Licorice Flavonoid Oil Effects Body Weight Loss by Reduction of Body Fat Mass in Overweight Subjects

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Licorice flavonoid oil (LFO) is a new dietary ingredient for functional foods consisting of licorice hydrophobic polyphenols in medium-chain triglycerides (MCT). In an effective dose finding study conducted previously, LFO has exhibited a dose-dependent body fat-reducing effect. Here we report the weight-reducing effect of LFO in a placebo-controlled, double-blind, long-term (12 weeks) ingestion study at 300 mg/day, the minimal effective dose observed in the dose finding study. A total of 103 overweight subjects [body mass index (BMI): 24–30] completed this study and were analyzed. Body weight increased in the placebo group, but was maintained at close to pre-ingestion level in the LFO group, resulting in significant ($p < 0.05$) differences in the changes in body weight and BMI between the LFO group and the placebo group at each time-point. Dual-energy X-ray absorptiometry (DXA) measurement of body fat indicated that the weight-reducing effect was attributable to reduced body fat. No clinically significant adverse events occurred during the 12-week ingestion period. To confirm the safety of LFO for practical use we also conducted a placebo-controlled, double-blind safety study in 40 overweight subjects with a 4-week excessive ingestion at 1800 mg/day; 6 times the dose of the 300 mg/day study that exhibited a weight-reducing effect. No clinically significant adverse events occurred during the 4-week ingestion period. Based on these findings in both human studies it was shown that LFO is a safe ingredient for functional foods even for long-term or excessive ingestion, with a potential weight-reducing effect.

Key words —— licorice, flavonoid, body fat, body weight, dual-energy X-ray absorptiometry, clinical trial

INTRODUCTION

Metabolic Syndrome is characterized by three or more metabolic risk factors occurring in one person.[1] In recent years human environmental conditions, behavior, and lifestyles have dramatically changed. These changes have resulted in an increasing number of people with metabolic syndrome.[2] Since central obesity is an essential component of metabolic syndrome, maintaining optimal body weight is important for avoiding the onset. In cases of borderline obesity, medication is not always useful primarily because of any problems such as side effects. On the contrary, evidence-based functional foods, in combination with lifestyle modification, have shown clinical benefits in preventing Metabolic Syndrome in the borderline obese, and these foods are associated with fewer side effects.[3]

For the purpose of finding such useful functional foods, we screened hundreds of food ingredients and found an anti-obese potential in the hydrophobic fraction of licorice, *Glycyrrhiza glabra*. By concentrating the licorice flavonoids, we developed licorice flavonoid oil (LFO; *Kaneka Glavonoid Rich Oil*™), which is a new dietary ingredient for functional foods with potential benefits for overweight subjects. We have already reported that LFO inhibits weight gain and reduces accumulation of visceral fat and elevation of blood glucose level in obese diabetic KK-A^y mice.[4]

Some pharmacological effects of licorice ethanolic extract and its main component of glabridin were reported by other researchers. Licorice ethanolic extract possesses well studied anti-oxida-

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tive properties,[5] and its clinical effects in moderately hypercholesterolemic patients have been reported.[6] Glabridin, a major polyphenolic flavonoid of *G. glabra*, has anti-oxidative,[7–11] anti-*Helicobacter pylori*,[12] as well as anti-nephritic and radical scavenging activities,[13] and inhibits serotonin re-uptake,[14] melanogenesis and inflammation.[15] However, an anti-obese effect of licorice has not previously been reported.

Licorice, the root of the leguminous *Glycyrrhiza* plant species, has been consumed for over 4000 years, since the era of ancient Egypt, and is among the botanicals most frequently employed in foods and traditional medicines in both Eastern and Western countries.[16] Although licorice has such a long history of consumption, safety information on the hydrophobic fraction of licorice (*G. glabra*) is limited. In order to confirm the safety of LFO, a series of nonclinical studies were conducted. As a result of these studies, it was concluded that, preclinically, LFO is safe and non-carcinogenic.

Prior to the present full-scale efficacy study, an 8-week placebo-controlled efficacy dose finding study at 300, 600, and 900 mg/day LFO was conducted in overweight subjects (56 men; 28 women). In the 900 mg group, significant decreases from baseline were observed in body weight and body mass index (BMI) after 4 and 8 weeks ingestion, and in visceral fat area measured by CT scan after 8 weeks ingestion. Furthermore, significant decreases from baseline in fat mass measured by dual-energy X-ray absorptiometry (DXA) were observed after 8 weeks ingestion in the 300, 600, and 900 mg groups, but not in the placebo group. Consequently, it was concluded that the minimum effective dose of LFO for reduction of body fat mass was 300 mg/day.

In the present study, we performed a long-term efficacy study of LFO at a daily dosage of 300 mg/day for 12 weeks, and demonstrated that LFO suppressed body weight gain by reducing body fat mass in overweight subjects. We also performed a safety study with excessive ingestion of LFO at a daily dose of 1800 mg, a 6-fold overdose of 300 mg/day, for 4 weeks.

MATERIALS AND METHODS

This study consisted of two parts: an efficacy study (Study A) and an excessive ingestion study (Study B), as described below.

Subjects ——

Study A: A total of 103 healthy subjects (63 men and 40 women) between 24 and 64 years of age were enrolled in this study. All subjects were moderately overweight, with BMI between 24.0 and 30.0, and body fat percentage of 20.0% or higher in men and 30.0% or higher in women, as measured using an 8-electrode impedance method.

Study B: A total of 40 healthy subjects (20 men and 20 women) between 22 and 58 years of age were enrolled in this study. All subjects were moderately overweight with BMI between 24.0 and 30.0.

In both studies A and B potential subjects were excluded from the studies: those who used licorice-containing medicinal products or health supplements; those who had a history of serious disease such as diabetes, hepatic disease, renal disease, or cardiac disease; those who had a history of food allergy (especially to licorice components), idiosyncrasy, or history of excessive alcohol use; those who underwent exercise programs for weight loss or were taking health supplements for weight loss; and women who were pregnant or who wanted to become pregnant during the study period. For all subjects in both studies, approval from the ethical committee of the medical institution (Kaiyuu Clinic for study A and Haradoi Hospital for study B) was given before the study commenced. All subjects signed informed consent forms in advance of entering this study, which was performed in accordance with the Declaration of Helsinki.

Study Food Product —— *Kaneka Glavonoid Rich Oil*™ that was used as LFO in the study was produced as follows: Root or rootstock of licorice (*Glycyrrhiza glabra*) was extracted with ethanol, filtered, concentrated, and treated with activated charcoal. After additional filtration and concentration, the ethanol extract was further extracted with medium-chain triglycerides (MCT) having a fatty acid composition of C8 : C10 = 99 : 1, then concentrated and filtered to separate insolubles. Prior to encapsulation the glabridin concentration was adjusted to 1% with MCT. The polyphenol content in LFO was approximately 8% when measured by the Folin-Ciocalteau method using glabridin as a standard compound. Active and placebo capsules were prepared using opaque brown-colored softgels. Active capsules contained 300 mg of LFO and 33 mg of beeswax and placebo capsules contained 300 mg of MCT and 33 mg of beeswax.

