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Original Article

Isohumulones, the bitter component of beer, improve hyperglycemia and decrease body fat in Japanese subjects with prediabetes

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SUMMARY

Background & aims: A recent study reported that isohumulones, the bitter component of beer, activate peroxisome proliferator-activated receptor alpha (PPARα) and PPARγ *in vitro* and decrease plasma glucose and lipid levels in diabetic mice. This study was to investigate the efficacy and safety of isohumulones for subjects with prediabetes.

Methods: Ninety-four subjects with prediabetes were randomly divided into four groups. A 12-week double-blind dose-finding study was performed in which subjects ingested placebo capsules or test capsules containing 16 mg, 32 mg or 48 mg of isohumulones per day.

Result: After treatment, fasting blood glucose was decreased in the 32 mg and 48 mg groups after 4 weeks, but did not change in the placebo group. HbA1c was also significantly decreased after 4 weeks in the 16 mg group and after 8 weeks in the 32 mg and 48 mg groups. Body mass index (BMI) was significantly decreased in the 48 mg group as compared with the placebo group at 12 weeks. The decrease in total fat area was also significantly greater in the 48 mg group than in the placebo group at 12 weeks.

Conclusion: The present study suggests that ingestion of isohumulones has beneficial effects in diabetes and obesity.

1. Introduction

Lifestyle-related diseases, such as diabetes, hyperlipidemia, hypertension, and obesity, are the main risk factors for metabolic syndrome.[1,2] Insulin resistance is a basic component of metabolic syndrome, and is caused by obesity. Recently, some reports have evaluated the efficacy of natural products on obesity.[3–5] Green tea, containing caffeine and catechins, has been reported to decrease body weight and fat in humans.[5]

Hops (*Humulus lupulus* L.) have been used as a main component of beer that provides bitterness, flavor, aroma and preservation. The bitterness of the beer stems from isohumulones, which mainly comprise isohumulone, isocohumulone and isoadhumulone. Isohumulones are present in beer at 10–50 mg/L.[6]

A recent study indicated that isohumulones have beneficial effects, improving hyperglycemia, hypercholesterolemia and hypertension.[7–10] It has also been reported that isohumulones activate peroxisome proliferator-activated receptor alpha (PPARα) and PPARγ *in vitro* and decrease plasma glucose, lipid levels and the size of hypertrophic adipocytes in diabetic mice. Treatment of type 2 diabetes with isohumulones decreases blood glucose and HbA1c levels. Isohumulones have also been shown to ameliorate renal injury and to exert antihypertensive effects in a Dahl salt-sensitive rat model.[11]

In the present study, we examined the efficacy and safety of isohumulones in subjects with prediabetes.

2. Material and methods

2.1. Test substance

Isomerized hop extract (ISOHOP) was obtained from Botanix Limited (Kent, UK). It is used for the post-fermentation control of beer bitterness and comprises about 30% isohumulones. A soft capsule was prepared for this study. The contents of the placebo and test capsules are shown in Table 1. One test capsule contained about 8 mg of isohumulones (isohumulone, isocohumulone, isoadhumulone ratio, 49:35:16; respectively).

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Table 1
Composition of the placebo and Hop capsules.

	Placebo capsule (%)	Hop capsule (%)
Hop extract	0	15
Safflower oil	86	72.9
Beeswax	14	12
Lactic acid (90%)	0	0.1

2.2. Subjects and design

The protocol was approved by the institutional review board at the Kirin Brewery Co., Ltd. The trial was conducted in accordance with the Helsinki Declaration under the supervision of clinical investigation. All subjects gave full, informed consent for this investigation. This study involved 94 subjects (age: 44–65, fasting glucose levels: 110–125 mg/dl, HbA1c: 5.2–6.4%, Body Mass Index: 24–30) with prediabetes diagnosed according to criteria of the Japanese Society of diabetes.[12] In a randomized double-blind placebo-controlled study, the 94 subjects were randomly assigned to receive 4 placebo capsules (group A), 2 test capsules containing 16 mg of isohumulones (group B), 4 test capsules containing 32 mg of isohumulones (group C), or 6 test capsules containing 48 mg of isohumulones (group D) daily for a 12-week period.

Anthropometry, fasting blood sampling for biochemical and hematological parameters, urine sampling for urinalysis, and interviews were performed at 0, 4, 8 and 12 weeks. Computed tomography was performed at 0 and 12 weeks to measure the abdominal fat area.

2.3. Anthropometric measurements

Body weight, waist, and hips were measured every 4 weeks. The BMI was calculated from height and body weight. The body fat ratio was measured by bioelectrical impedance analysis.

