Comparative study of Kampo medicines on diet-induced mouse obesity model

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Running title : Comparative Kampo study in DIO model

Abstract

Aim

Boiogito (BOT) and bofutsushosan (BTS) are major traditional Kampo medicines that have been widely used for obesity in Japan. In this study, we employed a high-fat diet-induced obesity (DIO) mouse model to conduct a comparative study of BOT and BTS for their therapeutic efficacy in obesity.

Methods

C57BL/6J mice were fed a high-fat diet (HFD) for 10 weeks for developing the DIO mouse model and were treated with Kampo medicines for 12 weeks. Body weight and food intake were monitored weekly and blood and tissue samples were collected.

Results

While neither BOT nor BTS showed any significant impact on body composition in the DIO mice, the serum level of leptin was significantly decreased in both BOT- and BTS-treated DIO mice. Contrary to blood leptin level, BOT suppressed, whereas BTS rather increased, the blood insulin level in the DIO mice. In addition to these serum obesity markers, BOT showed reduction of liver triglyceride and total cholesterol contents along with less steatosis. Importantly, mRNA expression of molecules associated with lipid metabolism was reduced in the DIO mice treated with BOT, whereas BTS treatment showed instead reduced

liver mRNA expression of TNF-α. Both BOT and BTS treatment inhibited adipocyte maturation, as seen in the enlarged size of adipocytes in the white adipose tissue of DIO mice.

Conclusion

We conclude that BOT and BTS have a differential therapeutic benefit in obesity through their modulating activity in lipid metabolism and/or anti-inflammatory effect.

Introduction

Traditional Japanese/Chinese (Kampo) medicines have been established through the accumulation of extensive knowledge and experience [1]. Kampo medicines have been used as formulations of several crude drugs, prepared by decoction, and mostly administered orally. Since 1976, the Ministry of Health, Labor and Welfare of Japan has approved 148 of Kampo medicines to be covered by the national health insurance system [2]. Boiogito (BOT) and bofutsushosan (BTS) are major formulations that have been widely used for obesity in Japan among approved Kampo medicines. While BOT is used for the treatment of hyperhidrosis, obesity, arthritis, edema, and a tendency toward being easily fatigued, BTS is used for the treatment of thick subcutaneous abdominal fat, constipation and obesity [3]. Although these two Kampo formulations are often used in the clinic to treat obesity, their mechanism of action and any distinction in their efficacy remain unsolved [1].

In this study, we employed one of the major pre-clinical animal models for studying obesity, which is the high-fat diet-induced obesity model, to conduct a comparative study of BOT and BTS for their therapeutic efficacy in obesity. The high-fat diet-induced obesity model has been known to share many of the same phenotypes as in human disease, including visceral adiposity, insulin resistance, hyperinsulinemia, hyperleptinemia, and leptin resistance [4, 5]. Although neither BOT nor BTS showed any significant impact on body

composition in the DIO mice, BOT and BTS treatment altered the serum level of leptin and insulin in the DIO mice. In addition, BOT treatment showed a reduction in liver lipid contents, mRNA expression of molecules associated with lipid metabolism, and less steatosis at pathology. Distinct from BOT, BTS treatment significantly reduced the liver mRNA expression of TNF-α, which is a typical inflammatory mediator and known to be an important risk factor in obesity [6]. Both BOT and BTS treatment inhibited adipocyte maturation, as seen in the enlarged size of the DIO mice. Collectively, BOT and BTS may have a differential therapeutic benefit in obesity through their modulating activity in lipid metabolism and anti-inflammatory effect, respectively.

Materials and methods

Preparation of boiogito (BOT) and bofutsushosan (BTS)

The extracts of BOT (TJ-20) and BTS (TJ-62) were kindly provided by Tsumura & Co. (Tokyo, Japan). The 3D-HPLC charts of the BOT and BTS extracts provided from Tsumura & Co. are shown in Figure 1 for quality reference.

Animal experiments

C57BL/6J mice (5-week old, male) were purchased from Japan SLC Inc. (Hamamatsu, Japan). All experiments were approved by and performed according to the guidelines of the Care and Use of Laboratory Animals of the University of Toyama. For developing the diet-induced obesity (DIO) mouse model, a group of C57BL/6J mice were fed a high-fat diet (HFD, D12492, Research Diets Inc., NJ, USA) for 10 weeks. Another group of mice were fed a normal diet (ND, D12450B, Research Diets Inc.) as a control. After 10 weeks of high-fat diet feeding, the DIO mice were divided into three groups with similar average of body weight and treated with BOT, BTS (orally with gavage, daily at 500mg/kg dose) or control water for 12 weeks. The dose for Kampo medicines was determined by following to previous studies [7]. Upon 8 weeks' treatment with BOT, BTS or water, an interim retro-orbital blood sample collection was conducted and then all mice were given a normal chow (Labo MR Stock) for another 4 weeks. On termination of the experiment, blood and tissue samples

were collected. Body weight and food intake were monitored weekly.

Serum measurements

Serum leptin or insulin levels were determined by using a specific enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. The ELISA kit for rat and mouse leptin (Lbis Leptin-Rat kit or Lbis Leptin-Mouse kit), and rat insulin kit (Lbis insulin-Rat kit) were purchased from Shibayagi Co. Ltd. (Shibukawa, Japan.) kit. The mouse insulin ELISA kit was purchased from Morinaga & Co. (Yokohama, Japan).

Liver lipid content measurements

The perfused liver tissues were weighed and homogenized in sodium chloride buffer and then the homogenates were extracted with 5 ml of chloroform and methanol (2:1, vol/vol) [8]. The chloroform layers were dried and then triglyceride (TG), total cholesterol (Cholesterol) or free fatty acids (FFA) were measured by using LabAssay Cholesterol kit, LabAssay triglyceride kit, LabAssay NEEA kit (Wako Chemical, Osaka, Japan).

Histological analysis

Liver and epididymal adipose tissues were collected upon termination of the experiment and immediately fixed with 4% PFA for 1-2 days. The fixed tissue samples were then sliced sequentially into sections 3-5 μm in thickness.

