Bioavailability of Acetate from Two Vinegar Supplements: Capsule and Drink

Shino SUGIYAMA1, Takashi FUSHIMI1,*, Mikiya KISHI1, Shin IRIE2, Shigeki TSUJI2, Natsuko HOSEKAWA3 and Takayuki KAGA1

1 Central Research Institute, Mizkan Group Corporation, Handa 475–8585, Japan
2 Nishikumamoto Hospital, Medical Co. LTA, Kumamoto 861–4157, Japan
3 Clinical Research Department, Mediscience Planning Inc., Tokyo 103–0004, Japan

(Received January 20, 2010)

Summary The bioavailability of acetate in various vinegar supplements, e.g. as capsules and drinks, remains unclear. Thus, we conducted a cross-over clinical study in 30 healthy subjects. After an overnight fast, subjects received each test sample in a randomised sequence: 9 vinegar capsules (containing 750 mg acetic acid in total) with 150 mL of water, 100 mL of vinegar drink (containing 750 mg acetic acid), and 150 mL of water as reference. Blood samples were collected before (defined as 0 min), at 15, 30, 45, 60, 90, 120 and 180 min after each test sample intake. In the vinegar drink group, serum acetate concentration increased immediately after intake, peaked at 15 min and returned to baseline at 90 min. That in the vinegar capsule group rose slowly, peaked at 30 min and returned to baseline at 120 min. The peak values in both groups exceeded 200 μmol/L, the physiologically active concentration confirmed by in vitro experiment. In the reference group, levels remained constant throughout the 180-min period. The amount of absorbed acetate from the vinegar capsule group and the drink group was evaluated by the difference value of the area under the serum acetate concentration-time curve (AUC) between in each vinegar group and in the reference group (expressed as AUCcapsule-ref and AUCdrink-ref, respectively). AUCcapsule-ref was about 80% of AUCdrink-ref, but there was no significant difference between them.

Key Words vinegar, acetate, supplements, absorption, bioavailability

Vinegar, whose main component is acetic acid, is an important seasoning in use all over the world. In the past decade, continuous intake (on a daily basis) of a drink containing 15 mL vinegar (750 mg acetic acid) was reported to improve lifestyle-related diseases such as hypertension (1), hyperlipidaemia (2) and obesity (3). Furthermore, animal studies demonstrated that acetic acid was the active ingredient responsible for these effects (4–9).

Acetic acid is absorbed after the consumption of a meal containing vinegar (10), and then circulates in the acetate form to be metabolized by the whole body (11). The effects of vinegar intake on the various lifestyle-related diseases are due to this acetate form, and the proposed mechanisms are as follows: anti-hypertension, enhancement of vasodilatation through adenosine receptor by acetate in blood vessels (12); anti-hyperlipidaemia and anti-obesity, inhibition of lipogenesis and enhancement of fatty acid oxidation via activation of AMP-activated protein kinase (AMPK) by acetate in the liver (5, 7–9). Therefore, to enjoy the benefits of vinegar, it is important that acetate is adequately absorbed by the body and that serum acetate levels rise above background levels.

There are various forms of vinegar supplements on the market, e.g. capsules and drinks. When it come to capsules, though, not all vinegar capsules contain an adequate amount of acetate. Moreover, there have been case reports of non-absorption of the active ingredient from such capsules (13, 14). Although, as discussed above, absorbed acetate plays an important role in the physiological functioning of vinegar, nevertheless, there have been no reports to date documenting the bioavailability of acetate after the intake of commercial vinegar supplements. Therefore, we considered it valuable to assess the change in serum acetate concentration over time after the intake of our products. Therefore, we report on our investigation into acetate bioavailability from both the vinegar capsule and the vinegar drink, using a cross-over, clinical study in healthy Japanese subjects.

