Supplementary Material

Black rice anthocyanins alleviate hyperlipidemia, liver steatosis and insulin resistance by regulating lipid metabolism ant gut microbiota in obese mice

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Supplementary methods

Preparation of BRAN

Black rice was obtained from a local market in Hangzhou, Zhejiang Province, China. Dried black rice was ground into powder, and subjected to extraction with 90% ethanol (v/v) containing 0.1% TFA and the liquid/solid ratio 12:1 (mL: g) for 90 minutes under the microwave power. Subsequently, the homogenate was kept in 4 ℃ for 24 h under continuous stirring. Then the extracts were centrifuged, filtered and concentrated under vacuum using a rotary evaporator. The concentrate was extracted six times with ethyl acetate, and concentrated by evaporation. Afterwards, the concentrate was loaded onto a column filled with XAD-7HP resins and eluted with distilled water, after which it was eluted with 90% ethanol containing 0.1% TFA. The elution was concentrated, and lyophilized to obtain BRAN. BRAN was analyzed by HPLC using a C18 column (4.6 mm × 250 mm, 5 μm), with A) water/formic acid (99.9:0.1, v/v) and B) acetonitrile/formic acid (99.9:0.1, v/v) as mobile phase, monitored at 520 nm. The operating conditions were listed as follows: column temperature, 30 °C; flow rate, 0.5 ml/ min; injection volume, 2 μL. The gradient program was as follows: 0 min, 5% B; 3 min, 5% B; 25 min, 40% B; 30 min, 40% B; 30.1 min, 95% B; 40 min, 95% B; 40.1 min, 5% B; 45 min, 5% B. As shown in Fig. 1, four anthocyanins were detected in BRAN. The total anthocyanin content in BRAN was 51.35 %, among of which the contents of cyanidin-3,5-diglucoside, cyanidin-3-glucoside, cyanidin-3-rutinoside and peonidin-3-glucoside were 1.01%, 89.42%, 3.10% and 6.47%, respectively (Fig. S1). Therefore, cyanidin-3-glucoside was the predominant anthocyanin in BRAN.

Supplementary Table S1. Ingredient and composition of diets (%)

Compositions	LFD (3.85 kcal/g)	HFD (4.73 kcal/g)
Ingredients (g %)		
Sucrose	33.17	20.14
Casein	18.96	23.31
Corn starch	29.86	8.48
Maltodextrin	3.32	11.65
L-cystein	0.28	0.35
Cellulose	4.74	5.83
Lard	1.9	20.68
Soybean oil	2.37	2.91
Phosphate dicalcium	1.23	1.51
Potassium citrate	1.56	1.92
Carbonate calcium	0.5	0.64
Vitamin Mix	0.95	1.16
Choline bitartrate	0.19	0.23
Mineral Mix	0.95	1.16
Energy (kcal %)		
Protein	20	20
Carbohydrate	70	35
Fat	10	45

LFD, low-fat diet; HFD, high-fat diet

Table S2. The primer sequences used for real-time PCR analysis.

Gene	Sequence of forward primers (5' to 3')	Sequence of reverse primers (5' to 3')
Pparγ	CAGGAGCAGAGCAAAGAGGT	TGGACACCATACTTGAGCAGA
Pparα	CCTGCTTCCTGCCACTTG	GTTCACCCTGATTCCTGATGTC
Fas	AGGGGTCGACCTGGTCCTCA	GCCATGCCCAGAGGGTGGTT
Scd1	CCGAAGAGGCAGGTGTAGAG	TTCTTACACGACCACCACCA
Acox1	GTTCTCACGATGCCAATGC	ATGCTGGGGTTACAGGTTTG
AdipoR2	TACCAAGGAGATTTGGAGCCC	GCCCATAAACCCTTCATCTTCC
HMGcoR	GAGCGTGAACAAGGACCAAG	CAGCCATTTTGCCAGAGTTT
β-actin	GTGCTATGTTGCTCTAGACTTCG	ATGCCACAGGATTCCATACC

Acox1, acyl-coenzyme A oxidase 1; *AdipoR2*, Adiponectin Receptor 2; *Fas*, fatty acid synthase; *HMGCoR*, 3-hydroxy-3-methyl-glutaryl CoA reductase; *Pparα*, peroxisome proliferator-activated receptor α; *Pparγ*, peroxisome proliferator-activated receptor γ; *Scd1*, stearoyl-CoA desaturase 1.

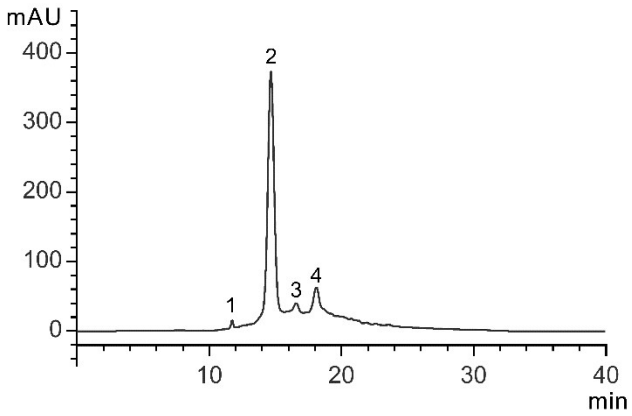

Fig. S1. HPLC chromatogram of black rice anthocyanins (BRAN). Detection was performed at 520 nm. Peak 1, cyanidin-3,5-diglucoside; peak 2, cyanidin-3-glucoside; peak 3, cyanidin-3-rutinoside; peak 4, peonidin-3-glucoside.