Representative sections of the liver and fat tissues 2-3 μm thick were selected and embedded in paraffin for routine histo-pathological analysis with hematoxylin and eosin (H&E) staining. Adipocytes in white adipose tissue were counted with a microscope at x200 magnification. Three visual fields were chosen randomly and the average number of adipocytes in the three fields was taken for each group. Macrovesicular steatosis was graded on a scale 0 to 4 based on the percentage of hepatocytes affected in each specimen. The score was 0 for none; 1 for up to 33%; 2 for 33–66%; 3 for more than 66%; and 4 for more than 66%-100%.

Real-time RT-PCR for quantitative assessment of mRNA expression

Total RNAs were prepared using the RNeasy Plus Mini kit (QIAGEN, Hilden, Germany). The expression level of targeted mRNAs was normalized to m*Gapdh* mRNA by using One Step SYBR PrimeScript RT-PCR kit II (Takara, Kyoto, Japan). The primers used in this experiment are listed in Table 1.

Statistics

Statistical analysis was performed with JMP (SAS Institute Japan, Tokyo). Data were expressed as mean ± S.E.M. One-way ANOVA followed by Dunnett's test was used to determine the statistical differences among groups.

Results

Effect of BOT and BTS on body weight changes and tissue weight in DIO model

In order to conduct a comparative study of the two major Kampo medicines, BOT and BTS, in a dietary obesity model, we employed a high-fat diet-induced obesity (DIO) mouse model in C57BL/6 mice. Mice were fed a high-fat diet (HFD) for 8 weeks before being subjected to BOT or BTS treatment. The HFD-treated mice significantly gained the body weight, compared with the ND group. Neither BOT nor BTS treatment affected on either body weight (Fig. 2A) in the chronic DIO mice (up to 8 weeks). We then further monitored the efficacy of BOT or BTS in combination with a diet modification by feeding mice a standard diet for a subsequent 4 weeks from the 8-week time point. Even in this situation, we did not observe any significant effect on body weight, epididymal fat (Fig. 2B), or liver weight (Fig. 2C) at the time of termination. Collectively, BOT and BTS did not show any dynamic efficacy on either body or tissue weight gain in the DIO model.

Effect of BOT and BTS on the level of serum biomarkers and liver lipid contents in the DIO model

We then investigated whether BOT or BTS treatment affects on the obesity-associated serum biomarkers. The DIO mice treated with both BOT and BTS showed significantly lower levels of serum leptin both in the chronic disease

state (Fig. 3A left) and in combination with the diet modification (Fig. 3 A left). While BOT treatment showed lower serum insulin levels in the DIO mice compared to the untreated NFD group, BTS treatment rather enhanced serum insulin level in the chronic disease state (Fig. 3 A right). Neither BOT nor BTS treatment affected serum insulin levels in combination with the diet modification (Fig. 3B right). To further explore a therapeutic effect of BOT or BTS in the DIO model, we examined the effect of BOT or BTS on the expression of liver lipids in the DIO mice. While BTS treatment did not show any significant effect on the liver lipid contents, the liver TG level and the total cholesterol, but not the free fatty acid (FFN), were significantly lower in BOT-treated DIO mice than in the HFD mice (Fig. 4).

Effect of BOT and BTS on lipid metabolisms in the DIO model

Along with the reduction in liver lipid contents in BOT-treated DIO mice (Fig. 4), we observed less fat deposition in the liver of DIO mice treated with BOT, but not BTS, as seen in the lower steatosis score (Fig. 5A and B). Furthermore, the size of adipocytes in white adipose tissue, which was enlarged in the DIO mice, was relatively smaller with BOT treatment or BTS treatment (Fig. 5A), as seen in the increased number of adipocytes on histological examination (Fig. 5C). We then examined the effect of BOT or BTS on the liver mRNA expression of molecules associated with obesity to understand the potential molecular mechanism for in vivo efficacy of BOT or BTS by using quantitative real-time polymerase chain

reaction (PCR). Among many molecules associated with obesity, we found that the wide spectrum of mRNA expression that associated with lipid metabolism, Lipe, Lpl, PPARα, Cpt1α and SREBP1, were significantly decreased by BOT treatment in DIO mice. Distinct from BOT treatment, BTS significantly reduced the expression of TNF-α mRNA, but did not affect the expression of mRNA associated with lipid metabolism.

Discussion

Boiogito (BOT) and Bofutsushosan (BTS) are major herbal medicines that have been widely used for obesity in Japan [3, 9, 10]. Although there are several reports studying the effect of these herbal medicines, it is not yet clear whether there is any difference between BOT and BTS in their efficacy in obesity. In this study, we employed one of the major pre-clinical animal models for studying obesity, which is the high-fat diet-induced obesity model, to conduct a comparative study of BOT and BTS for their therapeutic efficacy in obesity.

While neither BOT nor BTS showed any significant impact on the body composition of DIO mice, the serum level of leptin significantly decreased in BOT- and BTS-treated DIO mice (Fig. 3A). This effect of BOT and BTS in reducing blood leptin level was more significant in combination with food restriction (Fig. 3B). As leptin has been widely known as a potent lipid-lowering adipokine and is considered an important factor for preventing cellular lipotoxicity and insulin resistance [11, 12], the modulating effect of BOT or BTS on the systemic leptin level may be partially involved in their mechanism of action in vivo. Contrary to the similar effect on blood leptin level, BOT suppressed whereas BTS rather increased the blood insulin level in the DIO mice. These results imply a distinct effect of BOT and BTS on insulin resistance in the chronic obesity condition.

In addition to the serum obesity markers, BOT showed a reduction in

liver TG and total the cholesterol contents (Fig. 4), along with the lower steatosis score (Fig 5A and B). Importantly, we observed the reduction of liver mRNA expression of molecules associated with lipid metabolism (Lipe, Lpl, PPARα, Cpt1α and SREBP1) [13-15] in DIO mice treated with BOT (Fig. 6). BTS treatment did not show a significant effect on liver lipid metabolism in contrast to BOT treatment. Instead, there was a significant reduction in the liver mRNA expression of TNF-α, which is a typical inflammatory mediator and known to be an important risk factor in obesity. Recent studies have indicated that TNF-α plays an important role in mediating the insulin resistance of obesity through its over-expression in fat tissue [16, 17]. Considering both BOT and BTS treatment inhibited adipocyte maturation, as seen in their enlarged size in DIO mice, BOT and BTS may have a differential therapeutic benefit in obesity and such anti-obesity effect may be through their modulating activity in lipid metabolism and anti-inflammatory effect, respectively. Considering not only the body weight loss rather the improvement of obesity-related pathogenic status can be critical for treating obesity, we believe our presented results clearly indicate both BOT and BTS have anti-obesity effect without affecting the body composition of HFD mice. Nevertheless, considering that neither BOT nor BTS treatment was solely effective in DIO mice maintained on an HFD feeding condition (data not shown), we propose that the two major Kampo medicines, BOT and BTS, are better utilized for their optimal anti-obesity effect in the presence of diet therapy.

