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Effects of a Capsule Containing Licorice Extract on Body Fat: A Randomized, Double-Blind, Placebo-Controlled, Parallel-Group Study

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Abstract Metabolic syndrome is a condition based on the accumulation of visceral fat, which is associated with cardiovascular disease risk factors such as hyperglycemia, dyslipidemia, and hypertension. It has been reported that reducing body weight and visceral fat improves cardiovascular disease risk factors such as hyperglycemia, dyslipidemia, and hypertension. Therefore, functional food components that reduce visceral fat are expected to be developed. This clinical research evaluated the effect of licorice extract supplementation on visceral fat accumulation. A randomized, placebo-controlled, double-blind parallel group study was conducted in 81 healthy subjects (28-64 years old, body mass index (BMI) between 23 kg/m^2 and less than 30 kg/m^2, and visceral fat area greater than 100 cm^2). Subjects consumed either the test capsule that contained licorice extract (licorice extract group) or a placebo for 12 weeks. Compared with the placebo group, the licorice extract group showed a significant reduction in abdominal fat area (visceral fat area, subcutaneous fat area, and total fat area), body weight, and BMI in the BMI-based subgroup (BMI between 23 kg/m^2 and less than 25 kg/m^2). During this study period, no adverse events related to test capsules were observed. In conclusion, our results indicate that a capsule containing licorice extract reduces visceral fat in healthy subjects. (UMIN CTR ID: UMIN000048430)

Keywords: *glycyrrhiza uralensis, licorice extract, glyasperin B, body fat*

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1. Introduction

Metabolic syndrome is a condition based on the accumulation of visceral fat, which is associated with risk factors for cardiovascular disease such as hyperglycemia, dyslipidemia, and hypertension. Since risk factors for cardiovascular diseases such as hyperglycemia, dyslipidemia, and hypertension can be improved by reducing body weight and visceral fat through lifestyle modification, such as controlling energy intake and promoting exercise, the development of functional food components that reduce body weight and visceral fat has been expected in recent years.

Ural licorice (scientific name *Glycyrrhiza uralensis*) is a leguminous plant that is widely distributed mainly throughout all of Asia and the Mediterranean region. Licorice is used in a variety of ways, generally as a powder or extract from dried licorice root. Because of its unique sweetness, it has been used as a sweetener for foods and beverages and has also been used in Chinese herbal medicine and folk medicine due to its pharmacological effects. Several reports have described its anti-inflammatory [1], antiviral [2], antibacterial, immunomodulatory, and digestive-system-protecting effects [3].

We have previously reported that a root extract of Ural licorice (licorice extract) has antiobesity effects in high-fat high-sucrose (HFS) diet-induced obese mice and inhibits monocyte chemoattractant protein-1 (MCP-1) production in 3T3-L1 mouse adipocytes [4], but its clinical effects have not been investigated.

Therefore, the effects of the continuous intake of licorice extract in humans on visceral fat were investigated in this study for the purpose of developing foods with functional claims (FFCs) in Japan. A randomized, double-blind, parallel-group study was conducted for healthy volunteers with a tendency toward obesity (BMI between 23 kg/m^2 and less than 25 kg/m^2) and obesity level 1 (BMI between 25 kg/m^2 and less than 30 kg/m^2) in accordance with the guidelines of the Japan Society for the Study of Obesity (JASSO) and Food for specified health uses (FOSHU) in Japan.

2. Materials and Methods

2.1. Test Capsules

Both test capsules (licorice extract and placebo) were white hard capsules packaged in aluminum pouch bags of 30 capsules each, with an identification code for the test capsules. The licorice extract capsule contained 100 mg of licorice extract (manufactured by MG Pharma Inc.) and other excipients. The placebo capsule did not contain licorice extract and consisted of excipients colored with caramel coloring. The Institutional Review Board (IRB) reviewed and approved the test capsules and packaging for unidentifiability.

The nutritional composition and ingredients included in the test capsules are listed in Table 1. The amount of glyasperin B in the licorice extract capsule was 14 µg.

Table 1. Licorice extract content and nutritional composition of test capsules [per capsule (per day)]

	Licorice extract capsule	Placebo capsule
Licorice extract (mg)	100	0
Energy (kcal)	1.150	0.765
Protein (g)	0.016	0.000
Lipid (g)	0.006	0.004
Carbohydrate (g)	0.264	0.288
Salt equivalent (mg)	0.651	1.984

2.2. Subjects

The target number of subjects in this study was calculated as 34 subjects per group, for a total of 68 subjects, based on the assumption that the effect size of the test capsule was large (0.8), the significance level was 0.05, and the power of detection was 80-90% in a parallel group comparison study between two groups [5,6] and that 26-35 subjects per group were needed to be included in the study. In addition, the number of entries was set at 80 subjects (40 subjects per group) considering the possibility of dropouts or discontinuation of the study.

Subjects were recruited from the volunteers registered at the contract research organization, and 81 subjects (50 males and 31 females) who met the following inclusion criteria, did not meet the exclusion criteria and were judged by the investigator to be appropriate to participate in the study were included.

2.2.1. Inclusion Criteria

Subjects were selected based on the following conditions at the screening test: (1) male or female, aged 20-65 years; (2) BMI between 23.0 kg/m^2 and less than 30.0 kg/m^2; (3) visceral fat area more than 100 cm^2; and (4) fully informed of the purpose and content of the study, had the ability to consent, understood it well, volunteered to participate, and could give written consent to participate in this study.

2.2.2. Exclusion Criteria

Individuals with the following characteristics were excluded from the study: (1) suffering from or having a history of serious cardiovascular, hepatic, renal, respiratory, endocrine, or metabolic disorders; (2) receiving treatment for chronic diseases such as dyslipidemia, hypertension, or diabetes; (3) having psychiatric disorders such as depression, schizophrenia, or bulimia nervosa; (4) regularly using medicines or quasidrugs that may affect the assessment of body fat, triglycerides, body weight, cholesterol, energy metabolism, etc., in this study; (5) regularly using supplements or health foods (FOSHU, FFC, etc.) that may affect the assessment of body fat, triglycerides, body weight, cholesterol, energy metabolism, etc., in this study; (6) being potentially allergic to the test capsule; (7) having metal in the abdominal Computed Tomography (CT) scan site; (8) having implantable medical devices such as cardiac pacemakers and implantable defibrillators; (9) having claustrophobia; (10) having a smoking habit; (11) having habitual heavy alcohol consumption (more than 60 g/day of pure alcohol); (12) having an extremely irregular diet; (13) working night shifts or day/night shifts; (14) having not defecated for more than 5 days; (15) having menopausal symptoms; (16) having hypertension, pseudoaldosteronism, hypokalemia or myopathy; (17) regularly consuming medicines containing licorice or foods containing licorice as a main ingredient; (18) being prone to swelling and weakness; (19) having urinary dysfunction; (20) having had blood sampling (blood donation, etc.) exceeding 200 mL within 1 month or 400 mL within 3 months before the start of the study; (21) having participated in other clinical trials within the past 4 months, are currently participating in other clinical trials, or planning to participate in other clinical trials during the study period; (22) being pregnant or lactating, or planning to become pregnant; (23) and having any other characteristic for which participation in the study is deemed unsuitable by the investigator.

