Anti-obesity Effects of Escins Extracted from the Seeds of *Aesculus turbinata* Blume (Hippocastanaceae)

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To investigate the anti-obesity effects of escins extracted from the seeds of *Aesculus turbinata* Blume, anti-obesity models *in vitro* and *in vivo* were employed. In a preliminary experiment, different solvent fractions of *Aesculus turbinata* Blume as well as two isolated compounds were tested for their effects on pancreatic lipase (PL) *in vitro*. Subsequently, female ICR mice were fed a high fat diet with or without different concentrations of total escins for 11 weeks to examine body weight, parametrial adipose tissue weight, and hepatic triacylglycerol (TG) and total cholesterol (TC) contents. Plasma triacylglycerol levels (TG) after oral administration of lipid emulsions to rats were also investigated. The results showed that total escins (1 mg/ml) as well as two compounds isolated from total escins, namely escin Ib and IIa, showed inhibitory effects on PL activity. *In vivo*, total escins suppressed the increase in body weight, parametrial adipose tissue weight, TG content, and TC content in mice's liver; TG content in rat plasma was also reduced at 1, 2 and 3 h after oral administration of the lipid emulsion plus different concentrations of escins compared to those in the lipid emulsion groups. Meanwhile, mice fed a high fat diet plus 2% total escins for 3 d had an increased TG level in the feces compared to the HF group. The reason for this may be due to a delay in the intestinal absorption of dietary fat by inhibiting PL activity.

Key words escin; pancreatic lipase; high diet; obesity; mice

The prevalence of obesity has been increasing worldwide during the past years, and is reaching epidemic proportions in developed countries.[1)] Obesity, which has an important impact on life style-related diseases such as coronary heart disease,[2)] dyslipidemia,[3)] glucose intolerance,[4)] diabetics[5)] and elevated blood pressure[6)] is being given ever-increasing importance.

Aesculus turbinata Blume (Hippocastanaceae), is a medicinal plant widely distributed in northwestern China. Its dried ripe seeds have been used as a carminative, stomachic, and analgesic for the treatment of distention and pain in the chest and abdomen.[7)] The saponin mixture extracted from the seeds is called escins, which is a mixture of triterpene oligoglycosides. Escins have shown distinguished anti-inflammatory, anti-edema, capillary protective, hypoglycemic and ethanol absorption inhibitory activity.[8,9)] Recently, escins have been reported to show inhibitory effects on the elevation of blood glucose levels and inhibitory effects on pancreatic lipase activity in mice.[10)] Pancreatic lipase is well known to be critical lipase in the development of obesity. Until now, orlistat,[11)] a pancreatic lipase inhibitor, has been widely used clinically to treat obesity. However, it has been reported that orlistat causes gastrointestinal side effects during long-term pharmacotherapy for obesity and overweight.[12)] Therefore, it is necessary to develop new drugs with less side effects from natural plants to treat obesity and overweight. In order to provide further evidence as to whether escins possess anti-obesity effects, we investigated their effects in obese mice and rats. The results showed that escins have a strong inhibitory effect on obesity not only *in vitro* but also *in vivo*.

Experimental

Materials Pancreatic lipase and triolein were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Triglyceride E-test and Total Cholesterol E-test kits were purchased from Wako Pure Chemical Co. (Osaka, Japan). Laboratory pellet chow was purchased from CLEA Japan (Osaka, Japan). Beef tallow, casein, vitamin and mineral mixtures were purchased from Oriental Yeast Co. Ltd. (Tokyo, Japan). Two authentic compounds, escin Ib and IIa, were received as kind gifts from Dr. M. Yoshikawa and Dr. Y. Kimura. Other chemicals were of reagent grade.

Animals Female ICR strain mice (3 weeks old) and male Wistar King strain rats (6 weeks old) were obtained from CLEA Japan (Osaka, Japan) and Charles River Japan (Yokohama, Japan), respectively. The animals were housed for 1 week under a 12 h/12 h light/dark cycle in a temperature and humidity-controlled room. The animals were given free access to food and water. After adaptation to the lighting conditions for 1 week, the healthy animals were used in the following experiments. The experimental protocols were approved by the Ethical Committee of Jilin Agricultural University.

