Effects of Xanthohumol-rich Extract from the Hop on Fatty Acid Metabolism in Rats Fed a High-fat Diet

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Abstract: Xanthohumol is the major prenylated flavonoid of female inflorescences of the hop plant (Humulus lupulus L.) and is a hydrophobic flavonoid. We examined the effects of dietary xanthohumol-rich hop extract in obese rats that was induced by feeding a high-fat diet. Dietary xanthohumol-rich hop extract significantly lowered the body weight gain of these rats compared to rats fed a high-fat diet without the extract. The increase of body weight, liver weight, and triacylglycerol levels in the plasma and liver of the rats fed a high-fat diet was ameliorated by dietary xanthohumol-rich hop extract. Dietary xanthohumol-rich hop extract tended to reduce hepatic fatty acid synthesis through the reduction of hepatic SREBP1c mRNA expression in the rats fed a high-fat diet. The excreted of triacylglycerol into feces also was promoted by dietary xanthohumol-rich hop extract. Thus, xanthohumol-rich hop extract may inhibit the increase of body weight, liver weight, and triacylglycerol in the plasma and liver induced by feeding high-fat diet through the regulation of hepatic fatty acid metabolism and inhibition of intestinal fat absorption. Therefore, xanthohumol-rich hop extract may exert preventive function on the increase of body weight and tissue triacylglycerol levels by overnutrition.

Key words: fatty acid metabolism, hop, obesity, rat, xanthohumol

1 INTRODUCTION

Overnutrition results in obesity, which then leads to a higher risk of developing dyslipidemia, hepatic steatosis, type 2 diabetes, and cardiovascular disease. The prevention or amelioration of obesity by dietary components can reduce the risk of these diseases. Polyphenols are a large and diverse class of compounds, many of which occur naturally in components of the human diet including beans, cereals, fruits, vegetables, and red wine.

There is a growing interest in the useful functions of various polyphenolic compounds against lifestyle diseases, particularly in their preventive or ameliorative function on obesity and type II diabetes. In recent study, dietary curcumin was shown to suppress the onset of obesity in C57/BL mice fed a high-fat diet\(^1\). \((-\)-Epigallocatechin gallate from green tea inhibits the increase of body weight, white adipose tissue weight, formation of fatty liver, and rise of blood glucose levels in mice fed a high-fat diet\(^2\). Acacia polyphenol also suppresses the increase of body weight, plasma glucose, and insulin levels in KK-A\(^{n}\) mice fed a high-fat diet\(^3\). Moreover, we found that dietary apple polyphenol, which contains a large amount of procyanidins, suppressed the weight gain and increase of white adipose tissues weight by regulating fatty acid metabolism in the liver and inhibiting the intestinal absorption of dietary fat\(^5\). Thus, the anti-obesity effects of many polyphenolic compounds have been examined and demonstrated in various animal models.

Recently, we found that procyanidin-rich polyphenols from hop (Humulus lupulus L.) pomace suppressed the increase in white adipose tissue weight, plasma and liver triacylglycerol levels, and fasting blood glucose levels in Otsuka Long-EvansTokushima Fatty rats\(^5\). In addition to procyanidins, other bioactive substances are found in the hop. Xanthohumol is a prenylated flavonoid found in the female inflorescences of the hop. Stevens and colleagues showed various structures of xanthohumol and their useful functions in maintaining or increasing health in their detailed studies\(^6,7\). They found that xanthohumol exerted antiproliferative and cytotoxic effects against breast cancer, colon cancer, and ovarian cancer cells in vitro\(^8\). Xanthohumol prevents the hepatic injury induced by
carbon tetrachloride in rats through its antioxidant properties. Xanthohumol also acts on farnesoid X receptor through a selective bile acid receptor modulator, such as guggulsterone or polyunsaturated fatty acids, and thereby ameliorates lipid and glucose metabolism in KK-A mice. Moreover, inhibition of the differentiation of preadipocytes and the reduction of triacylglycerol synthesis by xanthohumol was shown in an in vitro assay. Therefore, we hypothesized that xanthohumol from the hop may act as a food component with anti-obesity properties.