Study Design ——

Study A: This study was designed as a random-

ized, double-blind, placebo-controlled trial. All subjects were randomly allocated to one of two groups. In the LFO group, subjects took 1 LFO capsule per day before supper with a glass of water for 12 weeks. In the placebo group, subjects took 1 placebo capsule in the same manner.

Study B: This study was also designed as a randomized, double-blind, placebo-controlled trial. All subjects were randomly allocated to one of two groups. In the LFO group, subjects took 6 LFO capsules per day, 3 before breakfast and 3 before supper, with a glass of water for 4 weeks. In the placebo group, subjects took 6 placebo capsules per day in the same manner.

Clinical Assessment ——

Study A: Study A required 6 visits to the clinic, once each for health screening, pre-ingestion, weeks 4, 8, and 12 of ingestion, and post-ingestion week 4. Measurements of waist and hip, body weight (and calculated BMI), blood pressure/pulse rate, and hematology, urinalysis, and medical history review were conducted at each visit. DXA (Discovery W; HOLOGIC, Massachusetts, U.S.A.) was conducted at pre-ingestion and week 12 of ingestion to measure body fat mass, bone mineral density, and lean body mass.

Study B: Study B required 5 visits to the clinic, once each for health screening, pre-ingestion, weeks 2 and 4 of ingestion, and post-ingestion week 2. Measurements of waist and hip, body weight (and calculated BMI), and blood pressure/pulse rate, plus hematology, urinalysis, and medical history review were conducted at each visit. Plasma glabridin concentrations were measured at weeks 2 and 4 of ingestion and post-ingestion week 2, in accordance with the method described by Aoki *et al*.[17] Briefly, approximately 10 ml of venous blood was collected in each tube containing heparin. After collection, the blood samples were centrifuged (himac CF7D2; HITACHI, Tokyo, JAPAN) for 10 minutes, and plasma samples were frozen until glabridin concentration measurement using solid-phase extraction and LC-MS/MS [an SIL-HTC or an LC-10A HPLC system (Shimadzu Corporation, Kyoto, Japan) coupled to an API 4000 mass spectrometer (Applied Biosystems/MDS Sciex, Foster City, CA)].

Statistical Analysis —— Data are presented as mean ± standard error. Statistical analysis was conducted using the paired *t*-test within each group for changes before and after the study. Comparison between placebo and LFO groups was conducted using the Student's *t*-test. Comparison of frequency between the placebo and LFO groups were analyzed by the Mantel-Haenszel correlation test. Relationships between body weight changes and changes in the three body components measured by DXA were analyzed by Fisher's exact test in a 2 by 2 contingency table according to positive or negative changes. A p value less than 0.05 was considered to be significant, unless otherwise specified in this report.

RESULTS

Subject characteristics are summarized in Table 1. There were no statistically significant differences between the placebo and LFO groups in any of the relevant parameters in either study.

Study A: Efficacy Study (300 mg/day for 12 weeks)

In total, 52 subjects (31 men and 21 women) in the placebo group and 51 subjects (32 men and 19 women) in the LFO group were included in the analysis, except that 4 fertile women in the LFO group were not measured by DXA.

Time-course changes in body weight and BMI are shown in Fig. 1. Body weight in the placebo group gradually increased and mean gain of body weight reached approximately 1 kg at post-ingestion week 4. In contrast, body weight in the LFO group was maintained at close to pre-ingestion level, and statistically significant differences in the change of body weight between the groups were observed at weeks 4, 8, and 12 of ingestion and post-ingestion week 4 (Fig. 1A). Accompanied by the change of body weight, BMI also gradually increased in the placebo group but not in the LFO group, and the change of BMI between the groups was statistically significant at weeks 4, 8, and 12 of ingestion and post-ingestion week 4 (Fig. 1B). A histogram of body weight change with an interval of 1 kg prepared during the 12-week ingestion period (Fig. 2A) showed that the number of subjects who lost 1 kg or more of body weight was 13 (25.5%) in the LFO group but 7 (13.5%) in the placebo group, while the number of subjects who gained 1 kg or more of body weight was 15 (29.4%) in the LFO group but 21 (40.4%) in the placebo group. Comparison of frequency by the Mantel-Haenszel correlation test indicated a significant difference between the placebo and LFO groups (p = 0.0425). Ingestion of LFO shifted the distribution of body weight change significantly to the lower side. A histogram of BMI

Table 1. Subject Characteristics

	Study A (300 mg/day for 12 weeks)					
	Placebo			LFO		
	All	Men	Women	All	Men	Women
N	52	31	21	51	32	19
Age	46.1 ± 1.2	46.4 ± 1.6	45.7 ± 1.8	44.9 ± 1.1	45.1 ± 1.4	44.6 ± 2.0
Hight (cm)	165.47 ± 1.17	171.04 ± 0.81	157.25 ± 1.24	165.82 ± 1.25	170.93 ± 1.11	157.23 ± 1.24
Body Weight (kg)	72.71 ± 1.13	77.39 ± 1.01	65.80 ± 1.37	72.69 ± 1.21	76.72 ± 1.28	65.91 ± 1.42
BMI (kg/m^2)	26.50 ± 0.20	26.44 ± 0.26	26.57 ± 0.32	26.37 ± 0.21	26.21 ± 0.24	26.62 ± 0.38
Body Fat Ratio (%)	29.89 ± 0.96	24.85 ± 0.56	37.33 ± 0.72	29.72 ± 1.01	24.57 ± 0.45	38.38 ± 0.62
	Study B (1800 mg/day for 4 weeks)					
	Placebo			LFO		
	All	Men	Women	All	Men	Women
N	20	10	10	20	10	10
Age	42.6 ± 2.2	37.6 ± 3.4	47.5 ± 1.8	43.1 ± 2.0	38.9 ± 3.1	47.2 ± 2.0
Hight (cm)	163.95 ± 1.90	170.28 ± 1.45	157.62 ± 2.05	163.40 ± 1.95	170.26 ± 1.69	156.54 ± 1.67
Body Weight (kg)	71.30 ± 1.68	75.28 ± 1.48	67.15 ± 2.49	70.65 ± 1.89	74.69 ± 2.26	66.60 ± 2.52
BMI (kg/m^2)	26.51 ± 0.42	25.97 ± 0.45	27.05 ± 0.70	26.41 ± 0.44	25.71 ± 0.42	27.12 ± 0.72
Body Fat Ratio (%)	28.27 ± 1.59	22.91 ± 1.63	33.62 ± 1.28	28.94 ± 1.53	23.33 ± 0.80	35.54 ± 1.48

There were no significant differences between placebo group and LFO group in any relevant parameters.

