〈Original Article〉

Evaluation of the effects of the ingestion of ajoene, a sulfur containing compound derived from oil-macerated garlic, on metabolic parameters, abdominal circumference, and blood pressure in Japanese metabolic syndrome patients. A pilot study

Hidehiko Beppu[1)], Yoshifuji Matsumoto[2)], Tsuchiyoshi Fujino[3)], Yoshinori Itani[2)], Toshiaki Sumitho[3)], Takashi Higashiguchi[2)], Takeshi Chihara[1)], Ikuko Tamai[1)] Shigeru Sonoda[1), 2)] and Kan Shimpo[1)]

Summary Oil-macerated garlic contains ajoene, which has been reported to have platelet aggregation inhibition, antibacterial, and antitumor activities. The present pilot study (single-blind, placebo-controlled trial) was conducted involving 17 males, average age 47.2 ± 7.72 years old, and 17 females, average age 49.3 ± 6.54 years old, patients with Japanese type metabolic syndrome. The subjects were stratified into four groups designated as hyperlipidemic, hyperglycemic, hypertensive, and visceral adiposity groups based on the results of clinical examinations during the pre-observation period, and then the patients were given ajoene extract to determine its effects on the laboratory test results.

As a result, the normal dose ingestion and 3 times normal dose ingestion periods showed significantly higher (P<0.05) HDL levels in the hyperlipidemic group as compared with those of the pre-observation period. In the hypertensive group, the normal dose ingestion and 3 times normal dose ingestion periods showed significantly lower systolic blood pressure (BP) (P<0.05 and P<0.001, respectively) and lower diastolic BP (P<0.001 and P<0.05,respectively) as compared with those of the pre-ingestion observation period. In the results of physical measurements of the visceral adiposity group, both female and male subjects showed significantly reduced (P<0.001) abdominal circumference (AC) at navel height in both the normal dose ingestion and 3 times normal dose ingestion periods as compared with those of the pre-observation period.

From these results it was suggested that the ingestion of ajoene extract was effective in reducing AC, improving HDL cholesterol levels, as determined by atheromatous indexes, and lowering BP in metabolic syndrome patients.

Key words: Garlic, Oil-macerated garlic, Ajoene, Clinical pilot study, Metabolic syndrome patients, HDL cholesterol levels

[1)]Fujita Memorial Nanakuri Institute, Fujita Health University, Hisai-isshiki-cho, Tsu, Mie 514-1296, Japan
[2)]Nanakuri Sanatorium Hospital, Fujita Health University Ohdori-cho, Tsu, Mie 514-1295, Japan
[3)]Nagoya Seiraku Co. Ltd., 310 Nakasuna, Tempaku, Nagoya, Aichi 468-0065, Japan
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1. Introduction

Garlic (*Allium sativum Linne*) has been used routinely as a popular flavoring and medicine for hundreds of years. Ajoene is derived from garlic in the course of low-temperature heating in vegetable oil[1]. It has been demonstrated in studies on laboratory animals that ajoene has platelet aggregation inhibition[2), 3)], antibacterial[4), 5)], and antitumor[6), 7)] activities. Foods containing ajoene have usually been referred to as oil-macerated garlic. Ajoene is obtained readily in the course of simple cooking of garlic, and it has been ingested as an easily accessible food for hundreds of years.

The present study prepared oil capsules containing ajoene, which was derived and stabilized in a unique way[8], and studies were performed on the effectiveness of the ajoene oil capsule in patients with metabolic syndrome. The effects of ajoene on metabolic syndrome, which has recently become an object of public concern as part of lifestyle-related diseases (hyperlipidemia, hyperglycemia, hypertension, and visceral adiposity was assessed).

The testing duration was divided into 3 periods, each consisting of 4 weeks, of a placebo ingestion period (ingestion of medium-chain triglyceride alone), an ingestion period of ajoene at normal dose (0.78 mg ajoene per day), and an ingestion period of ajoene at 3 times normal dose (2.34 mg ajoene per day).

Physical measurements and collections of blood and urine samples were carried out to monitor variations in the clinical observations observed between pre- and post-ajoene ingestion.

