Note

Bioavailability of Acetate from Two Vinegar Supplements: Capsule and Drink

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(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 $\mu\text{mol/L}$, 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 $\text{AUC}_{\text{capsule-ref}}$ and $\text{AUC}_{\text{drink-ref}}$, respectively). $\text{AUC}_{\text{capsule-ref}}$ was about 80% of $\text{AUC}_{\text{drink-ref}}$, 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.

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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 (Suntory Natural Water, Suntory 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 sequences: 1) A-B-C $\times$ 2, 2) B-C-A $\times$ 2, 3) C-A-B $\times$ 2, 4) A-C-B $\times$ 2, 5) C-B-A $\times$ 2, and 6) B-A-C $\times$ 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 “study supper” consisted of 130 g of rice (Sato-nogohann Karukuichizen, Sato Foods Co., Ltd., Niigata, Japan) and 270 g of a ready-prepared meal (Kikubari-rozen Gomokudofu-no-ankake set, Nichirei Foods Direct, Inc., Tokyo, Japan). The total nutritional content of the supper was as follows: energy (kJ) 2,160, carbohydrates (g) 76.5, fat (g) 15.0 and protein (g) 18.7. On the morning of the test period, subjects were to arrive at Nishikumamoto Hospital by 08:00 h. After the subject had rested for 30 min on a bed, a catheter was inserted in one arm, and the relevant test sample was taken within 3 min. Blood samples (4 mL) were immediately drawn from the antecubital vein into a glass vacuum tube before (defined as 0 min), at 15, 30, 45, 60, 90, 120 and 180 min after the test sample intake. Blood samples were stored at room temperature for 30 min, and then the tubes were centrifuged at 3,000 rpm for 10 min. The serum was immediately transferred to a screw-capped tube, and stored at -20°C until analysis. Subjects remained at rest on their bed in a quiet and air-conditioned room (a constant temperature of $26\text{--}27^{\circ}\text{C}$) until after the 180-min blood sample had been collected. Water was freely available except 30 min before and after the test sample intake.

Measurement of serum acetate levels. Serum acetate

levels ($\mu\text{mol/L}$) were measured by gas chromatography with isovaleric acid as an internal standard (15). The chromatograph was a GC-2010 (Simadzu, Kyoto, 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 $\pm$ 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 ($\mu\text{mol}\times\text{min/L}$)] 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 $\text{AUC}_{\text{capsule-ref}}$ and $\text{AUC}_{\text{drink-ref}}$, 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 11.5J; 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 $\times$ 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 time 0, 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

Fig. 1. Time-course changes of serum acetate concentration in the vinegar drink group, the vinegar capsule group and the reference group. Individual values are the means of values for each test sample repeated twice. Data points are means with their standard errors shown by vertical bars. There was a significant interaction (time $\times$ treatment) between the vinegar capsule and the vinegar drink group ($p<0.001$). ‡Mean value in the vinegar capsule or the vinegar drink group was significantly higher than that in the reference group ($p<0.05$). †Mean value in the vinegar capsule group was significantly higher than that in the vinegar drink group ($p<0.05$). §Mean value in the vinegar drink group was significantly higher than that in the vinegar capsule group ($p<0.05$). *Mean value at each time was significantly higher than that at time 0 ($p<0.05$). There was no significant difference in the reference group.

their initial value at 90 and 120 min, respectively.

Amount of absorbed acetate

$AUC_{\text{capsule-ref}}$ and $AUC_{\text{drink-ref}}$, as indexes of the amount of absorbed acetate, were $5,763\pm1,202$ and $7,477\pm1,067$, respectively. There was no significant difference between them.

Discussion

The present study is the first to demonstrate that acetate is absorbed after the intake of a vinegar supplement. The significant interaction indicated that the time course of the change of serum acetate levels differed between the capsules and the drink. The acetate absorption began immediately or gradually, after intake of the vinegar drink or capsule, respectively. Although the acetate absorption from the capsules was slower than seen with the vinegar drink, it continued for longer, and consequently $AUC_{\text{capsule-ref}}$ was about 80% of $AUC_{\text{drink-ref}}$.

Acetate is considered to be absorbed in the upper digestive tract, especially the stomach and jejunum (16–18), and to be metabolised by the whole body (11). Thus, it was not unexpected that the serum acetate concentration would increase immediately after intake of the vinegar drink. The vinegar capsule used in the present study was covered with soft gelatin. In preliminary examinations, these capsules were found to collapse within 20 min in the first fluid of the Japanese Pharmacopoeia XIV (artificial gastric juice) using the

disintegration test. It has been reported that no difference was observed between the gastric emptying time after intake of acetic acid and acetate. Thus, the start of acetate absorption from the vinegar capsule was delayed relative to the drink, not because of gastric emptying, but because of the protective gelatin coating.

Adenosine and AMP, which are produced when absorbed acetate is metabolised, induce vasodilatation (12) and activation of AMPK (8), respectively. The present study showed that 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 $>200\text{ }\mu\text{mol/L}$. Previous studies (5, 9) have reported that $200\text{ }\mu\text{mol/L}$ 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 high 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.

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