2.4. Blood sampling and clinical analysis

Eating and drinking anything other than water were prohibited from 21:00 on the day before sampling. The concentrations of the following variables were measured in fasting blood samples: glucose (hexokinase method), hemoglobin A1c (HbA1c; high performance liquid chromatography), 1,5-anhydroglucitol (enzymatic method), insulin (chemiluminesent enzyme immunoassay), total protein (TP; Biuret method), albumin (ALB; Nephelometry method), total bilirubin (T-Bil; Vanadate method), glutamic oxaloacetic transaminase (GOT; JSCC method), glutamic pyruvate transaminase (GPT; JSCC method), alkaline phosphatase (ALP; JSCC method), Gamma-glutamyl transpeptidase (GTP; JSCC method), lactate dehydrogenase (LDH; JSCC method), creatine phosphokinase (CPK; JSCC method), total cholesterol (T-chol; cholesterol oxidase ultraviolet method), triglyceride (TG; GK–GPO method), low-density lipoprotein cholesterol (LDL-chol; enzymatic method), high-density lipoprotein cholesterol (HDL-chol; selective inhibition method), free fatty acids (FFA; enzymatic method), phospholipid (enzymatic method), urea nitrogen (UN; urease and leucine dehydrogenase ultraviolet method), creatinine (enzymatic method), Na, Cl, K (electro-rode method) and Fe (nitroso-PSAP method). The JSCC methods were standard methods established by the Japan Society of Clinical Chemistry (JSCC).

Hematological parameters, white blood cells (WBC), red blood cells (RBC), hemoglobin (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelets were measured using a hematocytometer.

Table 2
Characteristics of the subjects before test capsule administration.

	Group A ($n=21$)	Group B ($n=22$)	Group C ($n=20$)	Group D ($n=21$)
Age (years)	52.3 ± 6.7	53.4 ± 8.2	53.3 ± 7.2	55.7 ± 6.8
Body weight (kg)	68.7 ± 10.6	68.2 ± 10.5	68.0 ± 19.9	69.1 ± 10.7
Body mass index (kg/m^2)	26.3 ± 2.4	26.6 ± 2.0	26.7 ± 2.3	25.9 ± 1.5
Body fat ratio (%)	31.2 ± 8.8	30.5 ± 6.2	29.9 ± 10.1	30.4 ± 4.9
Waist (cm)	84.9 ± 8.0	87.6 ± 9.8	83.9 ± 21.0	85.8 ± 8.8
Hip (cm)	94.5 ± 6.8	95.1 ± 7.0	91.6 ± 22.9	93.3 ± 6.9
Fasting blood sugar (mg/dl)	115.0 ± 4.1	114.2 ± 3.7	116.0 ± 5.4	116.0 ± 4.2
Hemoglobin A1c (%)	5.8 ± 0.5	5.8 ± 0.4	6.0 ± 0.4	5.8 ± 0.4

Values are mean ± SD.

2.5. Urinalyses

Urine samples were collected just before blood sampling. Glucose, protein, urobilinogen, bilirubin, and ketone bodies were assayed with urine test paper sticks.

2.6. Measurement of abdominal fat by computed tomography

The subjects underwent Computed Tomography imaging of the abdominal transverse section at the L4/L5 level. The X-ray conditions were a tube voltage of 120 kV and a tube current of 200 mAs. By using FatScan software,[13] the visceral fat area (VFA) and subcutaneous fat area (SFA) were obtained from the CT image, and these areas were summed to obtain the total fat area (TFA).

2.7. Statistical analysis

The values of all test parameters are presented as means and standard deviations. Statistical analyses were performed with SYSTAT version 11 (Systat Software, Inc., Chicago, USA). To evaluate

Table 3
Effect of isohumulones administration on body indices.

		Change from 0-week value				
	0 Week	4 Weeks	8 Weeks	12 Weeks	*P* for group	*P* for period
Body weight (kg)						
A	69.0 ± 11.1	−0.2 ± 0.8	−0.4 ± 1.0	−0.3 ± 1.5	0.010	0.006
B	68.1 ± 10.5	−0.1 ± 1.0	−0.2 ± 1.2	−0.3 ± 1.1		
C	71.6 ± 11.8	−0.2 ± 1.1	−0.1 ± 1.3	−0.4 ± 1.4		
D	69.4 ± 11.0	−0.4 ± 0.8*	−0.8 ± 1.4*	−1.3 ± 1.7**		
Body mass index (kg/m^2)						
A	26.4 ± 2.4	−0.1 ± 0.3	−0.2 ± 0.4	−0.1 ± 0.6	0.018	0.005
B	26.6 ± 2.0	0.0 ± 0.4	−0.1 ± 0.5	−0.1 ± 0.4		
C	26.8 ± 2.3	−0.1 ± 0.4	−0.1 ± 0.4	−0.2 ± 0.5		
D	26.0 ± 1.6	−0.1 ± 0.3*	−0.3 ± 0.5*	−0.5 ± 0.6**		
Body fat ratio (%)						
A	30.9 ± 8.6	−0.2 ± 0.9	−0.4 ± 2.0	−0.2 ± 1.8	0.241	0.072
B	30.8 ± 5.4	−0.4 ± 0.8*	−0.1 ± 1.3	0.0 ± 1.5		
C	32.3 ± 7.2	−0.5 ± 1.2	−0.5 ± 1.2	−0.8 ± 1.3*		
D	30.4 ± 5.0	−0.6 ± 1.5	−0.3 ± 1.6	−0.8 ± 1.6*		
Waist (cm)						
A	84.8 ± 8.0	−0.4 ± 0.9**	−0.7 ± 1.2**	−0.7 ± 1.1**	0.172	<0.001
B	87.1 ± 9.6	−0.5 ± 0.9*	−0.7 ± 1.0**	−1.1 ± 1.7**		
C	88.7 ± 7.0	−0.9 ± 1.4*	−1.1 ± 2.1*	−1.6 ± 2.4*		
D	86.0 ± 8.9	−0.6 ± 1.2*	−0.9 ± 1.6*	−1.1 ± 1.9*		
Hip (cm)						
A	94.5 ± 6.9	0.0 ± 0.9	−0.2 ± 1.6	−0.4 ± 1.4	0.033	0.168
B	95.2 ± 7.1	−0.3 ± 1.1	−0.6 ± 1.5	−0.7 ± 1.6*		
C	96.6 ± 7.4	0.1 ± 1.7	0.1 ± 1.6	0.1 ± 2.6		
D	93.0 ± 6.4	−0.6 ± 0.9**	−0.6 ± 1.8	−0.9 ± 2.3		