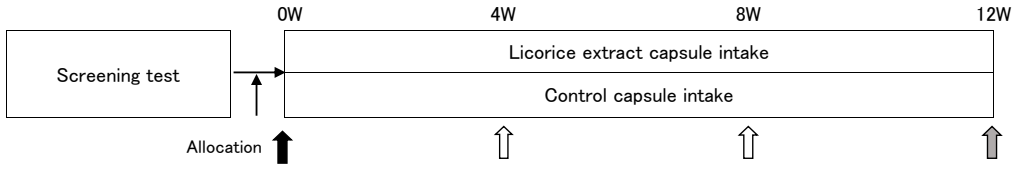
2.3. Study Protocol

This study was a randomized, placebo-controlled, double-blind, parallel-group study. Each examination was conducted at the beginning, 4 weeks, 8 weeks, and 12 weeks after intake of the test capsules. A summary of the study is shown in Figure 1. Subjects were incorporated by the principal investigator and assigned to subjects by an allocation manager who was not involved in the conduct of the study and generated a random number for randomization. At this time, it was confirmed that there were no significant differences between the groups in the allocation factors: sex at the time of consent, age, visceral fat area at the time of screening, BMI, hip circumference, and waist circumference.

During the study period, one capsule (containing licorice extract for the licorice extract group and placebo for the placebo group) was taken once a day with water or lukewarm water before dinner. However, if the subjects were unable to take the capsule before dinner, they were allowed to take one capsule once a day at possible time.

〔Test design〕

A randomized, placebo-controlled, double-blind, parallel-group study



〔Evaluation item〕

(Screening test)

Inspection content	(Prior inspection) height, InBody770 (weight, BMI, body fat percentage, muscle mass, body fat mass by region, basal metabolism, other body composition component analysis, etc.), waist circumference, hip circumference, VAS (fatigue), OSA sleep inventory MA version, blood test, urine test, blood pressure, pulse rate, diseases under treatment, medical history, other items related to exclusion criteria (During CT imaging) abdominal CT scan (visceral fat area, subcutaneous fat area, total fat area), weight, BMI

(Final examination)

⬆	Inspection content	no-show；VAS (fatigue), OSA sleep inventory MA version
⇧	Inspection content	abdominal CT scan (visceral fat area, subcutaneous fat area, total fat area), InBody770, blood test, urine test, waist circumference, hip circumference, VAS (fatigue), OSA sleep inventory MA version, blood pressure, pulse rate, medical interview (subjective symptoms, other symptoms, etc.), physician consultation
⇧	Inspection content	InBody770, blood test, waist circumference, hip circumference, VAS (fatigue), OSA sleep inventory MA version, blood pressure, pulse rate, medical interview (subjective symptoms, other symptoms, etc.), physician consultation

Figure 1. Test design

During the study period, the participants were needed to maintain their usual food and beverage habits as much as possible and were prohibited from consuming healthy foods (FOSHU, FFC, etc.) and grapefruit juice, which may affect the evaluation of body fat, neutral fat, body weight, cholesterol, and energy metabolism in this study. In addition, the participants were to maintain their normal habits regarding alcohol consumption and were prohibited from drinking in a manner that deviated from their normal intake. Alcohol consumption was prohibited on the day before the examination, and consumption of anything other than water was prohibited after 9:00 p.m. In addition, no intake of anything other than water was allowed for 4 hours prior to the abdominal CT scan. During the study period, normal exercise habits (frequency, type of exercise, etc.) were to be maintained as much as possible, and any change in exercise habits was prohibited. During the study period, the use of new medicines was to be approved by the principal investigator, except in case of emergency. Vaccinations were prohibited for 2 weeks prior to the test date. Blood donation was prohibited during the study period.

This study was conducted in the spirit of the Declaration of Helsinki (2013) - Tokyo, Venice, Hong Kong, Somerset West, Edinburgh Amendment, Washington, Tokyo Note added, Seoul Amendment, Fortaleza Amendment - and in accordance with the "Ethical Guidelines for Medical and Health Research Involving Human Subjects (formulated on March 23, 2005, enforced on June 30, 2021, and partially revised on March 10, 2022)". Approval was obtained from the IRB of the Fukuda Internal Medicine Clinic of the Koshokai Medical Corporation (Approval No.: IRB-20220716-2). In addition, the clinical trial was registered in the University Hospital Medical Information Network Clinical Trial Registration System (UMIN) (UMIN study ID: UMIN000048430).

2.4. Examination Items

2.4.1. Physical Examination and Abdominal CT Imaging

Physical examination items included height (at screening only), body composition evaluation (weight, BMI, body fat percentage, muscle mass, body fat mass by region, basal metabolism, and other body composition component analysis), waist circumference, hip circumference, blood pressure, and pulse rate using InBody770 (InBody Japan Inc.), and CT imaging was performed on those who met the inclusion criteria. For body weight, the DST-210 N (Muratec KDS Co., Ltd.) was used only during CT imaging. BMI was calculated from height and weight, and the BMI at the time of CT imaging was used as the value before the start of intake.

Abdominal CT imaging was performed using a Discovery 710 or Discovery MIDR (GE Healthcare Japan, Inc.). Three slices were taken at 1 cm intervals centered around the umbilical region, and the mean values of

visceral fat area, subcutaneous fat area, and total fat area were calculated. If the images taken at the umbilical location included the kidneys and ilium, reimaging was performed centering around the fourth lumbar vertebra.

2.4.2. Dietary and Activity Records

During the study period, subjects were asked to record their alcohol intake, use of medications, and diet in a daily logbook. During the test capsule intake period, subjects were asked to record their intake of the test capsules. They were also asked to record the amount of food they consumed in addition to their diet for the three days prior to the test date.

During the test period, the subjects were fitted with an activity meter (SAT-1, MediSync Inc.) except when sleeping, and the number of steps, active steps, and moderate-intensity activity time were checked every two weeks.

The subjects were interviewed and examined by a physician at 4, 8, and 12 weeks after intake to ascertain the occurrence of subjective and objective symptoms.

2.4.3. Fatigue and Sleep Evaluation

Fatigue was evaluated using the visual analog scale (VAS) method at the beginning of intake, 4, 8, and 12 weeks after intake, and upon waking up at the subject's home. Sleep was evaluated at the subject's homes using the OSA sleep inventory (MA version) upon waking and at the following time points: the beginning of intake and 4, 8, and 12 weeks after intake.

2.4.4. Blood and Urine Tests

Blood samples were collected before, 4, 8, and 12 weeks after intake, and the following analytes were measured: white blood cell (WBC), red blood cell (RBC), hemoglobin (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet (Plt), white blood cell fraction, total protein, albumin (Alb), albumin/globulin (A/G) ratio, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP), creatinine, blood glucose, total cholesterol (T-Cho), high-density lipoprotein cholesterol (HDL-Cho), low-density lipoprotein cholesterol (LDL-Cho), triglyceride (TG), creatine phosphokinase (CPK), uric acid (UA), urea nitrogen (UN), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), sodium (Na), potassium (K), chloride (Cl), calcium (Ca), magnesium (Mg), phosphorus (P), total bilirubin (T-Bil), hemoglobin A1c (HbA1c), glycoalbumin, insulin, and ketone body fraction (total ketone body, acetoacetate, and 3-hydroxybutyrate).

Urinalysis was performed for glucose, protein, occult blood, and urobilinogen before and 12 weeks after ingestion.

