Supplemental Information

Methods

The plasma levels of TC, LDL-c, TG and HDL-c during the 16-week treatment by BBR were determined using commercial kits. Serum homocyteine was measured by ABC-ELISA using rat homocysteine ELISA kit (Maibiotechnology, China). Hepatic DNMT1 and DNMT3b mRNA levels were analyzed by quantitative real-time RT-PCR. The specific primers of DNMT1 and DNMT3B was shown in Supplemental Table S1. The protein levels of DNMT1 and DNMT3b were detected by Western immunoblotting using DNMT1 (Santa Cruz) and DNMT3b (Cell Signaling Technology) antibodies. Total hepatic DNA methyl transferase (DNMT) activity was determined with a continuous cycle of colorimetry (GENMED DNMT total activity kit, Genmed Scientifics Inc, USA).

Measurement of S-adenosylmethionine (SAM) and S-adenosylhomocyteine (SAH) by high-performance liquid chromatography (HPLC)

Hepatic SAM and SAH levels were determined by HPLC. Liver specimens (300 mg) were homogenized in 10% perchiloric acid and centrifuged at 10000 rpm for 15 min at 4 ^{o}C. The supernatant was filtered through a 0.2-μm polypropylene syringe filter (0.4 mm diameter; Whatman, Clifton, NJ, USA). A 20-μl aliquot of the acid extract was applied directly onto the HPLC. SAM chloride and SAH standard were obtained from Sigma (St. Louis, MO, USA). Twenty μl of solution was injected directly onto an Agilent Eclipase C_{18} reversed-phase analytical column (250 mm X 4.6 mm, 5 μm particle). The mobile phase consisted of two solvents: Solvent A, 50 mM NaH_2PO_4

adjusted to pH 3.0 with H_3PO_4 and Solvent B, 100% methanol. The HPLC column was equilibrated with 90% Solvent A and 10% Solvent B. The flow-rate was 1 ml /min and detection was monitored at 254 nm. The HPLC was performed at room temperature. SAM and SAH were identified according to their retention time and co-chromatography with SAM and SAH standards. Quantification was done by integration of the peak areas and compared to the standard calibration curves. The results are expressed in μmol/gram wet tissue.

Supplemental Figures

Figure S1. Quantitative real-time RT-PCR and Western immunoblot analyses of hepatic mRNA/protein levels of DNMT1 (**A**) and DNMT3b (**B**) in the three indicated groups. Relative mRNA amounts of these genes were normalized to that of *beta-actin* and shown as mean ± SEM.

Supplemental Tables

Table S1: Primer sequences for real-time quantitative PCR and bisulphate genomic sequencing PCR of genes involved in hepatic lipid metabolism

	Forward primer (5'- 3')	Reverse primer (5'- 3')
Real-time quantitative PCR		
CPT-1α	GGACTGTGGTGCGGAGGA	GGCTCAGGCGGAGGTCAA
UCP-2	TACCAGAGCACTGTCGAAGC	GAAGTGAAGTGGCAAGGGAG
PPARα	TTCTGCGACATCATGGAACC	CACAATCCCCTCCTGCAACT
MTTP	TCTTGATGGAGCGGGAGT	TCTTGATGGAGCGGGAGT
apoB	TAAATGGAGCACTTTTCAAG	GGAACAGCAGCAGTAGCG
LDLR	CCAGTGCGGCGTAGGATT	GGGACTCATCGGAGCCAT
PPARγ	ACCCAATGGTTGCTGATTAC	CTCTTCATGTGGCCTGTTGT
ACC	GAGGACAAGAAGCAGGCACA	CAAATTCGGCTGGGGAAG
DGAT1	AGGATGTTCCGCCTCTGG	GGACTCAGGTGCCATCCC
DGAT2	AGGTGATCTTTGAGGAGGGC	CCAGCTTAGGGACGGTGA
GPAT	CAGAGCAGCAGTGGAACA	GACAAAGATGGCAGCAGA
SCD-1	GGGAAAGTGAAGCGAGCAA	GTGGTCGTGTAGGAACTGGAGA
DNMT1	AACGGAACACTC TCT CTCACTCA	TCA CTGTCCGACTTGCTCCTC
DNMT3b	CGTGGTAGGAGATGGAGATGG	TGGAGATACTGTTGCTGTTTCG
Beta-actin	GATTACTGCCCTGGCTCCTA	TCATCGTACTCCTGCTTGCT
Bisulphite genomic sequencing PCR of select genes' promoters		
MMTTP	GTGTATTGTTTTTGTTTGGTTT	TACTACCTAACTCCCCTCTACC
MCPT-1α	GGGTATGGTTTTAATGAGTTGGTT	AAAATACCCTCTACTTCTCCAATAC
MLDLR	TAGTTAAGTATTTGGGAATTGGTGAA	ACCTTACCTACAACTCCAACAAC