Values are mean ± SD. Asterisks indicate a significant difference from the 0-week value: *$P < 0.05$; **$P < 0.01$.

Table 4
Effect of isohumulones administration on biochemical blood parameters.

	0 Week	Change from 0-week value			*P* for group	*P* for period
		4 Weeks	8 Weeks	12 Weeks		
Fasting blood glucose (mg/dl)						
A	115.2 ± 12.8	−2.8 ± 8.4	−5.7 ± 12.6	−1.5 ± 9.6	0.030	0.001
B	109.1 ± 11.7	0.5 ± 16.4	−2.7 ± 10.1	−2.1 ± 10.5		
C	115.5 ± 8.8	−5.5 ± 7.8**	−6.4 ± 7.5**	−5.3 ± 8.4*		
D	115.5 ± 7.9	−5.1 ± 5.7**	−7.6 ± 10.8**	−6.7 ± 14.1*		
Hemoglobin A1c (%)						
A	5.79 ± 0.60	−0.02 ± 0.21	−0.06 ± 0.23	−0.07 ± 0.19	0.076	<0.001
B	5.75 ± 0.53	−0.07 ± 0.15*	−0.14 ± 0.19**	−0.16 ± 0.21**		
C	5.97 ± 0.55	−0.04 ± 0.17	−0.12 ± 0.23*	−0.27 ± 0.30**		
D	5.76 ± 0.44	−0.03 ± 0.18	−0.17 ± 0.34*	−0.29 ± 0.43**		
1,5-AG (μg/ml)						
A	16.7 ± 6.7	−0.1 ± 1.3	0.7 ± 1.6	0.5 ± 1.6	0.661	0.015
B	16.9 ± 7.8	−0.1 ± 1.4	0.3 ± 2.2	0.4 ± 3.0		
C	16.1 ± 8.3	0.1 ± 2.4	0.3 ± 2.0	0.8 ± 2.2		
D	18.2 ± 5.5	0.0 ± 1.5	0.8 ± 1.7	1.1 ± 2.9		
Insulin (μU/ml)						
A	10.7 ± 10.0	8.4 ± 7.0	7.9 ± 8.5	10.5 ± 14.4	0.945	0.268
B	9.7 ± 9.0	11.4 ± 12.8	7.7 ± 3.9	10.4 ± 9.4		
C	10.4 ± 7.8	12.1 ± 12.0	9.5 ± 5.6	11.6 ± 14.5		
D	8.7 ± 6.9	9.1 ± 6.5	9.9 ± 11.8	9.6 ± 7.7		
Total protein (g/dl)						
A	7.35 ± 0.45	0.03 ± 0.24	0.07 ± 0.25	−0.05 ± 0.33	0.489	0.701
B	7.31 ± 0.53	−0.01 ± 0.32	−0.07 ± 0.37	−0.05 ± 0.38		
C	7.40 ± 0.45	−0.06 ± 0.23	−0.07 ± 0.22	−0.06 ± 0.19		
D	7.42 ± 0.56	−0.09 ± 0.27	0.02 ± 0.30	−0.01 ± 0.25		
Albumin (g/dl)						
A	4.13 ± 0.42	0.00 ± 0.11	0.00 ± 0.17	−0.04 ± 0.20	0.833	0.499
B	4.29 ± 0.41	−0.05 ± 0.19	−0.07 ± 0.25	0.00 ± 0.27		
C	4.23 ± 0.42	−0.01 ± 0.18	−0.04 ± 0.18	−0.01 ± 0.20		
D	4.15 ± 0.42	−0.04 ± 0.12	−0.01 ± 0.12	0.02 ± 0.14		