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Disclosure statement

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References

1. Yamakawa, J., et al., *Significance of Kampo, Japanese traditional medicine, in the treatment of obesity: basic and clinical evidence.* Evid Based Complement Alternat Med, 2013. **2013**: p. 943075.

2. Hijikata, Y., *Analgesic treatment with Kampo prescription.* Expert Rev Neurother, 2006. **6**(5): p. 795-802.

3. Hioki, C., K. Yoshimoto, and T. Yoshida, *Efficacy of bofu-tsusho-san, an oriental herbal medicine, in obese Japanese women with impaired glucose tolerance.* Clin Exp Pharmacol Physiol, 2004. **31**(9): p. 614-9.

4. Birk, R.Z., et al., *Differential regulation of pancreatic digestive enzymes during chronic high-fat diet-induced obesity in C57BL/6J mice.* Br J Nutr, 2014. **112**(2): p. 154-61.

5. Hoevenaars, F.P., et al., *Adipose tissue metabolism and inflammation are differently affected by weight loss in obese mice due to either a high-fat diet restriction or change to a low-fat diet.* Genes Nutr, 2014. **9**(3): p. 391.

6. Ono, M., et al., *Bofutsushosan, a Japanese herbal (Kampo) medicine, attenuates progression of nonalcoholic steatohepatitis in mice.* J Gastroenterol, 2014. **49**(6): p. 1065-73.

7. Kimura, M., et al., *Traditional Japanese formula kigikenchuto accelerates healing of pressure-loading skin ulcer in rats.* Evid Based Complement Alternat Med, 2011. **2011**: p. 592791.

8. Zang, M., et al., *Polyphenols stimulate AMP-activated protein kinase, lower lipids, and inhibit accelerated atherosclerosis in diabetic LDL receptor-deficient mice.* Diabetes, 2006. **55**(8): p. 2180-91.

9. Yoshida, T., et al., *Thermogenic, anti-obesity effects of bofu-tsusho-san in MSG-obese mice.* Int J Obes Relat Metab Disord, 1995. **19**(10): p. 717-22.

10. Shimada, T., et al., *Preventive effects of Bofutsushosan on obesity and various metabolic disorders.* Biol Pharm Bull, 2008. **31**(7): p. 1362-7.

11. Borer, K.T., *Counterregulation of insulin by leptin as key component of autonomic regulation of body weight.* World J Diabetes, 2014. **5**(5): p. 606-29.

12. Munzberg, H. and C.D. Morrison, *Structure, production and signaling of leptin.* Metabolism, 2014.

13. Bauman, D.E., K.J. Harvatine, and A.L. Lock, *Nutrigenomics, rumen-derived bioactive fatty acids, and the regulation of milk fat synthesis.* Annu Rev Nutr, 2011. **31**: p. 299-319.

14. Usuda, D. and T. Kanda, *Peroxisome proliferator-activated receptors for hypertension.* World J Cardiol, 2014. **6**(8): p. 744-54.

15. Frisdal, E., et al., *Adipocyte Atp-Binding Cassette G1 Promotes Triglyceride Storage, Fat Mass Growth And Human Obesity.* Diabetes, 2014.

16. Oh, S., et al., *Abdominal obesity: causal factor or simply a symptom of obesity-related health risk.* Diabetes Metab Syndr Obes, 2014. **7**: p. 289-96.

17. Jin, D., et al., *TNF-alpha reduces g0s2 expression and stimulates lipolysis through PPAR-gamma inhibition in 3T3-L1 adipocytes.* Cytokine, 2014. **69**(2): p. 196-205.

Figure legends

Figure 1. Analysis by three-dimensional HPLC of major chemical compounds included in Kampo extracts.

The 3D-HPLC chart of major chemical compounds included in (A) boiogito (BOT) and (B) bofutsushosan (BTS) is shown.

Figure 2. Effect of BOT or BTS on body and tissue weight in obesity models.

C57BL/6 mice were pre-fed a normal diet (ND) or high-fat diet (HFD) for 10 weeks, and then administered control water or Kampo extracts (500 mg/kg, p.o., daily) for 12 weeks under the same feeding conditions. After 8 weeks of control or Kampo extract treatment, mice fed with HFD were changed to ND until termination of the experiment while maintaining the Kampo extract treatment for another 4 weeks. Body weight changes (A) and tissue weight (B: fat and C: liver) of DIO mouse are shown. Data are mean ± SEM (n=7-15).

Figure 3. Effect of BOT or BTS on serum levels of leptin and insulin in obesity models.

C57BL/6 mice were pre-fed a normal diet (ND) or high-fat diet (HFD) for 10 weeks, and then administered control water or Kampo extracts (500 mg/kg, p.o., daily) for 12 weeks under the same feeding conditions. After 8 weeks of control

or Kampo extract treatment, mice fed with HFD were changed to ND until termination of the experiment while maintaining the Kampo extract treatment for another 4 weeks. Serum samples were collected before the change in food (A) or upon termination (B), and levels of leptin (left) or insulin (right) were measured by using specific ELISA assay. Data are mean ± SEM (n=7-15). * $p<0.005$, ** $p<0.001$ as compared with the HFD group.

Figure 4. Effect of BOT or BTS on liver lipid levels in DIO mice.

C57BL/6 mice were pre-fed with a normal diet (ND) or high-fat diet (HFD) for 10 weeks, and then administered control water or Kampo extracts (500 mg/kg, p.o., daily) for 12 weeks under the same feeding conditions. After 8 weeks of control or Kampo extract treatment, mice fed with HFD were changed to ND until termination of the experiment while maintaining the Kampo extract treatment for another 4 weeks. Liver tissue samples were collected upon termination and levels of triglyceride (TG, left), total cholesterol (Cholesterol, middle) or free fatty acid (FAA, right) were measured. Data are mean ± SEM (n=7-15). * $p<0.005$ as compared with the HFD group.

Figure 5. Histopathological evaluation of fat and liver tissue in DIO mice.