2.5. Statistical Analysis

The primary endpoint was visceral fat area on CT abdominal imaging. Secondary endpoints were weight, BMI, subcutaneous and total fat area on abdominal CT imaging, body fat percentage, body fat mass, muscle mass, body fat mass by site, basal metabolic rate, waist circumference, hip circumference and waist-to-hip ratio on InBody770, VAS (fatigue), OSA sleep inventory MA version, ketone body fraction, T-Cho, LDL-Cho, HDL-Cho, TG, blood glucose, HbA1c, insulin and glycoalbumin on blood tests.

Statistical analysis was performed using the statistical processing software SPSS Ver. 26 (IBM Japan, Inc.), and the significance level was set at 5% with a two-tailed test. To identify trends by subject demographics, subgroup analyses were conducted for the primary endpoints by BMI (BMI between 23 kg/m^2 and less than 25 kg/m^2, BMI between 25 kg/m^2 and less than 30 kg/m^2 before the start of intake) and sex. Subgroups with significant differences in primary endpoints were also evaluated for secondary endpoints. The mean values and standard deviations of the values at each time point and the change from preconsumption to each time point were tabulated by group, and differences between groups were compared by performing an unpaired t test on the difference in mean values between the licorice extract group and the placebo group at each time point.

In addition, Dunnett's multiple comparison test was performed on the difference between the means of each test capsule group before the start of intake and at each examination time point to compare differences due to the duration of intake of the test capsules. For CT imaging of the abdomen, a paired t test was performed to compare the differences by the duration of intake of the test capsules. No correction for multiplicity was made for the paired t test.

3. Results

3.1. Subjects for Analysis

Although the total subject number enrolled in this study was 81 (41 for the licorice extract group, 40 for the placebo group), the study was started with 80 subjects because one subject in the licorice extract group was discontinued due to the start of treatment, which violated the exclusion criterion (2). The safety analysis population (Full Analysis Set (FAS)) consisted of 78 subjects (39 in the licorice extract group and 39 in the placebo group), excluding 1 subject in the licorice extract group who was found to be in violation of the exclusion criterion (15) after the start of the study and 1 subject in the placebo group who had never consumed the test capsules and discontinued the study for his own reason. The efficacy analysis population (per protocol set (PPS)) was 76 subjects (38 subjects in the licorice extract group and 38 subjects in the placebo group) because one subject in the licorice extract group discontinued the study due to autoimmune pancreatitis and one subject in the placebo group discontinued the study due to personal reasons during the intake period of the test capsules. Since the data from the following two cases in the placebo group were thought to be inaccurate, these data were excluded from analysis: in one case, LDH, K, and insulin in the blood test at 4 weeks after intake were excluded from the analysis due to hemolysis in the blood sample; in the other case, because food intake on the day of the test (noncompliance with control items) was suspected, blood glucose and insulin in the blood test at 8 weeks were excluded. A flowchart showing the subjects at each stage

of the study is shown in Figure 2, and the backgrounds of the subjects in the efficacy analysis are shown in Table 2.

There were no significant differences between the licorice extract group and the placebo group in sex at consent, age, visceral fat area at screening, BMI, waist circumference, and hip circumference, even when analyzed in any of the subgroups by FAS, PPS, BMI, or sex.

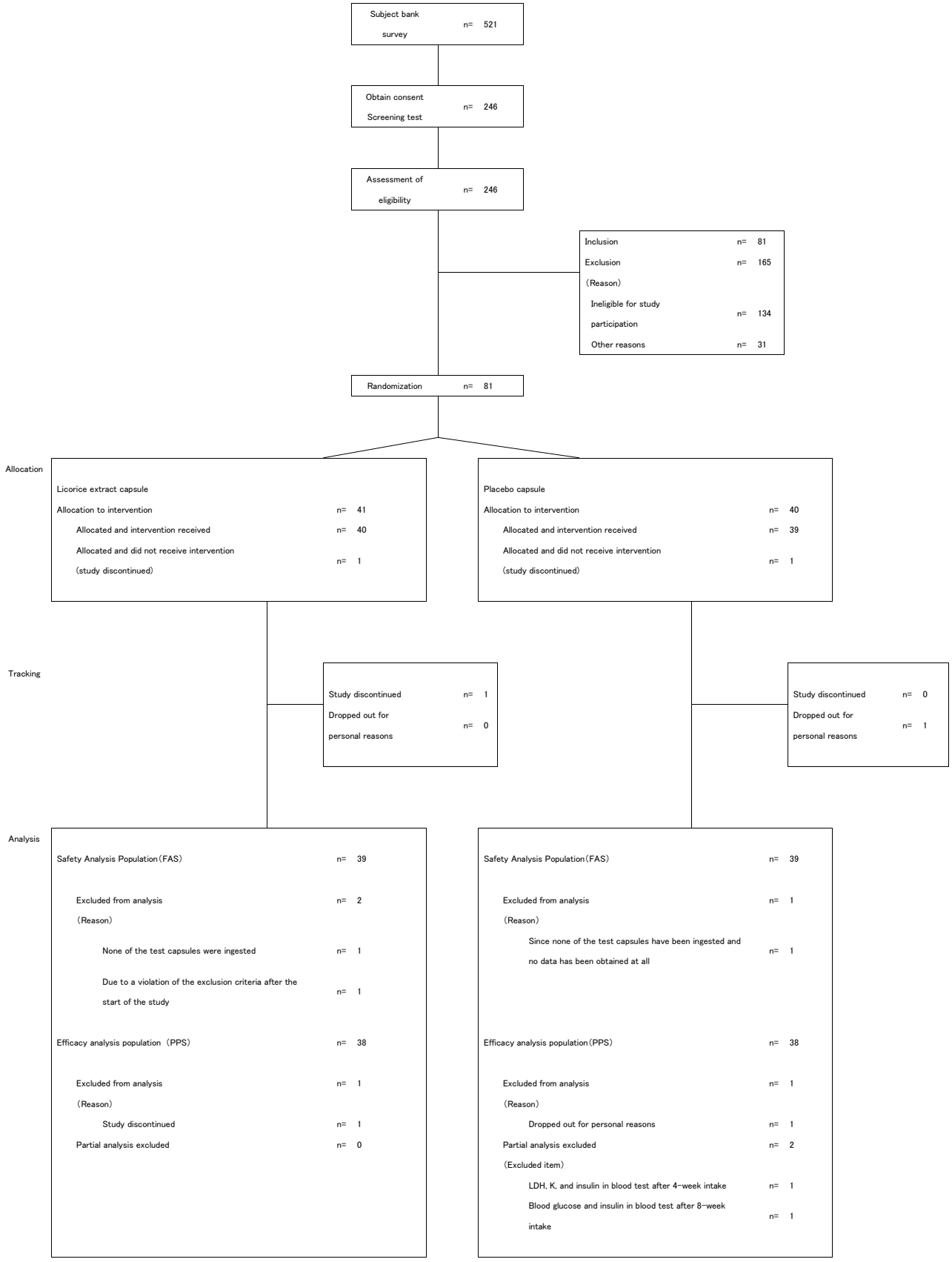


Figure 2. Flowchart showing the number of subjects in each phase

Table 2. Subject background

	n	Male	Female	Age(years)	Visceral fat area(cm^2) before intake	BMI(kg/m^2) before intake	Waist circumference(cm) before intake	Hip circumference(cm) before intake
Licorice extract	38	24	14	54.2 ± 6.0	129.91 ± 19.06	26.1 ± 1.5	93.9 ± 5.7	100.9 ± 4.4
Placebo capsule	38	23	15	53.7 ± 7.4	131.16 ± 19.66	26.2 ± 1.9	93.9 ± 7.0	101.1 ± 5.4

