Bioscience, Biotechnology, and Biochemistry

ISSN: 0916-8451 (Print) 1347-6947 (Online) Journal homepage: https://www.tandfonline.com/loi/tbbb20

Vinegar Intake Reduces Body Weight, Body Fat Mass, and Serum Triglyceride Levels in Obese Japanese Subjects

Tomoo KONDO, Mikiya KISHI, Takashi FUSHIMI, Shinobu UGAJIN & Takayuki KAGA

To cite this article: Tomoo KONDO, Mikiya KISHI, Takashi FUSHIMI, Shinobu UGAJIN & Takayuki KAGA (2009) Vinegar Intake Reduces Body Weight, Body Fat Mass, and Serum Triglyceride Levels in Obese Japanese Subjects, Bioscience, Biotechnology, and Biochemistry, 73:8, 1837-1843, DOI: 10.1271/bbb.90231

To link to this article: https://doi.org/10.1271/bbb.90231

Biosci. Biotechnol. Biochem., **73** (8), 1837–1843, 2009

Vinegar Intake Reduces Body Weight, Body Fat Mass, and Serum Triglyceride Levels in Obese Japanese Subjects

Tomoo KONDO,† Mikiya KISHI, Takashi FUSHIMI, Shinobu UGAJIN, and Takayuki KAGA

Central Research Institute, Mizkan Group Corporation, 2-6 Nakamura-cho, Handa, Aichi 475-8585, Japan

Received April 2, 2009; Accepted April 20, 2009; Online Publication, August 7, 2009
[doi:10.1271/bbb.90231]

Acetic acid (AcOH), a main component of vinegar, recently was found to suppress body fat accumulation in animal studies. Hence we investigated the effects of vinegar intake on the reduction of body fat mass in obese Japanese in a double-blind trial. The subjects were randomly assigned to three groups of similar body weight, body mass index (BMI), and waist circumference. During the 12-week treatment period, the subjects in each group ingested 500 ml daily of a beverage containing either 15 ml of vinegar (750 mg AcOH), 30 ml of vinegar (1,500 mg AcOH), or 0 ml of vinegar (0 mg AcOH, placebo). Body weight, BMI, visceral fat area, waist circumference, and serum triglyceride levels were significantly lower in both vinegar intake groups than in the placebo group. In conclusion, daily intake of vinegar might be useful in the prevention of metabolic syndrome by reducing obesity.

Key words: vinegar; acetic acid; obesity; body fat mass; serum triglyceride

The incidence of obesity has been increasing dramatically in the past decades and is recognized as a risk factor for lifestyle-related diseases.[1–3] Visceral fat obesity is more strongly correlated with lifestyle-related diseases than is subcutaneous fat obesity.[4] A cluster of obesity-related disorders in one individual has been designated syndrome X, the deadly quartet, and more recently, metabolic syndrome. In Japan, the percentage of the population with a body mass index (BMI) between 25 and 30 kg/m^2 exceeds 30%, and the percentage of people in Japan with a BMI greater than 30 $kg/m^2$is approximately 3%. In contrast, 10–20% of the population of Europe and the USA have a BMI greater than 30 kg/m^2.[5–7] Thus, although the World Health Organization defines obesity as a BMI greater than 30 kg/m^2, the Japanese criteria define it as a BMI greater than 25 kg/m^2.[8] Recent epidemiological studies have revealed that the incidence of obesity-related diseases such as diabetes mellitus, hypertension, and hyperlipidemia is significantly greater among Japanese with BMIs greater than 25 kg/m^2.[9] Therefore, it is necessary to develop substances that can reduce fat mass in Japanese people with BMIs between 25 and 30 kg/m^2. Moreover, it is important that these substances do not induce side effects and can be taken as part of the daily diet.

Vinegar has a very long history, going back to Babylonia in 5,000 BC. Today, various kinds of vinegar originating from different crops are consumed throughout the world as seasonings, preservatives, and ingredients in condiments such as ketchup, mayonnaise, and salad dressing. Especially in Japan, vinegar is a very common seasoning in popular foods such as sushi. Furthermore, beverages containing vinegar are also commonly consumed in Japan. Although vinegar has various ingredients, the main component is 4–8% acetic acid (AcOH).[10] We have found that intake of vinegar lowers blood pressure,[11,12] improves hypercholesterolemia,[13] and inhibits postprandial hyperglycemia[14] in humans. Furthermore, Yamashita *et al.* reported that AcOH inhibits the expression of lipogenic genes for fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) *via* AMP-activated protein kinase (AMPK)/carbohydrate responsive element-binding protein, resulting in decreased body weight in obese rats.[15] In addition, it has been shown that AcOH downregulates ATP citrate lyase (ATP-CL), FAS, and ACC gene expression *via* sterol regulatory element-binding protein-1 (SREBP-1), thereby decreasing serum triglyceride (TG) levels in hyperlipidemia rats.[16] These data suggest that intake of AcOH or vinegar might prevent and/or ameliorate obesity and hypertriglycemia, but it has been reported that results for humans and animals are not always consistent.[17]

In this study, we investigated the effects of continuous intake of vinegar on body weight, abdominal fat areas (visceral fat area [VFA] and subcutaneous fat area [SFA]), and serum TG levels in 175 obese but otherwise healthy Japanese subjects by a double-blind, placebo-controlled trial with a duration of 12 weeks.