Plant Materials The dried seeds of *Aesculus turbinata* Blume were obtained from Changchun Chinese Medical Material Co. in Changchun City, China, and were identified by Prof. H. Y. Bao. Voucher specimens (No. 0506) are deposited with the Faculty of Chinese Medical Material, Jinlin Agricultural University, China.

Preparation of Total Escins and Two Compounds from the Seeds of *Aesculus turbinata* Blume The dry powdered seeds of *Aesculus turbinata* Blume (5 kg) were soaked in 80% aqueous ethanol (10 l) overnight. Ultrasound-assisted extraction was applied three times for 30 min at room temperature. After filtration, the three extracts were combined and then evaporated to dryness by a rotary vacuum evaporator to yield an aqueous ethanol extract (0.85 kg). The ethanol extract was dissolved in distilled water (H_2O), passed through macroporous resin D101, and then eluted with H_2O, 80% ethanol. The 80% ethanol fraction was collected and evaporated to dryness using a rotary vacuum evaporator at 50 °C. The residue was re-dissolved in distilled H_2O, and extracted with acetic ether (EtOAc), water-saturated *n*-butanol (*n*-BuOH) to obtain an EtOAc fraction (38 g), an *n*-BuOH fraction (150 g) containing total escins, and an H_2O fraction (48 g). The *n*-BuOH fraction was further isolated several times by preparative HPLC (YMC-Pack, ODS, methanol–H_2O containing 10 mM sodium phosphate buffer,

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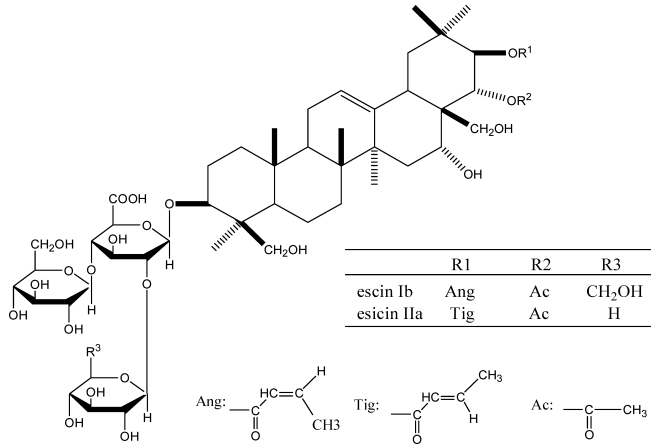

	R1	R2	R3
escin Ib	Ang	Ac	CH_2OH
esicin IIa	Tig	Ac	H

Fig. 1. The Structures of Escin Ib and IIa Isolated from the Seeds of *Aesculus turbinata* BLUME

Table 1. Composition of Experimental High Fat Diets

Composition	Treatment			
	HF+0	HF+0.35	HF+1	HF+2
Saponin	0	0.35	1	2
Beef tallow	40	40	40	40
Casein	36	35.65	35	34
Corn starch	10	10	10	10
Sugar	9	9	9	9
Vitamin mixture	4	4	4	4
Mineral mixture	1	1	1	1

HF+0: high fat diet+0% total escins; HF+0.35: high fat diet+0.35% total escins; HF+1: high fat diet+1% total escins; HF+2: high fat diet+2% total escins.

62 : 38, v/v) to give two known compounds escin Ib (50 mg) and IIa (230 mg). The two isolated compounds were identified by comparison of their physical data (IR, ^{1}H- and ^{13}C-NMR spectra) with those of authentic samples.[13] Figure 1 shows the structures of escins.

Diet Composition It has previously been reported that varying the casein concentration (22, 31, 34 and 36% diets) in a high fat diet containing 40% beef tallow did not affect body weight or parametrial adipose tissue weight.[14] Therefore, 0.35%, 1% or 2% escin extract was added into the high-fat diets as an equivalent mass of casein (Table 1). To avoid auto-oxidation of the fat components, food was stored at −30 °C. Laboratory chow pellet was used as a control diet.

Measurement of Pancreatic Lipase Activity Lipase activity in the porcine pancreas was assayed as described previously.[15] Lipase activity was expressed as μmol oleic acid released/ml of reaction mixture/min.