Mean ± standard deviation

Table 3. Abdominal fat area

			Before intake Licorice extract: n=38 Placebo capsule: n=38		After 12-week intake Licorice extract: n=37 Placebo capsule: n=38	
Visceral fat area (cm^2)	Measured value	Licorice extract	129.9 ± 19.1		124.0 ± 22.9	§
		Placebo capsule	131.2 ± 19.7		126.4 ± 24.9	
	⊿	Licorice extract			-6.5 ± 18.9	
		Placebo capsule			-4.7 ± 20.1	
Subcutaneous fat area (cm^2)	Measured value	Licorice extract	214.0 ± 52.8		211.2 ± 52.1	
		Placebo capsule	219.2 ± 73.1		221.8 ± 78.0	
	⊿	Licorice extract			-3.5 ± 17.9	
		Placebo capsule			2.5 ± 14.7	
Total fat area (cm^2)	Measured value	Licorice extract	344.0 ± 55.9		335.2 ± 57.2	§
		Placebo capsule	350.4 ± 77.6		348.2 ± 83.8	
	⊿	Licorice extract			-10.0 ± 27.9	
		Placebo capsule			-2.2 ± 27.8	

Mean ± standard deviation, ⊿; Mean ± standard deviation of change from before intake
Between-group comparison (unpaired t-test): No significant difference or trend
Comparison with before intake (paired t-test): § $p<0.05$

Table 4. Physical measurement item

			Before intake Licorice extract: n=38 Placebo capsule: n=38		After 4-week intake Licorice extract: n=38 Placebo capsule: n=38		After 8-week intake Licorice extract: n=36 Placebo capsule: n=37		After 12-week intake Licorice extract: n=37 Placebo capsule: n=38	
Body weight (kg)	Measured value	Licorice extract	72.3 ± 8.7		71.7 ± 8.6	§§	71.6 ± 8.4	§§	71.4 ± 8.8	§§
		Placebo capsule	73.1 ± 8.9		72.7 ± 9.2		72.8 ± 9.3		72.7 ± 9.3	
	⊿	Licorice extract			-0.6 ± 1.1		-0.7 ± 1.1		-0.7 ± 1.4	
		Placebo capsule			-0.4 ± 1.1		-0.4 ± 1.2		-0.4 ± 1.5	
BMI (kg/m^2)	Measured value	Licorice extract	26.1 ± 1.5		25.8 ± 1.5	§§	25.9 ± 1.5	§§	25.7 ± 1.6	§§
		Placebo capsule	26.2 ± 1.9		26.0 ± 2.0		26.0 ± 2.0		26.0 ± 2.0	
	⊿	Licorice extract			-0.2 ± 0.4		-0.2 ± 0.4		-0.3 ± 0.5	
		Placebo capsule			-0.2 ± 0.4		-0.2 ± 0.4		-0.1 ± 0.5	
Body fat mass (kg)	Measured value	Licorice extract	22.0 ± 3.9		21.9 ± 4.0		22.2 ± 4.1		22.1 ± 4.2	
		Placebo capsule	22.9 ± 5.2		22.7 ± 5.6		23.0 ± 5.6		23.0 ± 5.7	
	⊿	Licorice extract			-0.1 ± 1.1		0.0 ± 1.2		0.1 ± 1.4	
		Placebo capsule			-0.2 ± 1.0		0.1 ± 1.0		0.1 ± 1.3	
Muscle mass (kg)	Measured value	Licorice extract	47.5 ± 8.8		47.0 ± 8.4	§	46.7 ± 8.1	§§	46.5 ± 8.3	§§
		Placebo capsule	47.4 ± 8.4		47.2 ± 8.5		47.1 ± 8.5		46.9 ± 8.6	§
	⊿	Licorice extract			-0.5 ± 1.5		-0.7 ± 1.3		-0.8 ± 1.2	
		Placebo capsule			-0.2 ± 0.8		-0.5 ± 0.6		-0.4 ± 1.1	
Body fat mass (right arm) (kg)	Measured value	Licorice extract	1.5 ± 0.4		1.5 ± 0.4		1.5 ± 0.4		1.5 ± 0.4	
		Placebo capsule	1.6 ± 0.6		1.6 ± 0.6		1.6 ± 0.6		1.6 ± 0.6	
	⊿	Licorice extract			0.0 ± 0.1		0.0 ± 0.1		0.0 ± 0.1	
		Placebo capsule			0.0 ± 0.1		0.0 ± 0.1		0.0 ± 0.1	
Body fat mass (left arm) (kg)	Measured value	Licorice extract	1.5 ± 0.4		1.5 ± 0.4		1.5 ± 0.4		1.5 ± 0.4	
		Placebo capsule	1.6 ± 0.6		1.6 ± 0.6		1.6 ± 0.6		1.6 ± 0.6	
	⊿	Licorice extract			0.0 ± 0.1		0.0 ± 0.1		0.0 ± 0.1	
		Placebo capsule			0.0 ± 0.1		0.0 ± 0.1		0.0 ± 0.1	