C57BL/6 mice were pre-fed with a normal diet (ND) or high-fat diet (HFD) for 10 weeks, and then administered control water or Kampo extracts (500 mg/kg, p.o., daily) for 12 weeks under the same feeding conditions. After 8 weeks of control

or Kampo extract treatment, mice fed with HFD were changed to ND until termination of the experiment while maintaining the Kampo extract treatment for another 4 weeks. (A) Representative images (x 200) of H&E staining of liver (left panels) and white adipose tissue (right panels) of DIO mice are shown. (B) Steatosis scores of DIO mice. (C) Observed number of adipocyte counts of WAT in DIO mice. Data are mean ± SEM (n=7-15). ** $p<0.001$ as compared with the HFD group.

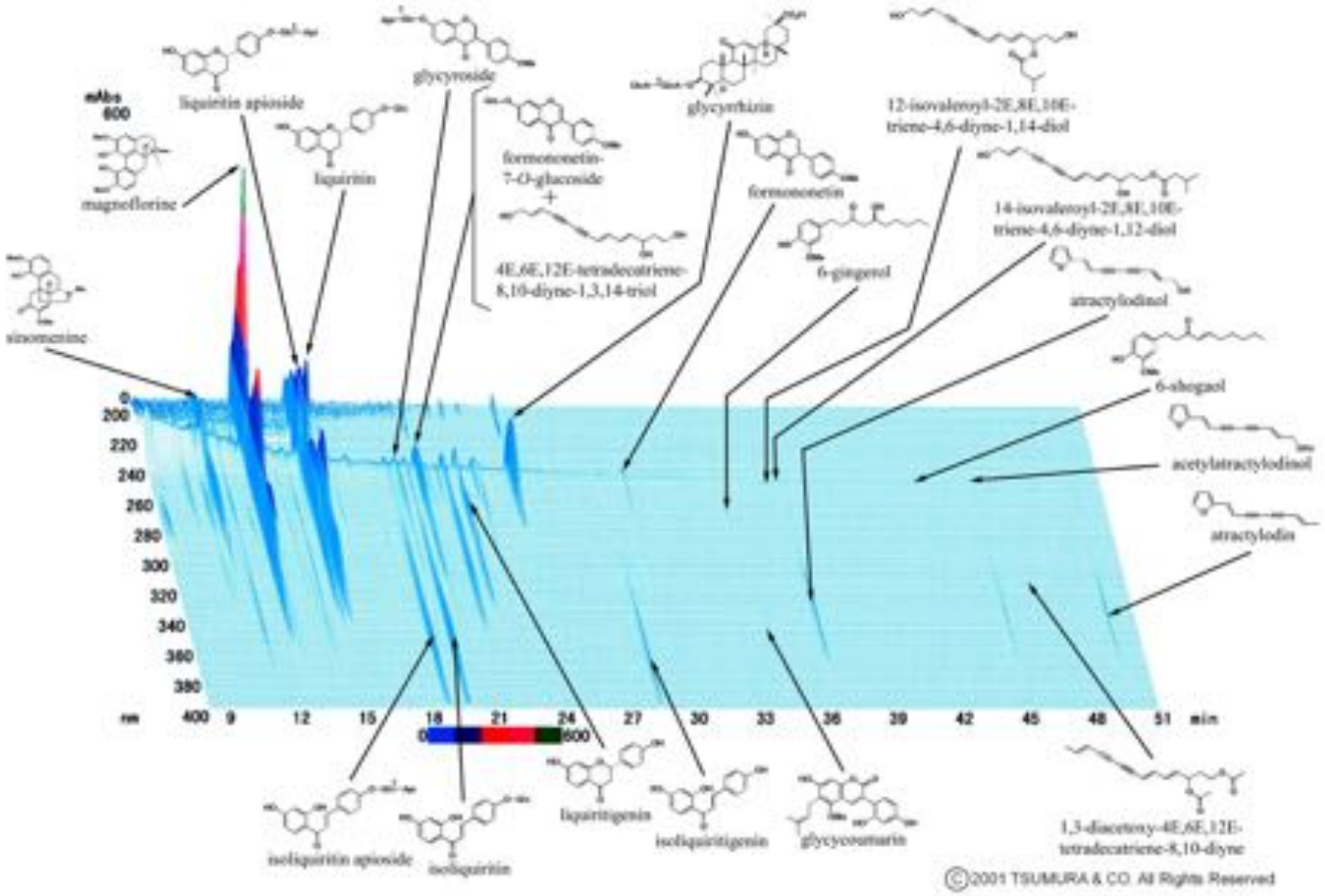
Figure 6. Effect of KBG on obesity-associated gene expression in liver tissue of DIO mice.

C57BL/6 mice were pre-fed with a normal diet (ND) or high-fat diet (HFD) for 10 weeks, and then administered control water or Kampo extracts (500 mg/kg, p.o., daily) for 12 weeks under the same feeding conditions. After 8 weeks of control or Kampo extract treatment, mice fed with HFD were changed to ND until termination of the experiment while maintaining the Kampo extract treatment for another 4 weeks. Liver tissue samples were collected upon termination and the relative expression of indicated obesity-associated genes was determined by RT-PCR and shown as relative expression to HFD group (HFD = 1). Data are mean ± SEM (n=7-15). * $p<0.005$, ** $p<0.001$ as compared with the HFD group.

Table 1. Sequences of the primers used in real-time PCR of the mouse tissue.