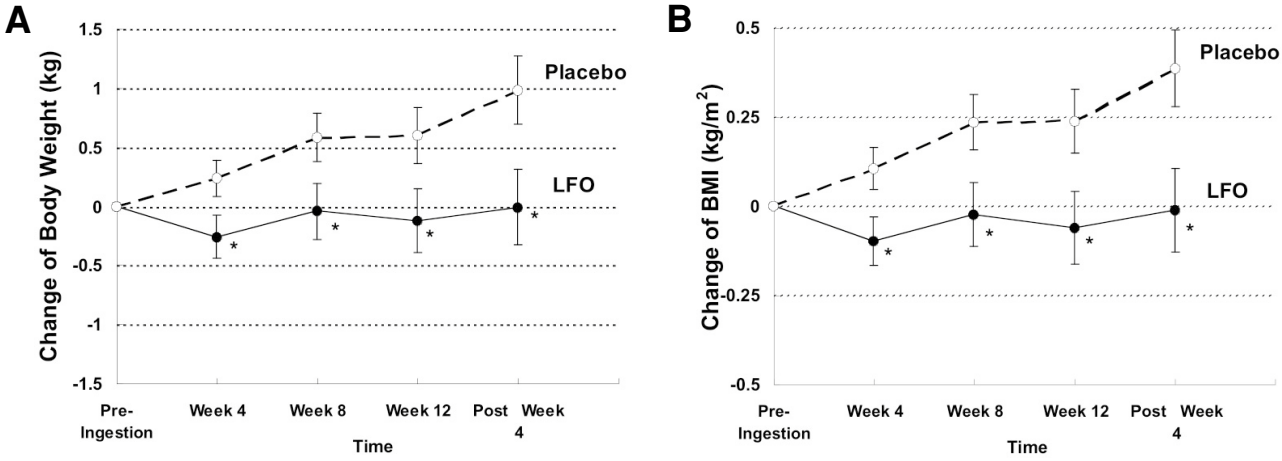

Fig. 1. Time-Course Changes in Body Weight (A) and BMI (B) in Overweight Subjects during and after LFO Ingestion

Subjects ingested placebo or LFO for 12 weeks, and body weight was measured and BMI calculated every 4 weeks. Vertical bars represent mean ± S.E. and asterisks indicate significant difference ($p < 0.05$) between placebo group and LFO group at each time-point.

change with an interval of 0.5 kg/m^2 prepared during the 12-week ingestion showed similar results (Fig. 2B). Comparison of frequency by the Mantel-Haenszel correlation test in BMI also indicated a significant difference between the placebo and LFO groups ($p = 0.0323$).

It is known that DXA can divide body composition into three components: fat mass, lean body mass, and bone mineral density. There was a very high correlation between the sum of these three components and body weight, with $y = 1.0094x$, $R^2 = 0.997$ at pre-ingestion and $y = 1.0112x$, $R^2 = 0.995$ at week 12 of ingestion (where y was the sum of the three weight components and x was body weight). Relationships between body weight changes and changes in the three body components measured by DXA during 12-week ingestion were analyzed using the data from 47 subjects (four fertile women in the LFO group were excluded) by Fisher's exact test in a 2 by 2 contingency table according to positive or negative changes (Table 2). A statistically significant ($p < 0.01$) relationship was observed between body

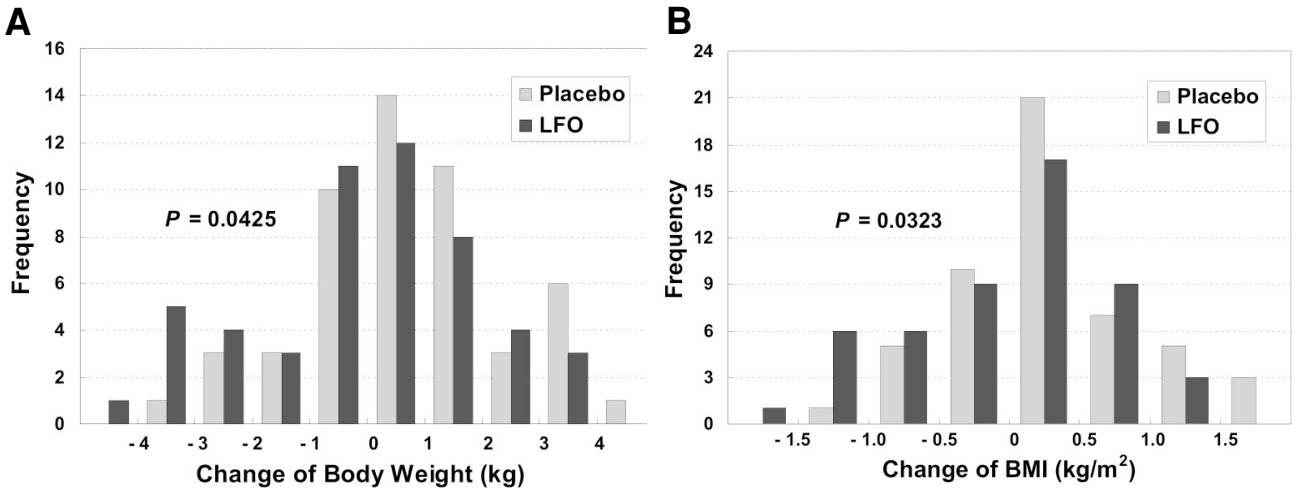

Fig. 2. A Histogram of Weight Change (A) and BMI Change (B) from Baseline to Week 12

Gray bars represent placebo and black bars represent LFO. (A): Body weight change interval is 1 kg. (B): BMI interval is 0.5 kg/m^2. Comparison of frequency by the Mantel-Haenszel correlation indicated significant differences ($p < 0.05$) between the placebo and LFO groups in body weight ($p = 0.0425$) and in BMI ($p = 0.0323$).

Table 2. Relationships between Weight Change and Changes Measured by DXA

	Body Weight			Body Weight			Body Weight		
	Increase	Decrease	Sum	Increase	Decrease	Sum	Increase	Decrease	Sum
	Fat Mass (DXA)			Lean Body Mass (DXA)			Bone Mineral Density (DXA)		
Increase	15	4	19	16	8	24	8	9	17
Decrease	10	18	28	9	14	23	17	13	30
Sum	25	22	47	25	22	47	25	22	47
	$p = 0.0067$			$p = 0.0820$			$p = 0.5583$		

Data were analyzed by using Fisher's exact test in 2 by 2 contingency table.

weight changes and changes in fat mass measured by DXA; however, there was no significant relationship between body weight changes and changes in lean body mass measured by DXA ($p = 0.0820$) or bone mineral density ($p = 0.5583$), suggesting that body weight changes in the LFO group were caused by changes in fat mass rather than changes in lean body mass or bone mineral density.

