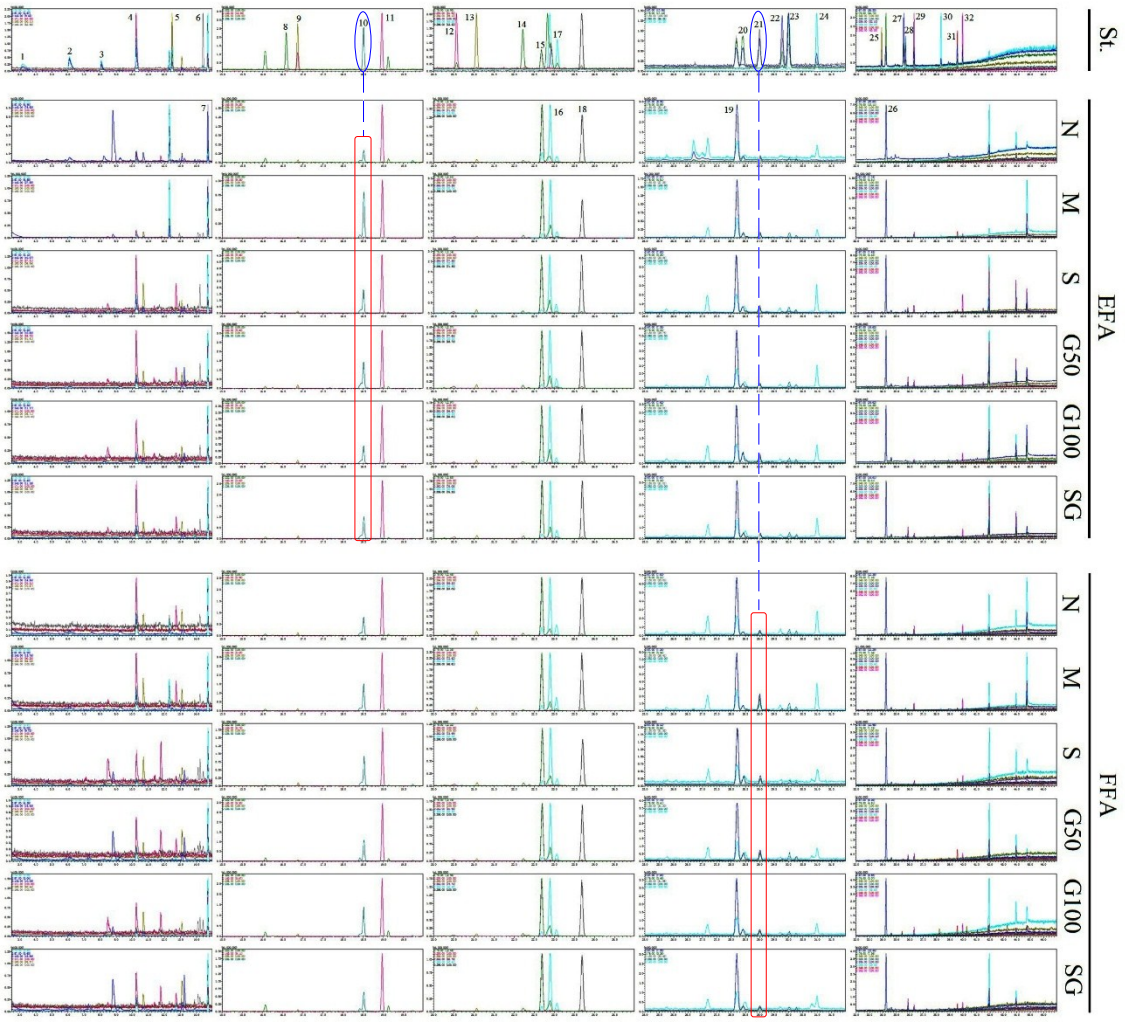
1 D. Ren, Y. Zhao, Y. Nie, N. Yang, and X. Yang, Hypoglycemic and hepatoprotective effects of polysaccharides from *Artemisia sphaerocephala Krasch* seeds, *Int. J. Biol. Macromol.,* 2014, **69**, 296-306.