In the present study, we examined the effects of long-term feeding of xanthohumol-rich hop extract (XRHE) on the growth parameters of rats with obesity induced by feeding them a high-fat diet.

2 EXPERIMENTAL PROCEDURES
2.1 Reagents
XRHE was purchased from Asama Chemical Co., Ltd. (Tokyo, Japan). The general component of XRHE is summarized in Table 1.

2.2 Analysis of XRHE
The level of phenolics in XRHE was dissolved in methanol and measured by Folin-Ciocalteu method. Phenolic compounds in XRHE were extracted by ethyl acetate, and analyzed by the Shimadzu LCMS 2010A series HPLC-UV-MSI system (Shimadzu Co., Kyoto, Japan) with YMC-Pack Pro C18 RS column (5 μm, 150 mm × 2.0 mmI.D., YMC Co. Ltd., Kyoto, Japan). Phenolic compounds were eluted using the mobile phase consisting of 1% (v/v) aqueous acetic acid (phase A) and 1% acetic acid in acetonitrile-methanol (phase B) at a flow rate of 0.2 mL/min. The linear gradient of phase B was 0% for the first 1 min, increased from 0% to 100% from 1 to 45 min, and maintained at 100% from 45 to 60 min. The detector wavelength was set at 280 and 317 nm. The mass spectra were acquired in negative ion mode for phenolics analysis. Other MS conditions for both were set as follows. Ions were scanned from 45 to 600 m/z. Xanthohumol was shown in an in vitro assay. Therefore, we hypothesized that xanthohumol from the hop may act as a food component with anti-obesity properties.

In the present study, we examined the effects of long-term feeding of xanthohumol-rich hop extract (XRHE) on the growth parameters of rats with obesity induced by feeding them a high-fat diet.

Table 1 Components of xanthohumol-rich hop extract.

<table>
<thead>
<tr>
<th>Component</th>
<th>Content (%)</th>
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<tbody>
<tr>
<td>Crude fats*</td>
<td>60.1</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>30.0</td>
</tr>
<tr>
<td>Protein</td>
<td>0.9</td>
</tr>
<tr>
<td>Water</td>
<td>4.2</td>
</tr>
<tr>
<td>Ash</td>
<td>4.8</td>
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</table>

*Hydrophobic flavonoids such as xanthohumol was contained.

was used as the nebulizing gas in a flow rate of 1.5 L/min. The drying gas pressure was 0.1 MPa.

Total ion chromatogram of flavonoid in XRHE is shown in Fig. 1. Procyanidins were not identified in XRHE. The m/z (H⁺) values of the other major flavonoid (peak 1) except for xanthohumol (peak 2) were 355 and 418. The identified component of flavonoids in XRHE is summarized in Table 2.

2.3 Animals and diet
All animal experiments were conducted according to the guidelines provided by the ethical committee of experimental animal care at Meiji University (approval code: IACUC 10-0008).

Wistar rats (3-week-old males; Japan Laboratory Animal, Inc., Tokyo, Japan) were housed individually in a temperature- (22–24°C) and light-controlled (07:00-19:00) room. After 6 days acclimation, 21 Wistar rats were divided into 3 groups: the low-fat (LF) group (7 rats) was fed a low-fat diet (LF group; 7% corn oil); the high-fat (HF) group (7 rats) was fed a high-fat diet (HF group; 7% corn oil and 20.8% lard), and the XRHE group (7 rats) was fed a high-fat diet supplemented with 1% XRHE. The diets were prepared according to the AIN93G recommendations; the detailed composition of the diets is shown in Table 3. The rats were pair-fed by measuring their daily food consumption. Body weight was measured every 7 days. After 41 days, the rats were anesthetized using diethyl ether and bled from the abdominal aorta; various tissues were then excised quickly. Plasma was prepared by centrifugation after allowing the blood to clot at room temperature. These samples were kept at −80°C until analyzed.