			Before intake Licorice extract: n=38 Placebo capsule: n=38	After 4-week intake Licorice extract: n=38 Placebo capsule: n=38	After 8-week intake Licorice extract: n=36 Placebo capsule: n=37	After 12-week intake Licorice extract: n=37 Placebo capsule: n=38
Body fat mass (trunk) (kg)	Measured value	Licorice extract	11.2 ± 1.9	11.1 ± 2.0	11.3 ± 2.1	11.3 ± 2.1
		Placebo capsule	11.5 ± 2.4	11.4 ± 2.6	11.6 ± 2.6	11.7 ± 2.7
	⊿	Licorice extract		-0.1 ± 0.6	0.1 ± 0.6	0.1 ± 0.8
		Placebo capsule		-0.1 ± 0.5	0.1 ± 0.6	0.2 ± 0.7
Body fat mass (right foot) (kg)	Measured value	Licorice extract	3.3 ± 0.6	3.3 ± 0.6	3.3 ± 0.6	3.3 ± 0.6
		Placebo capsule	3.5 ± 0.8	3.4 ± 0.9	3.5 ± 0.9	3.5 ± 0.9
	⊿	Licorice extract		0.0 ± 0.2	0.0 ± 0.2	0.0 ± 0.2
		Placebo capsule		-0.1 ± 0.2	0.0 ± 0.2	0.0 ± 0.2
Body fat mass (left foot) (kg)	Measured value	Licorice extract	3.3 ± 0.6	3.3 ± 0.6	3.3 ± 0.6	3.3 ± 0.6
		Placebo capsule	3.5 ± 0.8	3.4 ± 0.9	3.5 ± 0.9	3.4 ± 0.9
	⊿	Licorice extract		0.0 ± 0.2	0.0 ± 0.2	0.0 ± 0.2
		Placebo capsule		-0.1 ± 0.2	0.0 ± 0.2	-0.1 ± 0.2
Waist circumference (cm)	Measured value	Licorice extract	93.9 ± 5.7	93.9 ± 5.2	94.0 ± 5.6	93.2 ± 5.6
		Placebo capsule	93.9 ± 7.0	94.2 ± 7.4	94.6 ± 7.4 §	93.8 ± 7.2
	⊿	Licorice extract		0.0 ± 1.8	0.2 ± 1.8	-0.5 ± 1.9
		Placebo capsule		0.3 ± 1.5	0.7 ± 1.5	-0.1 ± 1.6
Hip circumference (cm)	Measured value	Licorice extract	100.9 ± 4.4	101.1 ± 4.2	101.1 ± 4.0	99.9 ± 4.2 §§
		Placebo capsule	101.1 ± 5.4	101.6 ± 5.6	102.2 ± 5.5 §§	100.9 ± 5.4
	⊿	Licorice extract		0.3 ± 1.7	0.4 ± 1.6	-0.8 ± 1.8
		Placebo capsule		0.5 ± 2.2	0.9 ± 2.1	-0.2 ± 2.5
Waist/Hip Ratio	Measured value	Licorice extract	0.93 ± 0.04	0.93 ± 0.03	0.93 ± 0.03	0.93 ± 0.04
		Placebo capsule	0.93 ± 0.05	0.93 ± 0.04	0.93 ± 0.04	0.93 ± 0.04
	⊿	Licorice extract		0.00 ± 0.02	0.00 ± 0.02	0.00 ± 0.02
		Placebo capsule		0.00 ± 0.02	0.00 ± 0.02	0.00 ± 0.03
Systolic blood pressure (mmHg)	Measured value	Licorice extract	120 ± 11	122 ± 15]*	119 ± 12	120 ± 12
		Placebo capsule	121 ± 12	115 ± 12 §§	115 ± 13 §§	118 ± 12
	⊿	Licorice extract		2 ± 13]**	-1 ± 11	1 ± 13
		Placebo capsule		-6 ± 9	-6 ± 11	-3 ± 11
Diastolic blood pressure (mmHg)	Measured value	Licorice extract	72 ± 8	76 ± 9 §§	73 ± 8	74 ± 8
		Placebo capsule	74 ± 8	73 ± 9	72 ± 9	73 ± 9
	⊿	Licorice extract		4 ± 8]**	1 ± 7	2 ± 8
		Placebo capsule		0 ± 6	-2 ± 6	-1 ± 7
Pulse rate (times/minute)	Measured value	Licorice extract	69 ± 9	74 ± 14 §	72 ± 10	74 ± 15 §§
		Placebo capsule	70 ± 10	70 ± 8	73 ± 12	75 ± 11 §§
	⊿	Licorice extract		5 ± 10]*	4 ± 9	5 ± 11
		Placebo capsule		0 ± 6	3 ± 7	5 ± 7

Mean ± standard deviation, ⊿; Mean ± standard deviation of change from before intake

※Systolic blood pressure, diastolic blood pressure, and pulse rate before intake of the Licorice extract capsule: n=39, the Placebo capsule: n=39, after 4-week intake, the Licorice extract capsule: n=38, the Placebo capsule: n=38, after 8-week intake, the Licorice extract capsule: n=37, the Placebo capsule: n=37, after 12-week intake, the Licorice extract capsule: n=37, the Placebo capsule: n=38

※Waist circumference, hip circumference, and waist/hip ratio after 8-week intake, the Licorice extract capsule: n=37, the Placebo capsule: n=37

Between-group comparison (unpaired t-test): ** $p<0.01$, * $p<0.05$

Comparison with before intake (Dunnett's multiple comparison test): §§ $p<0.01$, § $p<0.05$

3.2. Analysis Results

3.2.1. Abdominal Fat Area

Changes in visceral fat area, subcutaneous fat area, and total fat area in the efficacy analysis population (PPS) are shown in Table 3. There were no significant differences in visceral fat area, the primary endpoint, between the licorice extract and placebo groups. Within the test capsule groups, there was a significant decrease in visceral fat area and total fat area over time after 12 weeks of intake compared to the beginning of intake.

3.2.2. Physical Measurements

The changes in body weight, BMI, body fat mass, muscle mass, body fat mass by region, waist circumference and hip circumference in the PPS and the changes in blood pressure and pulse rate in the FAS are shown in Table 4. In terms of blood pressure and pulse rate, the licorice extract group showed significantly higher systolic and diastolic blood pressures and a significantly higher change in pulse rate than the placebo group after 4 weeks of consumption, but no significant changes were observed thereafter. There were no significant differences between the licorice extract group and the placebo group for the other endpoints.

3.2.3. Blood and Urine Tests

The changes in blood tests (secondary endpoints) during the PPS are shown in Table 1. After 4 weeks of intake, the licorice extract group showed a significant reduction in the change in T-Cho compared to the placebo

group. Blood glucose levels were significantly higher in the licorice extract group than in the placebo group before the start of consumption and remained significantly higher after 4 weeks of consumption, but there was no difference thereafter. The insulin level was significantly lower in the licorice extract group than in the placebo group.

No clinically relevant changes were observed in any of the other blood or urine test items in the FAS.

3.2.4. Activity Scale

The number of steps taken, the number of active steps taken, and the duration of moderate-intensity activity were checked every two weeks during the study period, and no significant between-group differences were observed.

3.2.5. Subgroup Analysis

The results of subgroup analysis by BMI (BMI between 23 kg/m^2 and less than 25 kg/m^2 before intake, BMI between 25 kg/m^2 and less than 30 kg/m^2 before intake) and sex are shown in Tables 5-1 through 5-4.

Table 5-1. Subgroup analysis Primary endpoint BMI between 23 kg/m^2 and less than 25 kg/m^2 before intake

			Before intake Licorice extract: n=10 Placebo capsule: n=12			After 12-week intake Licorice extract: n=10 Placebo capsule: n=12				
Visceral fat area(cm^2)	Measured value	Licorice extract	127.5	±	23.4	115.2	±	23.3	]*	§
		Placebo capsule	137.8	±	19.8	138.9	±	25.1		
	⊿	Licorice extract				-12.3	±	16.7		
		Placebo capsule				1.0	±	17.9		

Mean ± standard deviation, ⊿; Mean ± standard deviation of change from before intake
Between-group comparison (unpaired t-test): * $p<0.05$
Comparison with before intake (Dunnett's multiple comparison test): § $p<0.05$

Table 5-2. Subgroup analysis Primary endpoint BMI between 25 kg/m^2 and less than 30 kg/m^2 before intake

			Before intake Licorice extract: n=28 Placebo capsule: n=26			After 12-week intake Licorice extract: n=27 Placebo capsule: n=26		
Visceral fat area(cm^2)	Measured value	Licorice extract	130.8	±	17.7	127.3	±	22.3
		Placebo capsule	128.1	±	19.2	120.7	±	23.1
	⊿	Licorice extract				-4.3	±	19.4
		Placebo capsule				-7.4	±	20.8

Mean ± standard deviation, ⊿; Mean ± standard deviation of change from before intake
Between-group comparison (unpaired t-test): no significant difference
Comparison with before intake (paired t-test): no significant difference