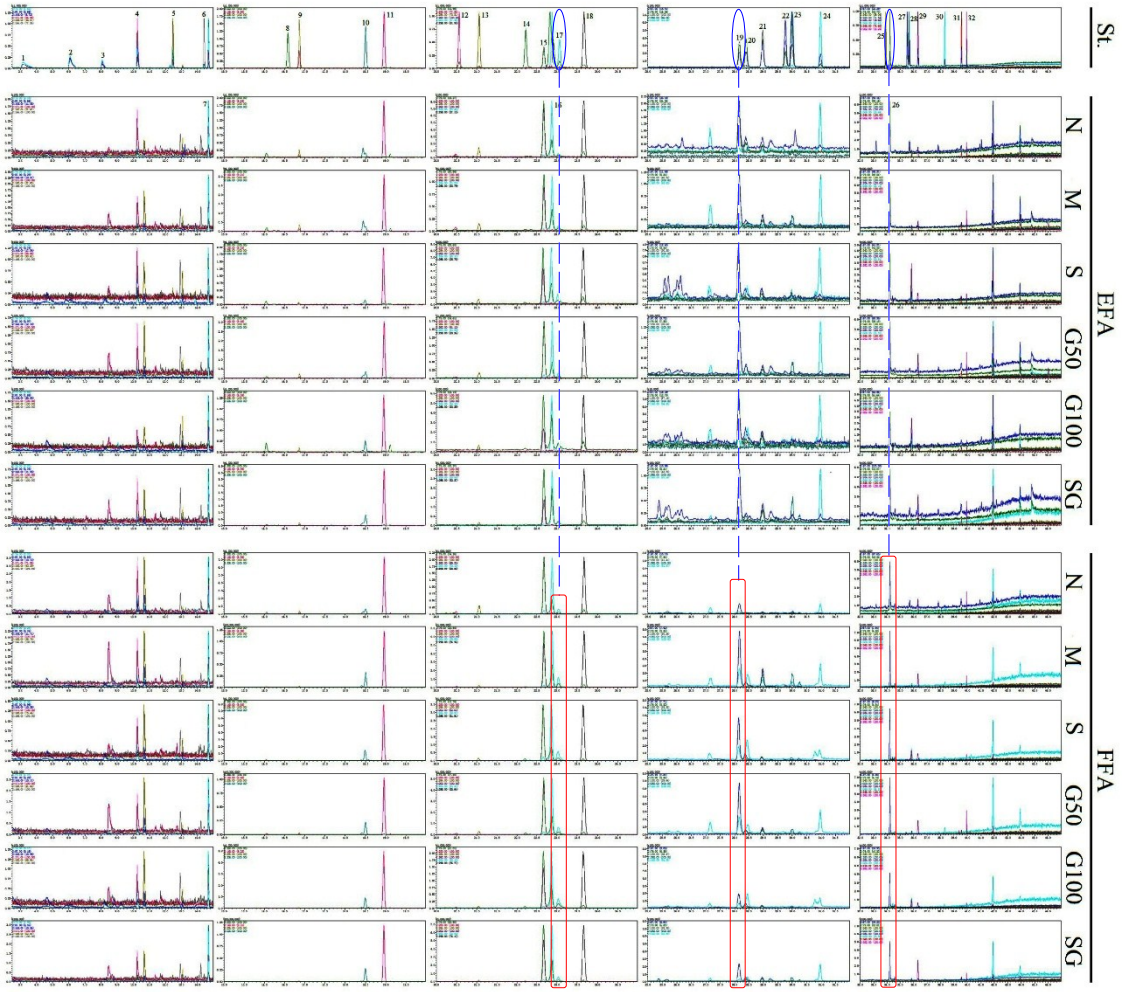
Supplemental Figure 1 The partial ion chromatograms (m/z = 368) of epicatechin (IS, internal standard) and serum, urine and feces samples without IS (A). The GC-MS chromatograms of epicatechin (IS) and genistein derivatives for standard compounds, and serum, urine and feces samples of HF-fed mice (B).

Although epicatechin was found in many food, its typical ion peak (m/z = 368) was no found in serum, urine and feces samples in this study (Supplemental Figure 1A). Thus, epicatechin as internal standard was applied for determination of genistein in serum, urine