2.4 Plasma and liver lipid analysis
The levels of plasma triacylglycerol, free fatty acids, total cholesterol, and high-density lipoprotein (HDL)-cholesterol were measured using commercial kits (Wako Pure Chemical Industries, Ltd., Osaka, Japan). After the rats were killed, liver lipids were extracted by the method described by Folch et al. The levels of liver triacylglycerol, cholesterol, and phospholipids were measured according to the methods of Ide et al., Burchard et al., and Rouser et al., respectively.

2.5 Plasma adipocytokine levels
The plasma levels of adiponectin, leptin, and monocyte chemoattractant protein-1 (MCP-1) were measured using an Adiponectin (Rat) ELISA Kit (AdipoGen Co., CA, USA), Leptin (Mouse/Rat) ELISA Kit (Enzo Life Sciences International, Inc., NY, USA), and RayBio Rat MCP-1 ELISA Kit (RayBio tech, Inc., GA, USA), respectively.

2.6 Preparation of enzyme solution from liver
After sacrifice, a section of liver (approximately 3 g) was
Fig. 1 Analysis of flavonoids in XRHE. A: Total ion chromatogram of XRHE by high performance liquid chromatography mass spectrometry analysis. B: The mass spectrum of peak 1. C: The mass spectrum of peak 2 (Xanthohumol).
The supernatant acquired in the 9,000 × g centrifugation step was centrifuged at 105,000 × g for 1 h to isolate mitochondria. The mitochondria were washed twice with the homogenizing solution and finally resuspended in 3 mL of this solution. The supernatant was again centrifuged at 9,000 × g for 10 min at 4°C to isolate mitochondria. The mitochondria were washed twice with the homogenizing solution and finally resuspended in 3 mL of this solution. The supernatant from the 9,000 × g centrifugation step was centrifuged at 105,000 × g for 60 min at 4°C to obtain a pellet containing microsomes; the supernatant from this step represented the cytosolic fraction.

### Enzyme activity assay

Fatty acid synthase (FAS) activity was determined spectrophotometrically according to the method of Nepokroeff et al. The reaction solution was 0.2 M potassium phosphate buffer (pH 7.0) containing 0.4 mM EDTA, 200 μM malonyl-CoA, 50 μM acetyl-CoA, 300 μM NADPH, and the sample (i.e., the supernatant acquired in the 9,000 × g centrifugation step). One unit of FAS was defined as the amount of enzyme required to synthesize 1 nmol palmitic acid per min (equivalent to the oxidation of 14 nmol NADPH) at 30°C.

Glucose-6-phosphate dehydrogenase (G6PDH) activity was measured as described previously. The reaction mixture (pH 7.6) contained 0.1 M Tris-HCl, 30 mM MgCl₂, 3.3 mM glucose-6-phosphate, 1.2 mM NADP, 0.5 μM mL 6-phosphogluconate dehydrogenase, and the sample (i.e., the supernatant acquired in the 105,000 × g centrifugation step). One unit of G6PDH activity was expressed as the production of 1 nmol NADPH per min.

Malic enzyme (ME) activity was assayed by measuring palmitoyl-CoA-dependent H₂O₂ production as described by Pongrats et al. The reaction mixture (pH 7.4) contained 1.5 M Tris-HCl, 0.12 M MgCl₂, 0.03 M L-malate, 3.4 mM NADP, and the sample (i.e., the supernatant acquired in the 105,000 × g centrifugation step). One unit of malic enzyme was defined as the amount of enzyme that yielded 1 nm of NADPH per min.

### 2.8 RNA extraction

Total RNA was extracted from rat liver tissue using RNAiso Plus (TAKAi RA Bio, Inc., Kyoto, Japan). The RNA concentration was determined by measuring the absorbance at 260 nm using a UV photometer. RNA samples were treated with DNase (RQ1 RNase-Free DNase; Promega, Fitchburg, WI, USA). After phenol-chloroform extraction, aliquots of the purified RNA samples were stored at −80°C.