Gene	**Forward primer**	**Reverse primer**
Lipase (Lipe)	CAGGCTCACAGTTACCATCTC	TGTCCTTCCCGTAGGTCATA
LPL	GCCCGAGGTTTCCACAAATA	GCTGAAGTAGGAGTCGCTTATC
PPARγ	GAACCTGCATCTCCACCTTATT	TGGAAGCCTGATGCTTTATCC
PPARα	CGGTGTGTATGAAGCCATCT	TAAGGAACTCGCGTGTGATAAA
Cpt1α	CTCTGCTGCATGGTAGATGTT	GCTCTGCGTTTATGCCTATCT
SREBP1	CATCGACTACATCCGCTTCTT	CACCAGGTCCTTCAGTGATTT
TNF-α	AAGCCTGTAGCCCACGTCGTA	GGCACCACTAGTTGGTTGTCTTTG

A


liquiritin apioside
glycyroside
glycyrrhizin
12-isovaleroyl-2E,8E,10E-triene-4,6-diyne-1,14-diol
liquiritin
formononetin-7-O-glucoside
formononetin
magnoflorine
4E,6E,12E-tetradecatriene-8,10-diyne-1,3,14-triol
6-gingerol
14-isovaleroyl-2E,8E,10E-triene-4,6-diyne-1,12-diol
sinomenine
atractylodinol
6-shogaol
acetylatractylodinol
atractylodin
isoliquiritin apioside
isoliquiritin
liquiritigenin
isoliquiritigenin
glycycoumarin
1,3-diacetoxy-4E,6E,12E-tetradecatriene-8,10-diyne
mAbs 600
nm
min
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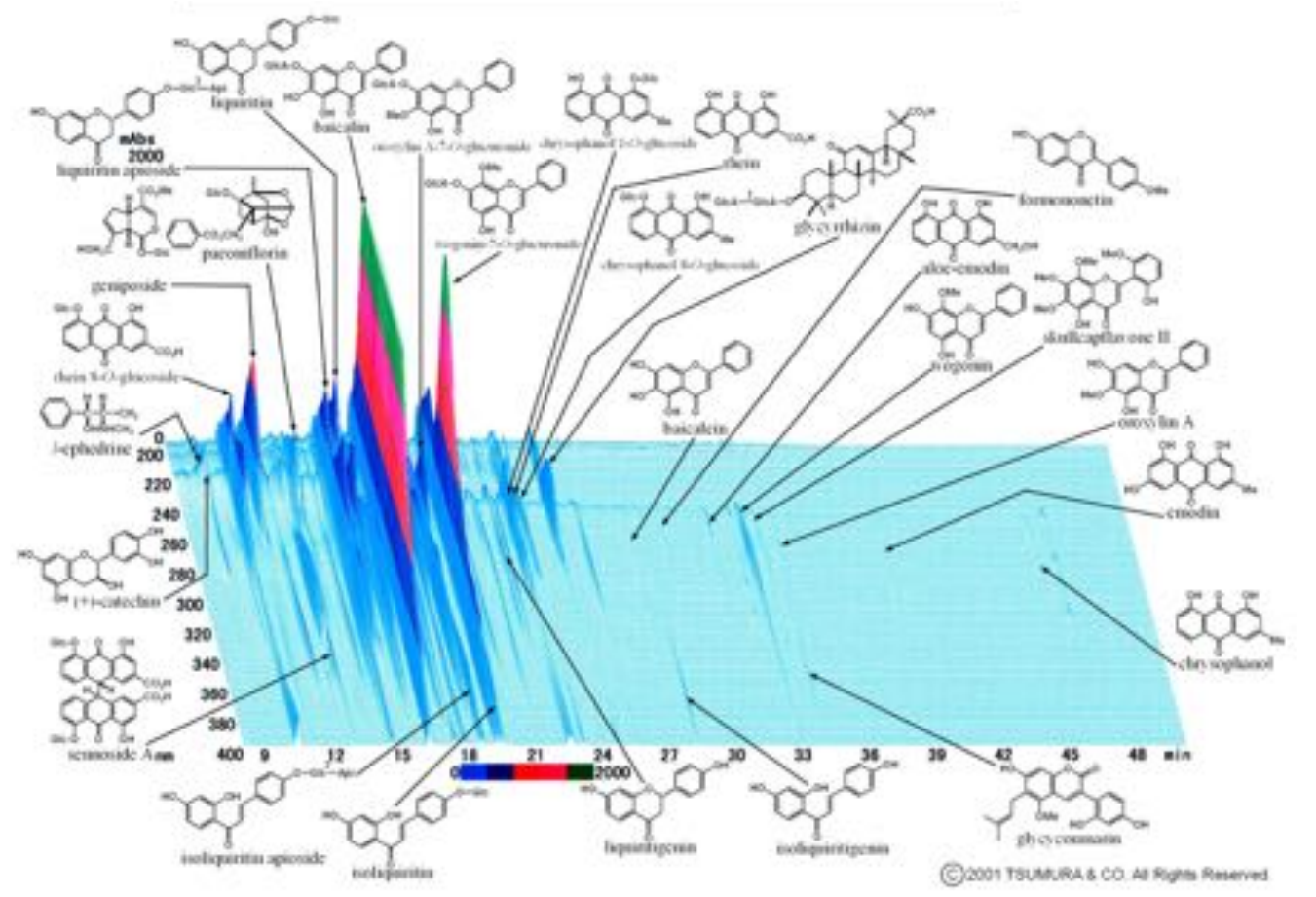
B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glycyrrhizin
formononetin
aloe-emodin
wogonin
skullcapflavone II
oroxylin A
emodin
chrysophanol
isoliquiritin apioside
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isoliquiritigenin
glycycoumarin
nm
min
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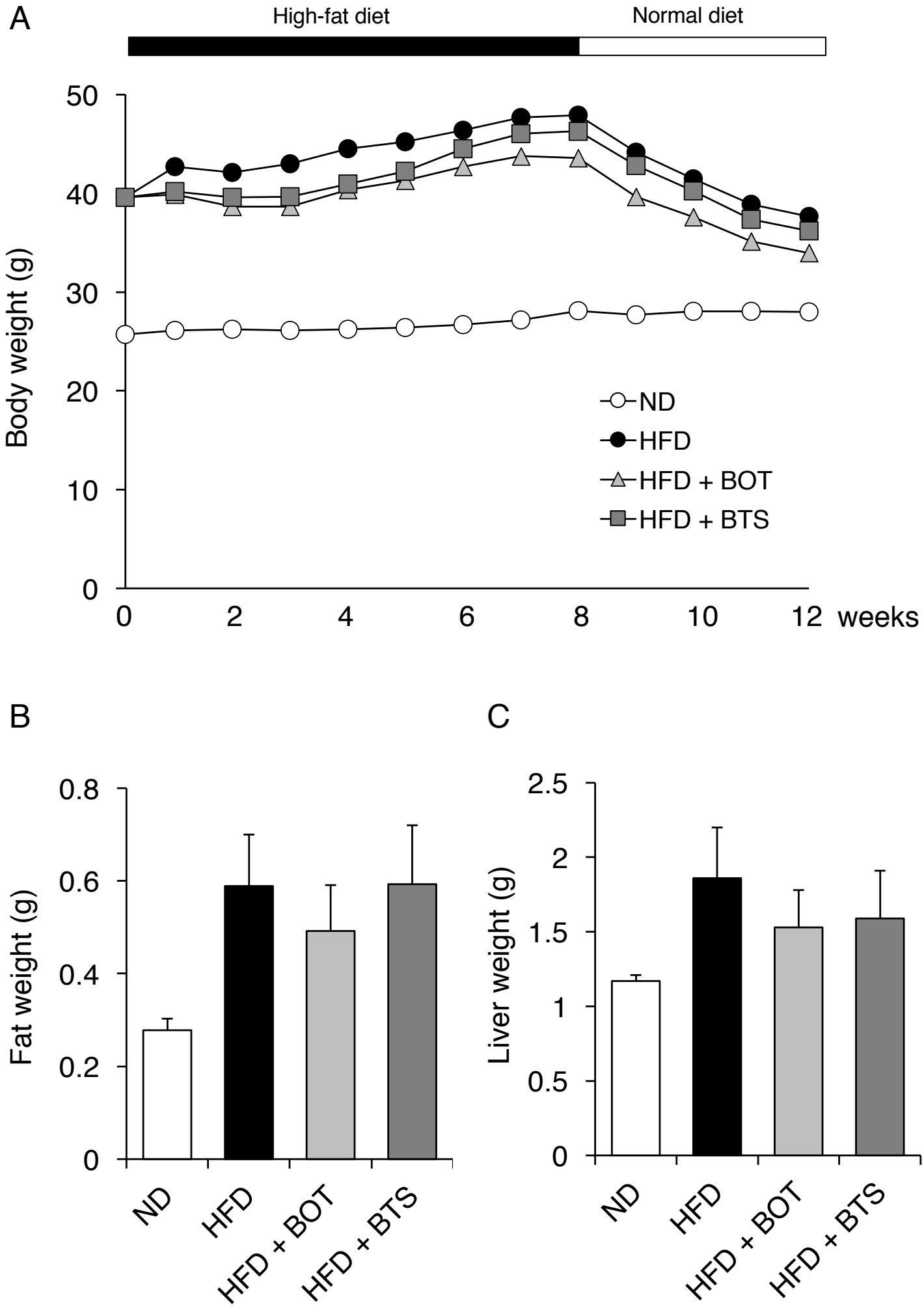
Figure 1