The following parameters were examined blood glucose, HbA1c, body weight, body mass index (BMI), body fat percentage, and abdominal circum-

Table 1 Background information on the metabolic syndrome patients: results of medical checkups in the pre-ingestion period

a: Hyperlipidemia group

Parameter	In total	Male	Female
Subject	26	14	12
Age	48.2±7.60	47.6±8.18	48.9±7.15
Height	164.5±8.39	171.2±3.25	156.7±4.82
BW	70.3±12.08	76.8±7.45	62.8±12.31
BFR	28.8±4.65	25.2±1.71	33.0±3.13
BMI	25.9±3.39	26.2±2.50	25.5±4.29
Waist	89.3±7.82	89.9±6.35	88.7±9.51
SBP	130.9±22.81	132.9±23.50	128.7±22.80
DBP	83.9±15.90	87.8±16.13	79.4±15.04
FBG	103.0±22.49	109.9±28.82	94.8±5.86
TG	183.8±83.64	209.7±75.29	153.7±85.76
HDL	46.9±12.43	42.3±12.72	52.3±10.09

＊Mean ± Standard deviation

b: Hyperglycaemic group

Parameter	In total	Male	Female
Subject	6	4	2
Age	53.3±7.92	52.5±10.08	55.0±0.00
Height	165.2±10.80	171.4±4.24	152.9±8.63
BW	70.8±9.74	74.9±9.58	62.7±0.42
BFR	28.1±7.06	24.0±4.06	36.2±0.57
BMI	26.0±3.14	25.6±3.61	26.9±2.83
Waist	90.1±5.10	89.8±6.40	90.8±2.47
SBP	146.8±23.28	136.3±13.72	168.0±28.28
DBP	89.2±8.47	87.8±8.88	92.0±9.90
FBG	149.3±24.25	163.3±11.50	121.5±14.85
TG	191.8±64.53	208.0±57.37	159.5±88.39
HDL	43.2±13.42	37.8±3.50	54.0±22.63

＊Mean ± Standard deviation

c : Hypertension group

Parameter	In total	Male	Female
Subject	14	5	9
Age	49.8±6.58	51.2±9.20	49.0±5.10
Height	162.2±6.97	169.3±1.00	158.2±5.33
BW	72.6±11.80	72.9±8.13	72.4±13.89
BFR	31.6±6.63	24.3±3.58	35.7±3.62
BMI	27.7±4.42	25.6±3.26	28.9±4.68
Waist	93.0±8.32	89.6±5.82	94.9±9.18
SBP	155.5±19.69	154.4±27.17	156.1±16.12
DBP	96.1±14.45	99.8±22.70	94.1±8.30
FBG	115.6±30.74	141.0±38.34	101.4±13.13
TG	165.1±77.95	174.4±46.14	159.9±93.41
HDL	54.6±14.25	49.2±16.87	57.6±12.64

＊Mean ± Standard deviation

d : Visceral adiposity group (BMI)

Parameter	In total	Male	Female
Subject	20	10	10
Age	45.0±5.20	44.0±5.64	45.9±4.82
Height	164.5±8.85	171.6±3.74	157.4±6.32
BW	77.8±10.00	81.6±5.17	74.1±12.39
BFR	30.9±5.60	25.9±1.98	35.9±2.80
BMI	28.8±2.90	27.8±1.78	29.8±3.53
Waist	95.5±6.67	93.9±5.21	97.1±7.80
SBP	138.7±26.90	134.0±25.85	143.4±28.45
DBP	88.7±16.10	90.1±18.06	87.2±14.71
FBG	103.9±19.44	108.9±26.77	98.9±5.02
TG	196.4±86.36	226.2±78.58	166.5±87.12
HDL	45.1±12.93	38.9±9.72	51.2±13.21

＊Mean ± Standard deviation

e: Visceral adiposity group (Navel high)

Parameter	In total	Male	Female
Subject	25	15	10
Age	46.7±6.82	47.4±8.17	45.6±4.25
Height	166.1±7.29	170.9±3.42	158.9±5.22
BW	76.1±9.59	77.3±7.65	74.3±12.19
BFR	29.5±5.73	25.6±2.80	35.5±2.98
BMI	27.6±3.43	26.4±2.57	29.3±3.90
Waist	93.9±6.85	91.6±5.40	97.2±7.69
SBP	135.7±22.55	133.9±22.36	138.4±23.75
DBP	88.4±14.57	89.5±15.04	86.9±14.47
FBG	107.7±25.36	114.5±31.20	97.5±2.68
TG	180.6±82.38	198.5±76.61	153.8±87.38
HDL	47.0±13.77	42.9±13.44	53.1±12.44