Methods

Subjects. The study consisted of obese Japanese, as defined by the Japanese criteria for obesity, with BMIs between 25 and 30 kg/m^2.[18] The subjects were 25–60 years old and reported a stable body weight for 1 month before the study. The subjects did not use any medications. Before the pre-treatment period, we excluded those subjects who had

† To whom correspondence should be addressed. Tel: +81-569-24-5128; Fax: +81-569-24-5029; E-mail: t-kondo@mizkan.co.jp

Abbreviations: ACC, acetyl-CoA carboxylase; ACO, acyl-CoA oxidase; AcOH, acetic acid; ALT, alanine aminotransferase; AMPK, AMP-activated protein kinase; AST, aspartate aminotransferase; ATP-CL, ATP citrate lyase; BFR, body fat ratio; BMI, body mass index; CHD, coronary heart disease; CT, computed tomography; FAS, fatty acid synthase; FPG, fasting plasma glucose; γ-GTP, γ-glutamyl transpeptidase; HbA1c, glycohemoglobin; HDL-cho, high density lipoprotein cholesterol; HOMA-R, homeostasis model assessment of insulin resistance; LDL-cho, low density lipoprotein cholesterol; SFA, subcutaneous fat area; SREBP-1, sterol regulatory element-binding protein-1; T-chol, total cholesterol; TFA, total fat area; TG, triglyceride; VFA, visceral fat area

Table 1. Compositions of Test Beverages[1]

	Placebo	Low-dose	High-dose
Acetic acid (mg/100 ml)	ND	150	300
Lactic acid (mg/100 ml)	250	1	1
Energy (kJ/100 g)[2]	ND	4.2	8.4
Water (g/100 ml)	100.0	99.7	99.5
Protein (g/100 ml)	0.0	0.0	0.0
Fat (g/100 ml)	0.0	0.0	0.0
Carbohydrate (g/100 ml)	ND	0.3	0.5
Ash (g/100 ml)	0.0	0.0	0.0
Sodium (mg/100 ml)	ND	4.3	4.3
Phosphorus (mg/100 ml)	ND	ND	ND
Calcium (mg/100 ml)	ND	ND	ND
Potassium (mg/100 ml)	ND	ND	2.0
Magnesium (mg/100 ml)	ND	ND	ND

[1]ND, not detectable.
[2]Calculated from the amounts of protein, fat, and carbohydrate except for organic acids.

serum TG levels <1.13 mmol/l or >2.83 mmol/l, serum total cholesterol (T-cho) >6.73 mmol/l, fasting plasma glucose (FPG) >6.94 mmol/l, aspartate aminotransferase (AST) >61 IU/l, alanine aminotransferase (ALT) >61 IU/l, or γ-glutamyl transpeptidase (γ-GTP) >101 IU/l in males and >51 IU/l in females. Furthermore, those who experienced heartburn due to vinegar or were pregnant were also excluded. The remaining 175 subjects (111 males and 64 females; age, 44.1 ± 9.7 years; body weight, 74.4 ± 9.9 kg; BMI, 27.2 ± 1.7 kg/m^2) participated in the study.

In compliance with the Helsinki Declaration, the study protocol, purpose, and methodology were explained to the subjects, and they were informed of their right to withdraw at any time. Written informed consent for participation was obtained from all subjects. The study was performed under the supervision of an occupational health physician. The ethics committees of two separate groups, the Mizkan Group Corporation (Aichi, Japan) and the Health Functional Food Evaluation Center Co., Ltd. (Saitama, Japan), approved the study protocol.

Test samples. The compositions of the three test beverages are shown in Table 1. The 500-ml beverages for the placebo, low-, and high-dose groups contained 0, 15, and 30 ml of apple vinegar (0, 750, and 1,500 mg AcOH) respectively. Apple vinegar is generally preferred for drinking in Japan because of its flavor and taste. In order to mimic the taste of vinegar, the placebo group's beverage contained 1,250 mg of lactate. For easy drinking, each test sample contained the same amount of flavor and no-calorie artificial sweetener.

Study protocol. This study was a parallel-group, randomized, double-blind, placebo-controlled trial. There were three test periods: a 3-week pre-treatment period, a 12-week treatment period, and a 4-week post-treatment period. The first day of the treatment period was defined as week zero. Measurement days were set at weeks -3 (the initial day of the pre-treatment period), 0, 4, 8, 12, and 16 (the final day of the post-treatment period). Before the pre-treatment period, the 175 subjects were randomly assigned to three groups: a low-dose group ($n = 59$), a high-dose group ($n = 58$), and a placebo group ($n = 58$). During the treatment period, the subjects drank the test beverage (500 ml) in two equal portions after breakfast (250 ml) and after supper (250 ml). A diet diary was kept 3 d before each measurement day, and the number of steps taken was recorded every day throughout the test period. On the day before the measurement day, the subjects were prohibited from drinking alcohol and performing strenuous exercise. They were also asked to refrain from eating or drinking after 9:00 pm. Consequently, they fasted overnight for more than 12 h. On the measurement day, blood samples were collected, blood pressure was recorded, and body weight and waist-hip circumferences were measured. On the same day, a medical checkup by the occupational health physician was conducted, and the subjects were advised to continue on isocaloric diets and to maintain their accustomed degree of physical activity throughout the test period. Computed tomography (CT) was performed at weeks 0 and 12.

The subjects were required to adhere to the following restrictions: daily alcoholic beverage intake not to exceed the equivalent of approximately 25 ml of alcohol during the test period, no other vinegar beverages to be consumed, functional foods and drugs to treat obesity or hyperlipidemia to be avoided during the test period, and smoking prohibited on the measurement day.

Diet diary and record of number of steps taken. The contents of daily meals and snacks were recorded in a diet diary. Intake of energy, protein, fat, carbohydrates, cholesterol, fiber, and salt was analyzed using Healthy Maker Pro (Mushroomsoft, Okayama, Japan). To minimize the confounding effects of dietary vinegar, the subjects were instructed not to consume foods that included large quantities of vinegar. The number of steps walked daily was recorded with a pedometer during the test period.