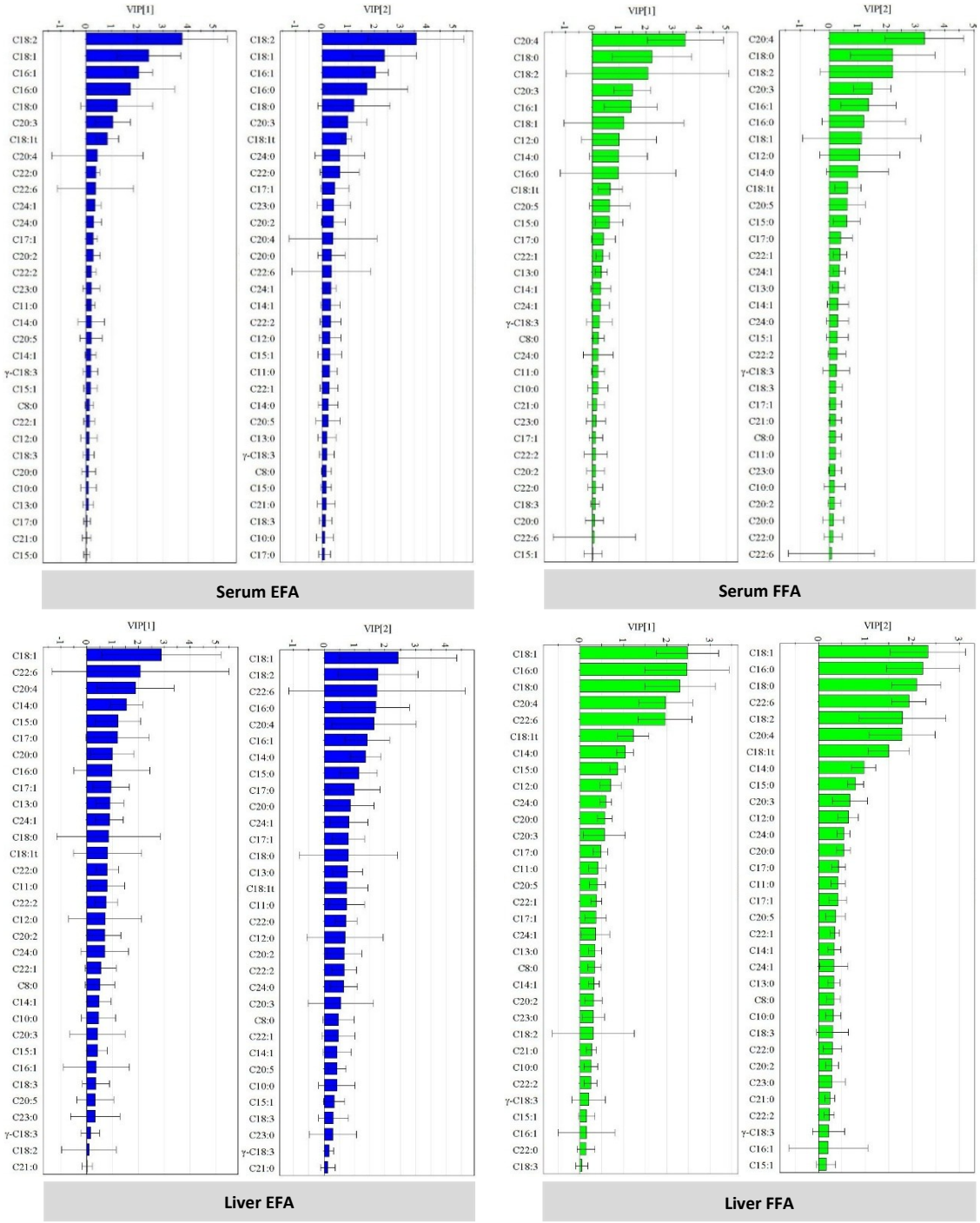
and feces samples in current research.