We also investigated the safety of the 12-week long-term ingestion of LFO in this study. There were no clinically significant findings in physiology, hematology, blood biochemistry, or urinalysis tests either in the LFO group or placebo group. Typical data are shown in Table 3. Although some statistically significant changes were observed in both groups, the changes were slight, within a physiological variance and without time dependency, and thus considered to be clinically insignificant. Subjective symptoms were reported in both the placebo and LFO groups; however, all symptoms were mild, or were considered unrelated or only possibly related to LFO. Symptoms that were considered possibly related to placebo or LFO were the following: diarrhea (3 in placebo), soft stools (1 in placebo), stomachache (1 in LFO), constipation (1 in placebo), and urticaria (1 in placebo). These symptoms were clinically insignificant.

Study B: Excessive Ingestion Study (1800 mg/day for 4 weeks)

A total of 40 subjects participated in the study, comprising 20 subjects (10 men and 10 women) in the placebo group and 20 subjects (10 men and 10 women) in the 1800 mg LFO group. One subject in the LFO group failed to ingest the study food product on the day before the hospital visit at week 2 of ingestion. Another subject failed to ingest the study food product on the day before the hospital visit at week 4 of ingestion. Data from these 2 subjects during the periods in which they failed to ingest the

Table 3. Physical Examination, Hematology and Blood Chemistry (Study A)

	Standard Value		Baseline	Week 4	Week 8	Week 12	Post-Week 4
Physical Examination							
Pulse Rate		Placebo	72.9 ± 1.2	71.0 ± 1.3	73.1 ± 1.1	71.9 ± 1.3	72.9 ± 1.4
(/min)		300 mg	71.8 ± 1.5	69.6 ± 1.5	72.5 ± 1.5	71.0 ± 1.4	72.1 ± 1.6
Systolic Blood		Placebo	121.3 ± 2.3	123.4 ± 2.1	125.6 ± 2.3*	125.4 ± 2.3*	123.4 ± 2.1
Pressure (mmHg)		300 mg	118.8 ± 2.2	119.6 ± 1.9	121.8 ± 2.2*	121.7 ± 2.4*	121.9 ± 2.2*
Diastolic Blood		Placebo	72.1 ± 1.5	71.1 ± 1.3	72.2 ± 1.4	74.0 ± 1.5	71.7 ± 1.3
Pressure (mmHg)		300 mg	68.8 ± 1.3	70.4 ± 1.3	71.5 ± 1.3**	72.2 ± 1.3**	70.0 ± 1.4
Hematology							
White Blood Cell	3000–9000	Placebo	6076.9 ± 238.8	6046.2 ± 231.7	5459.6 ± 220.8**	5973.1 ± 224.6	5767.3 ± 220.7
Count (/μl)		300 mg	6207.8 ± 265.6	6366.7 ± 256.1	5803.9 ± 213.8	5933.3 ± 204.8	5870.6 ± 234.8
Red Blood Cell	M 400–570	Placebo	471.6 ± 6.0	476.4 ± 5.8	475.8 ± 6.1	475.6 ± 5.8	474.8 ± 5.9
Count ($\times 10^4/\mu$l)	W 350–500	300 mg	475.6 ± 5.5	480.8 ± 5.3*	478.4 ± 5.6	480.3 ± 5.9	482.3 ± 6.0*
Platelet Count	M 10.0–35.0	Placebo	25.25 ± 0.85	24.85 ± 0.89	25.14 ± 0.84	25.27 ± 0.89	25.57 ± 0.93
($\times 10^4/\mu$l)	W 11.0–40.0	300 mg	26.29 ± 0.79	26.51 ± 0.86	26.98 ± 0.90	26.96 ± 0.86	27.63 ± 1.03**
Hemoglobin Level	M 12.0–18.0	Placebo	14.44 ± 0.19	14.57 ± 0.19	14.56 ± 0.20	14.56 ± 0.19	14.48 ± 0.20
(g/dl)	W 11.0–16.0	300 mg	14.40 ± 0.23	14.55 ± 0.22*	14.50 ± 0.23	14.54 ± 0.24	14.54 ± 0.24
PT Time	9.5–12.0	Placebo	11.24 ± 0.07	11.20 ± 0.08	11.03 ± 0.12*	10.98 ± 0.07**	11.06 ± 0.07*
(sec)		300 mg	11.20 ± 0.08	11.12 ± 0.08	10.90 ± 0.10**	10.93 ± 0.08**	11.06 ± 0.08*
APTT	23.5–42.5	Placebo	34.07 ± 0.42	33.77 ± 0.47	33.09 ± 0.45**	32.72 ± 0.38**	32.75 ± 0.48**
(sec)		300 mg	34.20 ± 0.47	33.94 ± 0.57	32.88 ± 0.46**	33.18 ± 0.45**	32.65 ± 0.40**
Blood Chemistry							
Total Protein	6.0–8.3	Placebo	7.41 ± 0.04	7.55 ± 0.05*	7.49 ± 0.05	7.36 ± 0.05	7.46 ± 0.05
(g/dl)		300 mg	7.41 ± 0.05	7.63 ± 0.06**	7.55 ± 0.05**	7.49 ± 0.06	7.58 ± 0.05**
Albumin	3.8–5.3	Placebo	4.63 ± 0.03	4.71 ± 0.04	4.67 ± 0.03	4.66 ± 0.03	4.78 ± 0.04**
(g/dl)		300 mg	4.62 ± 0.03	4.74 ± 0.04**	4.71 ± 0.04**	4.73 ± 0.04**	4.82 ± 0.04**
A/G Ratio	1.0–2.2	Placebo	1.69 ± 0.03	1.68 ± 0.03	1.68 ± 0.03	1.74 ± 0.03**	1.79 ± 0.03**
		300 mg	1.68 ± 0.03	1.66 ± 0.03	1.68 ± 0.03	1.74 ± 0.03**	1.78 ± 0.04**
AST	0–40	Placebo	21.1 ± 1.1	22.6 ± 1.4*	22.7 ± 1.3*	22.5 ± 1.4	23.9 ± 1.6**
(IU/l)		300 mg	20.7 ± 0.8	26.5 ± 4.9	23.5 ± 1.5*	21.8 ± 1.0	21.4 ± 0.8
ALT	0–45	Placebo	27.0 ± 3.1	29.8 ± 3.6*	30.0 ± 3.7*	31.0 ± 3.7*	32.9 ± 3.8**
(IU/l)		300 mg	24.3 ± 1.8	28.1 ± 3.4	25.9 ± 1.9	24.4 ± 1.8	24.5 ± 1.6#
LDH	60–240	Placebo	210.2 ± 5.4	224.7 ± 9.3	210.2 ± 5.4	200.2 ± 5.0**	208.0 ± 6.4
(IU/l)		300 mg	200.7 ± 4.5	211.4 ± 7.0*	201.2 ± 5.7	192.0 ± 4.2**	196.6 ± 5.9
ALP	40–325	Placebo	217.0 ± 8.0	221.0 ± 7.8	217.9 ± 8.8	215.4 ± 8.9	218.4 ± 7.8
(IU/l)		300 mg	203.3 ± 6.8	210.7 ± 7.0**	203.2 ± 7.1	203.1 ± 6.5	208.6 ± 7.1

Mean ± S.E., M: Men, W: Women. Paired *t*-test, significantly different from baseline: *$p < 0.05$, **$p < 0.01$. One-way analysis of variance (between groups): #$p < 0.05$.

study food product were therefore excluded from analysis of blood glabridin concentration.