＊Mean ± Standard deviation

1)Units of inspection items and diagnostic criteria for metabolic syndrome

Subject		
Age	(year)	
Height (cm)	(cm)	
Body weight	BW (kg)	
Body fat ratio	BFR (%)	
Body mass index	BMI (kg/m^2)	
Waist	Waist (cm)	M≧85；F≧90
Systolic blood pressure	SBP (mmHg)	≧130
Diastolic blood pressure	DBP (mmHg)	≧85
Fasting blood sugar	FBG (mg/dL)	≧110
Triglyceride	TG (mg/dL)	≧150
High density lipoprotein cholesterol	HDL-C (mg/dL)	＜40

ference, which are associated with contributing factors for metabolic syndrome, such as accumulation of visceral fat, diabetes mellitus, and adipositas. The effects of ajoene ingestion on the correlation between adiponectin/leptin levels and parameters related to diabetes and adipositas were also studied.

2. Materials and Methods

1. Test subjects

The subjects of the study were residents of the outskirts of Tsu-city, Mie prefecture, Japan.Thirty-four males and females (17 males, average age 47.2 ± 7.72 years old, 17 females, average age 49.3 ± 6.54 years old) who were diagnosed with metabolic syndrome (hyperlipidemia, diabetes mellitus, hyperpiesia, and visceral adiposity) by routine physical checkups at their workplaces were divided into the following groups for stratified analysis.

1) Hyperlipidemic group

Subjects showing either more than 150 mg/dL of neutral fat or less than 40 mg/dL of HDL or subjects showing acceptable levels of both neutral fat and HDL during the pre-ingestion observation period were incorporated into this group, although the average HDL-C level did not fall into this category. Physical checkup data of the subjects are summarized in Table 1a.

2) Hyperglycemic group

Subjects showing more than 110 mg/dL of blood glucose during the pre-ingestion observation period were enrolled in this group. Physical checkup data of the subjects are summarized in Table 1b.

3) Hypertensive group

Subjects showing either a systolic BP level of more than 130 mmHg or diastolic BP level of more than 85 mmHg or subjects showing acceptable levels of both systolic BP and diastolic BP levels were enrolled in this group. Table 1c presents the summarized physical checkup data of the subjects.

4) Visceral adiposity group

①Subjects with BMI higher than 25 kg/m^2 during the pre-ingestion observation period were enrolled in this group. Table 1d presents the summarized physical checkup data of the subjects.

②Subjects with AC at navel height of more than 85 cm in males or more than 90 cm in females were enrolled in this group. Table 1e presents the summarized physical checkup data of the subjects.

Approvals were received from the Ethical Committee of Fujita Health University (Recept number 05-033; approval date 2005/09/21) and the Ethical Committee of Nanakuri Sanatorium (Nanakuri Ethical Committee No.17; approval date of 2006/01/26) for the present clinical study. The study was performed under supervision of a physician in accordance with the Helsinki Declaration, subsequent to sufficient explanation of the trial contents to the subjects and the receipt of written consent to participate in the study from each subject.

2. Test material

Soft capsules containing ajoene, which was extracted from garlic, with medium-chain triglyceride as the main ingredient were used for the study. Soft capsules containing only medium-chain triglyceride were used as placebo test material. Tables 2a and 2b present the compositions and nutrition information of the test materials.

3. Test schedule and mode of ingestion

The ingestion of 3 ajoene capsules per day was set as the normal ingestion dose for the 34 test subjects. Each subject took the respective experimental capsules with ordinary meals over a 12-week ingestion duration, which was divided into three periods, each consisting of 4 weeks, comprising a placebo ingestion period, an ingestion period of ajoene at normal dose, and an ingestion period of ajoene at 3 times normal dose. Pre- and post-ingestion observation periods, each consisting of 4 weeks, were provided before and after the ingestion periods.

Physical measurements and collections of blood and urine samples were carried out to monitor clinical variations between before and after ajoene ingestion. Prior to medical checkups, the subjects were kept

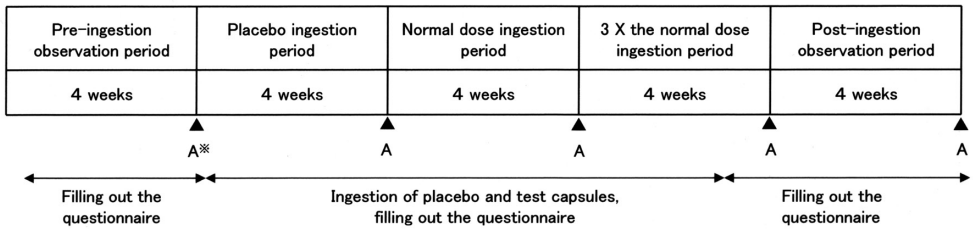

Fig. 1 Trial schedule

Table 2a Compositions of placebo and test capsules

	Placebo capsule	Normal dose capsule	3 × normal dose capsule
Combination per soft capsule			
Gelatin	100 mg	100 mg	100 mg
Glycerol	30 mg	30 mg	30 mg
Medium chain triglyceride (MCT)	180 mg	–	–
Oil-macerated garlic	–	180 mg	180 mg
(Ajoene)	–	(0.28 mg)	(0.85 mg)