Anthropometric measurements. Body weight (to the nearest 0.01 kg) and height (to the nearest 0.1 cm) were measured while the subject was fasting and wearing only undergarments. The body fat ratio (BFR) was measured by the bioimpedance analysis method (Tanita Body Fat Analyzer, Model no. BC-300; Tanita, Tokyo). The BMI was calculated as body weight divided by the square of the height. Waist circumference was measured according to the criteria of the Japan Society for the Study of Obesity. The measurement was taken between the lowest rib margin and the iliac crest while the subject was in a standing position (to the nearest 0.1 cm). Hip circumference was measured at the widest point of the hip (to the nearest 0.1 cm).

Measurement of visceral and subcutaneous fat areas. Cross-sectional abdominal VFA and SFA were measured by CT at the level of the fourth to fifth lumbar vertebrae (L4–L5) with the subject in a supine position. This is a standardized method used to measure body fat mass. This examination was performed within 3 d of the anthropometric measurements. The x-ray conditions were a tube voltage of 120 kVp and 360 mAs, and the film was processed at a window level of 0 and a window width of 1,000. Using FatScan (N2 System, Ibaraki, Japan), the VFA and SFA were obtained from the abdominal CT image. These areas were added to obtain the total fat area (TFA).

Measurement of blood values and blood pressure. Fasting blood was collected from a vein on the flexor side of the arm. Blood samples were analyzed by SRL (Tokyo). The levels of the following variables were measured: TG (Pureauto S TG-N; Sekisui Medical, Tokyo), T-cho (T-CHO Reagent KL; Sysmex International Reagents, Hyogo, Japan), low density lipoprotein cholesterol (LDL-cho, Cholestest LDL; Sekisui Medical), high density lipoprotein cholesterol (HDL-cho, Cholestest HDL), FPG (Quick Auto Neo GLU-HK; Shino-Test, Tokyo), insulin (Lumipulse Presto Insulin; Fujirebio, Tokyo), glycohemoglobin (HbA1c, Papidiaauto HbA1c; Fujirebio), HOMA-R (calculated as FBP $\times$ insulin/405), AST (Cica Liquid AST; Kanto Chemical, Tokyo), ALT (Cica Liquid ALT), γ-GTP (Cica Liquid γ-GT J; Kanto Chemical), creatinine (Cr, Pureauto S CRE-L; Sekisui Medical), blood urea nitrogen (BUN, Cica Liquid-N UN; Kanto Chemical), and uric acid (UA, Pureauto S UA; Sekisui Medical). Blood pressure was measured after a 10-min rest with the subject in sitting position.

Statistical analysis. All experimental data were analyzed with SPSS for Windows (version 11.5 J; SPSS, Inc., Chicago, IL). Analyses of various nutrient intakes and the number of steps taken were conducted by ANOVA with a *post hoc* Tukey test. In this study, because the assignment of the subjects was conducted before the pre-treatment period, it was necessary to correct for the change from week -3 to week 0. Therefore, in intergroup comparisons, we performed ANCOVA with a *post hoc* Bonferroni test using the value at week 0 as the covariate. For intragroup comparisons, we performed one-way repeated ANOVA with a *post hoc* Dunnett test using the value at week 0 as the reference. Since the fat areas (VFA, SFA, and TFA) were measured only at weeks 0 and 12, the differences in values between week 0 and week 12 were analyzed by performing ANOVA with a *post hoc* Dunnett test using the placebo group's value as the reference. Differences were considered significant at $p < 0.05$. Tables 2–5 are presented as mean $\pm$ SD and Fig. 1 is presented as mean $\pm$ SE.

Table 2. Characteristics of Subjects[1]

	Placebo	Low-dose	High-dose
n (male/female)	50 (32/18)	54 (34/20)	51 (31/20)
Age (years)	44.1 ± 9.6	44.7 ± 9.7	43.4 ± 9.5
Height (cm)	165.5 ± 9.4	165.6 ± 9.1	164.4 ± 7.5
Weight (kg)	74.2 ± 11.0	74.9 ± 10.1	73.1 ± 8.6
BMI (kg/m^2)	26.9 ± 1.6	27.2 ± 1.8	27.0 ± 1.7
Waist (cm)	90.2 ± 6.8	90.9 ± 6.4	90.5 ± 6.5
Hip (cm)	99.9 ± 4.9	100.7 ± 6.1	100.1 ± 5.3
Waist hip ratio	0.901 ± 0.041	0.904 ± 0.053	0.904 ± 0.039
VFA (cm^2)	101.3 ± 33.2	112.1 ± 38.5	104.2 ± 33.6
SFA (cm^2)	210.3 ± 67.7	221.0 ± 86.4	220.6 ± 80.1
TFA (cm^2)	311.6 ± 79.6	333.1 ± 103.9	324.8 ± 97.0
SBP (mmHg)	127.5 ± 13.6	126.6 ± 12.0	125.5 ± 12.6
DBP (mmHg)	76.6 ± 9.4	77.0 ± 9.3	76.9 ± 9.2
TG (mmol/l)	1.71 ± 0.50	1.71 ± 0.60	1.78 ± 0.55
T-cho (mmol/l)	5.53 ± 0.82	5.31 ± 0.82	5.70 ± 0.83

[1]All values are mean ± SD.

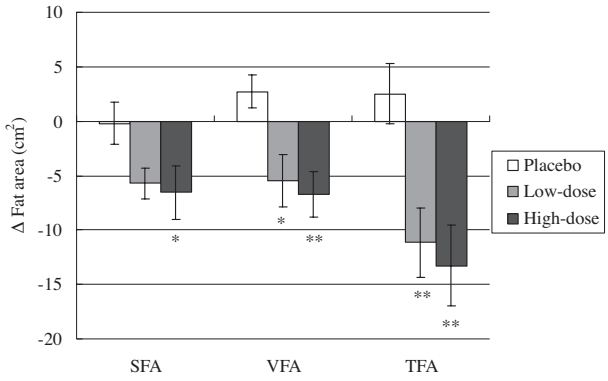

Fig. 1. Degrees of Change from Week 0 to Week 12 in Fat Areas (SFA, VFA, TFA).