There were no clinically significant findings in physiology, hematology, blood biochemistry, or urinalysis tests either in the LFO group or placebo group. Typical data are shown in Table 4. Although some statistically significant changes were observed in both groups, the changes were slight and within physiological variance and without time dependency, and thus considered to be clinically insignificant. Subjective symptoms were reported in both the placebo and LFO groups; however, all symptoms were mild or clinically insignificant and judged to be unrelated to LFO. Symptoms considered possibly related or probably related to placebo or LFO were as follows: enlarged abdomen feeling (1 events in placebo), diarrhea (2 in LFO), soft stools (1 in placebo), headache (1 in placebo), and dull headache (2 in placebo). These symptoms were clinically insignificant. Plasma glabridin concentrations in the 1800 mg group are shown in Table 5. At weeks 2 and 4 of ingestion, plasma glabridin concentrations were 1.95 ± 1.38 and 2.08 ± 1.17 ng/ml (mean ± S.D.), respectively. The ratio of mean concentration at week 4 against that at week 2 was 1.06, indicating a nearly steady state level was reached at week 2 of ingestion. For individual subjects, the accumulation factor exceeded 1.6 in 5 of 18 subjects, suggesting that the time to reach a steady state tended to be delayed in certain subjects.

Table 3. Continued

	Standard Value		Baseline	Week 4	Week 8	Week 12	Post-Week 4
Blood Chemistry							
γ-GTP	M 0–80	Placebo	39.0 ± 4.3	38.9 ± 3.9	39.0 ± 4.5	37.8 ± 4.2	38.5 ± 3.9
(IU/l)	W 0–50	300 mg	38.8 ± 4.6	37.5 ± 4.4	37.4 ± 5.0	35.4 ± 4.6	38.1 ± 5.5
Total Bilirubin	M 0.1–1.4	Placebo	0.66 ± 0.04	0.62 ± 0.05	0.63 ± 0.05	0.62 ± 0.04	0.67 ± 0.04
(mg/dl)	W 0.1–1.3	300 mg	0.69 ± 0.04	0.61 ± 0.04*	0.64 ± 0.04	0.64 ± 0.04	0.62 ± 0.03
Creatinine	M 0.7–1.5	Placebo	0.88 ± 0.02	0.90 ± 0.02	0.89 ± 0.02	0.91 ± 0.02**	0.92 ± 0.02**
(mg/dl)	W 0.5–1.2	300 mg	0.90 ± 0.02	0.90 ± 0.02	0.90 ± 0.02	0.91 ± 0.02	0.92 ± 0.02*
Urea Nitrogen	M 8.0–23.0	Placebo	13.27 ± 0.42	12.96 ± 0.42	13.09 ± 0.39	13.20 ± 0.37	13.75 ± 0.44
(mg/dl)	W 6.0–23.0	300 mg	13.60 ± 0.63	13.40 ± 0.45	13.28 ± 0.49	13.43 ± 0.45	13.29 ± 0.45
Uric Acid	M 3.0–7.9	Placebo	5.82 ± 0.19	5.77 ± 0.17	5.70 ± 0.18	5.68 ± 0.17	5.84 ± 0.19
(mg/dl)	W 2.5–7.0	300 mg	6.04 ± 0.20	5.96 ± 0.18	5.84 ± 0.17	5.70 ± 0.19**	5.85 ± 0.19
Total Cho	100–239	Placebo	219.1 ± 4.2	221.8 ± 3.5	220.8 ± 4.4	220.1 ± 4.7	230.7 ± 5.5**
(mg/dl)		300 mg	212.9 ± 5.4	221.3 ± 5.6**	215.8 ± 4.9	216.4 ± 5.6	224.8 ± 5.5**
HDL-Cho	30–100	Placebo	49.6 ± 1.3	51.1 ± 1.3	50.7 ± 1.2	51.7 ± 1.3**	52.8 ± 1.4**
(mg/dl)		300 mg	51.3 ± 1.7	52.8 ± 1.7	52.2 ± 1.8	53.1 ± 1.8	54.6 ± 1.9**
LDL-Cho	65–139	Placebo	151.4 ± 4.2	150.3 ± 3.6	146.2 ± 4.0*	144.5 ± 4.3*	153.3 ± 5.1
(mg/dl)		300 mg	143.8 ± 5.3	148.3 ± 5.2*	139.5 ± 4.5	137.0 ± 5.2**	144.3 ± 4.8
Triglycerid	30–200	Placebo	150.7 ± 10.8	152.7 ± 11.0	145.4 ± 10.9	136.4 ± 9.1	147.6 ± 9.5
(mg/dl)		300 mg	141.5 ± 10.8	145.8 ± 10.9	135.3 ± 10.5	149.1 ± 20.5	141.8 ± 13.5
Free Fatty Acid	0.10–0.90	Placebo	0.517 ± 0.023	0.557 ± 0.025	0.533 ± 0.024	0.500 ± 0.023	0.496 ± 0.024
(mEq/l)		300 mg	0.514 ± 0.024	0.522 ± 0.022	0.521 ± 0.030	0.521 ± 0.026	0.494 ± 0.028
Phospholipid	150–220	Placebo	233.9 ± 3.7	234.8 ± 3.4	238.0 ± 4.3	233.2 ± 3.7	238.6 ± 4.3
(mg/dl)		300 mg	231.2 ± 5.3	237.5 ± 4.5	235.9 ± 4.4	233.9 ± 5.5	235.4 ± 5.0
Na	135–148	Placebo	141.3 ± 0.2	143.0 ± 0.3**	143.7 ± 0.3**	143.6 ± 0.3**	144.2 ± 0.3**
(mEq/l)		300 mg	141.0 ± 0.2	143.0 ± 0.4**	143.0 ± 0.3**	142.9 ± 0.3**	144.2 ± 0.2**
K	3.5–5.0	Placebo	4.23 ± 0.05	4.39 ± 0.06**	4.41 ± 0.05**	4.40 ± 0.06**	4.42 ± 0.05**
(mEq/l)		300 mg	4.24 ± 0.04	4.42 ± 0.05**	4.43 ± 0.04**	4.57 ± 0.08**	4.49 ± 0.06**
Cl	98–108	Placebo	104.9 ± 0.2	104.9 ± 0.3	105.3 ± 0.2*	105.0 ± 0.3	105.1 ± 0.3
(mEq/l)		300 mg	105.0 ± 0.3	105.0 ± 0.3	104.6 ± 0.3#	104.2 ± 0.3*,#	105.1 ± 0.3
Blood Glucose	70–110	Placebo	93.9 ± 1.6	96.8 ± 2.0*	94.9 ± 1.5	99.7 ± 1.8**	99.6 ± 2.4**
(mg/dl)		300 mg	93.7 ± 1.2	94.7 ± 1.4	94.2 ± 1.4	97.8 ± 1.3**	98.0 ± 1.5**
HbA1c	4.3–5.8	Placebo	5.46 ± 0.07	5.30 ± 0.05**	5.37 ± 0.05*	5.37 ± 0.05*	5.46 ± 0.07
(%)		300 mg	5.36 ± 0.05	5.23 ± 0.05**	5.33 ± 0.05	5.28 ± 0.05**	5.34 ± 0.05
Insulin	2.7–10.4	Placebo	7.56 ± 0.79	6.69 ± 0.56	6.61 ± 0.38	7.54 ± 0.49	7.60 ± 0.59
(μU/ml)		300 mg	7.90 ± 1.33	7.26 ± 1.03	7.53 ± 0.72	8.51 ± 1.37	8.74 ± 1.43