Total bilirubin (mg/dl)						
A	0.76 ± 0.22	−0.02 ± 0.12	−0.01 ± 0.14	0.00 ± 0.16	0.041	0.417
B	0.75 ± 0.20	−0.02 ± 0.12	−0.02 ± 0.14	−0.04 ± 0.16		
C	0.76 ± 0.23	−0.01 ± 0.20	−0.11 ± 0.26	−0.08 ± 0.13**		
D	0.76 ± 0.21	0.00 ± 0.11	0.01 ± 0.13	0.02 ± 0.18		
Glutamic oxaloacetic transaminase (IU/L)						
A	23.5 ± 7.3	0.2 ± 2.9	2.0 ± 5.8	0.7 ± 4.9	0.004	0.321
B	25.3 ± 13.3	−0.7 ± 3.0	0.5 ± 5.6	−0.8 ± 6.2		
C	31.6 ± 13.2	−2.7 ± 7.3	−2.3 ± 4.1*	−3.7 ± 8.7		
D	28.8 ± 22.2	−0.1 ± 4.4	−0.1 ± 7.2	−0.9 ± 7.8		
Glutamic pyruvate transaminase (IU/L)						
A	26.6 ± 22.0	−0.2 ± 2.0	0.5 ± 5.9	0.3 ± 11.3	0.713	0.965
B	29.6 ± 18.3	−0.3 ± 6.2	1.1 ± 6.3	−0.6 ± 8.2		
C	36.3 ± 22.0	−0.6 ± 11.9	−1.6 ± 7.0	−0.1 ± 14.1		
D	30.7 ± 29.5	−0.5 ± 8.7	−2.2 ± 10.6	−1.7 ± 10.0		
Alkaline phosphatase (IU/L)						
A	192.1 ± 38.8	6.3 ± 13.7*	7.5 ± 12.0**	5.5 ± 15.8	0.721	0.212
B	206.5 ± 38.7	6.3 ± 18.6	3.1 ± 16.9	6.9 ± 21.5		
C	212.4 ± 49.4	−0.9 ± 16.6	4.2 ± 29.3	5.6 ± 29.5		
D	207.6 ± 59.9	0.0 ± 25.2	5.1 ± 29.7	3.6 ± 14.7		
Y-glutamyl transpeptidase (IU/L)						
A	34.8 ± 28.6	−1.7 ± 6.9	−2.5 ± 7.7	−2.7 ± 9.2	0.185	0.481
B	72.3 ± 121.5	2.6 ± 28.5	3.7 ± 41.6	0.2 ± 27.8		
C	46.9 ± 49.9	−3.6 ± 8.8	−5.5 ± 11.8*	−5.6 ± 15.1		
D	50.2 ± 57.6	−0.4 ± 5.6	−4.3 ± 11.8	−7.4 ± 21.4		
Lactate dehydrogenase (IU/L)						
A	190.7 ± 26.3	5.0 ± 14.5	15.7 ± 45.5	6.3 ± 21.3	0.009	0.071
B	184.5 ± 29.2	−3.3 ± 14.4	−1.7 ± 15.5	−2.1 ± 17.7		
C	205.0 ± 35.7	−4.9 ± 19.2	−1.5 ± 14.7	−6.7 ± 19.3		
D	191.5 ± 34.3	−5.9 ± 11.4*	8.8 ± 41.1	−4.0 ± 20.1		
Creatine phosphokinase (IU/L)						
A	100.3 ± 64.5	6.2 ± 42.2	14.4 ± 50.7	11.0 ± 38.2	0.002	0.947
B	99.2 ± 73.8	−2.5 ± 12.8	−3.8 ± 13.1	1.7 ± 12.6		
C	114.6 ± 52.9	−7.3 ± 28.2	−10.4 ± 33.0	−12.8 ± 28.1		
D	93.1 ± 25.2	2.5 ± 13.7	5.0 ± 20.6	7.4 ± 42.9		

