Metabolomic investigation into molecular mechanisms of a clinical herb prescription against metabolic syndrome by a systematic approach

WDD decoction preparation

WDD decoction was prepared as follows. First, herb drugs, including 3g Radix Glycyrrhizae Preparata, 9g Pericarpium Citri Reticulatae, 4.5g Poria Cocos, 6g Citrus Aurantium, 6g Pinellia Ternata and 6g Caulis Bambusae in Taeniam, were decocted with 414 ml distilled water for twice in the pots, 1.5 hour for first time and 1 hour for second time. Then, two filtered decoctions were combined and concentrated into 14.4ml [1].

Animal, diets, maintenance and sample collection [2, 3]

All animal experiments were approved by the Animal Care and Use Committee, Fujian University of Traditional Chinese Medicine, Fuzhou, P. R. China, and all experiments were performed in accordance with relevant guidelines and regulations. Forty male Wistar rats weighing 200±20g, purchased from Shanghai SLAC Laboratory Animal Ltd. Corp. (Permission#SCXK (Shanghai) 2012-0002, China), were first randomly divided into two groups: ten rats in normal control group and thirty rats in Mets model group. All rats were housed in a 12/12 h light/dark cycle at an ambient temperature of 20 ± 2 °C and relative humidity of 60% - 65%. Control group were fed with normal diet. Mets model group were fed with high-sugar-fat-diet (50% of normal diet, 18% sugar, 10% lard, 12% egg yolk powder, 2% cholesterol, 7.5% salt and 0.5% bile salt). After 15 weeks, Mets model group were fed with high-fat emulsion (ingredients: 10% of cholesterol, 30% of lard, 2% of sodium cholate, 0.5% of propylthiouracil, 10% of tween 80 and 10% of propylene glycol) by intragastric at a dose of 10 mL/kg/d for 2 weeks [4]. The metabolic disorder of the animals was accessed by measuring abdominal perimeters, serum levels of HDL-C and insulin, and insulin-resistances (HOMA-IR, estimated using the homeostasis model assessment) according to the WHO definition [5]. Subsequently, rats in Mets model group were randomized into 3 groups (ten rats in each group): Mets model group, herb treated group and metformin treated group. Rats in herb treated group were administrated with WDD by intragastric at a dose of 10 ml/kg/d, and rats in metformin group were administrated with metformin by intragastric at a dose of 100 mg/kg/d for two weeks, respectively [6]. The dose of WDD and metformin that we employed in the rat experiments was within the human therapeutic range according to the Guidance of FDA-CDER and Chinese pharmacopoeia [1, 6].Then, all rats were sacrificed and blood samples were isolated by centrifugation at 2,500 rpm at 4 °C. And, serum samples were separated into 200 μL sub- aliquots and stored at -80 °C until analysis.

Reference


**Figure S1.** Biochemistry values of Wistar rats during 17 weeks diet feeding: (A) abdominal perimeter; (B) serum HDL level; (C) serum insulin level; (D) HOMA-IR. $^a$P < 0.05 vs. Mets model group, $^b$P < 0.05 vs. control group by t-test.

**Figure S2.** Biochemistry values of Wistar rats after 2 weeks intervention of herb (WDD) and
metformin, respectively: (A) abdominal perimeter; (B) serum HDL level; (C) serum insulin level; (D) HOMA-IR. a P < 0.05 vs. Mets model group, b P < 0.05 vs. control group, c P < 0.05 vs. herb treated group, d P < 0.05 vs. low-dose herb treated group by one-way ANOVA.