Materials and Methods

Subjects Thirty healthy Japanese subjects (17 men and 13 women) were enrolled in this study. Their ages ranged from 23 to 59 y (40.4±2.2). None had a history of alimentary allergy or serious disease, and none were currently taking any medication. Written informed consent for participation was obtained from all subjects.

*To whom correspondence should be addressed.
E-mail: tfushimi@mizkan.co.jp
The Ethics Committees of the Mizkan Group Corporation and Nishikumamoto Hospital approved the study protocol and found it to be in accordance with the Declaration of Helsinki.

**Test samples.** There were three types of test samples: 150 mL of non-carbonated mineral water (Suntoy Natural Water, Suntoy Foods Co., Ltd., Tokyo, Japan), which acted as the reference group [Sample A]; a bottle (100 mL) of the vinegar drink, approved as Food for Specified Health Uses (MAINZ, Mizkan Co., Ltd., Aichi, Japan) [Sample B]; or 9 vinegar capsules (MICARA, Mizkan Group Corporation, Aichi, Japan) with 150 mL of the reference non-carbonated mineral water [Sample C]. One hundred milliliters of the vinegar drink and 9 vinegar capsules contained 750 mg acetate [the other nutritional composition is as follows: energy (kJ) 66.9, 125.5, carbohydrates (g) 3.9, 1.02, fat (g) 0, 2.28, protein (g) 0, 1.56 and calcium (mg) 65, 300 in the drink or the 9 capsules, respectively].

**Study design.** This was a single-blind, randomized cross-over study, performed between July and September 2007. The investigators were blinded to the treatments. To examine acetate bioavailability twice (the first and the second set) per test sample, six test periods were included in this study. Therefore, the 30 subjects were randomly assigned to one of six treatment groups: 1) A-B-C×2, 2) B-C-A×2, 3) C-A-B×2, 4) A-C-B×2, 5) C-B-A×2, and 6) B-A-C×2. The washout interval between the first and the second test periods was 6–13 d. On the night preceding the test period, the subjects ate a “study supper,” which they had to consume before 21:00 h. Following their supper, the subjects fasted until after the 180-min blood sample had been taken the following day. In addition, they were instructed not to consume any alcohol on the day preceding the test period. The subjects were instructed to refrain from consuming foods that might influence the evaluation of serum acetate levels.

Following their supper, the subjects fasted until after the 180-min blood sample had been taken the following day. In addition, they were instructed not to consume any alcohol on the day preceding the test period. The “study supper” consisted of 130 g of rice (Sato-nogohann Karukichizen, Sato Foods Co., Ltd., Niigata, Japan) and 270 g of a ready-prepared meal (Kikubari-gozen Gomokudofu-no-ankake set, Nichirei Foods Japan) and 270 g of a ready-prepared meal (Kikubari-gozen Gomokudofu-no-ankake set, Nichirei Foods Japan) and 270 g of a ready-prepared meal (Kikubari-gozen Gomokudofu-no-ankake set, Nichirei Foods Japan). All reagents for the measurement were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

**Statistical analysis.** All data except nutrient composition are shown as mean±SE. The transitions of serum acetate concentrations in the first set of each treatment were the same as those in the second set. Therefore, the averaged value of the two sets was analyzed. The area under the serum acetate concentration-time curve [AUC (μmol×min/L)] was calculated by the linear trapezoidal rule. The amount of absorbed acetate from the vinegar capsule group and the drink group was evaluated by the difference value of AUC between in each vinegar group and in the reference group (expressed as AUCcapsule-ref and AUCdrink-ref, respectively).

Serum acetate levels in the reference group remained constant throughout the 180-min period. Thus, repeated measures multivariate ANOVA was used to examine the effect of treatment over time between the vinegar capsule and the vinegar drink group (SPSS for Windows version 11.5; SPSS, Inc., Chicago, IL). For inter-group comparisons at each time point, intra-group comparisons in each treatment group and comparisons in AUC among the three groups, one-way repeated measures ANOVA, followed by the Bonferroni test, was used. The difference in the peak value and the amount of absorbed acetate between the vinegar capsule and the vinegar drink groups was analyzed by a paired t-test. Differences were considered significant when p<0.05.