DISCUSSION

Previously, we conducted a dose finding study with 8-week repeated LFO ingestion at 300, 600, and 900 mg/day in overweight subjects. Body composition was measured by DXA, which provides a very useful method for measuring body composition because of its convenience, high reproducibility, and low dose of X-ray exposure.[18,19] Owing to higher sensitivity and reproducibility, DXA measurements showed a dose-dependent body-fat-reducing effect of 300 mg/day LFO, while CT scans showed significant reduction in body weight and visceral fat only at 900 mg/day LFO. As a result, in consideration of safety, we used a daily dose of LFO at 300 mg, which is the minimal body-fat-reducing dose, in the dose finding study. Thus, the current study was designed to confirm the efficacy and safety of a daily dose of 300 mg with a longer ingestion period and more subjects than the previous study.

This study was conducted for 12 weeks without strict dietary restriction and physical exercise for weight loss. Body weight was measured and BMI was calculated every 4 weeks. The mean body weight of the placebo group gradually increased over time while that of the LFO group was significantly suppressed at weeks 4, 8, and 12 of ingestion (Fig. 1A). Body weight did not further decrease 4 weeks after completion of ingestion in the LFO group. In the present study, which was conducted during the period from fall to winter, the observed average body weight increase in the placebo group could be attributed to the generally known seasonal variation in body weight as reported by other researchers.[20–22]

Table 4. Physical Examination, Hematology and Blood chemistry (Study B)

Standard Value			Baseline	Week 2	Week 4	Post-Week 2
Physical Examination						
Pulse Rate		Placebo	69.4 ± 1.9	68.5 ± 2.0	69.4 ± 2.5	68.1 ± 2.0
(/min)		1800 mg	67.7 ± 1.5	68.2 ± 1.4	69.2 ± 2.2	64.9 ± 1.5*
Systolic Blood		Placebo	124.6 ± 3.3	122.0 ± 3.2	124.5 ± 3.0	128.1 ± 3.0
Pressure (mmHg)		1800 mg	125.8 ± 3.5	123.3 ± 3.5	124.8 ± 3.7	126.2 ± 2.8
Diastolic Blood		Placebo	84.6 ± 2.2	84.5 ± 2.3	85.9 ± 2.2	87.8 ± 2.2*
Pressure (mmHg)		1800 mg	86.0 ± 2.6	85.3 ± 2.6	83.4 ± 2.6	84.9 ± 2.9
Hematology						
White Blood Cell	3500–9700	Placebo	5884.5 ± 365.0	6011.0 ± 421.2	5814.5 ± 419.3	5565.0 ± 358.8
Count (/μl)		1800 mg	6549.0 ± 395.1	6008.0 ± 311.9*	5952.0 ± 383.8	6070.0 ± 371.8
Red Blood Cell	M 438–577	Placebo	475.6 ± 9.4	468.2 ± 11.2	473.1 ± 8.9	475.4 ± 9.3
Count ($\times 10^4/\mu$l)	W 376–516	1800 mg	476.1 ± 10.3	470.1 ± 9.9	470.4 ± 10.6	472.6 ± 10.1
Platelet Count	14.0–37.9	Placebo	25.70 ± 1.18	24.89 ± 1.23	25.98 ± 1.07	25.39 ± 1.07
($\times 10^4/\mu$l)		1800 mg	24.81 ± 1.58	24.78 ± 1.45	24.87 ± 1.47	24.61 ± 1.51
Hemoglobin Level	M 13.6–18.3	Placebo	14.25 ± 0.39	14.06 ± 0.42	14.23 ± 0.37	14.32 ± 0.35
(g/dl)	W 11.2–15.2	1800 mg	14.35 ± 0.37	14.25 ± 0.36	14.36 ± 0.34	14.45 ± 0.35
PT Time	8.0–12.0	Placebo	10.19 ± 0.13	10.23 ± 0.15	10.21 ± 0.15	10.22 ± 0.12
(sec)		1800 mg	9.97 ± 0.12	9.83 ± 0.10	10.02 ± 0.10	9.94 ± 0.09
APTT	26.0–38.0	Placebo	35.43 ± 1.02	37.01 ± 1.80	33.23 ± 0.78**	32.09 ± 0.8**
(sec)		1800 mg	35.49 ± 1.28	33.96 ± 1.29	33.04 ± 1.28	31.97 ± 1.05**
Blood Chemistry						
Total Protein	6.5–8.2	Placebo	7.67 ± 0.07	7.48 ± 0.08*	7.53 ± 0.10	7.55 ± 0.07
(g/dl)		1800 mg	7.42 ± 0.10	7.35 ± 0.10	7.39 ± 0.09	7.38 ± 0.10
Albumin	3.7–5.5	Placebo	4.61 ± 0.05	4.53 ± 0.05	4.55 ± 0.06	4.54 ± 0.05
(g/dl)		1800 mg	4.54 ± 0.06	4.50 ± 0.06	4.53 ± 0.06	4.53 ± 0.06
A/G Ratio	1.30–2.00	Placebo	1.516 ± 0.033	1.544 ± 0.032	1.533 ± 0.029	1.515 ± 0.027
		1800 mg	1.596 ± 0.042	1.594 ± 0.043	1.605 ± 0.054	1.609 ± 0.043
AST	10–40	Placebo	25.0 ± 2.4	24.0 ± 1.7	24.7 ± 1.9	23.4 ± 1.8
(IU/l)		1800 mg	25.9 ± 2.0	24.8 ± 1.5	24.2 ± 2.0	22.7 ± 1.4
ALT	5–45	Placebo	29.9 ± 3.8	29.5 ± 3.7	30.3 ± 3.9	28.6 ± 3.6
(IU/l)		1800 mg	31.6 ± 4.0	31.3 ± 4.1	31.2 ± 5.1	27.7 ± 4.0
LDH	120–245	Placebo	177.5 ± 7.2	175.3 ± 7.3	179.6 ± 6.9	177.3 ± 6.6
(IU/l)		1800 mg	176.6 ± 4.9	177.1 ± 5.6	175.6 ± 4.2	175.4 ± 4.1
ALP	104–338	Placebo	222.5 ± 16.9	208.7 ± 13.1*	217.5 ± 14.8	217.4 ± 15.6
(IU/l)		1800 mg	225.6 ± 11.8	217.4 ± 10.9	212.1 ± 9.6*	216.3 ± 11.5