A
High-fat diet
Normal diet
Body weight (g)
50
40
30
20
10
0
0
2
4
6
8
10
12
weeks
ND
HFD
HFD + BOT
HFD + BTS
B
Fat weight (g)
0.8
0.6
0.4
0.2
0
ND
HFD
HFD + BOT
HFD + BTS
C
Liver weight (g)
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2
1.5
1
0.5
0
ND
HFD
HFD + BOT
HFD + BTS

Figure 2

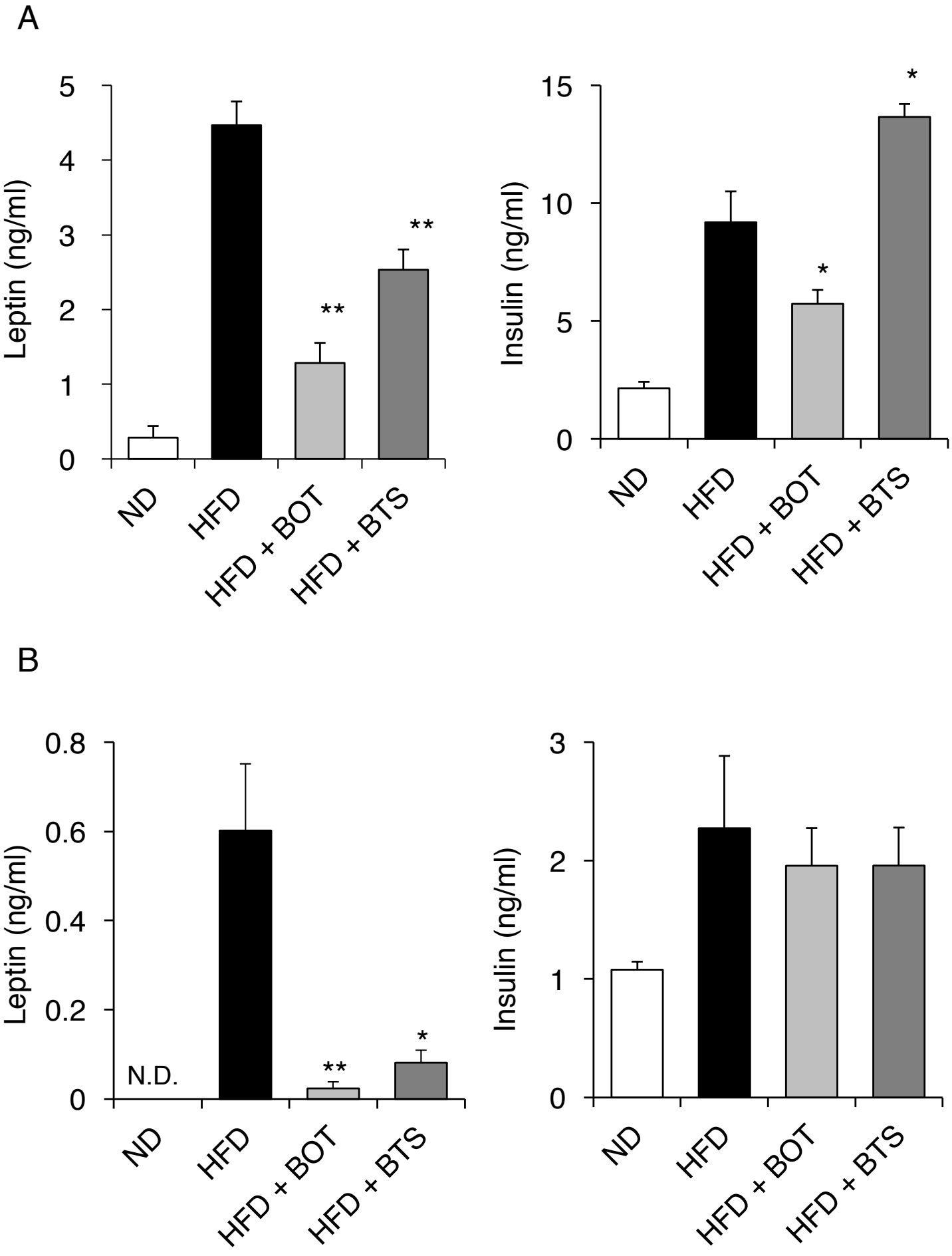

A
Leptin (ng/ml)
0
1
2
3
4
5
**
**
ND
HFD
HFD + BOT
HFD + BTS
Insulin (ng/ml)
0
5
10
15
*
*
ND
HFD
HFD + BOT
HFD + BTS
B
Leptin (ng/ml)
0
0.2
0.4
0.6
0.8
N.D.
**
*
ND
HFD
HFD + BOT
HFD + BTS
Insulin (ng/ml)
0
1
2
3
ND
HFD
HFD + BOT
HFD + BTS