### 2.9 Oligonucleotide primer sequences

The primers for reverse transcription polymerase chain reaction (RT-PCR) amplification of the rat sterol regulatory element-binding protein 1c (SREBP1c) gene were designed using Primer3Plus software (http://www.bioinformatics.nl/cgi-bin/primer3plus.cgi). The primers, synthesized by Operon Biotecnologies (Tokyo, Japan), were designed to flank known or putative introns of this gene, thereby preventing the amplification of any contaminating genomic DNA. The primer sequences were as follows: SREBP1c (Gene ID: 78968), forward 5′-GGAGCCATTGGATTGCA-CATT-3′ and reverse 5′-AGGAAGGCCCAAGAGGAGGA-3′; and glyceraldehyde 3-phosphate dehydrogenase (GAPDH, Gene ID: 24383), forward 5′-CTCATGACCACAGTCCATCAGC-3′ and reverse: 5′-TTCAGCTCTGGGATGACCTT-3′, which was used as a control.

### 2.10 Real-time quantitative polymerase chain reaction

One microgram of RNA was incubated at 65°C for 5 min, and then quickly cooled on ice. Reverse transcription of RNA was performed using a ReverTra Ace qPCR RT Kit (Toyobo Co., Ltd., Osaka, Japan) and by heating the sample to 37°C for 15 min, followed by heating at 98°C for 5 min. An aliquot of the generated cDNA samples was mixed with 5 μL THUNDERBIRD SYBR qPCR Mix (Toyobo Co., Ltd.,
Osaka, Japan) in the presence of 0.3 μmol of each of the forward and reverse primers for GAPDH and SREBP1c. This reaction mix was then subjected to the following cycling conditions in a Chromo 4 Sequence Detection System (Bio-Rad Laboratories, Inc., Hercules, CA, USA): 1 cycle at 95°C for 1 min, and thereafter, 40 cycles each of 95°C for 15s and 58.5°C for 1 min. The results (fold-changes) were expressed as relative-fold by comparing the amount of RNA of the target gene to that of GAPDH as an internal control, as determined by the equation $2^{-\Delta\Delta Ct}$.

2.11 Statistical analysis

The data are expressed as the mean ± standard error (SE). The data were analyzed statistically using one-way analysis of the variance and the Tukey-Kramer test to evaluate significant differences among the means of the 3 groups. Differences were considered significant at $p < 0.05$.

3 RESULTS

3.1 Effects of dietary XRHE on growth parameters

Body weight was higher in the rats fed a high-fat diet (HF and XRHE groups) than in those receiving a normal diet (LF group) from days 7 to 41 (Fig. 2). However, dietary XRHE significantly reduced the body weight gain from days 21 to 41 compared to the HF group. Therefore, body weight gain at 41 days was also significantly lower in the XRHE group than in the HF group (Table 4). Liver weight was significantly lower in the XRHE group than in the LF and HF groups. Moreover, the weight of mesenteric white adipose tissue in the XRHE group tended to be lower than in the HF group ($p = 0.17$). However, the weights of epididymal, perirenal, and subcutaneous white adipose tissues were not significantly different between the 3 groups.

3.2 Effects of dietary XRHE on plasma and liver lipid levels

Plasma triacylglycerol levels were higher in the HF group than in the LF group; however, they were significantly lower in the XRHE group than in the HF group (Table 5). Plasma free fatty acids levels were the highest in the HF group, and they tended to be lower in the XRHE group than in the HF group. Plasma total cholesterol and HDL-cholesterol levels were not significantly different between

### Table 4 Effects of dietary XRHE on growth parameters.

<table>
<thead>
<tr>
<th></th>
<th>Group</th>
<th></th>
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<tr>
<td></td>
<td>LF</td>
<td>HF</td>
<td>XRHE</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Food intake (g)</td>
<td>552 ± 1</td>
<td>550 ± 1</td>
<td>547 ± 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial body weight (g)</td>
<td>78 ± 1</td>
<td>78 ± 1</td>
<td>79 ± 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>264 ± 2*</td>
<td>342 ± 4*</td>
<td>324 ± 3*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight gain (g)</td>
<td>185 ± 3*</td>
<td>263 ± 3*</td>
<td>246 ± 3*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Final waist (cm)</td>
<td>13.7 ± 2.4</td>
<td>15.4 ± 3.7</td>
<td>14.9 ± 2.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>9.74 ± 0.21*</td>
<td>12.6 ± 0.48*</td>
<td>10.9 ± 0.26*</td>
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</tbody>
</table>

