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Strawberry, Garlic and Kale Consumption Increase Urinary Excretion of Dimethylamine and Trimethylamine in Humans

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Abstract

Dimethylamine (DMA) is the immediate precursor of carcinogenic N-nitrosodimethylamine (NDMA). *In vitro* and *in vivo* experiments using whole strawberries, and garlic and kale juices were conducted to determine concentrations of DMA and trimethylamine (TMA) in foods and urine. Experimental diets [an amine-rich diet as nitrosatable precursors in combination with added nitrate-containing drinking water without (TD1) or with whole strawberries or garlic or kale juices (TD2, TD3 and TD4, respectively), or a diet of low in nitrate and amine (TD5) were incubated in simulated saliva and gastric juices at 37°C for 1 hour. We also studied the urinary excretion of DMA and TMA after consumption of the experimental diets (TD1~TD5). Urine samples were obtained for 18 hrs after consumption of experimental diets and concentrations of DMA and TMA were measured in the digested diet and urine. The DMA concentration after incubation in experimental diets (TD1~TD5) was 4.7±0.3, 6.7±0.2, 7.9±0.2, 7.1±0.2 and 0.3±0.1 mg/kg, respectively. Urinary excretion of DMA (TD1~TD5) was 22.0±5.0, 28.3±4.3, 29.2±4.1, 27.4±4.5 and 20.4±3.1 mg/18 hr, respectively. Consumption diets with added strawberries or juices of kale or garlic increased urinary TMA and DMA, suggesting that those precursors were excreted and not converted to the carcinogen, NMDA.

Key words: dimethylamine, trimethylamine

INTRODUCTION

Eating foods rich in nitrates and amines increases the risk of endogenous formation of carcinogenic N-nitroso compounds (NOCs) (1,2). Dimethylamine (DMA) is the immediate precursor of carcinogenic N-nitrosodimethylamine (NDMA). DMA occurs naturally within the tissues of fish and sea creatures such as squid and octopus (3,4). Trimethylamine oxide and trimethylamine (TMA) are major dietary sources of DMA. Owing to quantitative recoveries following oral administration, urinary DMA levels provide a good overall indication of body exposure (5-8).

Nitrite can react with secondary amines under the acidic conditions of the stomach to form N-nitrosamine (NA). Ascorbic acid and ascorbate inhibit nitrosation because they react faster than the amine with the nitrosating agents (9,10). Strawberries contains the highest amount of vitamin C (99 mg/100 g) of commonly eaten fruits (11).

Phenolic compounds, which are found many plant derived human foods and beverages, are potent blocking agents of NOCs formation (12-15). Kale juice has been demonstrated to have anti-mutagenic activity with color reactions characteristic of protein, carbohydrate, and phenolic compounds (16).

Shenoy and Choughley (17) reported that onion and garlic juices are effective in reducing the chemical formation of NAs and suggested that allyl sulfur compounds in garlic juice might act as nitrite scavengers.

The objective of this study was to evaluate the effect of strawberry, garlic and kale on the urinary excretion of DMA and TMA in human volunteers consuming an amine-rich and nitrate-containing diet. Urine was collected to determine excretion levels of DMA and TMA.

MATERIALS AND METHODS

Sample preparation

Strawberry (*Fragaria ananassa*), garlic (*Allium sativum L.*), and kale (*Brassica oleracea* var. *acephala*) were obtained from a local supermarket and washed with tap water to eliminate external pesticides, soil, and other contaminants. Garlic and kale juices were used in the experimental diets (TD3 and TD4).

Preparation of digested diet

Simulated saliva was composed of calcium (3.1

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mEq/L), chloride (15.5 mEq/L), inorganic phosphate, (4.8 mEq/L), potassium (14.1 mEq/L), sodium (17.4 mEq/L), ammonia (3.5 mM), glucose (196.0 mg/L), urea (88.0 mg/L), α-amylase (100.0 units/mL) and lysozyme (670.0 units/L) and adjusted to pH 6.7. Simulated gastric juice was composed of calcium (3.6 mEq/L), potassium (11.6 mEq/L), sodium (49.0 mEq/L), free HCl (57.5 mEq/L), total chloride (119.0 mEq/L), pepsin (36.4 units/mL) and adjusted to pH 2.0.

For the meals (TD1~TD5) listed in Table 1, about 20 g of food samples were minced into small pieces and accurately weighed and homogenized in a mixing blender with 10 mL of simulated saliva. The mixture was then incubated at 37°C for 5 minute before adding 40 mL of simulated gastric juice. The volumetric ratio of saliva to gastric juice was 1 : 4 (10 mL and 40 mL, respectively), in conformity with the approximately five-fold dilution of saliva in the stomach as estimated by Boyland and Walker (18). The mixture was adjusted to pH 2.5 with 3 N HCl and then incubated at 37°C for one hour while gently shaking. The sample was referred to as "digested diet".