Table 4 (*continued*)

	0 Week	Change from 0-week value			*P* for group	*P* for period
		4 Weeks	8 Weeks	12 Weeks		
Total cholesterol (mg/dl)						
A	220.0 ± 29.4	−0.7 ± 18.2	−2.7 ± 19.0	−1.6 ± 23.0	0.012	0.019
B	203.6 ± 41.3	−1.2 ± 15.0	0.6 ± 19.6	−7.6 ± 14.9*		
C	226.8 ± 39.1	−8.3 ± 13.0*	−12.6 ± 13.4**	−12.6 ± 14.3**		
D	218.6 ± 49.7	−3.0 ± 15.4	−3.2 ± 17.9	−7.2 ± 20.2		
Triglyceride (mg/dl)						
A	120.8 ± 57.1	5.3 ± 31.6	10.9 ± 33.1	5.1 ± 30.8	0.010	0.201
B	133.1 ± 80.6	23.5 ± 46.9*	−8.2 ± 24.6	−18.6 ± 39.8*		
C	167.6 ± 105.5	−3.8 ± 61.8	54.7 ± 166.1	17.6 ± 73.9		
D	196.4 ± 163.9	−23.4 ± 114.5	−5.9 ± 79.7	−42.1 ± 103.3		
LDL-cholesterol (mg/dl)						
A	133.0 ± 24.4	−1.9 ± 15.2	−1.4 ± 15.2	0.8 ± 18.3	0.001	0.156
B	120.9 ± 32.9	−5.1 ± 11.3*	−0.3 ± 15.2	−6.3 ± 13.0*		
C	137.4 ± 35.4	−7.3 ± 16.3	−13.8 ± 14.6**	−11.4 ± 13.7**		
D	126.0 ± 39.2	0.6 ± 16.4	−0.4 ± 18.3	−0.6 ± 22.5		
HDL-cholesterol (mg/dl)						
A	61.8 ± 13.5	−1.6 ± 4.8	−2.3 ± 5.1	−1.3 ± 5.9	0.038	0.431
B	58.1 ± 11.0	−0.6 ± 4.7	1.0 ± 5.6	1.4 ± 5.0		
C	58.6 ± 13.1	−0.3 ± 5.1	−1.7 ± 5.6	−3.0 ± 7.6		
D	53.6 ± 11.2	−0.1 ± 4.3	−1.7 ± 5.4	1.3 ± 4.7		
Free fatty acids (mEq/L)						
A	0.50 ± 0.18	0.03 ± 0.20	0.06 ± 0.23	0.05 ± 0.24	0.272	0.474
B	0.56 ± 0.20	0.00 ± 0.17	0.06 ± 0.17	−0.02 ± 0.20		
C	0.56 ± 0.19	0.02 ± 0.26	−0.02 ± 0.26	−0.04 ± 0.20		
D	0.56 ± 0.25	−0.05 ± 0.16	0.03 ± 0.20	−0.04 ± 0.25		
Phosphlipid (mg/dl)						
A	213.6 ± 28.4	4.9 ± 21.9	3.2 ± 23.9	5.8 ± 26.2	0.161	0.659
B	216.9 ± 32.7	4.9 ± 15.9	3.3 ± 14.8	−1.0 ± 17.7		
C	219.2 ± 31.5	0.6 ± 20.4	6.5 ± 21.3	0.3 ± 22.2		
D	219.1 ± 39.9	−4.5 ± 21.2	−0.8 ± 18.0	−4.6 ± 20.0		
Urea nitrogen (mg/dl)						
A	12.5 ± 3.4	0.6 ± 3.5	−0.2 ± 3.6	0.3 ± 3.2	0.092	0.189
B	14.1 ± 4.2	−0.9 ± 3.0	−1.2 ± 2.7*	−0.3 ± 3.6		
C	12.5 ± 4.1	0.0 ± 2.4	−0.7 ± 1.7	0.6 ± 2.4		
D	12.2 ± 3.4	0.3 ± 1.8	0.1 ± 1.5	0.4 ± 2.4		
Creatinine (mg/dl)						
A	0.88 ± 0.18	0.01 ± 0.09	−0.01 ± 0.11	−0.02 ± 0.10	0.502	0.411
B	0.86 ± 0.17	0.00 ± 0.08	−0.02 ± 0.10	0.00 ± 0.09		
C	0.84 ± 0.16	−0.01 ± 0.05	−0.01 ± 0.06	0.02 ± 0.08		
D	0.85 ± 0.16	−0.03 ± 0.07	−0.03 ± 0.08	−0.01 ± 0.10		
Na (mEq/L)						
A	143.1 ± 2.6	−0.5 ± 1.9	−0.1 ± 2.0	0.0 ± 2.4	0.110	0.162
B	143.6 ± 1.9	−0.2 ± 2.4	−0.8 ± 2.1	0.4 ± 2.3		
C	142.5 ± 2.2	0.5 ± 1.4	0.4 ± 2.0	1.0 ± 2.0*		
D	143.3 ± 2.0	−0.3 ± 1.5	−0.1 ± 2.0	0.2 ± 2.4		
Cl (mEq/L)						
A	104.0 ± 2.3	−0.4 ± 2.3	−0.4 ± 2.1	−0.6 ± 2.1	0.454	0.429
B	103.5 ± 1.9	0.3 ± 2.4	−0.4 ± 2.2	0.3 ± 1.6		
C	103.9 ± 1.8	−0.3 ± 1.6	−0.8 ± 2.1	−0.5 ± 2.9		
D	103.6 ± 1.7	−0.1 ± 2.0	−0.2 ± 2.6	−0.3 ± 2.7		
K (mEq/L)						
A	4.30 ± 0.29	0.02 ± 0.26	0.03 ± 0.25	0.07 ± 0.39	0.032	0.209
B	4.27 ± 0.43	0.05 ± 0.29	0.05 ± 0.34	0.15 ± 0.36		
C	4.30 ± 0.36	−0.02 ± 0.25	−0.03 ± 0.24	−0.02 ± 0.19		
D	4.30 ± 0.32	0.11 ± 0.23*	0.17 ± 0.32*	0.12 ± 0.36		
Fe (μg/dl)						
A	88.9 ± 25.0	4.7 ± 24.8	6.1 ± 28.0	11.3 ± 35.8	0.017	0.641
B	116.6 ± 37.6	−3.2 ± 26.7	−2.5 ± 32.1	−12.2 ± 30.9		
C	104.9 ± 36.9	−4.8 ± 35.2	−18.0 ± 31.5*	−5.4 ± 24.6		
D	105.6 ± 37.3	−1.6 ± 28.2	−6.0 ± 34.0	−4.4 ± 36.2		

Values are mean ± SD. Asterisks indicate a significant difference from the 0-week value: *$P < 0.05$; **$P < 0.01$.

the changes from the 0-week values in each group, a paired Student's *t*-test was used. The interaction between the test group (intake of isohumulones) and the experimental period (change from 0-week value) was tested by using a two-way repeated-measures analysis of variance (ANOVA). When significant differences were observed, a Dunnett's post hoc test was used to examine the difference between the placebo and the treatment. Differences were considered significant at $P < 0.05$.