All values are mean ± SE. Significantly different from placebo, $^*p < 0.05$, $^{**}p < 0.01$ (ANOVA followed by the Dunnett test).

Results

Subjects

Fourteen subjects declined participation (three before the pre-treatment period and 11 during the treatment period). Six subjects were excluded because of deviation from the x-ray conditions. The background information on the remaining 155 subjects who participated in the entire study period is shown in Table 2. The distribution of subjects with BMIs between 25 and 30 kg/m^2 was similar to that of the general Japanese population. The following percentages correspond to the subjects and to the general population of Japan respectively: BMI 25–26: 35% and 35%; BMI 26–27: 21% and 25%; BMI 27–28: 20% and 18%; BMI 28–29: 13% and 13%; and BMI 29–30: 12% and 9%.[18)]

Diet diary and number of steps taken

No significant differences among the three groups were observed with regard to intake of energy, protein, fat, carbohydrates, cholesterol, and fiber. Likewise, there were no significant differences among the three groups with regard to the number of steps taken (Table 3). Furthermore, no changes occurred in these parameters throughout the test period in any group, indicating that all the subjects maintained a consistent lifestyle during the test period.

Effects on anthropometric variables

Body weight, BMI, and BFR values in both the low- and high-dose groups began to decrease from week 4 (Table 4). Furthermore, body weight and BMI values in the high-dose group were significantly lower than those in the low-dose group at weeks 8 and 12, indicating dose dependency. The circumferences of the waist and hip in the high- and low-dose groups started to decrease from weeks 4 and 8 respectively. As compared with the placebo group, both the high- and low-dose groups had significantly lower body weights, BMIs, and BFRs at weeks 4, 8 and 12, circumferences of waist and hip at weeks 8 and 12, and waist-hip ratios at week 12.

Abdominal fat mass analysis

The differences in VFA, SFA, and TFA between week 0 and week 12 are presented in Fig. 1. No significant differences in any variable among the three groups were observed at week 0 (pooled data of 155 subjects; VFA, 106 ± 35 cm^2; SFA, 217 ± 78 cm^2; TFA, 323 ± 94 cm^2). Both the low- and high-dose groups had more significant decreases in VFA and TFA than the placebo group. With regard to SFA, the value for the high-dose group was significantly lower and that for the low-dose group tended to be lower (p-value = 0.085) than for the placebo group. The percentage changes in VFA values between treatment periods were 4.4 ± 1.8, −2.8 ± 2.4, and −4.9 ± 2.0; the percentage changes in SFA values between treatment periods were −0.2 ± 0.9, −2.3 ± 0.7, and −2.8 ± 1.0; and the percentage changes in TFA values between treatment periods were 1.0 ± 0.9, −2.8 ± 1.0, and −3.5 ± 1.1 in the placebo, low-, and high-dose groups respectively.

Effects on blood values and blood pressure

Serum TG levels in both the low- and high-dose groups began to decrease from week 4 (Table 5). As compared with the placebo group, the low-dose group had significantly lower serum TG levels at weeks 4, 8, and 12, and a significantly lower T-cho levels at week 12. The high-dose group had significantly lower serum TG values than the placebo group at weeks 4 and 12. Systolic blood pressure in the high-dose group began to decrease from week 8. As compared with the placebo group, the high-dose group had significantly lower systolic blood pressure at week 12. No significant differences were observed in the other measurements among the three groups, and no significant changes in measurements of liver function (AST, ALT, and γ-GTP) or kidney function (Cr, BUN, and UA) were found throughout the test period (data not shown).

Discussion

The present study is the first to demonstrate that vinegar reduces body weight, BMI, and body fat mass in obese Japanese subjects. Furthermore, continuous vinegar intake was found to lower serum TG levels.

Body weight, BMI, and BFR in both the low- and high-dose groups significantly decreased from week 4 in a dose-dependent manner. In addition, waist circumference, waist-hip ratio, and serum TG values also fell from week 8. These results can be considered to be due

Table 3. Daily Intake of Energy, Protein, Fat, Carbohydrate, Cholesterol, and Fiber, and Steps Walked[1]

	Treatment period				Post-treatment period
	week 0	week 4	week 8	week 12	week +4
Energy (kJ/d)[2]					
Placebo	7393 ± 1536	7435 ± 1318	7343 ± 1368	7351 ± 1226	7339 ± 1272
Low-dose	7774 ± 1356	7531 ± 1247	7627 ± 1230	7577 ± 1209	7544 ± 1251
High-dose	7895 ± 1414	7753 ± 1284	7661 ± 1247	7740 ± 1234	7719 ± 1234
Protein (g/d)[2]					
Placebo	63.7 ± 14.5	66.5 ± 13.5	67.0 ± 14.5	65.5 ± 13.4	64.4 ± 12.6
Low-dose	68.6 ± 15.1	67.1 ± 14.4	68.0 ± 12.3	67.4 ± 12.6	68.0 ± 12.9
High-dose	69.5 ± 13.1	68.9 ± 12.4	68.6 ± 11.6	68.6 ± 11.9	68.0 ± 11.5
Fat (g/d)[2]					
Placebo	52.3 ± 14.8	50.7 ± 11.1	50.9 ± 12.1	49.0 ± 12.5	51.3 ± 11.2
Low-dose	53.1 ± 15.8	50.2 ± 14.3	49.3 ± 13.6	50.7 ± 13.8	48.7 ± 12.7
High-dose	55.7 ± 14.2	54.4 ± 12.4	52.9 ± 12.3	52.7 ± 11.8	52.2 ± 12.2
Carbohydrates (g/d)[2]					
Placebo	246.1 ± 57.2	252.1 ± 48.8	244.3 ± 53.2	252.1 ± 47.7	245.3 ± 48.8
Low-dose	260.8 ± 47.3	256.2 ± 40.0	263.7 ± 43.4	257.9 ± 43.2	260.6 ± 48.6
High-dose	262.2 ± 49.3	259.2 ± 44.9	256.8 ± 44.5	261.9 ± 45.0	262.2 ± 43.0
Cholesterol (mg/d)[2]					
Placebo	272.4 ± 113.7	282.7 ± 105.7	293.0 ± 107.2	277.6 ± 118.1	280.2 ± 117.1
Low-dose	294.2 ± 131.2	306.0 ± 132.3	282.9 ± 115.9	299.0 ± 121.2	283.2 ± 93.7
High-dose	301.3 ± 113.9	292.8 ± 117.4	306.2 ± 98.7	291.7 ± 107.9	292.9 ± 114.5
Fiber (g/d)[2]					
Placebo	11.2 ± 3.1	12.6 ± 4.1	12.4 ± 4.1	12.5 ± 4.2	12.1 ± 3.4
Low-dose	12.3 ± 3.1	12.5 ± 3.5	13.0 ± 4.2	12.1 ± 3.6	12.6 ± 4.1
High-dose	12.0 ± 3.3	12.4 ± 3.3	12.6 ± 3.7	12.8 ± 3.5	12.8 ± 3.7
Steps taken (steps/d)[3]					
Placebo	8269 ± 2850	8068 ± 3002	7963 ± 3026	8166 ± 3434	7559 ± 3003
Low-dose	7305 ± 2347	7303 ± 2672	7413 ± 2708	7233 ± 2502	6961 ± 2497
High-dose	8304 ± 3416	8605 ± 2522	8174 ± 2457	8145 ± 2577	7564 ± 2281