Table 5-3. Subgroup analysis Primary endpoint Male

			Before intake Licorice extract: n=24 Placebo capsule: n=23			After 12-week intake Licorice extract: n=23 Placebo capsule: n=23		
Visceral fat area(cm^2)	Measured value	Licorice extract	129.8	±	20.5	125.5	±	24.6
		Placebo capsule	127.9	±	16.5	127.3	±	25.5
	⊿	Licorice extract				-5.3	±	21.6
		Placebo capsule				-0.6	±	23.0

Mean ± standard deviation, ⊿; Mean ± standard deviation of change from before intake
Between-group comparison (unpaired t-test): no significant difference
Comparison with before intake (paired t-test): no significant difference

Table 5-4. Subgroup analysis Primary endpoint Female

			Before intake Licorice extract: n=14 Placebo capsule: n=15			After 12-week intake Licorice extract: n=14 Placebo capsule: n=15			
Visceral fat area(cm^2)	Measured value	Licorice extract	130.1	±	17.1	121.6	±	20.5	§
		Placebo capsule	136.2	±	23.4	125.2	±	24.7	§§
	⊿	Licorice extract				-8.4	±	13.6	
		Placebo capsule				-11.0	±	12.8	

Mean ± standard deviation, ⊿; Mean ± standard deviation of change from before intake
Between-group comparison (unpaired t-test): no significant difference
Comparison with before intake (paired t-test): §§ $p<0.01$, § $p<0.05$

Table 5-5. Subgroup analysis Secondary endpoint BMI between 23 kg/m^2 and less than 25 kg/m^2 before intake

			Before intake Licorice extract: n=10 Placebo capsule: n=12		After 4-week intake Licorice extract: n=10 Placebo capsule: n=12		After 8-week intake Licorice extract: n=10 Placebo capsule: n=12		After 12-week intake Licorice extract: n=10 Placebo capsule: n=12	
Subcutaneous fat area (cm^2)	Measured value	Licorice extract	189.4 ± 44.0						179.5 ± 40.6	§
		Placebo capsule	164.4 ± 30.6						168.1 ± 32.1	
	⊿	Licorice extract							-9.9 ± 12.6	]*
		Placebo capsule							3.7 ± 10.2	
Total fat area (cm^2)	Measured value	Licorice extract	317.0 ± 57.5						294.7 ± 54.0	§
		Placebo capsule	302.2 ± 38.9						306.9 ± 44.2	
	⊿	Licorice extract							-22.3 ± 26.5	]*
		Placebo capsule							4.7 ± 22.7	
Body weight (kg)	Measured value	Licorice extract	65.7 ± 7.0		65.3 ± 7.0		65.8 ± 7.0		64.7 ± 7.1	§§
		Placebo capsule	68.3 ± 7.4		68.0 ± 7.3		68.1 ± 7.3		68.3 ± 7.2	
	⊿	Licorice extract			-0.3 ± 0.9		-0.6 ± 1.0		-1.0 ± 1.0	]*
		Placebo capsule			-0.3 ± 1.0		-0.2 ± 0.7		0.0 ± 1.0	
BMI (kg/m^2)	Measured value	Licorice extract	24.2 ± 0.3		24.1 ± 0.5		24.0 ± 0.5		23.9 ± 0.5	§§
		Placebo capsule	24.2 ± 0.5		24.1 ± 0.6		24.1 ± 0.5		24.2 ± 0.6	
	⊿	Licorice extract			-0.1 ± 0.3		-0.2 ± 0.3		-0.4 ± 0.3	]*
		Placebo capsule			-0.1 ± 0.4		-0.1 ± 0.3		0.0 ± 0.4	
Total ketone body (μmoL/L)	Measured value	Licorice extract	117 ± 119		157 ± 198		135 ± 129		154 ± 156	
		Placebo capsule	125 ± 158		121 ± 140		91 ± 98		68 ± 83	
	⊿	Licorice extract			40 ± 91		18 ± 82		37 ± 70	]*
		Placebo capsule			-5 ± 66		-34 ± 188		-57 ± 87	
Acetoacetic acid (μmoL/L)	Measured value	Licorice extract	31 ± 28		44 ± 42		43 ± 37		42 ± 37	
		Placebo capsule	32 ± 33		37 ± 37		30 ± 26		21 ± 20	
	⊿	Licorice extract			13 ± 24		12 ± 23		11 ± 19	]*
		Placebo capsule			5 ± 19		-2 ± 39		-11 ± 20	
3-hydroxybutyric acid (μmoL/L)	Measured value	Licorice extract	86 ± 92		113 ± 158		92 ± 93		112 ± 119	
		Placebo capsule	94 ± 126		84 ± 103		61 ± 73		47 ± 64	
	⊿	Licorice extract			27 ± 72		6 ± 61		26 ± 53	]*
		Placebo capsule			-10 ± 53		-32 ± 150		-47 ± 69	
T-Cho (mg/dL)	Measured value	Licorice extract	219 ± 37		225 ± 32		224 ± 41		228 ± 38	
		Placebo capsule	207 ± 20		210 ± 22		211 ± 23		216 ± 27	
	⊿	Licorice extract			6 ± 17		5 ± 15		9 ± 16	
		Placebo capsule			3 ± 15		3 ± 17		9 ± 22	
LDL-Cho (direct method) (mg/dL)	Measured value	Licorice extract	134 ± 24		133 ± 26		131 ± 31		137 ± 29	
		Placebo capsule	132 ± 18		134 ± 17		132 ± 15		134 ± 24	
	⊿	Licorice extract			-1 ± 15		-3 ± 14		3 ± 14	
		Placebo capsule			2 ± 14		0 ± 16		2 ± 21	
HDL-Cho (mg/dL)	Measured value	Licorice extract	65 ± 18		67 ± 17	]*	69 ± 17	]*	72 ± 16	]** §§
		Placebo capsule	54 ± 8		52 ± 9		54 ± 10		55 ± 11	
	⊿	Licorice extract			2 ± 8		4 ± 6		7 ± 6	
		Placebo capsule			-2 ± 5		0 ± 5		1 ± 7	
TG (mg/dL)	Measured value	Licorice extract	96 ± 25		113 ± 50		100 ± 22		77 ± 15	]*
		Placebo capsule	124 ± 59		141 ± 92		120 ± 62		137 ± 86	
	⊿	Licorice extract			18 ± 62		5 ± 30		-19 ± 27	]*
		Placebo capsule			17 ± 44		-4 ± 27		13 ± 40	
Blood glucose (mg/dL)	Measured value	Licorice extract	98 ± 12	]*	98 ± 12	]*	99 ± 9	]*	98 ± 10	
		Placebo capsule	88 ± 8		89 ± 6		92 ± 5		92 ± 6	§
	⊿	Licorice extract			0 ± 7		1 ± 7		0 ± 6	
		Placebo capsule			0 ± 5		3 ± 6		4 ± 7	
HbA1c (%)	Measured value	Licorice extract	5.5 ± 0.3		5.4 ± 0.3		5.4 ± 0.4	§	5.3 ± 0.3	§§
		Placebo capsule	5.6 ± 0.3		5.5 ± 0.2		5.4 ± 0.2	§§	5.4 ± 0.2	§§
	⊿	Licorice extract			-0.1 ± 0.2		-0.1 ± 0.2		-0.2 ± 0.2	
		Placebo capsule			-0.1 ± 0.2		-0.2 ± 0.2		-0.2 ± 0.2	