Figure 3

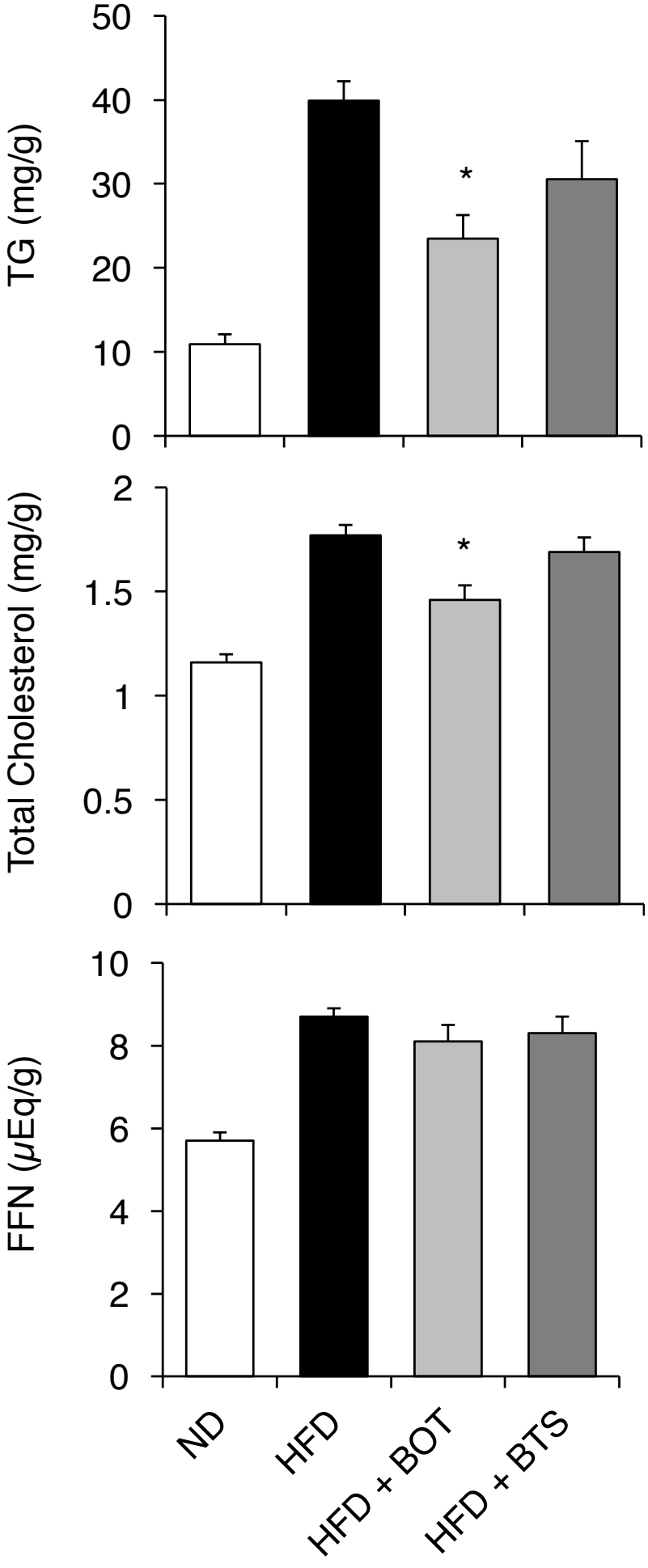
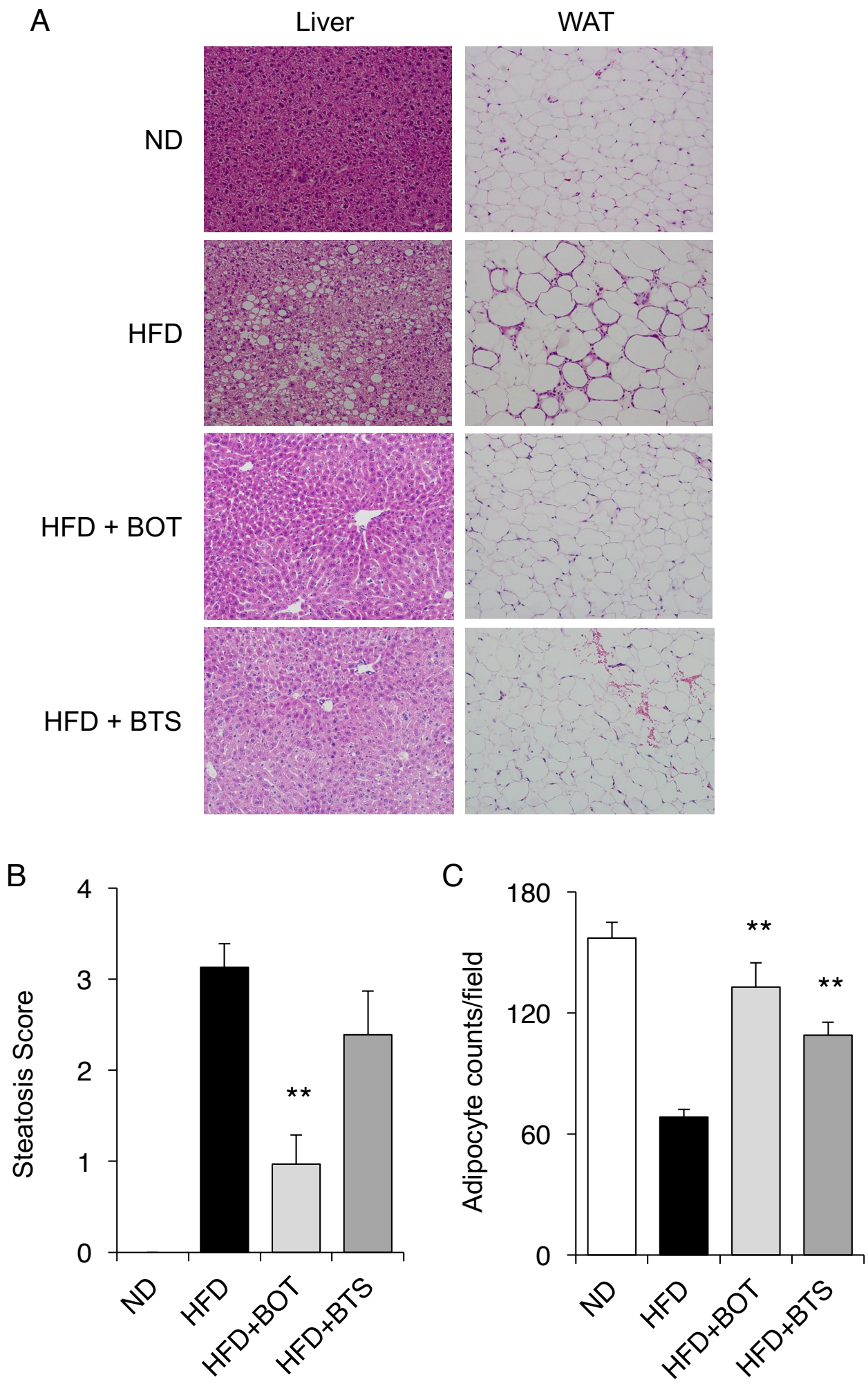