No significant differences were observed among three groups.
[1]All values are mean ± SD.
[2]Average of 3 d before measurement day.
[3]Average of the days between measurement days (for example, week 0: average between week −3 to week 0).

Table 4. Anthropometric Variables and Body Composition[1]

	Treatment period				Post-treatment period
	week 0	week 4	week 8	week 12	week +4
Body weight (kg)					
Placebo	74.2 ± 11.0	74.3 ± 11.0	74.4 ± 11.2	74.6 ± 11.3#^{2}	74.5 ± 11.4
Low-dose	74.9 ± 10.1	74.5 ± 10.1a^3#^{2}	74.0 ± 10.2a^3#^{3}	73.7 ± 10.3a^3#^{3}	74.9 ± 10.5
High-dose	73.1 ± 8.6	72.6 ± 8.5a^{3}b^3#^{2}	71.4 ± 8.3a^{3}b^3#^{3}	71.2 ± 8.3a^{3}b^2#^{3}	72.7 ± 8.3a^{3}b^3#^{1}
BMI (kg/m^2)					
Placebo	26.9 ± 1.6	27.0 ± 1.7	27.0 ± 1.7	27.1 ± 1.8#^{1}	27.0 ± 1.8
Low-dose	27.2 ± 1.8	27.1 ± 1.9a^2#^{2}	26.9 ± 1.9a^3#^{3}	26.8 ± 2.0a^3#^{3}	27.2 ± 2.0
High-dose	27.0 ± 1.7	26.8 ± 1.6a^3#^{3}	26.4 ± 1.6a^{3}b^3#^{3}	26.3 ± 1.6a^{3}b^2#^{3}	26.8 ± 1.7#^{1}
BFR (%)					
Placebo	29.9 ± 6.8	29.9 ± 6.9	29.9 ± 7.0	29.9 ± 6.9	30.0 ± 7.0
Low-dose	30.3 ± 7.2	30.0 ± 7.2a^1#^{2}	29.8 ± 7.1a^2#^{3}	29.6 ± 7.1a^3#^{3}	30.2 ± 7.1
High-dose	30.2 ± 7.6	29.8 ± 7.5a^2#^{3}	29.6 ± 7.6a^3#^{3}	29.3 ± 7.5a^3#^{3}	29.8 ± 7.5a^1#^{3}
Waist (cm)					
Placebo	90.2 ± 6.8	90.2 ± 6.9	90.4 ± 7.0	90.4 ± 6.9	90.6 ± 7.1
Low-dose	90.8 ± 6.4	90.4 ± 6.5	89.7 ± 6.5a^3#^{3}	89.4 ± 6.5a^3#^{3}	90.1 ± 6.5a^1#^{3}
High-dose	90.5 ± 6.5	89.9 ± 6.7#^{1}	89.3 ± 6.3a^3#^{3}	88.6 ± 6.3a^3#^{3}	89.8 ± 6.1a^2#^{2}
Hip (cm)					
Placebo	99.9 ± 4.9	99.7 ± 4.8	99.8 ± 4.8	99.7 ± 4.8	100.1 ± 5.0
Low-dose	100.7 ± 6.1	100.3 ± 6.2	99.9 ± 6.0a^1#^{3}	99.6 ± 6.2a^2#^{3}	100.2 ± 6.1#^{1}
High-dose	100.1 ± 5.3	99.5 ± 5.3#^{3}	99.0 ± 5.3a^3#^{3}	98.7 ± 5.2a^3#^{3}	99.4 ± 5.0a^2#^{3}
Waist-hip ratio					
Placebo	0.903 ± 0.042	0.904 ± 0.045	0.905 ± 0.042	0.906 ± 0.043	0.905 ± 0.045
Low-dose	0.903 ± 0.052	0.903 ± 0.051	0.899 ± 0.049	0.898 ± 0.052a^1	0.900 ± 0.051
High-dose	0.904 ± 0.041	0.903 ± 0.044	0.902 ± 0.042	0.898 ± 0.042a^2#^{1}	0.903 ± 0.038

[1]All values are mean ± SD.
a Significantly different from placebo, a^1 $p < 0.05$, a^2 $p < 0.01$, a^3 $p < 0.001$ (ANCOVA followed by the Bonferroni test).
b Significantly different between low-dose and high-dose, b^1 $p < 0.05$, b^2 $p < 0.01$, b^3 $p < 0.001$ (ANCOVA followed by the Bonferroni test).
Significantly different from the value at week 0, #^{1} $p < 0.05$, #^{2} $p < 0.01$, #^{3} $p < 0.001$ (one-way repeated ANOVA followed by the Dunnett test).