Estimation of Body Weight, Parametrial Adipose Tissue Weight, Hepatic TG and TC in Mice Fed a High-Fat Diet for 11 Weeks Female ICR mice (3 weeks old) were randomly divided into five groups matched for body weight after 1 week of being fed laboratory pellet chow. The control group continued to be fed laboratory pellet chow *ad libitum*. The remaining mice consumed HF diet or HF diet plus 0.35%, 1% or 2% total escins (*n*-BuOH fraction) for 11 weeks. The body weight of each mouse was measured once a week and the total amount of food consumption was recorded every day for 11weeks. After the mice had been fed these diets for 11 weeks, blood was taken by venous puncture under anesthesia with diethyl ether, and the mice were then killed with an overdose of diethyl ether. Experiments were performed in a ventilated room. The plasma was prepared and frozen at −80 °C until analysis. The liver and parametrial adipose tissue were dissected and weighed. Liver TG and TC concentrations were measured using Wako Triglyceride E-Test and Total Cholesterol E-Test kits.

Fat Excretion in Feces of Mice As has been reported previously,[14] female ICR mice (3 weeks old) were housed for 1 week in a room maintained at 25±1 °C with 60% relative humidity and given free access to standard laboratory pellet chow and water. The mice consumed the HF diet or the HF diet plus 0.35%, 1% or 2% total escins for 3 d. TG content in the feces obtained during the last 24 h was measured using a Wako Triglyceride E-Test kit.

Table 2. Effect of Fractions from the Seeds of *Aesculus turbinata* BLUME on PL Activity *in Vitro*

Sample	mg/ml	Activity of PL 100% *vs.* control
Aqueous ethanol extract	1	17.13±1.9*
EtOAc extract	1	91.30±10.9
H_2O extract	1	91.97±10.9
n-Butanol extract	1	6.38±2.1*
Orlistat	0.2	0.85±0.1*

* $p<0.05$, *vs.* control.

Plasma TG Levels after Oral Administration of Lipid Emulsions to Rats Male Wistar rats were also housed for 1 week under the same conditions as described above. After the rats had been deprived of food overnight, they were orally administered 1 ml of lipid emulsion consisting of corn oil (3 ml), cholic acid (40 mg), cholesteryloleate (1 g) and physiological saline (3 ml), plus total escins (final concentration 250 mg/kg body or 1 g/kg body). Blood samples were taken from the tail vein at 0, 1, 2, 3, 4 and 5 h after administration of the lipid emulsion with or without total escins using a capillary tube (heparinized), and centrifuged at 5500×***g*** for 5 min in a Model KH-120M (Kubota Co., Osaka, Japan) centrifuge to obtain the plasma. The plasma TG concentration was determined using a Wako Triglyceride E-Test Kit.

Statistical Analysis All values are expressed as means±S.E. Data were analyzed by one-way ANOVA, and then differences among means were analyzed using Scheffe's test. Differences were considered significant at $p<0.05$.

Results

Effects of Different Fractions and Isolated Compounds on Pancreatic Lipase Activity *in Vitro* As one of the critical indexes, inhibiting pancreatic lipase should be thoroughly considered in the clinic treatment of obesity. After solvent fractionation of the seeds of *Aesculus turbinata* BLUME, the inhibitory effects of different fractions were compared (Table 2). Aqueous ethanol and *n*-BuOH fractions were found to inhibit the PL activity at a concentration of 1 mg/ml (17.13±1.9, 6.38±2.05, respectively); whereas the EtOAc and H_2O fractions did not exhibit an inhibitory effect on PL. Figure 2 shows that total escins (*n*-BuOH fraction) as well as two isolated compounds, escin Ib and IIa, dose-dependently inhibited the pancreatic lipase activity in an assay system using triolein emulsified with lecithin. The activity of pancreatic lipase was 38.21%, 2.07% and 3.27%, respectively, compared to that in control at a concentration of 0.5 mg/ml.

Plasma TG Levels after Oral Administration of Lipid Emulsions to Rats Total escins reduced the elevation of rat plasma TG levels after oral administration of lipid emulsion to rats. Figure 3 shows the time course of the plasma TG level after oral administration of lipid emulsion. Total escins (250 mg/kg body) prevented the increase in rat plasma TG concentration at 60 and 120 min after oral administration of lipid emulsion. However, total escins (1 g/kg body) inhibited the increase in rat plasma TG concentrations at 60, 120 and 180 min. At 120 min after consumption of the high-fat diet plus 1 g/kg total escins, the TG content in rat plasma was reduced to 0.88±0.13 compared to that in the lipid emulsion group (1.83±0.73).