under fasted conditions with the exception of water intake for 12 to 13 hours from 9 pm of the previous day to 9 to 10 am of the observation day in the pre- and post-ingestion observation periods and observation days in the 4th week of each ingestion period, and also kept under resting condition for more than 30 minutes after visiting hospital on the observation day (Fig. 1).

Each subject recorded food intake, consumption of test capsules, and certain symptoms in a daily questionnaire report throughout the ingestion periods. Subjects were requested to avoid excessive alcohol consumption and atypical activity levels throughout the ingestion periods, but they were given instructions not to change their daily habits including dietary habits and physical exercise.

4. Observations and assessment procedure

The following items were observed and examined in the present study. The clinical laboratory of Nanakuri Sanatorium Hospital, Fujita Health University, carried out physical examinations, biochemical examinations of blood, hematological tests, and urinalysis. Analyses of certain parameters were entrusted to Mitsubishi BCL.

Table 2b Nutrient composition of the normal dose capsule

Nutrient composition[a)]	
Water	4.9 g/100 g
Protein	30.0 g/100 g
Fat	58.7 g/100 g
Ash	0.2 g/100 g
Carbohydrate	6.2 g/100 g
Energy	673 kcal/100 g

a) Japan Food Research Laboratories No.304030738-001

1) Observations

① Doctor's questions: subjective symptoms (headache, heaviness of the head, fatigue, mouth dryness, and digestive symptoms etc).

② Questionnaire items: meal contents, consumption of test capsules, and subjective symptoms.

③ Physical examination: body height, body weight, BP, pulse, body fat percentage, BMI, and AC at navel height.

④ Biological sample analysis: biochemical examinations of blood, hematological tests, urinalysis.

5. Assessment procedure

All the measured values of the respective tests were expressed as mean ± standard deviation. Comparative results between ingestion periods were interpreted with reference to the results of two-sided paired *t*-tests. All statistical analysis was performed using a statistical software package, Statcel[9], with a level of significance of 5%.

3. Results

1. Questionnaires, doctor's questions and subjective symptoms

No test subjects showed abdominal symptoms (diarrhea, abdominal pain, or nausea) attributable to the ingestion of the test capsules or deconditioning due to fatigue.

2. Results of stratified analysis

1) Hyperlipidemic group

Changes in lipid related metabolic test results as a result of ajoene ingestion in the hyperlipidemic group are given in Table 3. There were no significant changes in the levels of neutral fat, total cholesterol, or LDL-C due to ajoene ingestion at the normal or 3 times normal dose levels.

HDL-C levels measured in the normal dose ingestion period (49.0 ± 11.31 mg/dL), 3 times normal dose ingestion period (49.4 ± 11.68 mg/dL), and post-ingestion observation period (51.5 ± 12.93 mg/dL) were significantly higher (P<0.01 to P<0.05) than those of the pre-ingestion observation period (46.9 ± 12.43 mg/dL).

HDL-C levels measured in the normal ingestion dose period, 3 times normal dose ingestion period, and post-ingestion period were also significantly higher (P<0.001 to P<0.005) than those of the placebo ingestion period.

In male subjects, high HDL-C levels found in the normal dose ingestion period were significantly (P<0.005) different from those in the pre-ingestion observation period and high HDL-C levels found during the normal dose ingestion period and post-ingestion observation period were significantly (P<0.001) different from those of the placebo ingestion period.

In female subjects, high HDL-C levels found in the 3 times normal dose ingestion period and the post-ingestion observation period significantly (P<0.001 to P<0.05) out-performed the placebo ingestion period, and the highest HDL-C levels found in the post-ingestion observation period significantly (P<0.05 to P<0.05) differed from those of the normal dose ingestion and 3 times normal dose ingestion periods. The highest HDL levels observed during the post-ingestion observation period in female subjects suggested that the effect of ajoene on HDL-C level was carried over to the post-ingestion period unlike with male subjects.