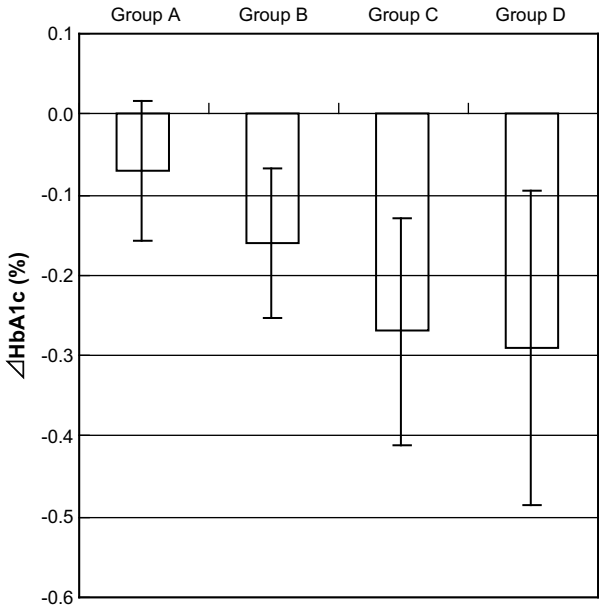

Fig. 1. Effect of isohumulone administration on the change in HbA1c after the 12-week treatment period. Changes were determined as the 12-week value minus the 0-week value. Values are the mean and 95% confidence interval. HbA1c was decreased as compared with groups A and group D ($P = 0.0505$) by Dunnett's post hoc test.

3. Results

3.1. Background of the subjects

Four subjects dropped out of the study because they moved during the experiment. Six subjects were excluded because of abnormal values before the test period. Consequently, the statistical analyses were conducted on 84 subjects. The characteristics of the subjects measured 4 weeks before test capsule administration are shown in Table 2. No significant differences were found in the data among the groups before the experiment.

3.2. Effects on anthropometric values

Table 3 shows the changes in anthropometric values. There was no significant difference in the value of any variable among the four groups at 0 weeks. Body weight significantly decreased in group D between 0 weeks and 4 ($P = 0.0229$), 8 ($P = 0.0175$) and 12 ($P = 0.0020$) weeks. Repeated-measures ANOVA indicated a significant effect of group and period in this decrease in body weight.

BMI significantly decreased in group D between 0 weeks and 4 ($P = 0.0156$), 8 ($P = 0.0140$) and 12 ($P = 0.0014$) weeks. Repeated-measures ANOVA indicated a significant effect of group and period in this decrease in BMI. In group D, the decrease in BMI was significant as compared with group A ($P = 0.0411$) at 12 weeks.

Body fat ratio was significantly decreased from the 0-week value after 4 weeks in group B ($P = 0.0496$), and after 12 weeks in groups C ($P = 0.0106$) and D ($P = 0.0483$). However, repeated-measures ANOVA showed no significant effects in these decreases in body fat ratio.

Waist circumference decreased significantly from the 0-week value after 4 weeks in groups B, C and D, and repeated-measures ANOVA showed a significant effect of period in these decreases in waist circumference.

Hip circumference decreased significantly from the 0-week value after 4 weeks in group D ($P = 0.0062$), and after 12 weeks in group B ($P = 0.0398$). Repeated-measures ANOVA showed a significant effect of group in these decreases in hip circumference.

3.3. Effect on blood values

Table 4 shows the changes in biochemical blood values.

Fasting blood glucose significantly decreased in group C between 0 weeks and 4 ($P = 0.0051$), 8 ($P = 0.0013$) and 12 ($P = 0.0117$) weeks; and in group D between 0 weeks and 4 ($P = 0.0005$), 8 ($P = 0.0045$) and 12 ($P = 0.0416$) weeks. Repeated-measures ANOVA showed significant effects of group and period in these decreases in fasting blood glucose.

HbA1c significantly decreased in group B between 0 weeks and 4 ($P = 0.0440$), 8 ($P = 0.0027$), and 12 ($P = 0.0015$) weeks; in group C between 0 weeks and 8 ($P = 0.0284$) and 12 ($P = 0.0009$) weeks; and in group D between 0 weeks and 8 ($P = 0.0345$) and 12 ($P = 0.0054$) weeks. Repeated-measures ANOVA indicated a significant effect of period in the decrease in HbA1c. HbA1c was decreased as dose dependently at 12 weeks (Fig. 1).

1,5-Anhydroglucitol, insulin, TP, albumin, T-Bil, GPT, ALP, GTP, CPK, HDL-cho, FFA, phospholipid, creatinine, and Cl showed no significant changes in value.

GOT, LDH, T-cho, TG, LDL-cho, UN, Na, K, and Fe showed some changes over the treatment period. But there were no significant differences among the groups.

Table 5 shows the changes in hematological values.

WBC, RBC, Hb, Ht, MCV, MCH, MCHC and platelets showed some changes over the treatment period, but there were no significant differences among the groups.

3.4. Urinalyses

No subjects had abnormal results in the urine test during the test period.

3.5. Effect of abdominal fat

Table 6 and Fig. 2 show the changes in abdominal fat. There was no significant difference in any abdominal fat area among the four groups at 0 weeks.

Total fat area (TFA) was significantly decreased in groups C ($P = 0.0010$) and D ($P < 0.0001$) at 12 weeks (Table 6). ΔTFA (the 12-week value minus the 0-week value) was significantly greater in groups C and D versus group A ($P = 0.0415$ and $P = 0.0001$, respectively) (Fig. 2).

Visceral fat area (VFA) was significantly decreased in group D ($P \leq 0.0001$) at 12 weeks. ΔVFA (the 12-week value minus the 0-week value) was significantly greater in group D versus group A ($P = 0.0047$).