TG (mg/g)
50
40
30
20
10
0
*
Total Cholesterol (mg/g)
2
1.5
1
0.5
0
*
FFN (μEq/g)
10
8
6
4
2
0
ND
HFD
HFD + BOT
HFD + BTS

Figure 4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Liver
WAT
ND
HFD
HFD + BOT
HFD + BTS
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Steatosis Score
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1
2
3
4
**
ND
HFD
HFD+BOT
HFD+BTS
C
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60
120
180
**
**
ND
HFD
HFD+BOT
HFD+BTS

Figure 5

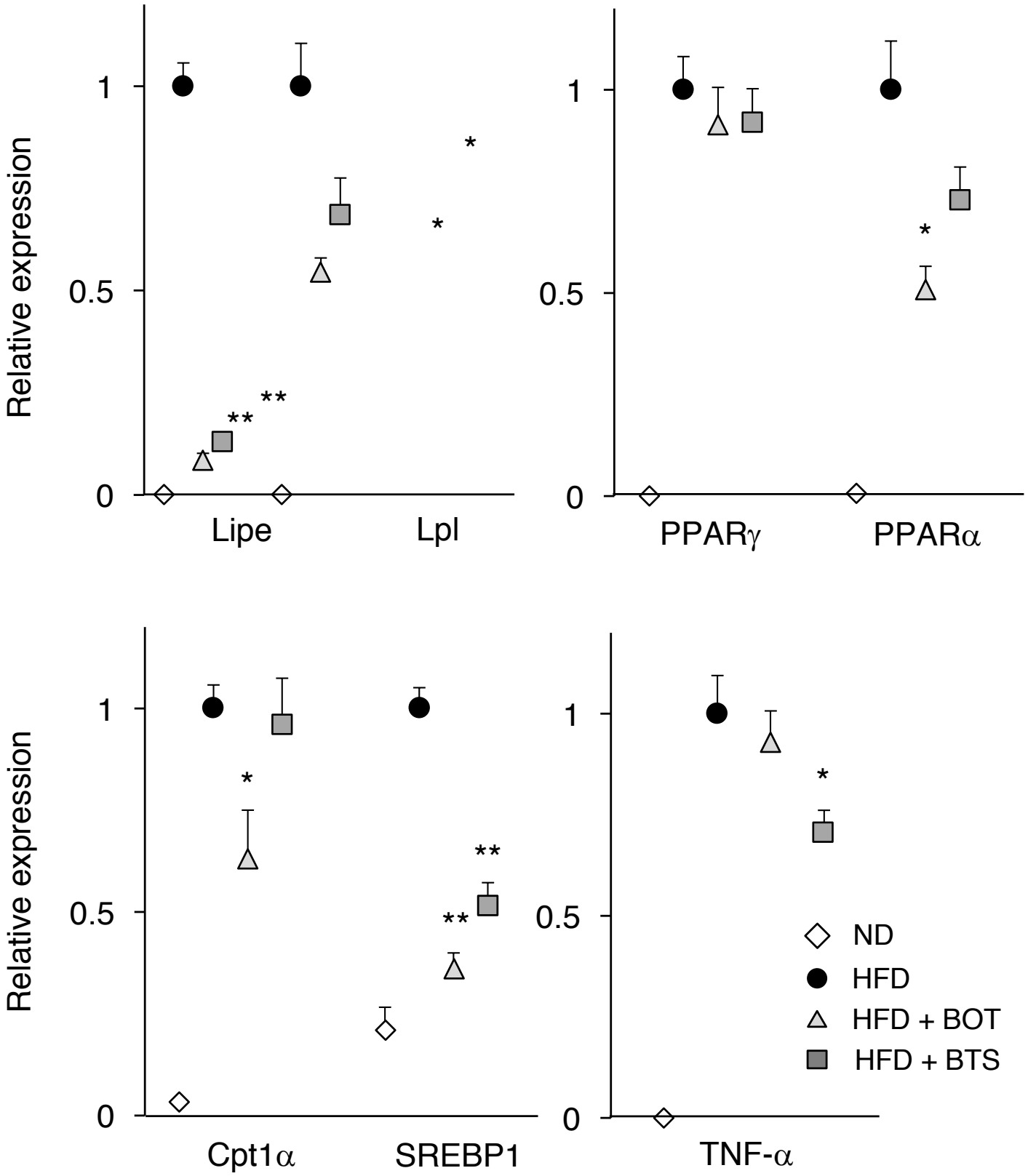
Relative expression
1
0.5
0
Lipe
Lpl
PPARγ
PPARα
Cpt1α
SREBP1
TNF-α
ND
HFD
HFD + BOT
HFD + BTS

Figure 6