Table 3 Changes in lipid related metabolic test results as a result of ajoene ingestion in the hyperlipidemia group

Lipid type		Pre-ingestion observation period	Placebo ingestion period	Normal dose ingestion period	3 X the normal dose ingestion period	Post-ingestion observation period
TG	A	183.8±83.64^{a}	163.0±100.31	159.8±102.36^{a}	184.7±134.42	171.9±112.62
30–149 (mg/dL)	B	209.7±75.29	202.5±118.67	185.6±122.58	206.0±114.37	213.2±133.36
	C	153.7±85.76	116.9±43.87	129.8± 65.02	159.8±156.04	123.7±55.65
Total cholesterol	A	235.8±29.30^{h}	222.5±32.18^{aehi}	232.4±30.04^{e}	233.6±33.00^{a}	235.7±28.79^{j}
150–219 (mg/dL)	B	237.2±37.94^{a}	226.1±38.05^{ae}	232.3±33.18	236.4±38.38	240.6±32.78^{e}
	C	234.1±12.63^{a}	218.2±24.58^{abcd}	232.5±27.38^{b}	230.3±26.70^{c}	229.9±23.38^{d}
LDL-C	A	150.4±30.37^{e}	142.6±35.59^{ae}	148.4±32.41^{a}	146.4±36.92	149.7±31.00
70–139 (mg/dL)	B	152.8±37.41^{a}	144.6±43.52^{ab}	147.7±39.98	150.8±41.63	154.0±39.44^{b}
	C	147.7±20.67	140.3±25.10	149.2±22.32	141.3±31.58	144.6±17.14
HDL-C	A	46.9±12.43^{abh}	46.3±11.06^{fgj}	49.0±11.31^{acf}	49.4±11.68^{bg}	51.5±12.93^{chi}
40–96 (mg/dL)	B	42.3±12.72^{f}	41.1±10.82^{aeh}	44.9±11.54^{fh}	44.1±10.03^{a}	44.7±10.49^{e}
	C	52.3±10.09^{h}	52.3±8.20^{ai}	53.8±9.36^{j}	55.6±10.64^{ab}	59.3±11.14^{bhij}

Mean ± Standard deviation [A: In total (n = 26), B: Males (n = 14), C: Females (n = 12)]
Numbers bearing the same superscript letter are statistically significant at
P<0.05 for a, b, c, and d, P<0.01 for e, P<0.005 for f and g, and P<0.001 for h, i, and j (Paired *t*-test).

Table 4 Changes in carbohydrate related metabolic test results as a result of ajoene ingestion in the hyperglycemia group

Blood parameter		Pre-ingestion observation period	Placebo ingestion period	Normal dose ingestion period	3 X the normal dose ingestion period	Post-ingestion observation period
Fasting blood sugar 70-109 (mg/dL)	A	149.3±24.25	152.7±34.78^{c}	153.7±35.46	158.2±35.58^{c}	182.5±24.28
	B	163.3±11.50	172.8±19.74^{a}	173.0±23.62	179.0±18.81^{a}	160.0±39.65
	C	121.5±14.85	112.5± 6.36	115.0±11.31	116.5± 7.78	115.0± 4.24
HbA1c 4.2-5.7 (%)	A	6.80±0.888	6.85±1.164^{a}	7.27±1.464^{a}	7.15±1.471	7.25±1.443
	B	7.20±0.783^{a}	7.43±0.936^{b}	7.98±1.195^{ab}	7.85±1.277	7.93±1.274
	C	6.00±0.424	5.70±0.424	5.85±0.636	5.75±0.212	5.90±0.283

Mean ± Standard deviation [A: In total (n = 6), B: Males (n = 4), C: Females (n = 2)]
Numbers bearing the same superscript letter are statistically significant at
P<0.01 for a and b and P<0.001 for c (Paired t-test).

Table 5 Changes in carbohydrate related metabolic test results as a result of ajoene ingestion in the hyperpiesia group

Parameter		Pre-ingestion observation period	Placebo ingestion period	Normal dose ingestion period	3 X the normal dose ingestion period	Post-ingestion observation period
Systolic blood pressure (mmHg)	A	155.5±19.69^{adef}	145.9±18.27^{be}	143.2±17.73^{d}	140.5±19.42^{bf}	146.2±16.67^{a}
	B	154.4±27.17^{ab}	141.2±24.41^{a}	140.2±20.27^{b}	141.0±29.80	139.4±20.79
	C	156.1±16.12^{abe}	148.4±14.94^{ac}	144.9±17.22^{b}	140.2±12.99^{cde}	150.0±13.79^{d}
Diastolic blood pressure (mmHg)	A	96.1±14.45^{abe}	90.4±15.12^{a}	90.8±13.65^{e}	90.7±14.47^{b}	91.9±10.20
	B	99.8±22.70^{a}	95.0±22.64	93.6±20.79^{a}	97.2±18.74	94.0±15.46
	C	94.1±8.30^{ac}	87.9±9.74	89.2±8.89^{c}	87.1±11.13^{a}	90.8±6.74