WAT weight

|          | Epididymal WAT (g) | 4.51 ± 0.32* | 6.19 ± 0.43* | 6.67 ± 0.46* |        |     |
| Perirenal WAT (g) | 5.01 ± 0.63* | 8.31 ± 0.74* | 8.89 ± 1.00* |        |     |     |
| Mesenteric WAT (g) | 2.01 ± 0.20* | 3.44 ± 0.31* | 2.86 ± 0.32* |        |     |     |

Date are presented as the mean ± SE of 6–7 rats in each group.

*Values without a common superscript letter are significantly different at $p < 0.05$.

WAT, white adipose tissue. The other abbreviations are the same as in Table 2.
Liver triacylglycerol levels were significantly higher in the HF and XRHE groups than in the LF group; however, they were significantly lower in the XRHE group than in the HF group. Liver total cholesterol levels were significantly higher in the HF group than in the LF group. Dietary XRHE significantly lowered the total cholesterol level in the rats fed a high-fat diet.

### 3.3 Effects of dietary XRHE on plasma adiponectin, MCP-1, and leptin levels

Plasma adiponectin levels were significantly lower in the HF and XRHE groups than in the LF group; however, they tended to be higher in the XRHE group than in the HF group. Dietary XRHE significantly lowered the total cholesterol level in the rats fed a high-fat diet.

### 3.4 Effects of dietary XRHE on hepatic fatty acid synthesis activity

Hepatic FAS activity in the HF group was the highest among the 3 groups; however, it tended to be lower in the XRHE group than in the HF group (Fig. 4). Hepatic G6PDH activity was significantly lower in the HF and XRHE groups than in the LF group, and it tended to be lower in the XRHE group than in the HF group. There was no significant difference in hepatic ME activity between the groups, although it was lowest in the XRHE group.
Effects of dietary XRHE on SREBP1c expression in the liver. Data are presented as the mean±SE of 6–7 rats in each group. a,bValues without a common superscript letter are significantly different at p<0.05. FAS, fatty acid synthase; G6PDH, glucose-6-phosphate dehydrogenase; ME, malic enzyme. The other abbreviations are the same as in Table 2.

Effect of dietary XRHE on SREBP1c expression in the liver

The HF group had the highest expression levels of hepatic SREBP1c mRNA; however, dietary XRHE significantly lowered its levels in the rats fed a high-fat diet (Fig. 5).

4 DISCUSSION

We examined the effects of a high-dose of dietary XRHE on the growth parameters of rats fed a high-fat diet. Dietary XRHE significantly reduced the body weight gain in the rats fed a high-fat diet from days 21 to 41, suggesting that dietary XRHE inhibits the increase of body weight induced by a high-fat diet. Contrary to this observation, dietary XRHE did not have an effect on epididymal, perirenal, or mesenteric white adipose tissue; however, liver weight was significantly lowered compared to that of rats fed a high-fat diet. The weights of organs such as cecum other than the liver and other subcutaneous white adipose tissue may be lower in the XRHE group than in the HF group. Effect on the mass of total abdominal subcutaneous white adipose tissue must be examined in the next study because dietary hydrophobic flavonoid such as kaempferol decreased thickness of subcutaneous fat in broiler chickens.

We found that dietary XRHE lowered plasma triacylglycerol levels in the rats fed a high-fat diet. Cho et al. reported that dietary caffeic acid and chlorogenic acid lowered plasma triacylglycerol level in mice fed a high fat diet through inhibition of hepatic fatty acid synthesis and activation of hepatic fatty acid β-oxidation. Moreover, azuki-bean extract containing 50% polyphenols suppressed serum triacylglycerol level in rats fed a high fat diet through the activation of hepatic peroxisome proliferator-activated receptor alpha. Xanthohumol in XRHE may also regulate plasma triacylglycerol level through the modulation of hepatic fatty acid synthesis as described below; although xanthohumol is hydrophobic and its structure is different from the above-mentioned phenolics.