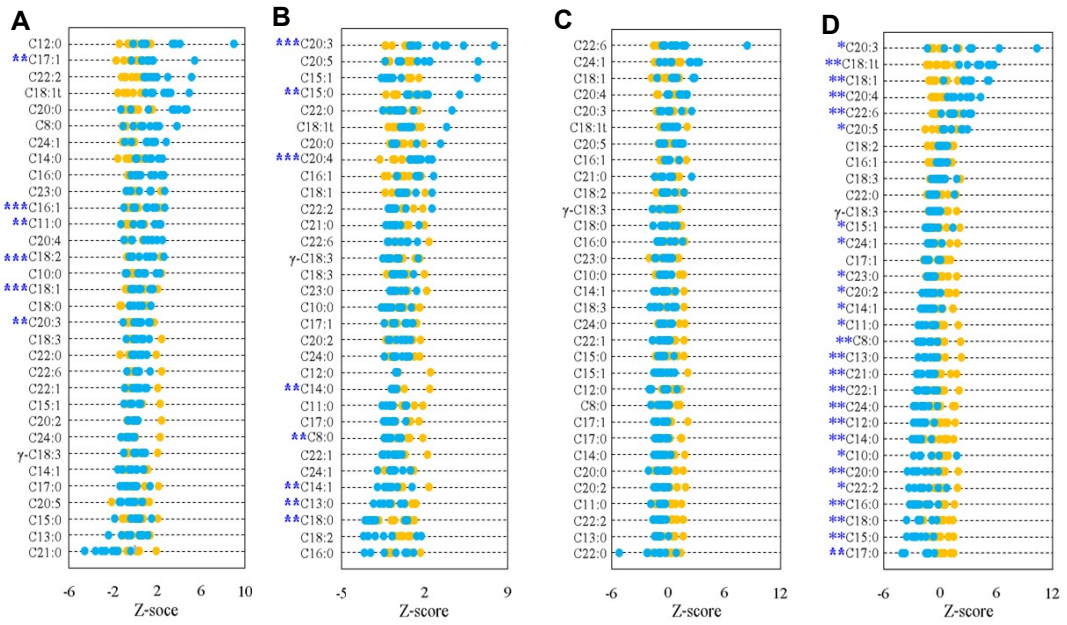
Supplemental Figure 2 GC–MS chromatograms of EFA and FFA obtained from mice serum and standard substance (St.) at selected ion monitoring (SIM) model. Peaks: (1) C8:0, (2) C10:0, (3) C11:0, (4) C12:0, (5) C13:0, (6) C14:1, (7) C14:0, (8) C15:1, (9) C15:0, (10) C16:1, (11) C16:0, (12) C17:1, (13) C17:0, (14) γ-C18:3, (15) C18:2, (16) C18:1, (17) C18:1t, (18) C18:0, (19) C20:4, (20) C20:5, (21) C20:3, (22) C20:2, (23) C18:3, (24) C20:0, (25) C21:0, (26) C22:6, (27) C22:2, (28) C22:1, (29) C22:0, (30) C23:0, (31) C24:1, (32) C24:0.



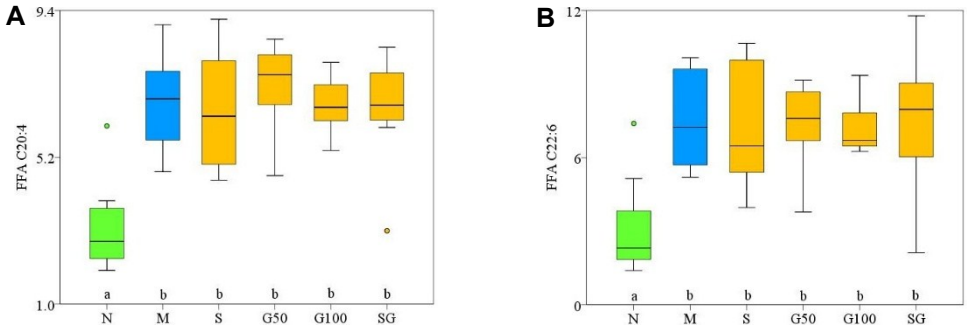
Supplemental Figure 3 GC–MS chromatograms of EFA and FFA obtained from mice liver and standard substance (St.) at selected ion monitoring (SIM) model. Peaks: (1) C8:0, (2) C10:0, (3) C11:0, (4) C12:0, (5) C13:0, (6) C14:1, (7) C14:0, (8) C15:1, (9) C15:0, (10) C16:1, (11) C16:0, (12) C17:1, (13) C17:0, (14) γ-C18:3, (15) C18:2, (16) C18:1, (17) C18:1t, (18) C18:0, (19) C20:4, (20) C20:5, (21) C20:3, (22) C20:2, (23) C18:3, (24) C20:0, (25) C21:0, (26) C22:6, (27) C22:2, (28) C22:1, (29) C22:0, (30) C23:0, (31) C24:1, (32) C24:0.



Supplemental Figure 4 Column plots of VIP value with jack-knifed confidence intervals for multivariate statistical analysis of serum fatty acid.



Supplemental Figure 5 Z-score plots of serum EFA (A) and FFA (B), and hepatic EFA (C) and FFA (D), respectively. The blue *, ** and *** means the statistical significance (p < 0.05, 0.01 and 0.001, respectively) between the normal group and model group by Wilcoxon Mann-Whitney Test.



Supplemental Figure 6 Potential biomarkers about fatty acid metabolism in HF-diet fed mice: C20:4 (A) and C22:6 (B) in FFA of mice liver. ^{a,b} Values having different superscripts are significantly different (p < 0.05).