Mean ± Standard deviation [A: In total (n = 14), B: Males (n = 5), C: Females (n = 9)]
Numbers bearing the same superscript letter are statistically significant at
P<0.05 for a and b, P<0.01 for c, and P<0.005 for e and f (Paired t-test).

2) Hyperglycemic group

Changes in carbohydrate-related metabolic test results as a result of ajoene ingestion in the hyperglycemic group are given in Table 4. Blood glucose levels found in male subjects during the 3 times normal dose ingestion period were significantly (P<0.05) higher than those of the placebo ingestion period, and no suppressive effect of ajoene on blood glucose levels was demonstrated.

Meanwhile, in female subjects, blood glucose levels measured in the placebo ingestion period (112.5 ± 6.36 mg/dL), normal dose ingestion period (115.0 ± 11.31 mg/dL), and 3 times normal dose ingestion period (116.5 ± 7.78 mg/dL) were lower than those of the pre-ingestion observation period (121.5 ± 14.85 mg/dL), but these differences were not statistically significant.

HbA1c levels in male subjects in the normal dose ingestion period were significantly (P<0.01) higher than those of the pre-ingestion observation period. In female subjects, HbA1c levels measured during the placebo ingestion period (5.70 ± 0.424%), normal dose ingestion period (5.85 ± 0.636%), and the times normal dose ingestion period (5.75 ± 0.212%) were lower than those of the pre-ingestion observation period (6.00 ± 0.424%) without showing any statistical difference.

3) Hypertensive group

Changes in BP measurements as a result of ajoene ingestion in the hypertensive group are presented in Table 5. Systolic BP values measured in total during the 3 ingestion periods and the post-ingestion observation period were significantly (P<0.001 to P<0.05) lower than those of the pre-ingestion observation period. Systolic BP values of male subjects measured during the placebo ingestion period and the normal dose ingestion period were significantly (P<0.05) lower than those of the pre-ingestion observation period. In female test subjects, systolic BP values measured during the placebo ingestion period, normal dose ingestion period, and the 3 times normal dose ingestion period were significantly (P<0.001 to

Table 6 Changes in BMI test values as a result of ajoene ingestion in the visceral adiposity group

Parameter		Pre-ingestion observation period	Placebo ingestion period	Normal dose ingestion period	3 X the normal dose ingestion period	Post-ingestion observation period
BMI	A	28.8±2.90	28.6±2.90	28.7±3.00	28.8±2.89	28.7±2.94
(kg/m^2)	B	27.8±1.78	27.6±1.55	27.7±1.60	27.8±1.23	27.6±1.56
	C	29.8±3.53	29.5±3.65[a]	29.6±3.81	29.7±3.75[a]	29.8±3.64

Mean ± Standard deviation [A: In total (n = 20), B: Males (n = 10), C: Females (n = 10)]
Numbers bearing the same superscript letter are statistically significant at
$P<0.05$ for a (Paired t-test).

Table 7 Changes in abdominal circumference at navel height as a result of ajoene ingestion in the visceral adiposity group

Parameter		Pre-ingestion observation period	Placebo ingestion period	Normal dose ingestion period	3 X the normal dose ingestion period	Post-ingestion observation period
Abdominal circumference	A	93.9±6.85[efgh]	90.9±7.31[ae]	90.4±7.60[f]	90.0±7.02[ag]	90.5±7.16[h]
	B	91.6±5.40[efgh]	88.2±5.52[e]	88.3±5.96[f]	88.3±5.17[g]	88.5±5.59[h]
(cm)	C	97.2±7.69[adef]	94.9±8.08[abc]	93.5±8.99[e]	92.5±8.83[cd]	93.5±8.43[bf]

Mean ± Standard deviation [A: In total (n = 25), B: Males (n = 15), C: Females (n = 10)]
Numbers bearing the same superscript letter are statistically significant at
$P<0.05$ for a and b, $P<0.01$ for c, $P<0.005$ for d, and $P<0.001$ for e, f, g, and h (Paired t-test).