Table 5. Blood Values and Blood Pressure[1]

	Treatment period				Post-treatment period
	week 0	week 4	week 8	week 12	week +4
TG (mmol/l)					
Placebo	1.71 ± 0.50	1.69 ± 0.60	1.69 ± 0.73	1.68 ± 0.67	1.56 ± 0.65
Low-dose	1.70 ± 0.60	1.40 ± 0.57a[2]#[3]	1.41 ± 0.55a[1]#[3]	1.39 ± 0.58a[1]#[3]	1.46 ± 0.70#[1]
High-dose	1.78 ± 0.55	1.50 ± 0.57a[1]#[1]	1.49 ± 0.66#[1]	1.31 ± 0.54a[3]#[3]	1.78 ± 0.82
T-cho (mmol/l)					
Placebo	5.53 ± 0.82	5.63 ± 0.85	5.70 ± 0.80	5.45 ± 0.87	5.71 ± 0.88
Low-dose	5.31 ± 0.82	5.44 ± 0.88	5.39 ± 0.73	4.99 ± 0.82a[1]#[3]	5.51 ± 0.99
High-dose	5.70 ± 0.83	5.64 ± 0.84	5.58 ± 0.79	5.37 ± 0.83#[1]	5.65 ± 0.84
LDL-cho (mmol/l)					
Placebo	3.39 ± 0.83	3.51 ± 0.78	3.55 ± 0.83	3.44 ± 0.73	3.52 ± 0.80
Low-dose	3.12 ± 0.73	3.35 ± 0.75#[1]	3.33 ± 0.73#[1]	3.00 ± 0.76	3.39 ± 0.75#[3]
High-dose	3.48 ± 0.74	3.47 ± 0.67	3.50 ± 0.72	3.37 ± 0.77	3.46 ± 0.81
HDL-cho (mmol/l)					
Placebo	1.36 ± 0.24	1.40 ± 0.30	1.36 ± 0.27	1.38 ± 0.26	1.42 ± 0.31
Low-dose	1.35 ± 0.32	1.38 ± 0.33	1.40 ± 0.32	1.38 ± 0.34	1.41 ± 0.35
High-dose	1.41 ± 0.34	1.39 ± 0.28	1.40 ± 0.29	1.41 ± 0.33	1.43 ± 0.37
FPG (mmol/l)					
Placebo	4.99 ± 0.45	5.06 ± 0.60	5.12 ± 0.60	5.20 ± 0.68	5.17 ± 0.83
Low-dose	5.15 ± 0.68	5.05 ± 0.68	5.22 ± 0.93	5.15 ± 0.78	5.25 ± 1.11
High-dose	5.01 ± 0.50	5.05 ± 0.63	5.06 ± 0.70	5.04 ± 0.65	5.16 ± 0.77
Insulin (pmol/l)					
Placebo	58.2 ± 31.1	58.3 ± 44.0	72.1 ± 54.7	62.4 ± 38.2	73.5 ± 70.3
Low-dose	75.7 ± 96.3	65.4 ± 37.7	77.2 ± 75.3	67.1 ± 53.5	75.5 ± 56.3
High-dose	58.4 ± 52.6	68.5 ± 55.8	66.8 ± 60.5	59.6 ± 48.6	65.0 ± 40.1
HbA1c (%)					
Placebo	5.34 ± 0.46	5.32 ± 0.45	5.28 ± 0.48	5.28 ± 0.49	5.27 ± 0.47
Low-dose	5.35 ± 0.67	5.39 ± 0.65	5.36 ± 0.65	5.32 ± 0.63	5.28 ± 0.66
High-dose	5.29 ± 0.45	5.34 ± 0.47	5.22 ± 0.50	5.23 ± 0.47	5.23 ± 0.47
HOMA-R					
Placebo	1.87 ± 1.01	1.93 ± 1.57	2.44 ± 2.07	2.14 ± 1.49	2.52 ± 2.55
Low-dose	2.61 ± 3.59	2.13 ± 1.23	2.64 ± 2.61	2.23 ± 1.72	2.62 ± 2.17
High-dose	1.90 ± 1.72	2.30 ± 2.21	2.33 ± 2.67	1.98 ± 1.78	2.18 ± 1.45
SBP (mmHg)					
Placebo	127.5 ± 13.6	127.5 ± 12.6	127.3 ± 14.0	127.6 ± 13.9	127.9 ± 15.0
Low-dose	126.6 ± 12.0	125.6 ± 13.7	124.7 ± 13.9	124.8 ± 12.8	126.1 ± 13.3
High-dose	125.5 ± 12.6	122.5 ± 11.0	122.0 ± 10.5#[1]	121.0 ± 13.0a[1]#[2]	123.3 ± 13.6
DBP (mmHg)					
Placebo	76.6 ± 9.4	76.7 ± 8.8	76.7 ± 9.5	77.1 ± 8.6	77.0 ± 9.1
Low-dose	77.0 ± 9.3	77.0 ± 8.7	75.8 ± 8.7	75.9 ± 8.5	76.8 ± 8.2
High-dose	76.9 ± 9.2	75.2 ± 8.7	74.9 ± 7.0	74.7 ± 9.4	75.5 ± 9.3

[1]All values are mean ± SD.
a Significantly different from placebo, a[1] $p < 0.05$, a[2] $p < 0.01$, a[3] $p < 0.001$ (ANCOVA followed by the Bonferroni test).
Significantly different from the value at week 0, #[1] $p < 0.05$, #[2] $p < 0.01$, #[3] $p < 0.001$ (one-way repeated ANOVA followed by the Dunnett test).