TG Content in Feces of Mice Figure 4 shows that consumption of the HF diet for 3 d could elevate the TG level in

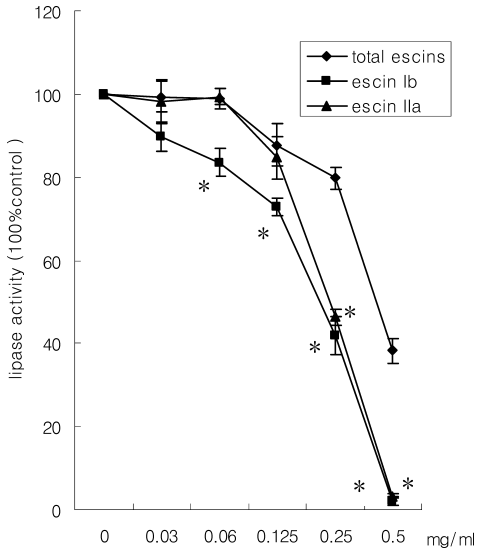

Fig. 2. Effect of Escins Extracted from the Seeds of *Aesculus turbinata* Blume on Pancreatic Lipase Activity (*in Vitro*)

Values are mean±S.E. of three experiments. The effects of escins at concentrations ranging from 0 to 0.5 mg/ml on pancreatic lipase activity were evaluated. $*p<0.05$, significantly different from total escins saponin fractions.

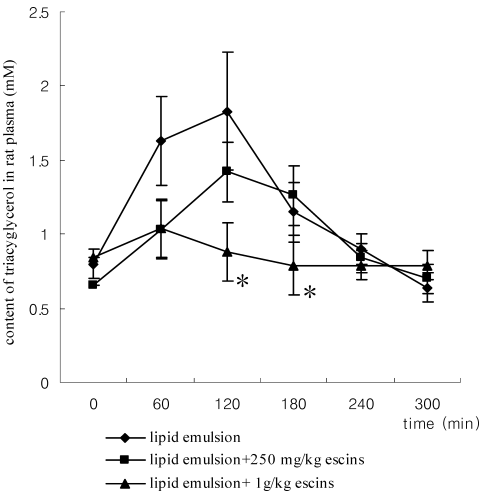

Fig. 3. Effects of Total Escins on Rat Plasma Triacylglycerol Levels after Oral Administration of a Lipid Emulsion

Values are mean±S.E. of 4 rats. $*p<0.05$, significantly different from lipid emulsion group.

the feces of mice compared to that of the control group. Mice fed the HF diet plus 2% total escins had a significantly higher TG level in the feces compared to the HF group. However, HF diet plus 0.35% and 1% total escins did not increase TG content.

Food Consumption; Body, Parametrial Adipose Tissue Weight, Content of Hepatic Triacylglycerol and Total Cholesterol in Mice Fed a High-Fat Diet for 11 Weeks The mean food consumption was significantly different between the control group (4.8±0.3 g/d/mouse) and HF group (3.0±0.3 g/d/mouse). However, there was no obvious difference in food consumption among the HF group (3.0±0.3 g/d/mouse), HF diet plus 0.35% total escins group (3.4±0.5 g/d/mouse), HF diet plus 1% total escins group (3.6±0.5 g/d/mouse), and HF plus 2% total escins group

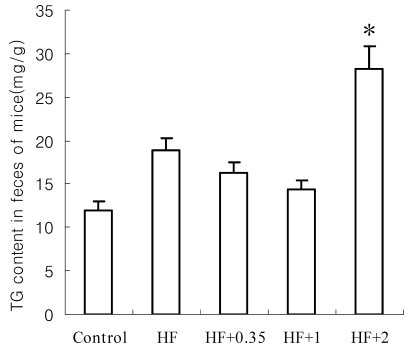

Fig. 4. Effects of Total Escins on TG Content in Feces of Mice That Consumed One of the Diets for 3 d

Control: normal diet; HF: high fat diet; HF+0.35: high fat diet plus 0.35% total escins; HF+1: high fat diet plus 1% total escins; HF+2: high fat diet plus 2% total escins. Values are mean±S.E. of 10—14 mice. $*p<0.05$, significantly different from HF group.