$P<0.05$) lower in comparison with those of the pre-ingestion observation period. Systolic BP values measured in females during 3 times normal dose ingestion period were also significantly ($P<0.001$) lower compared to those of the placebo ingestion period.

Diastolic BP values measured during the 3 ingestion periods were significantly ($P<0.05$) lower than those of the pre-ingestion period. Diastolic BP values measured in male subjects during the normal dose ingestion period showed significantly ($P<0.05$) lower values compared to those of the pre-ingestion observation period and the values measured in female subjects during the normal dose ingestion and the 3 times normal dose ingestion periods were significantly ($P<0.01$ to $P<0.05$) lower than those of the pre-ingestion observation period.

4) Visceral adiposity group

① Changes in BMI measurements as a result of ajoene ingestion in the visceral adiposity group are shown in Table 6. There was no significant change in the BMI measurements in the male subjects between each ingestion period. BMI measured in female subjects in the 3 times normal dose ingestion period (29.7 ± 3.75 kg/m^2) were significantly ($P<0.05$) higher than those of the placebo ingestion period (29.5 ± 3.65 kg/m^2). However, BMI values measured in female subjects never exceeded those of the pre-ingestion observation period.

② Changes in the values of AC at navel height as a result of ajoene ingestion in the visceral adiposity group are shown in Table 7. AC at navel height measured in the placebo ingestion period, 3 times normal dose ingestion period, and post-ingestion observation period were significantly ($P<0.001$) lower than those of the pre-ingestion observation period. Compared with the placebo ingestion period, AC at navel height measured in the 3 times normal dose ingestion period showed significantly lower values. AC at navel height measured in male subjects during the placebo ingestion period, normal dose ingestion period, 3 times normal dose ingestion period, and the post-ingestion observation period were significantly ($P<0.001$) lower than those of the pre-ingestion observation period. AC at navel height measured in female subjects in the placebo ingestion period, normal dose ingestion period, 3 times normal dose ingestion period, and post-ingestion observation period were significantly ($P<0.001$ to $P<0.05$) lower than those of the

pre-ingestion observation period. AC at navel height measured in females during the 3 times normal dose ingestion period and post-ingestion observation period showed significantly ($P<0.01$ to $P<0.05$) lower values compared with those of the placebo ingestion period. AC values measured in female subjects decreased at a lower rate compared with the values in males.

4. Discussion

The present study was conducted to determine the effects of encapsulated ajoene, as extracted from garlic with medium-chain triglyceride, on 34 metabolic syndrome cases with equal numbers of both sexes.

The results indicated that the ingestion of ajoene capsules improved HDL-C levels in the hyperlipidemic group, as shown in Table 3. The positive effects of garlic on HDL-C levels have been reported previously in several papers. Holzgartner et al.[10] reported increased levels of HDL-C in 98 hyperlipoteinemia patients following the ingestion of dried garlic powder preparation for 12 weeks as compared with a control group receiving placebo powder for 6 weeks. Rotzsch et al.[11] observed a decrease in serum triglyceride levels and an upward trend in HDL-C levels in dietary hypertriglycemia patients that received an experimental fat diet including 100 g of butter following ingestion of dried garlic powder at 900 mg/day for 6 weeks. Warshafsky et al.[12] concluded that dietary garlic significantly decreased the level of total cholesterol, as determine by meta-analyses with 5 cases. In addition, Lau et al.[13] reported that dietary treatment of condensed aged-garlic extract (AGE) at 4 mL/day for 6 months decreased serum triglyceride levels and LDL and/or VLDL and increased HDL levels. Steiner[14] carried out a 10-month double-blind test with 41 moderately severe hypercholesterolemia patients with 200 to 290 mg/dL of serum cholesterol to determine the effect of dietary garlic and reported a significant decrease in serum cholesterol levels. Gebhardt et al.[15] studied the effects of ajoene on fat metabolism using rat liver and found depressive effects on the synthesis of cholesterol and fatty acids. Sendl et al.[16] reported that approximation of the cholesterol synthesis suppression concentration of ajoene was 234 μ g/mL as determined using a liver homogenate system. The cholesterol synthesis suppression concentration of allicin was reported to be 208 μ g/mL. Therefore, sulfur compounds that did not contain allicin also have the potential to suppress the synthesis of cholesterol.