Subcutaneous fat area (SFA) was significantly decreased in groups C ($P = 0.0113$) and D ($P = 0.0001$) at 12 weeks. ΔSFA (the 12-week value minus the 0-week value) was not significantly changed.

4. Discussion

In this study, we confirmed the beneficial effect of isohumulones for subjects with prediabetes. The results showed that intake of isohumulones decreased fasting blood glucose level. In addition, we demonstrated an improvement in long-term glucose control as indicated by the decrease in HbA1c. In a previous pilot study, an intake of about 160 mg of isohumulones per day for 8 weeks reduced fasting serum glucose and HbA1c in subjects with mild type 2 diabetes.[7] The current findings suggest that a dose of only 32 mg of isohumulones per day improves hyperglycemia.

Table 5
Effect of isohumulones administration on blood parameters.

	0-week	Change from 0-week value			*P* for group	*P* for period
		4 weeks	8 weeks	12 weeks		
White blood cells (/μl)						
A	6086.2 ± 1090.8	150.0 ± 920.8	143.8 ± 747.4	307.1 ± 1285.4	0.597	0.756
B	5885.5 ± 853.7	136.4 ± 556.7	−93.2 ± 809.0	−59.5 ± 718.5		
C	6106.5 ± 980.0	253.0 ± 1448.9	233.0 ± 671.3	−297.5 ± 574.3		
D	5624.8 ± 1031.3	−57.1 ± 668.2	175.7 ± 816.0	350.0 ± 1421.9		
Red blood cells ($\times 10^4$/μl)						
A	450.2 ± 44.6	4.1 ± 12.1	7.7 ± 17.0	6.5 ± 18.1	0.003	0.354
B	460.5 ± 45.6	−0.4 ± 14.0	2.6 ± 17.2	1.7 ± 20.2		
C	457.8 ± 44.0	−4.8 ± 17.0	−6.2 ± 14.5	−1.1 ± 20.6		
D	449.4 ± 43.4	1.7 ± 13.7	5.3 ± 11.6*	5.4 ± 12.6		
Hemoglobin (g/dl)						
A	13.8 ± 1.6	0.0 ± 0.4	0.2 ± 0.5	−0.1 ± 0.5	0.342	0.62
B	14.0 ± 1.7	0.1 ± 0.5	0.1 ± 0.7	0.1 ± 0.7		
C	14.4 ± 1.4	−0.1 ± 0.5	−0.1 ± 0.5	0.0 ± 0.6		
D	14.1 ± 1.2	0.1 ± 0.4	0.1 ± 0.4	0.1 ± 0.4		
Hematocrit (%)						
A	41.1 ± 4.8	0.4 ± 1.4	0.5 ± 1.7	0.5 ± 1.9	0.095	0.586
B	42.0 ± 5.9	0.0 ± 1.4	0.0 ± 1.7	0.2 ± 2.2		
C	42.6 ± 4.7	−0.3 ± 1.6	−0.5 ± 1.7	−0.1 ± 1.9		
D	42.1 ± 4.9	0.0 ± 1.9	0.3 ± 1.5	0.5 ± 1.7		
Mean corpuscular volume (fl)						
A	91.3 ± 6.5	−0.2 ± 2.1	−0.3 ± 2.5	−0.2 ± 2.4**	0.817	0.918
B	91.2 ± 8.1	0.0 ± 1.3*	−0.4 ± 1.6	0.3 ± 1.9		
C	93.1 ± 4.9	0.2 ± 2.7	0.2 ± 4.0	0.1 ± 4.0		
D	93.9 ± 8.6	−0.3 ± 2.6	−0.4 ± 3.1	0.0 ± 2.9		
Mean corpuscular hemoglobin (pg)						
A	30.6 ± 1.7	−0.3 ± 0.7	−0.1 ± 0.7	−0.7 ± 0.9*	0.001	0.196
B	30.4 ± 2.3	0.3 ± 0.7*	0.2 ± 0.7	0.1 ± 1.1		
C	31.4 ± 1.7	0.2 ± 0.8	0.2 ± 1.1	0.2 ± 1.1		
D	31.4 ± 1.6	0.1 ± 0.7	0.0 ± 1.0	−0.1 ± 0.8		
Mean corpuscular hemoglobin concentration (%)						
A	33.6 ± 2.0	−0.3 ± 0.9	0.0 ± 1.0	−0.6 ± 0.9**	0.051	0.082
B	33.4 ± 2.2	0.4 ± 0.7*	0.3 ± 0.8	−0.1 ± 1.3		
C	33.8 ± 2.1	0.1 ± 0.8	0.1 ± 1.1	0.1 ± 1.2		
D	33.6 ± 2.7	0.2 ± 1.0	0.0 ± 1.5	−0.2 ± 1.3		
Platelets ($\times 10^4$/μl)						
A	22.6 ± 5.1	0.4 ± 1.7	1.4 ± 2.5*	0.4 ± 2.2	0.346	0.107
B	20.1 ± 3.1	0.8 ± 1.8*	0.5 ± 1.5	0.5 ± 2.0		
C	22.0 ± 4.5	0.1 ± 1.5	0.2 ± 2.8	0.4 ± 1.7		
D	23.4 ± 5.5	−0.3 ± 1.2	0.6 ± 2.8	0.2 ± 3.1		

Values are mean ± SD. Asterisks indicate a significant difference from the 0-week value: $^*P < 0.05$; $^{**}P < 0.01$.