Table S2. Plasma lipid profiles of HFD-fed rats during 16 weeks of BBR treatment.

	ND	HFD	BBR+HFD
TG (mmol/l)			
0 weeks	2.0±0.17	2.26±0.22	2.27±0.28
4 weeks	2.07±0.14	2.39±0.20	2.35±0.16
8 weeks	2.16±0.18	1.84±0.14	1.91±0.12
16 weeks	2.01±0.51	1.02±0.10*	0.95±0.12*
TC (mmol/l)			
0 weeks	2.0±0.13	2.3±0.14	2.4±0.15
4 weeks	2.06±0.10	2.27±0.14	2.16±0.13
8 weeks	2.05±0.11	2.34±0.12	2.15±0.15
16 weeks	2.17±0.13	2.81±0.28*	2.02±0.21$^{\#}$
LDL-c (mmol/l)			
0 weeks	0.60±0. 13	1.34±0.10**	1.45±0.15**
4 weeks	0.7±0.18	1. 54±0.14**	1.40±0.12**
8 weeks	0.50±0.06	1.67±0.12**	1.37±0.14**
16 weeks	0.54±0.04	2.10±0.31**	1.22±0.19$^{\#}$
HDL-c (mmol/l)			
0 weeks	1.13±0.07	0.73±0.04**	0.75±0.03**
4 weeks	1.05±0.10	0.65±0.04**	0.66±0.02**
8 weeks	1.09±0.06	0.64±0.03**	0.74±0.04**
16 weeks	1.33±0.12	0.74±0.08**	0.79±0.03**