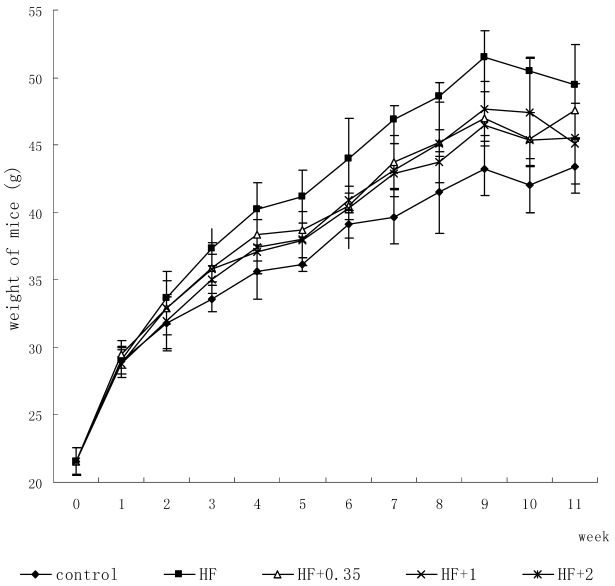

Fig. 5. Effects of Total Escins on Body Weight in Mice Fed High Fat Diet for 11 Weeks

Control: normal diet; HF: high fat diet; HF+2: high fat diet plus 2% total escins; HF+1: high-fat diet plus 1% total escins; HF+0.35: high fat diet plus 0.35% total escins. Values are mean±S.E. of 10—14 mice.

(3.4±0.6 g/d/mouse). Figure 5 shows the changes in body weight of each group during the experimental period. The body weight gain was reduced in groups treated with either HF diet plus 0.35%, 1% or 2% total escins in comparison to the HF group, but the relationship did not appear to be dose-dependent. The final parametrial adipose tissue weight was measured for the reason it is where fat is easily accumulated. As shown in Fig. 6, it was increased to 2.81±0.13 by consumption of the HF diet compared to the control group (1.5±0.21), whereas that in the animals fed the HF diet plus 2% total escins was significantly reduced to 2.22±0.15. Furthermore, the mice fed the HF diet for a long period of time developed fatty liver, and had an increase in liver weight and the accumulation of hepatic TG and TC compared to the control group. Consumption of the HF diet containing 0.35%, 1% or 2% total escins could significantly reduce the hepatic TG content, and it could also reduce liver weight and hepatic TC content compared with the HF group (Fig. 7).

Discussion

It is well known that obesity results from an imbalance between energy intake and energy expenditure, processes in which lipase and pancreatic lipase are critical. Fat tissue is hydrolyzed by lipase which releases fatty acid as well as triolein; on the other hand, a high fat diet can not be directly absorbed across the intestinal mucosa. At first, it must be decomposed into one 2-monoglyceride and two free fatty acids by pancreatic lipase, which is delivered into the lumen of the gut as a constituent of pancreatic juice.[16,17] Therefore, the anti-obesity mechanism of a drug should be in its decomposition of unnecessary fat or inhibition of absorption of fat from food. Activating lipase or inhibiting pancreatic lipase would have an anti-obesity effect. It has been reported that orlistat, one pancreatic lipase inhibitor, prevented obesity and hyperlipidemia through the enhancement of fat excretion in feces and inhibition of pancreatic lipase.[18]

There are several reports showing that a single oral dose of a total escins fraction was found to effectively inhibit pancreatic lipase activity,[10] lower blood glucose levels and lower plasma glucose levels.[19,20] In this study, firstly we attempted to examine the effect on pancreatic lipase using 80% aqueous ethanol fractions extracted from the seeds of *Aesculus turbinata* BLUME. *in vitro*. We found an aqueous ethanol extract had an inhibitory effect on pancreatic lipase. Next, total escins (*n*-BuOH fraction) as well as two major compounds, escin Ib and IIa isolated from total escins, were further tested and found to have inhibitory effects on PL activity (38.21%, 2.07%, 3.27%, respectively at concentration of 0.5 mg/ml). *In vivo*, the consumption of a high fat diet containing 2% total escins elevated the TG level in mouse feces at day 3 compared to the high fat diet groups. The reason for this appears to be due in part to inhibition of the absorption of dietary fat by inhibiting pancreatic lipase in the intestinal mucosa. Another likely reason was that total escins increased gastrointestinal motility,[21] resulting in a reduction of the absorption of dietary fat in the gastrointestinal tract. The results suggest that total escins suppressed fat accumulation *in vivo* as well as *in vitro*.