**Results**

**Time course of serum acetate concentrations**

Serum acetate concentration in the vinegar drink group increased immediately after intake (Fig. 1), while that in the vinegar capsule group rose gradually after intake. The peaks were found at 15 and 30 min, respectively. The peak in the vinegar drink group was significantly higher than in the vinegar capsule group (349±21 vs. 216±14, p<0.001). There was a significant interaction (time×treatment) between the vinegar capsule and the vinegar drink group (p<0.001). The serum acetate levels in the reference group remained stable throughout the 180-min measurement period. Both the vinegar drink group and the vinegar capsule group had higher serum acetate levels than the reference group at 15–60 min, and at 30–90 and 180 min, respectively. The levels in the vinegar drink group were significantly higher than those in the vinegar capsule group at 15 min (349±21 vs. 142±7, p<0.001) and 30 min (265±12 vs. 216±14, p=0.013), but were significantly lower at 90 min (131±5 vs. 157±6, p=0.008). Both the vinegar drink group and the vinegar capsule group had higher serum acetate levels than the reference group at 15–45 min, and at 30–90 min, respectively.

Serum acetate concentrations in the vinegar drink group and in the vinegar capsule group returned to
Pharmacopoeia XIV (artificial gastric juice) using the present study was covered with soft gelatin. In preliminary of the vinegar drink. The vinegar capsule used in the concentration would increase immediately after intake. Thus, it was not unexpected that the serum acetate was absorbed by intake of the vinegar supplement and that the amount of absorbed acetate from the vinegar capsule was about 80% of that from the vinegar drink. Thus, the vinegar supplements might be expected to be beneficial for control of blood pressure and lipid metabolism via production of these two metabolites. Also it was found that serum acetate concentration rose above baseline after intake of both the vinegar drink and the capsules, and that the peak values in both groups were >200 μmol/L. Previous studies (5, 9) have reported that 200 μmol/L of acetate reduced mRNA expression of sterol-regulatory element binding protein-1, a fatty acid synthesis enhancer, and increased mRNA expression of peroxisome proliferator-activated receptor α and uncoupling protein 2, mediators of fatty acid oxidation and thermogenesis, respectively, through activation of AMPK in hepatocytes. These actions result in a reduction of serum lipid levels and body fat mass. Taken together, these results indicate that the vinegar capsule as well as the vinegar drink might be beneficial for lifestyle-related diseases such as hypertension, hyperlipidaemia, and obesity via the same mechanism.

In conclusion, the vinegar drink and the capsules containing 750 mg acetic acid led to an acute and moderate rise of serum acetate concentration, respectively. The peak of serum acetate levels in both vinegar supplement groups exceeded the physiologically active concentration. The amount of absorbed acetate was not so different between these groups. Therefore, continuous intake of the vinegar capsule is thought to be as useful as the vinegar drink for achieving the health benefits of vinegar for lifestyle-related diseases. It is important to confirm the effect of the vinegar capsules by further clinical studies.

**REFERENCES**

1) Kajimoto O, Tayama K, Hirata H, Takahashi T, Tsukamoto Y. 2001. Effect of a drink containing vinegar on blood pressure in mildly and moderately hypertensive subjects. *Kenko Eiyo Shokuhin Kenkyu (J Nutr Food)* 4(4): 47–60 (in Japanese with English summary).

2) Fushimi T, Ohshima Y, Kishi M, Nishimura A, Kajimoto O, Tsukamoto Y. 2005. Effects of a drink containing vinegar on serum total cholesterol and assessment of its safety. *Kenko Eiyo Shokuhin Kenkyu (J Nutr Food)* 8(1): 13–26 (in Japanese with English summary).