Previously, it was reported that isohumulones activate PPARγ mediated transcription and that treatment with isohumulones decreased plasma glucose and lipid levels in diabetic mice. Thiazolidinediones, which are ligands for PPARγ, regulate the transcription of many genes involved in glucose and fatty acid metabolism[14–16] and have been used to treat hyperglycemia in obese type 2 diabetes.[17–20] Those studies reported that the main side-effects of thiazolidinediones included body weight gain and edema. In this study, such side-effects were not detected. In addition, isohumulones raise plasma HDL-chol levels and reduce liver cholesterol and triacylglycerol content in C57BL/6 mice by activating PPARα.[9] Therefore, we expected the effects of isohumulones to be similar to those of fibrates: namely, to lower LDL-chol and improve HDL-chol and TG levels in serum.[21–23] However, the intake of isohumulones decreased LDL-chol and TG levels, but had no effect on HDL-chol in this study.

Moreover, the ingestion of isohumulones for 12 weeks led to a significant decrease in body weight, BMI and abdominal fat area. Isohumulones were found to reduce body weight gain and adipose tissue mass in C57BL/6 N mice with high-fat diet-induced obesity.[8] In this study, a significant decrease in VFA and SFA was observed. These findings suggest that intake of isohumulones at 48 mg per day improves obesity.

Recently, treatment with anti-obesity drugs, such as rimonabant, has been shown to reduce and control body weight in subjects with type 2 diabetes and obesity.[24,25] Thus, the simultaneous use of

Table 6
Effect of isohumulones administration on body fat area.

	Group A	Group B	Group C	Group D
	(*n* = 21)	(*n* = 19)	(*n* = 19)	(*n* = 20)
Total fat area (%)				
0 Week	318.2 ± 127.5	312.7 ± 102.9	332.6 ± 111.9	317.9 ± 79.2
12 Weeks	312.2 ± 124.8	309.0 ± 103.0	310.3 ± 114.3**	283.6 ± 76.2**
Visceral fat area (%)				
0 Week	116.9 ± 60.4	98.3 ± 40.0	120.6 ± 56.1	139.2 ± 51.8
12 Weeks	112.6 ± 53.9	101.0 ± 42.7	112.4 ± 61.9	116.8 ± 49.4**
Subcutaneous fat area (%)				
0 Week	201.3 ± 91.6	214.3 ± 86.2	212.0 ± 93.4	178.7 ± 66.6
12 Weeks	199.6 ± 95.2	208.1 ± 86.9	197.9 ± 88.9*	166.8 ± 67.2**

Values are mean ± SD. Asterisks indicate a significant difference from the 0-week value: $^*P < 0.05$; $^{**}P < 0.01$.

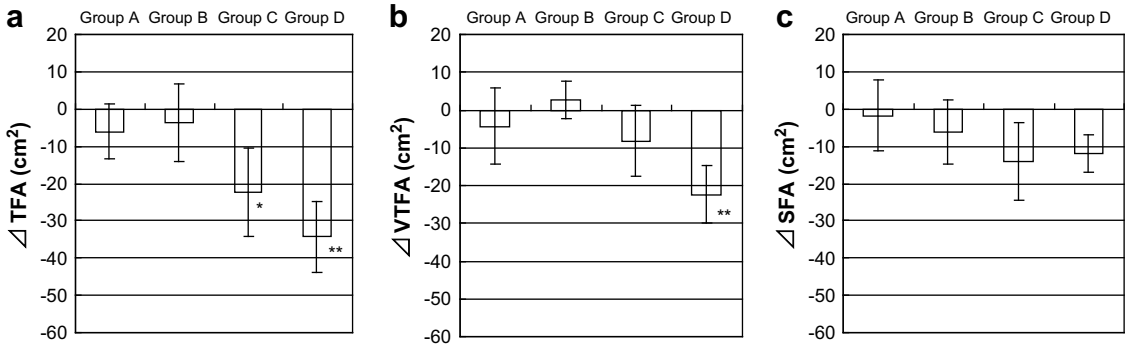

Fig. 2. Effect of isohumulones administration on the change in body fat area after the 12-week treatment period. a) Change in TFA. b) Change in VFA c) Change in SFA. Changes were determined as the 12-week value minus the 0-week value. Values are the mean and 95% confidence interval. Asterisks indicate a significant difference from placebo by Dunnett's post hoc test: $^{*}P < 0.05$; $^{**}P < 0.01$.

isohumulones and anti-obesity drugs should be the subject of future study.

In conclusion, ingestion of isohumulones decreased fasting glucose level, HbA1c, body weight and abdominal body fat in subjects with prediabetes. No side-effects related to the study were reported. These results suggest that isohumulones improve life-style-related diseases, such as diabetes and obesity.

Conflict of interest

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Acknowledgments

Kuniaki Obara and Mai Mizutani contributed to study design and data analysis. Yoshitaka Hitomi carried out samples preparation and analysis. Kuniaki Obara, Hiroaki Yajima and Keiji Kondo contributed to data discussion and drafted the manuscript. All authors read and approved the final manuscript.

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