Recently, it has also been reported that the saponin in Panax ginseng,[22] the saponin in Platycodi radix,[23] and the saponin in Panax japonicus rhizomes,[24] all belonging to the family of triterpenoid saponins, showed strong inhibitory effects on pancreatic lipase *in vitro* and suppressed the increase of body weight induced by a high fat diet *in vivo*. In this study, total escins, which also belong to the triterpenoid saponins, have shown the same effect as mentioned above. Based on those results, compounds that possess the structure of triterpenoid saponins may have a similar anti-obesity effect. Further experiments should be conducted to confirm this.

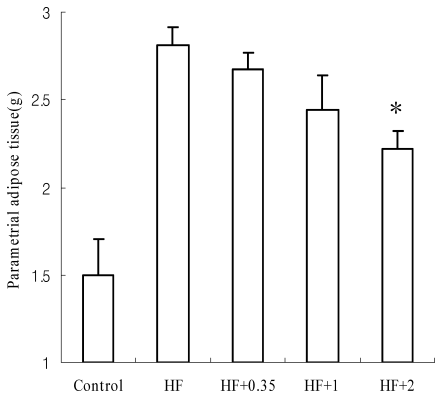

Fig. 6. Effects of Total Escins on Parametrial Adipose Tissue in Mice Fed High Fat Diet for 11 Weeks

Control: normal diet; HF: high fat diet; HF+0.35: high fat diet plus 0.35% total escins; HF+1: high fat diet plus 1% total escins; HF+2: high fat diet plus 2% total escins. Values are mean±S.E. of 10—14 mice. $*p<0.05$, significantly different from HF group.

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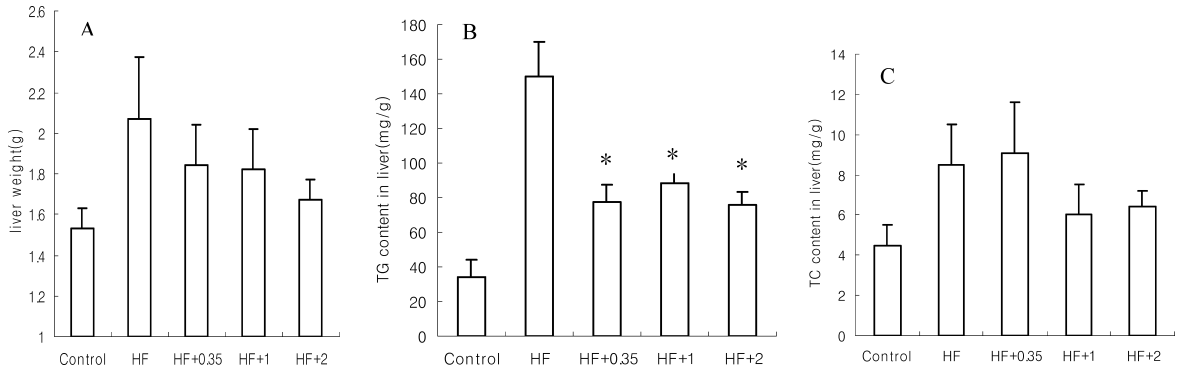

Fig. 7. Effects of Total Escins on Liver Tissue in Mice Fed High Fat Diet for 11 Weeks

(A) Effect on liver weight; (B) effect on TG content in liver; (C) effect on TC content in liver. Control: normal diet; HF: high fat diet; HF+0.35: high fat diet plus 0.35% total escins; HF+1: high fat diet plus 1% total escins; HF+2: high fat diet plus 2% total escins. Values are mean±S.E. of 10—14 mice. $*p<0.05$, significantly different from HF group.

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