3) Kondo T, Kishi M, Fushimi T, Ugaishii S, Kaga T. 2009. Vinegar intake reduces body weight, body fat mass, and serum triglyceride levels in obese Japanese subjects. *Bio Sci Biotechnol Biochem* 73: 1837–1843.

4) Kondo S, Tayama K, Tsukamoto Y, Ikeda K, Yamori Y.
Acetate Absorption from Vinegar Supplements

2001. Antihypertensive effects of acetic acid and vinegar on spontaneously hypertensive rats. *Biosci Biotechnol Biochem* 65: 2690–2694.

5) Sakakibara S, Yamauchi T, Oshima Y, Tsukamoto Y, Kadowaki T. 2006. Acetic acid activates hepatic AMPK and reduces hyperglycemia in diabetic KK-A(y) mice. *Biochem Biophys Res Commun* 344: 597–604.

6) Tanizawa H, Suzuki Y, Komatsu (Serita) A, Takino Y. 1983. Acute toxicity of Komezu and its effects on lipid metabolism in male mice. *Nippon Eiyo Shokuryo Gakkai-shi* (J Jpn Soc Nutr Food Sci) 36: 283–289 (in Japanese with English summary).

7) Fushimi T, Suruga K, Oshima Y, Fukiharu M, Tsukamoto Y, Goda T. 2006. Dietary acetic acid reduces serum cholesterol and triacylglycerols in rats fed a cholesterol-rich diet. *Br J Nutr* 95: 916–924.

8) Yamashita H, Fujisawa K, Ito E, Idei S, Kawaguchi N, Kimoto M, Hiemori M, Tsuji H. 2007. Improvement of obesity and glucose tolerance by acetate in Type 2 diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats. *Biosci Biotechnol Biochem* 71: 1236–1243.

9) Kondo T, Kishi M, Fushimi T, Kaga T. 2009. Acetic acid increases gene expression of fatty acid oxidation enzymes in liver and suppresses body fat accumulation. *J Agric Food Chem* 57: 5982–5986.

10) Brighenti F, Castellani G, Benini L, Casiraghi MC, Leopardi E, Crovetti R, Testolin G. 1995. Effect of neutralized and native vinegar on blood glucose and acetate responses to a mixed meal in healthy subjects. *Eur J Clin Nutr* 49: 242–247.

11) Cummings JH, Pomare EW, Branch WJ, Naylor CP, Macfarlane GT. 1987. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut* 28: 1221–1227.

12) Carmichael FJ, Saldivia V, Varghese GA, Israel Y, Orrego H. 1988. Ethanol-induced increase in portal blood flow: role of acetate and A1- and A2-adenosine receptors. *Am J Physiol* 255: G417–G423.

13) Miles MV, Horn P, Miles L, Tanga P, Steelec P, DetGrauwa T. 2002. Bioequivalence of coenzyme Q10 from over-the-counter supplements. *Nutr Res* 22: 919–929.

14) Leonard SW, Good CK, Gugger ET, Traber MG. 2004. Vitamin E bioavailability from fortified breakfast cereal is greater than that from encapsulated supplements. *Am J Clin Nutr* 79: 86–92.

15) Brighenti F. 1997. Simple method for quantitative analysis of short chain fatty acids in serum by gas-liquid chromatography. In: Plant Polysaccharides in Human Nutrition: Structure, Function, Digestive Fate and Metabolic Affects (Guillon F, Abraham G, Amado R, eds), p 114–119. INRA, Nantes.

16) Schmitt MG Jr, Soergel KH, Wood CM. 1976. Absorption of short chain fatty acids from the human jejunum. *Gastroenterology* 70: 211–215.

17) Saunders D. 1991. Absorption of short chain fatty acids in human stomach and rectum. *Nutr Res* 11: 841–847.

18) Argenzio RA, Southworth M. 1975. Sites of organic acid production and absorption in gastrointestinal tract of the pig. *Am J Physiol* 228: 454–460.