Mean ± S.E., M: Men, W: Women. Paired t-test, significantly different from baseline: *$p < 0.05$, **$p < 0.01$.

Similar results were obtained from BMI data (Fig. 1B). Because of this seasonality, in the placebo group, 21 subjects (40.4%) showed a weight gain of 1 kg or more during the 12 weeks. A total of 13 subjects (25.5%) showed a weight loss of 1 kg or more in the LFO group, but only 7 subjects (13.5%) in the placebo group (Fig. 2A). If this study was conducted in a season when the body weight in the placebo group was expected not to increase, such as spring or summer, the body weight in the LFO group would likely decrease compared to the pre-ingestion level. As a result, the difference in body weight between the LFO and placebo group, without strict dietary restriction, at the third month (12 weeks) was 0.72 kg. If body weight reduction were to continue at the same rate, body weight may be reduced by about 3 kg in one year. According to some clinical reports,[23] this effect would be slightly weaker than some medicinal drugs. However, with mild dietary restriction and/or physical activity, LFO could potentially be even more effective in overweight subjects.

In recent years, the DXA method became popular in body composition analysis. In fact, the DXA

Table 4. Continued

Standard Value			Baseline	Week 2	Week 4	Post-Week 2
Blood Chemistry						
γ-GTP	16–73	Placebo	53.0 ± 11.1	49.4 ± 9.4	54.6 ± 12.5	50.9 ± 11.0
(IU/l)		1800 mg	54.6 ± 9.5	51.8 ± 7.9	48.4 ± 7.7	48.3 ± 8.8
Total Bilirubin	0.2–1.0	Placebo	0.66 ± 0.04	0.70 ± 0.05	0.61 ± 0.04	0.59 ± 0.05
(mg/dl)		1800 mg	0.63 ± 0.05	0.69 ± 0.06	0.71 ± 0.04*	0.60 ± 0.05
Creatinine	M 0.65–1.09	Placebo	0.673 ± 0.029	0.656 ± 0.032	0.680 ± 0.029	0.701 ± 0.038
(mg/dl)	W 0.46–0.82	1800 mg	0.685 ± 0.031	0.685 ± 0.029	0.726 ± 0.032**	0.685 ± 0.030
Urea Nitrogen	8–20	Placebo	14.04 ± 0.71	12.93 ± 0.73	13.15 ± 0.61*	14.17 ± 0.57
(mg/dl)		1800 mg	12.61 ± 0.77	12.40 ± 0.59	12.84 ± 0.64	12.60 ± 0.76
Uric Acid	7.0 or below	Placebo	5.69 ± 0.30	5.62 ± 0.33	5.63 ± 0.29	5.79 ± 0.36
(mg/dl)		1800 mg	5.63 ± 0.31	5.76 ± 0.33	5.66 ± 0.28	5.71 ± 0.33
Total Cho	150–219	Placebo	220.1 ± 10.9	216.3 ± 9.6	216.2 ± 10.2	219.4 ± 10.6
(mg/dl)		1800 mg	224.2 ± 7.4	223.0 ± 7.7	227.2 ± 7.1	221.8 ± 7.5
HDL-Cho	M 40–80	Placebo	57.3 ± 2.7	55.3 ± 2.4	55.9 ± 2.6	56.4 ± 2.9
(mg/dl)	W 40–90	1800 mg	58.0 ± 2.2	58.3 ± 2.8	56.5 ± 2.5	57.4 ± 2.1
LDL-Cho	70–139	Placebo	142.0 ± 9.1	141.7 ± 8.3	139.6 ± 9.1	142.7 ± 9.4
(mg/dl)		1800 mg	147.0 ± 5.9	148.0 ± 6.7	150.4 ± 5.9	145.7 ± 6.6
Triglycerid	50–149	Placebo	130.5 ± 17.8	130.2 ± 19.9	142.8 ± 16.7	124.4 ± 14.0
(mg/dl)		1800 mg	137.1 ± 12.7	126.6 ± 10.6	141.8 ± 20.2	136.2 ± 12.8
Free Fatty Acid	0.10–0.81	Placebo	0.516 ± 0.052	0.508 ± 0.038	0.482 ± 0.045	0.427 ± 0.049*
(mEq/l)		1800 mg	0.468 ± 0.052	0.490 ± 0.066	0.500 ± 0.053	0.428 ± 0.046
Phospholipid	150–250	Placebo	233.1 ± 9.1	225.2 ± 8.1	232.4 ± 6.7	233.2 ± 7.3
(mg/dl)		1800 mg	238.3 ± 8.3	233.4 ± 6.9	237.7 ± 8.2	237.3 ± 6.5
Na	135–145	Placebo	141.7 ± 0.3	141.7 ± 0.3	141.9 ± 0.4	141.4 ± 0.4
(mEq/l)		1800 mg	141.7 ± 0.4	142.0 ± 0.4	142.1 ± 0.4	141.2 ± 0.3
K	3.5–5.0	Placebo	4.22 ± 0.08	4.19 ± 0.07	4.34 ± 0.07*	4.43 ± 0.10**
(mEq/l)		1800 mg	4.32 ± 0.05	4.23 ± 0.04	4.39 ± 0.10	4.32 ± 0.09
Cl	98–108	Placebo	104.8 ± 0.3	105.3 ± 0.5	104.4 ± 0.4	103.8 ± 0.4*
(mEq/l)		1800 mg	105.2 ± 0.6	105.4 ± 0.5	104.5 ± 0.5	103.8 ± 0.5**
Blood Glucose	70–109	Placebo	90.4 ± 2.2	92.3 ± 2.1	94.6 ± 2.4**	91.2 ± 2.6
(mg/dl)		1800 mg	95.2 ± 2.1	93.9 ± 1.6	93.6 ± 1.6	93.8 ± 1.9
HbA1c	4.3–5.8	Placebo	4.98 ± 0.07	4.91 ± 0.07**	4.93 ± 0.08	5.01 ± 0.07
(%)		1800 mg	5.03 ± 0.06	5.01 ± 0.06	4.98 ± 0.05**	5.05 ± 0.06
Insulin	2.2–12.4	Placebo	6.84 ± 0.79	7.15 ± 0.78	7.00 ± 0.78	7.27 ± 0.71
(μU/ml)		1800 mg	7.26 ± 0.75	6.49 ± 0.86	7.51 ± 0.89	7.83 ± 1.18