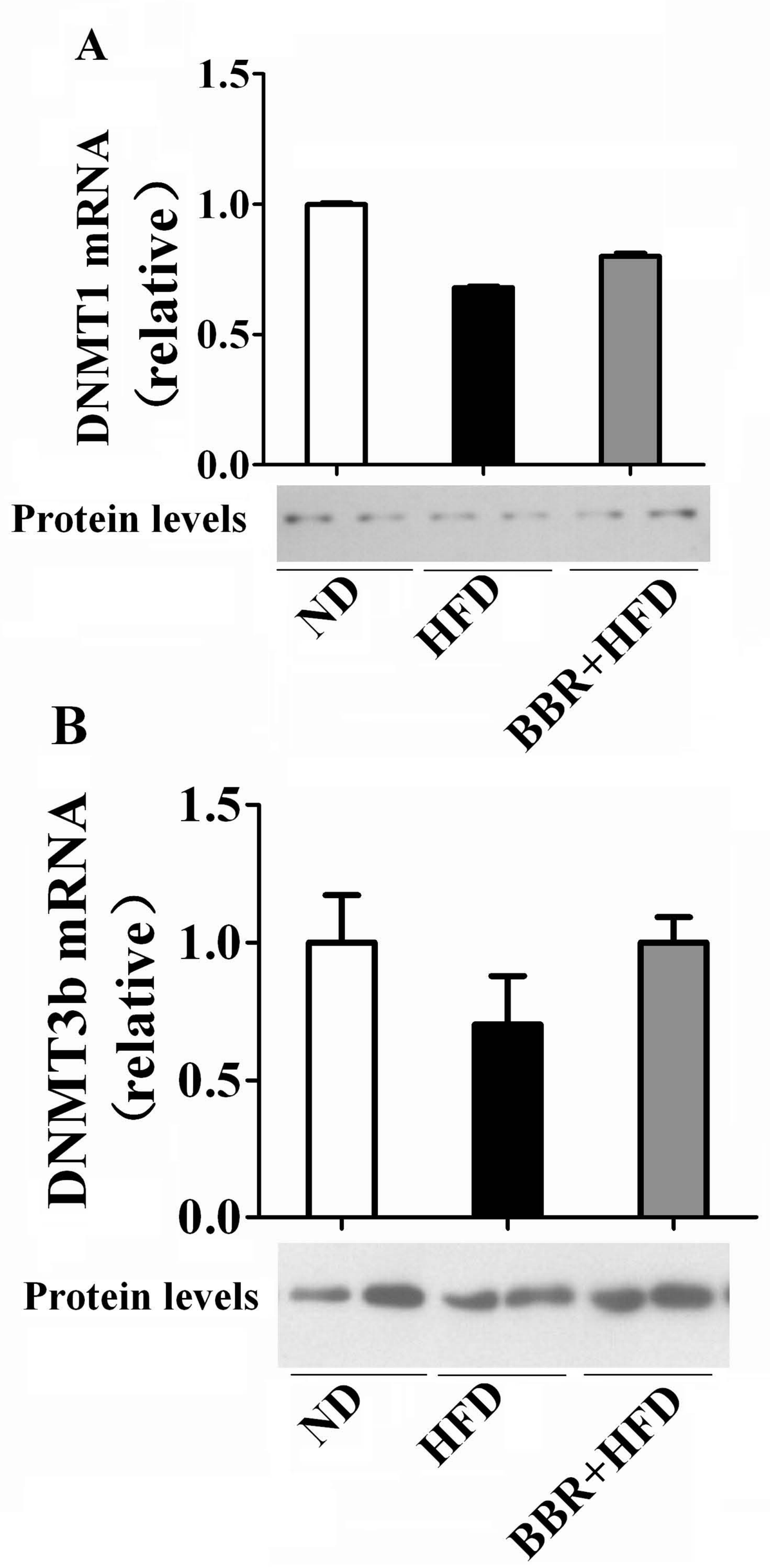
Data are expressed as mean ± SEM (n=8). $^{*}p<0.05$, $^{**}p<0.01$ *vs.* ND-fed control group (ND); $^{\#}p<0.05$ *vs.* HFD-fed vehicle group (HFD).

Table S3. The effect of BBR treatment for 16 weeks on serum homocysteine (Hcy), hepatic SAM and SAH levels, and hepatic DNMT activity.

	ND	HFD	BBR+HFD
Serum Hcy (μmol/l)	23.19 ± 3.45	48.44 ± 4.87**	21.10 ± 4.32$^{\#\#}$
Hepatic SAM (μmol/g liver)	177.3 ± 10.3	136.1 ± 21.1	107.5 ± 17.9
SAH (μmol/g liver)	12.3 ± 1.2	7.2 ± 0.7	8.3 ± 2.5
SAM/SAH	15.9 ± 0.4	18.6 ± 1.0	18.6 ± 0.2
DNMT activity (IU/g liver)	0.84±0.14	0.60±0.22	0.64±0.16

Data are expressed as mean ± SEM (n=8). $^{*}p<0.05$, $^{**}p<0.01$ *vs.* ND; $^{\#}p<0.05$, $^{\#\#}p<0.05$ *vs.* HFD.

Fig S1


A
DNMT1 mRNA (relative)
1.5
1.0
0.5
0.0
Protein levels
ND
HFD
BBR+HFD
B
DNMT3b mRNA (relative)
1.5
1.0
0.5
0.0
Protein levels
ND
HFD
BBR+HFD