An antihyperglycemic effect of ajoene was not observed in the hyperglycemic group in the present study (Table 4). It was reported in experiments with experimental animals[17), 18)] that a dry powder of garlic or s-allylcysteinsulfoxide lowered blood glucose levels and stimulated insulin secretion. No significant effect of the ingestion of garlic preparation on the blood glucose level was detected in non-diabetic test subjects in studies where glycemic control was evaluated as a secondary evaluation item among many clinical trials[19)-21)]. Consequently, ajoene may have no beneficial effect on blood glucose levels in humans.

The ingestion of ajoene significantly ($P<0.01$ to $P<0.05$) lowered systolic BP in the hypertension group (Table 5). The survey results of McMahon[22] stated that the BP-lowering effect of garlic was predominantly attributed to the improvement of atheroma index based on the platelet aggregation inhibition action of garlic, although no reports on clinical trials with ajoene have been found. Block et al.[23] reported 100% inhibition of platelet aggregation was achieved in rabbits that were subjected to the collagen-induced platelet aggregation inhibition test 24 hours after the ingestion of ajoene at a rate of 20 mg/kg. Bordia et al.[3] observed a pronounced inhibiting action of ajoene against epinephrine-induced platelet aggregation in humans following ingestion of ajoene capsules that were fortified with increased contents of ajoene (including extract equivalent to 1 g of fresh garlic) at a rate of 8 to 14 capsules a day and reported that the inhibiting action of ajoene was observed in platelet aggregation induced by either adenosine 5'-diphospate (ADP), epinephrine, or collagen when patients with a history of coronary ailment were given the capsules for a period of 1 month. Block et al.[23] further reported an increase in quantity of *Z*-ajoene (cis form) which has a 1.6 times stronger platelet aggregation inhibition activity compared with *E*-ajoene (trans form) when ajoene has been retained at a temperature of less than

110℃. As described, ajoene had the strongest platelet aggregation inhibition activity among the components derived from garlic. The strong inhibition action of ajoene may have an effect on the BP-lowering effect based on the improvement of atheromatous index. Several reports have been published recently suggesting the possible involvement of NO in the BP-lowering effect and platelet aggregation inhibition activity[24), 25)] of ajoene. Dirsch et al.[26)] stated in their report concerning iNOS that allicin and ajoene, thioallyl compounds, constrained the concentration of NO metabolites in a cell line of lipopolysaccharide (LPS)-stimulated macrophages and repressed the appearance of iNOS at the protein and mRNA levels. Elrod et al.[27)] conducted an experiment to demonstrate the efficacy of garlic on cardiovascular diseases. They observed the generation of hydrogen sulfide from erythrocyte membranes when a small quantity of erythrocytes was added to the garlic extract solution. They also observed the onset of tissue spreading while generating hydrogen sulfide when pieces of the main artery and heart blood vessel of rats were immersed in a solution containing polysulfide. These findings were different from the previous mode of action, which was based on the hypotensive effect derived from the platelet aggregation inhibition activity, and were considered to be the direct action of polysulfides. With the above active ingredients, Benavides et al.[28)] conducted an experiment to determine the generation of H_2S when garlic was reacted with human red blood cells using a H_2S sensor and reported that diallyl trisulfide showed the strongest H_2S generation activity followed by diallyl disulfide, which generated H_2S in approximately one third the quantity of that produced by diallyl trisulfide, but diallyl sulfide produced no effect on H_2S production. It is considered necessary to confirm the ability of ajoene to produce H_2S, because ajoene is similar in structure to diallyl sulfides.

A BMI lowering effect of ajoene was not observed in the visceral adiposity group (Table 6), but the ingestion of ajoene produced excellent results on the AC at navel height of both sexes, as indicated by values measured during the normal dose ingestion and 3 times normal dose ingestion periods, which were significantly (P<0.001) lowered compared with those of the pre-ingestion period. The present study is the first report in which ajoene was evaluated using physical examination results. In Japan, experimental plans have recently been reviewed to take measures to assess the improvement of metabolic syndrome as a result of the functionality of ingested foods and to evaluate the performance of a test materials in reference to measured values of visceral fat or BMI. Further studies are planned to measure visceral fat using CT scans in order to determine whether changes in subcutaneous or visceral fat are attributed to the lower AC at navel height values observed in the present study.

The results show the ingestion of soft capsules, whose main ingredient was ajoene, was found to be effective in improving atheromatous index, which is an indicator of *arteriosclerosis*, in the hyperlipidemic group, decreasing BP in the hypertension group, and decreasing AC in the visceral adiposity group. It is anticipated that the effectiveness of ajoene ingestion will be further ascertained by the accumulation of validated data in humans and studies on the mechanism of action of ajoene in the body after ingestion.

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