to the reduction in body fat mass caused by vinegar intake, because the VFA, SFA, and TFA values were significantly lower in the vinegar intake groups than in the placebo group. Although visceral fat is reported to decrease more readily than subcutaneous fat through exercise and dietary restriction,[19–22] reductions in both the VFA and SFA were observed in the present study. Body weight, abdominal fat, and serum TG values are known to vary greatly depending on diet and exercise. However, energy intake, meal content, and physical activity did not differ among the three groups throughout the test period. Therefore, vinegar intake was considered to decrease the BMI of obese subjects *via* a reduction in body fat mass, regardless of the type of adipose tissue. In Japan, the percentage of the population with a BMI between 25 and 30 kg/m^2 exceeds 30%, and the percentage of the population with a BMI of 30 kg/m^2 or more is approximately 3%. The results of the present study show that in obese Japanese subjects who had BMIs ranging from 25 to 30 kg/m^2, body weight and BMI were reduced by 1–2 kg and about 0.4–0.7 points. Although the degree of reduction was not very high, even mildly obese Japanese tend to have obesity-related diseases,[8] and a slight decrease in body weight has been reported to be beneficial for this group of people.[23] In addition, each 1-kg increase in body weight is reported to increase the risk of coronary heart disease (CHD) mortality by between 1 and 1.5%.[24] Therefore, intake of 15 ml of vinegar (750 mg AcOH) per d might well have clinical significance.

Yamashita *et al.* reported that suppression of body fat mass by AcOH is due to inhibition of lipogenesis, mediated by decreases in gene expression of FAS, ACC, and malic enzyme, among other factors.[15] Our previous animal experiment also showed that AcOH lowered high serum TG values by down-regulation of the genes for ATP-CL, FAS, and ACC.[16] These reactions were induced by phosphorylated AMPK. After ingestion of vinegar, the plasma acetate level reaches several hundred μmol/l in humans,[25] and phosphorylation of AMPK in hepatocytes has been found with the addition of 100–200 μmol/l of acetate.[26] In addition to inhibition

of lipogenesis, it is reported that phosphorylated AMPK induces gene expression of peroxisome proliferator-activated receptor alpha,[27] which regulates mRNA expression of fatty acid oxidation enzymes, such as acetyl-CoA oxidase[28] and carnitine palmitoyl transferase-1,[29] and thermogenic protein, uncoupling protein-2.[30] Hence the acetate in vinegar is thought to inhibit lipogenesis and possibly to stimulate fatty acid oxidation. The test beverages used in this study for the low- and high-dose groups contained 750 and 1,500 mg of AcOH respectively, and reductions in body weight and BMI were found to be dose dependent. All together, although it is necessary to conduct further research to confirm the contribution of the two mechanisms in clinical study, AcOH was considered to be the active ingredient in vinegar that effected reductions in BMI, body fat mass, and serum TG levels.

Significant decreases in VFA in both the low- and high-dose groups were observed. Reduction of VFA has a positive effect on metabolic risk factors: elevated blood pressure, dyslipidemia, and dysglycemia/impaired glucose tolerance.[31–37] The decrease of more than 5 cm^2 in VFA induced by vinegar intake in this study might lead a 0.1 decrease in the number of metabolic risk factors.[38] In addition, it has been reported that the risk of CHD dcreased by a factor of 1.34 when serum TG levels decrease by 1 mmol/l in Japanese.[39] Therefore, using calculations from that report, daily consumption of vinegar not only decreases serum TG values to the normal range (1.0–1.7 mmol/l), but might also lead to a 10% reduction in CHD risk.[39] Moreover, Yusuf *et al.* found that lowering the waist-hip ratio reduced the risk of myocardial infarction.[40] As mentioned above, vinegar consumption decreased the waist-hip ratio, suggesting a possible concomitant decrease in the occurrence of myocardial infarction.

During the test period, neither abnormality of liver and kidney functions nor adverse effects were observed. Since daily intake of 90 ml of vinegar (4500 mg AcOH) for 4 weeks in healthy subjects was reported not to cause side effects, vinegar consumption is thought to be safe.[41] Body weight, BMIs, and waist-hip ratios returned to their initial values at the end of the post-treatment period, indicating that continuous administration of vinegar is necessary in order to maintain the positive effects discussed above.

In conclusion, vinegar intake reduced body weight, visceral and subcutaneous fat mass, and serum TG levels without causing adverse effects in our obese Japanese study subjects. Intake of 15 ml of vinegar (750 mg AcOH) per d was sufficient to achieve these effects. Vinegar can perhaps be considered beneficial for preventing metabolic syndrome by reducing obesity in Japanese people. Hereafter, we intend to investigate the effects of vinegar intake on the expression of genes for fatty acid oxidation enzymes and on energy consumption.