			Before intake Licorice extract: n=10 Placebo capsule: n=12	After 4-week intake Licorice extract: n=10 Placebo capsule: n=12	After 8-week intake Licorice extract: n=10 Placebo capsule: n=12	After 12-week intake Licorice extract: n=10 Placebo capsule: n=12	
Insulin (mU/mL)	Measured value	Licorice extract	5.7 ± 2.1	7.8 ± 4.5	7.0 ± 3.3	6.5 ± 2.1	
		Placebo capsule	5.7 ± 2.5	7.9 ± 3.2	§ 8.7 ± 3.0	§§ 8.6 ± 2.9	§§
	⊿	Licorice extract		2.1 ± 3.9	1.3 ± 2.0	0.8 ± 1.4	]**
		Placebo capsule		2.2 ± 2.1	2.9 ± 1.8	2.9 ± 1.4	
Glycoalbumin (%)	Measured value	Licorice extract	13.8 ± 1.0	13.7 ± 1.1	14.0 ± 1.1	13.8 ± 1.2	
		Placebo capsule	13.5 ± 0.9	13.3 ± 0.8	13.5 ± 0.7	13.3 ± 0.6	
	⊿	Licorice extract		-0.2 ± 0.4	0.1 ± 0.4	0.0 ± 0.4	
		Placebo capsule		-0.1 ± 0.5	0.0 ± 0.4	-0.2 ± 0.5	

Mean ± standard deviation, ⊿; Mean ± standard deviation of change from before intake
Between-group comparison (unpaired t-test): ** p<0.01, * p<0.05
Comparison with before intake (Dunnett's multiple comparison test): §§ p<0.01, § p<0.05

Table 6. Adverse events

Adverse events	Licorice extract capsule (number of patients)	Placebo capsule (number of patients)
Headache	2	0
Headache, general malaise, chill	0	1
Chill	0	1
Slight fever	1	0
Fever	1	2
Hyperemia	0	1
Eye discomfort	0	1
Herpes labialis	0	1
Gum pain	1	0
Sore throat	1	0
Cough, phlegm	2	0
Lumbago	2	0
Abdominal symptoms (abdominal pain, soft stools, dysentery)	1	4
Autoimmune pancreas	1	0
CPK High	1	0
Itching, redness, rash(due to wearing activity meters, insect bites, etc.)	0	11
Arthralgia	1	0
Fever, joint pain	1	0
Abrasion	1	0
Bone fracture	2	0
Ill mood	1	0
Tired feeling	0	1

Licorice extract group: n=39; Placebo group: n=39

The value of visceral fat area on CT scan of the abdomen, the primary endpoint, was significantly lower in the licorice extract group than in the placebo group at 12 weeks after intake in the subgroup analysis for BMI between 23 kg/m^2 and less than 25 kg/m^2 before intake. On the other hand, there was no significant difference between the licorice extract and placebo groups in the subgroup analysis of BMI between 25 kg/m^2 and less than 30 kg/m^2 before intake. In a subgroup analysis by sex, there were no significant differences between the licorice extract and placebo groups.

The results of the analysis performed on the secondary endpoints for the subgroup of BMI between 23 kg/m^2 and less than 25 kg/m^2, where a significant difference between licorice extract and placebo groups was observed in the primary endpoint, are shown in Table 5-5. In the change in subcutaneous fat area and total fat area in abdominal CT from before intake, significant decreases were observed between the groups after 12 weeks of intake. In the anthropometric parameters, the licorice extract group showed significant decreases from before intake in the change in body weight and BMI after 12 weeks of intake compared to the placebo group. In addition, in the blood test endpoints after 12 weeks of intake, the licorice extract group showed significant increases from before intake in total ketones, acetoacetic acid, and 3-hydroxybutyric acid compared to the placebo group; a significantly higher value for HDL-Cho after 4, 8, and 12 weeks of intake than that in the placebo group; significant decreases in TG after 12 weeks of intake compared to that in the placebo group; a significantly higher blood glucose value before, 4 and 8 weeks after intake than that in the placebo group; and a significantly lower insulin value than that in the placebo group after 12 weeks of intake.

4. Safety

Adverse events observed during the study period are listed in Table 6. Although there were 19 adverse events

in 13 subjects in the licorice extract group and 23 events in 14 subjects in the placebo group, none of the adverse events were diagnosed as causally related to the test capsules. One serious adverse event (autoimmune pancreatitis) was reported by the study investigator to the IRB and the head of the study institution according to the study protocol.

5. Discussion

A randomized, placebo-controlled, double-blind, parallel-group study was conducted to investigate the body fat-reducing effects of capsules containing licorice extract on healthy adult volunteers with a BMI between 23 kg/m^2 and 30 kg/m^2 and an abdominal visceral fat area of more than 100 cm^2 who were given capsules containing 100 mg of licorice extract (licorice extract group) or capsules containing no licorice extract (placebo group) once a day for 12 weeks to investigate the body fat-reducing effects of capsules containing licorice extract. In the analysis with all subjects included, visceral fat area, the primary endpoint, was not significantly different between the licorice extract group and the placebo group; however, there was a significant decrease in visceral fat area within the licorice extract group after 12 weeks of intake. Subgroup analysis of the primary endpoint showed no significant differences between groups in the subjects with a BMI between 25 kg/m^2 and less than 30 kg/m^2, but in the subjects with a BMI between 23 kg/m^2 and less than 25 kg/m^2, the visceral fat area of the licorice extract group was significantly lower than that of the placebo group after 12 weeks of intake. Analysis of secondary endpoints in the subgroup with BMI between 23 kg/m^2 and less than 25 kg/m^2 showed significant decreases in subcutaneous fat area, total fat area, body weight, and BMI in the licorice extract group. The reason for the significant difference in the subgroup with BMI between 23 kg/m^2 and less than 25 kg/m^2 may be because the licorice extract ingested in this study was developed as a food and its efficacy was relatively mild. Therefore, it may be useful for improving the above variables in individuals in the normal range with a tendency toward obesity.

Regarding the mechanism of the body fat reduction occurring with licorice extract consumption, it has been confirmed that licorice extract promotes the breakdown of TG in adipocytes in cellular tests (unpublished data). In animal studies, inhibition of body fat accumulation and reduction in adipocyte size were also confirmed, supporting the results of cellular studies [4].

In the present study, body fat reduction was also observed, with significant increases in total ketone bodies, acetoacetic acid, and 3-hydroxybutyric acid in the licorice extract group compared to the placebo group. The increase in blood ketone bodies is an indicator of fatty acid consumption by β-oxidation and is also seen when insulin resistance is indicated, but no increase in blood glucose or insulin levels was observed, suggesting that the increase was not due to worsening of insulin resistance but rather to increased energy consumption, which has been reported in other functional foods [7].

These results suggest that when TG degradation is promoted in adipocytes by intake of licorice extract, free fatty acids should be released and quickly consumed as an energy source by β-oxidation, resulting in a body fat reduction effect.

Regarding TG degradation in adipocytes, it has been confirmed that glyasperin B in licorice extract shows an almost 100% contribution rate (in-house test report by MG Pharma Inc.). Based on the above, it was considered that the body fat reduction effect of licorice extract intake was due to glyasperin B.