A high-fat diet also increased fat accumulation in the liver and induced fatty liver; however, dietary XRHE lowered fat accumulation in the liver of the rats fed a high-
fat diet. Excessive fat accumulation in the liver could be initiated by the increased synthesis of fatty acids via the de novo pathway, and increased hepatic fatty acid synthesis from carbohydrates is attributed to the progression of obesity. Dietary xanthohumol reduced hepatic SREBP1c mRNA expression in KKA-Δ mice. Moreover, this observation was well correlated with the decreased plasma and hepatic triacylglycerol levels and the decreased liver weight in the XRHE group. Thus, dietary XRHE inhibits hepatic fatty acid synthesis in the rats fed a high-fat diet, similar to other polyphenols.

We also found that the XRHE group excreted significantly larger amounts of feces than the other 2 groups (LF group: 1.11 ± 0.07 g/day; HF group: 1.24 ± 0.05 g/day; XRHE group: 1.60 ± 0.08 g/day). In addition, the triacylglycerol levels in the feces of the XRHE group were the highest among the 3 groups (LF group: 7.63 ± 1.08 g/day; HF group: 9.75 ± 0.95 g/day; XRHE group: 33.75 ± 3.56 g/day). Tea catechin inhibits pancreatic lipase activity in vitro; therefore, dietary XRHE may exert anti-obesity effect in the rats fed a high-fat diet through the reduction of lipid absorption from the small intestine by inhibiting lipase activity. Effects of XRHE on intestinal absorption of fat must be elucidated in future.

Adipocytes secrete a variety of bioactive molecules that affect the insulin sensitivity of other tissues and regulate energy homeostasis, thereby functioning as an important endocrine organ. We observed that dietary XRHE tended to increase plasma adiponectin levels in the rats fed a high-fat diet (p = 0.08). Adiponectin improves the insulin sensitivity of the liver and skeletal muscle, resulting in a reduction of the progression of atherosclerosis. Yamauchi et al. reported that adiponectin decreases insulin resistance by decreasing triacylglycerol levels in the muscle and liver of obese mice by increasing the expression of molecules involved in fatty acid combustion and energy dissipation in muscle. Therefore, dietary XRHE may exert its anti-obesity effect by reducing fatty acid synthesis in the liver and promoting fatty acid oxidation in the skeletal muscle.

The bioavailability of xanthohumol has not yet been completely elucidated. Yilmazer et al. reported the metabolism of xanthohumol using rat and human microsomes. More recently, Legette et al. elucidated a part of bioavailability of xanthohumol and its metabolism in rats after oral or intravenous administration. Xanthohumol is a hydrophobic flavonoid, although many polyphenolic compounds are hydrophilic; therefore, the bioavailability of xanthohumol may be higher than that of hydrophilic polyphenols. Legette et al. also reported that dietary xanthohumol lowered body weight and fasting plasma glucose in obese Zucker rats. They speculate that xanthohumol may exert anti-obesity effect through mitochondrial uncoupling and stress response induction in the newest report. Their hypothesis also may relate to our observed results. However, our used XRHE contains unknown flavonoids and other components in addition to xanthohumol. Therefore, the effects of the other components of XRHE on the phenomena observed in this study cannot be ignored. In a future study, we will examine the effects of the other components of XRHE to determine the main component responsible for the anti-obesity action of XRHE.

5 CONCLUSION

We found that dietary XRHE, containing approximately 18% xanthohumol, inhibited the increase of body weight, liver weight, and triacylglycerol levels in the plasma and liver of rats fed a high-fat diet. The modulation of hepatic fatty acid synthesis and secretion of adiponectin induced by dietary XRHE may contribute to these observations, although these were not necessarily significant effects. The inhibition of intestinal triacylglycerol absorption by dietary XRHE may also be involved in the reduction of body weight gain and triacylglycerol levels in the plasma and liver. Thus, xanthohumol-rich XRHE may inhibit the increase of body weight associated with consuming a high-fat diet.

References

Xanthohumol and fatty acid metabolism


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