References

1) Visscher TL and Seidell JC, *Annu. Rev. Public Health*, **22**, 355–375 (2001).
2) Flegal KM, Carroll MD, Ogden CL, and Johnson CL, *JAMA*, **288**, 1723–1727 (2002).
3) Seidell JC, *J. Endocrinol. Invest.*, **25**, 816–822 (2002).
4) Fox CS, Massaro JM, Hoffmann U, Pou KM, Maurovich-Horvat P, Liu CY, Vasan RS, Murabito JM, and Meigs JB, *Circulation*, **116**, 39–48 (2007).
5) Report of a WHO Expert Committee, *World Health Organ. Tech. Rep. Ser.*, **854**, 1–452 (1995).
6) Yanai M, Kon A, Kumasaka K, and Kawano K, *Int. J. Obes. Relat. Metab. Disord.*, **21**, 484–488 (1997).
7) Seidell JC, Verschuren WM, and Kromhout D, *Int. J. Obes. Relat. Metab. Disord.*, **19**, 924–927 (1995).
8) Examination Committee of Criteria for 'Obesity Disease' in Japan, Japan Society for the Study of Obesity, *Circ. J.*, **66**, 987–992 (2002).
9) Collaborative study of Japan Society for the Study of Obesity and Japanese Ministry of Health and Welfare, Epidemiological studies on obesity: Research report in 1996 (in Japanese) (1997).
10) Ren H, Endo H, Watanabe E, and Hayashi T, *J. Tokyo Univ. Fish.*, **84**, 1–11 (1997).
11) Kajimoto O, Tayama K, Hirata H, Takahashi T, and Tsukamoto Y, *J. Nutr. Food* (in Japanese), **4**, 47–60 (2001).
12) Kajimoto O, Ohshima Y, Tayama K, Hirata H, Nishimura A, and Tsukamoto Y, *J. Nutr. Food* (in Japanese), **6**, 51–68 (2003).
13) Fushimi T, Ohshima Y, Kishi M, Nishimura A, Kajimoto O, and Tsukamoto Y, *J. Nutr. Food* (in Japanese), **8**, 13–26 (2005).
14) Inage H, Sato Y, Sakakibara S, Sakuma M, and Kimura S, *J. Jpn. Soc. Clin. Nutr.*, **27**, 321–325 (2006).
15) Yamashita H, Fujisawa K, Ito E, Idei S, Kawaguchi N, Kimoto M, Hiemori M, and Tsuji H, *Biosci. Biotechnol. Biochem.*, **71**, 1236–1243 (2007).
16) Fushimi T, Suruga K, Oshima Y, Fukiharu M, Tsukamoto Y, and Goda T, *Br. J. Nutr.*, **95**, 916–924 (2006).
17) Perel P, Roberts I, Sena E, Wheble P, Briscoe C, Sandercock P, Macleod M, Mignini LE, Jayaram P, and Khan KS, *BMJ*, **334**, 197 (2007).
18) Ministry of Health, Labour and Welfare, The National Nutrition Survey in Japan (2005).
19) Ross R, Rissanen J, Pedwell H, Clifford J, and Shragge P, *J. Appl. Physiol.*, **81**, 2445–2455 (1996).
20) Ross R and Rissanen J, *Am. J. Clin. Nutr.*, **60**, 695–703 (1994).
21) Chowdhury B, Kvist H, Andersson B, Bjorntorp P, and Sjostrom L, *Int. J. Obes. Relat. Metab. Disord.*, **17**, 685–691 (1993).
22) Stallone DD, Stunkard AJ, Wadden TA, Foster GD, Boorstein J, and Arger P, *Int. J. Obes.*, **15**, 775–780 (1991).
23) Japan Society for the Study of Obesity, Guideline for the treatment of obesity, *J. Jpn. Soc. Study Obesity* (in Japanese), (2006).
24) Jousilahti P, Tuomilehto J, Vartiainen E, Pekkanen J, and Puska P, *Circulation*, **93**, 1372–1379 (1996).
25) Brighenti F, Castellani G, Benini L, Casiraghi MC, Leopardi E, Crovetti R, and Testolin G, *Eur. J. Clin. Nutr.*, **49**, 242–247 (1995).
26) Sakakibara S, Yamauchi T, Oshima Y, Tsukamoto Y, and Kadowaki T, *Biochem. Biophys. Res. Commun.*, **344**, 597–604 (2006).
27) Suzuki A, Okamoto S, Lee S, Saito K, Shiuchi T, and Minokoshi Y, *Mol. Cell. Biol.*, **27**, 4317–4327 (2007).
28) Tugwood JD, Issemann I, Anderson RG, Bundell KR, McPheat WL, and Green S, *EMBO J.*, **11**, 433–439 (1992).
29) Brandt JM, Djouadi F, and Kelly DP, *J. Biol. Chem.*, **273**, 23786–23792 (1998).
30) Nakatani T, Tsuboyama-Kasaoka N, Takahashi M, Miura S, and Ezaki O, *J. Biol. Chem.*, **277**, 9562–9569 (2002).
31) Eckel RH, Grundy SM, and Zimmet PZ, *Lancet*, **365**, 1415–1428 (2005).
32) Isomaa B, Almgren P, Tuomi T, Forsen B, Lahti K, Nissen M, Taskinen MR, and Groop L, *Diabetes Care*, **24**, 683–689 (2001).
33) Alberti KG, Zimmet P, and Shaw J, *Lancet*, **366**, 1059–1062 (2005).

34) Despres JP and Lemieux I, *Nature*, **444**, 881–887 (2006).
35) Lean ME, Han TS, and Seidell JC, *Lancet*, **351**, 853–856 (1998).
36) Nakamura T, Tokunaga K, Shimomura I, Nishida M, Yoshida S, Kotani K, Islam AH, Keno Y, and Kobatake T, *Atherosclerosis*, **107**, 239–246 (1994).
37) Lemieux I, Pascot A, Couillard C, Lamarche B, Tchernof A, Almeras N, Bergeron J, Gaudet D, and Tremblay G, *Circulation*, **102**, 179–184 (2000).
38) Okauchi Y, Nishizawa H, Funahashi T, Ogawa T, Noguchi M, Ryo M, Kihara S, Iwahashi H, and Yamagata K, *Diabetes Care*, **30**, 2392–2394 (2007).
39) Iso H, Naito Y, Sato S, Kitamura A, Okamura T, Sankai T, Shimamoto T, Iida M, and Komachi Y, *Am. J. Epidemiol.*, **153**, 490–499 (2001).
40) Yusuf S, Hawken S, Ounpuu S, Bautista L, Franzosi MG, Commerford P, Lang CC, Rumboldt Z, and Onen CL, *Lancet*, **366**, 1640–1649 (2005).
41) Kishi M, Fushimi T, and Tsukamoto Y, *J. Jpn. Soc. Clin. Nutr.*, **27**, 313–320 (2006).