In this study, the safety of licorice extract was also examined. As a result, no adverse events, including abnormalities in laboratory test values, were observed, suggesting that there is no problem with the safety of long-term intake of licorice extract.

In this study, the body fat-reducing effect of licorice extract and its safety were confirmed.

A limitation of this study is that the results of the subgroup analysis were obtained in a small number of cases, and another study using an additional number of cases would provide more details.

6. Conclusions

In a randomized, placebo-controlled, double-blind, parallel-group study, capsules containing licorice extract showed no effect on body fat reduction in all subjects (BMI between 23 kg/m^2 and less than 30 kg/m^2), while in healthy, obese adult men and women (BMI between 23 kg/m^2 and less than 25 kg/m^2), it was shown to have a body fat reducing effect, with associated weight and BMI reductions.

Statement of Competing Interests

The cost of conducting this study was funded by ROHTO Pharmaceutical Co., Ltd. The authors, Kosei Tsukamoto, Kazutake Fukada, Yukiko Sekii, Chiaki Ogura, and Yasuo Sumida, are employees of ROHTO Pharmaceutical Co., Ltd. The licorice extract used in the test capsule was provided by the manufacturer, MG Pharma Inc., and the authors, Keiichi Ishido, Yuka Sasakawa, and Yasuo Sumida, are employees of MG Pharma Inc. There are no other personal interests or other matters to disclose.

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Appendix

Table 1. Blood measurements (secondary endpoint)

			Before intake Licorice extract: n=38 Placebo capsule: n=38				After 4-week intake Licorice extract: n=38 Placebo capsule: n=38				After 8-week intake Licorice extract: n=37 Placebo capsule n=37				After 12-week intake Licorice extract: n=37 Placebo capsule: n=38			
Total ketone body (μmoL/L)	Measured value	Licorice extract	91	±	88		113	±	130		95	±	84		103	±	105	
		Placebo capsule	92	±	102		107	±	118		86	±	72		103	±	107	
	⊿	Licorice extract					22	±	78		3	±	78		12	±	62	
		Placebo capsule					15	±	93		-5	±	126		11	±	128	
Acetoacetic acid (μmoL/L)	Measured value	Licorice extract	25	±	21		33	±	33		31	±	24		30	±	24	
		Placebo capsule	26	±	23		33	±	31		30	±	20		33	±	31	
	⊿	Licorice extract					9	±	23		6	±	21		5	±	16	
		Placebo capsule					7	±	25		5	±	29		8	±	35	
3-hydroxybutyric acid (μmoL/L)	Measured value	Licorice extract	66	±	68		79	±	99		64	±	61		73	±	82	
		Placebo capsule	67	±	81		74	±	87		56	±	53		70	±	77	
	⊿	Licorice extract					13	±	58		-3	±	57		7	±	47	
		Placebo capsule					8	±	70		-9	±	98		4	±	94	
T-Cho (mg/dL)	Measured value	Licorice extract	211	±	29		218	±	27	§	218	±	31	§	222	±	29	§§
		Placebo capsule	211	±	32		209	±	30		209	±	32		215	±	33	
	⊿	Licorice extract					7	±	18	]*	7	±	20		10	±	16	
		Placebo capsule					-3	±	23		-2	±	25		4	±	27	
LDL-Cho (direct method) (mg/dL)	Measured value	Licorice extract	130	±	22		132	±	21		131	±	25		136	±	23	
		Placebo capsule	133	±	27		130	±	25		127	±	24		133	±	26	
	⊿	Licorice extract					2	±	15		0	±	15		4	±	13	
		Placebo capsule					-3	±	22		-6	±	22		-1	±	24	
LDL-Cho (F formula) (mg/dL)	Measured value	Licorice extract	129	±	24		133	±	24		134	±	26		137	±	23	§§
		Placebo capsule	129	±	27		129	±	28		127	±	26		133	±	29	
	⊿	Licorice extract					4	±	17		4	±	15		7	±	13	
		Placebo capsule					-1	±	21		-2	±	22		3	±	25	
HDL-Cho (mg/dL)	Measured value	Licorice extract	58	±	13		60	±	15		63	±	16	§§	64	±	17	§§
		Placebo capsule	56	±	14		56	±	14		57	±	14		59	±	16	§§
	⊿	Licorice extract					2	±	7		4	±	8		5	±	9	
		Placebo capsule					0	±	5		1	±	5		3	±	6	
TG (mg/dL)	Measured value	Licorice extract	118	±	59		125	±	71		109	±	45		109	±	56	
		Placebo capsule	129	±	64		120	±	62		122	±	66		121	±	72	
	⊿	Licorice extract					6	±	68		-7	±	39		-11	±	43	
		Placebo capsule					-9	±	47		-7	±	47		-9	±	58	
Blood glucose (mg/dL)	Measured value	Licorice extract	98	±	11	]**	97	±	11	]*	97	±	12		98	±	10	
		Placebo capsule	92	±	7		91	±	7		93	±	8		94	±	7	§
	⊿	Licorice extract					0	±	9		-1	±	9		0	±	8	
		Placebo capsule					0	±	5		2	±	6		3	±	6	
HbA1c (%)	Measured value	Licorice extract	5.5	±	0.3		5.5	±	0.3		5.4	±	0.3	§§	5.4	±	0.3	§§
		Placebo capsule	5.5	±	0.3		5.5	±	0.2		5.4	±	0.2	§§	5.4	±	0.2	§§
	⊿	Licorice extract					-0.1	±	0.2		-0.1	±	0.2		-0.2	±	0.2	
		Placebo capsule					0.0	±	0.2		-0.1	±	0.2		-0.1	±	0.2	
Insulin (mU/mL)	Measured value	Licorice extract	8.7	±	6.2		9.3	±	6.6		9.0	±	4.1		8.0	±	3.0	]*
		Placebo capsule	9.2	±	4.9		9.0	±	4.2		9.7	±	4.1		10.0	±	4.3	
	⊿	Licorice extract					0.5	±	8.3		0.2	±	6.2		-0.9	±	6.0	
		Placebo capsule					-0.2	±	3.2		0.8	±	2.7		0.8	±	3.1	

			Before intake Licorice extract: n=38 Placebo capsule: n=38			After 4-week intake Licorice extract: n=38 Placebo capsule: n=38				After 8-week intake Licorice extract: n=37 Placebo capsule n=37			After 12-week intake Licorice extract: n=37 Placebo capsule: n=38			
Glycoalbumin (%)	Measured value	Licorice extract	13.5	±	1.2	13.4	±	1.1	§	13.6	±	1.2	13.5	±	1.2	
		Placebo capsule	13.4	±	0.9	13.1	±	0.9	§§	13.4	±	0.9	13.2	±	0.8	§
	⊿	Licorice extract				-0.2	±	0.4		0.1	±	0.4	0.0	±	0.4	
		Placebo capsule				-0.2	±	0.4		0.0	±	0.4	-0.2	±	0.4	

Mean ± standard deviation, ⊿; Mean ± standard deviation of change from before intake
※Insulin, LDH, and K after 4-week intake, the Placebo capsule: n=37, blood glucose, insulin after 8-week intake, the Placebo capsule: n=36
Between-group comparison (unpaired t-test): ** $p<0.01$, * $p<0.05$
Comparison with before intake (Dunnett's multiple comparison test): §§ $p<0.01$, § $p<0.05$