method has been frequently used in development of functional foods or drugs.[24–26] Our previous data indicated that the sum of body fat mass, lean body mass, and bone mineral density measured by DXA was highly correlated with the body weight measured on a scale suggesting that the DXA method is applicable to measure these three components of the body. We therefore used DXA to investigate which of these three components contributed to the reduction of body weight. Statistically significant ($p < 0.01$) correlation was observed between body weight changes and changes in fat mass measured by DXA, but not between body weight and either change in lean body mass or bone mineral density. It was thus concluded that the weight-reducing effect of LFO was attributable to reduced fat mass. These results are in agreement with those obtained in the dose finding study. Total body weight reduction might be considered an adverse effect in some cases. Given the above-mentioned result, however, it is unlikely that the observed weight loss was due to such an adverse effect.

Suppression of lipid absorption,[27] reduction in calorie intake,[28] reduction in biosynthesis of fatty acid, and enhancement of fatty acid oxidation[29] are possible mechanisms of the reduction in body fat.

Table 5. Blood Glabridin Concentration (Study B)

Glabridin Concentration (ng/ml)			Ratio of Means
	Week 2	Week 4	Week 4/Week 2
Mean	1.95	2.08	1.06
S.D.	1.38	1.17	
N	19	19	

There was no appreciable change in calorie intake for any subject in the present study. Unfortunately, changes in lipid absorption was not analyzed in the present study, but blood triglyceride did not change, suggesting that suppression of lipid absorption was probably not a relevant factor; further experiments would be needed in regard to this point. We established a hypothesis that the weight loss by LFO is due to reduction in fatty acid synthesis and enhancement of fatty acid oxidation in the liver, and there are lines of evidence supporting this hypothesis. For example, we have observed that the body weight and white adipose tissue mass of obese C57BL/6J mice fed with a high-fat diet for 8 weeks were suppressed, compared with the control, by administration of LFO (in preparation). A preliminary microarray study using these mice showed that LFO induced genes in some fatty acid oxidation pathways and reduced some fatty acid synthesis pathways in the liver. Further experiments are needed to confirm the mechanism of action.

There are three major varieties of licorice, namely *Glycyrrhiza uralensis* FISCHER, *G. glabra* LINNE, and *G. inflata* BATALIN, each containing species-specific flavonoids.[30] Of these varieties, *G. glabra* is the most popular species used as a food ingredient worldwide. LFO is prepared *via* a sequence of processes: extracting the roots of licorice *G. glabra* with ethanol, re-extracting with MCT, and standardizing the final concentration of glabridin, a major polyphenol flavonoid, to 1%. The concentration of glycyrrhizic acid, which induces a mineral corticoid action as the major side effect of licorice root,[31,32] is reduced to below 0.005% by the above processing. Licorice root and its extracts are highly safe food ingredients that have been widely consumed since ancient times and also are registered as Generally Recognized As Safe (GRAS) by the FDA in the U.S. However, LFO is manufactured by a novel production method as described above and its safety has therefore been assessed in a step-wise manner. We have demonstrated the safety of LFO in nonclinical studies including a 90-day subchronic toxicity study in rats, genotoxicity studies such as reverse mutation assay, bone marrow and liver micronucleus test and a rat medium-term liver bioassay for carcinogens. As a result of these studies, it was concluded that, preclinically, LFO is safe and non-carcinogenic.

In addition, the following clinical studies were conducted that support the safety of LFO: In a stepwise fashion, single-dose, 1- and 4-week placebo-controlled studies at daily doses of 300, 600, and 1200 mg LFO were carried out in healthy humans. In these three human studies, there were no clinically significant findings in hematology or blood biochemistry. All adverse events were mild and considered unrelated to the LFO administration. The results showed that LFO has little or no adverse events in healthy humans receiving daily doses up to 1200 mg for 4 weeks. On the basis of these results, LFO was accepted as a New Dietary Ingredient (NDI) in the U.S.

In this study, we evaluated the safety of long-term (12 weeks) ingestion of LFO at a dose of 300 mg in overweight subjects (Study A). We also conducted a 4-week ingestion study in overweight subjects at 1800 mg/day (Study B), which corresponds to 6 times as much as the effective dose of 300 mg/day, to confirm the safety of LFO at high dose levels. In both studies, no clinically significant findings were obtained in hematology, blood biochemistry, physiology, or urinalysis; all adverse events were mild and/or not related to LFO ingestion, and were not clinically significant. As a result, as in the studies that we conducted in healthy subjects with daily doses up to 1200 mg, the safety at an dose of 1800 mg of LFO was verified in overweight subjects. We expect a daily dose of 300 mg could be safe even for some consumers sensitive to LFO, and higher doses to be safe for those who accidentally overdose LFO.

Because glabridin is the major component of LFO, we measured plasma glabridin concentration level as part of an attempt to estimate the bioaccumulation potential of LFO. The ratio of mean plasma glabridin concentration at week 4 versus week 2 was 1.06 indicating that the plasma glabridin concentration had already reached a nearly steady state level at week 2 of ingestion. For individual subjects, the factor exceeded 1.6 in 5 of 18 subjects suggesting that the time to reach a steady state tended to be delayed in certain subjects. In the previous 8-week dose-finding study, that time was longer in subjects receiving a higher dose of LFO, but accumula-

tion was not observed. Therefore, it is conceivable that plasma glabridin concentration at a daily dose of 1800 mg will reach steady state even for an ingestion period of over one month. We are now determining glabridin concentration levels in major organs including the liver and kidney in rats.

Since central obesity is an essential component of metabolic syndrome, maintaining optimal body weight is important for avoiding the onset. Weight loss strategies in current use include dietary therapy, physical therapy, pharmacotherapy, and weight loss surgery. For overweight subjects with BMI of 25 to 30, dietary and physical therapy are considered suitable. Combined with these therapies, LFO may reduce body weight more rapidly and effectively.

In conclusion, ingestion of 300 mg/day of LFO significantly suppressed body weight gain in overweight subjects, probably due to reduction of body fat. LFO was also found to be sufficiently safe when ingested for 12 weeks at 300 mg/day and even for 4 weeks at 1800 mg/day, corresponding to 6 times the effective dose of 300 mg/day. LFO would be a promising safe food ingredient which suppresses weight gain due to an unhealthy lifestyle such as lack of exercise and overeating, and its long-term ingestion could contribute to the maintenance of optimal weight.

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