Study population and protocol

Fifty healthy volunteers (10 in each group; thirty-two males and eighteen females) were selected from Gyeongsang National University students and supervised by the Center for Food and Nutrition. Study participants agreed to collect urine specimens and answer a questionnaire on food consumption and lifestyle habits. All volunteers were nonsmokers and used no medicines or vitamin preparations. The mean body weight of the volunteers [±standard deviation (SD)] was 56.0±5.0 kg and the mean age was 25.0±3.0 years. The study lasted 4 days; the first to third days were control days (samples of control diet groups are labeled CD in this study) and the last day was the experimental day. During the control days the subjects refrained from high NDMA, nitrate, amine, sulfur compound, ascorbic acid and phenolic compound-containing foods. The composition of the meals are shown in Table 1 (designated TD1, TD2, TD3, TD4 and TD5; experiment day 1, 2, 3, 4 and 5 diets).The different amount of cooked rice between male and female subjects were calculated by referring to the Food Composition Table in Korea (11). The amount of food materials was calculated from typical daily intakes of carbohydrate, protein and fat for Koreans (11). For the evening meal on the fourth day, the experimental the diet group 1 (TD1) participants received a 400 mg dose of nitrate ten minutes prior to an amine rich, low nitrate meal (dried squid) as a source of nitrosatable precursors. Experimental diet groups 2, 3 and 4 (TD2, TD3 and TD4) subjects received the same meal as diet group 1 with the addition of whole strawberries (300 g), garlic juice (200 g; 75 g garlic juice in drinking water), or kale juice (200 g), respectively, at the end of the meal as a substitute for drinking water. Experimental diet group 5 (TD5) subjects received a low amine and nitrate diet. The subjects themselves provided the breakfast and lunch. The following morning breakfast was provided at 8 AM. It was consumed within about 15 minutes, and consisted of three slices (100 g) of whole-wheat toast and 200 mL of whole milk. The urine samples were collected for 18 hrs after experimental diets (TD1, TD2, TD3, TD4 and TD5) were consumed.

Table 1. Composition of TD1, TD2, TD3, TD4 and TD5 diets (g)

Food materials	TD1		TD2		TD3		TD4		TD5	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Cooked rice	250	200	250	200	250	200	250	200	250	200
Egg soup	200	200	200	200	200	200	200	200	-	-
Brown-seaweed soup	-	-	-	-	-	-	-	-	200	200
Braised squid (amine precursor)	50	50	50	50	50	50	50	50	-	-
Pan-fried rolled egg	-	-	-	-	-	-	-	-	80	80
Fried lotus root	48	48	48	48	48	48	48	48	-	-
Braised lotus root	-	-	-	-	-	-	-	-	48	48
Macaroni salad	65	65	65	65	65	65	65	65	65	65
Distilled water ($NaNO_3$ 548 mg)[1)]	100	100	100	100	100	100	100	100	-	-
Drinking water	200	200	-	-	-	-	-	-	200	200
Whole strawberries	-	-	300	300	-	-	-	-	-	-
Garlic juice (75 g garlic juice in drinking water)	-	-	-	-	200	200	-	-	-	-
Kale juice	-	-	-	-	-	-	200	200	-	-

[1)]$NaNO_3$ 548 mg corresponding to 400 mg nitrate dissolved in 100 mL of distilled water. Designated TD1, TD2, TD3, TD4 and TD5: experiment day 1~4 and 5 diets. TD1, TD2, TD3 and TD4: amine rich diet+nitrate (400 mg/day)+drinking water (200 mL), whole strawberries (300 g), garlic juice (75 g garlic juice in drinking water; 200 mL) and kale juice (200 mL), respectively. TD5: low amine and nitrate diet.

Analysis of dimethylamine (DMA) and trimethylamine (TMA)

10 g of the digested diets and urine samples were put in a 50 mL flask, 25 mL of isopropanol was added, mixed, and allowed to stand at room temperature for 10 minute and then filtered through a filter paper (Whatman No. 2). The extracted sample was injected into a gas chromatograph (GC, a Hewlett Packard 5890 series II) with a glass column (length 3 m, inside diameter 2 mm) and stationary phase Chromosorb 103 (60~80 mesh). The injector temperature was 180°C and FDI 250°C. The initial column temperature was held at 50°C for 5 minutes and programmed to increase by 5°C/min to 130°C and then remain constant for 5 minutes. Nitrogen was used as the carrier gas (40 mL/min).

Statistical methods

Results are expressed as mean±SD. The statistical analysis was performed by analysis of variance with Duncan's multiple range test, one-way analysis of variance (ANOVA) and Person's correlation coefficient (r). A p-value <0.05 was considered significant.

RESULTS AND DISCUSSION

Effect of whole strawberries, garlic and kale juices on concentrations of DMA and TMA following simulated gastric digestion

In vitro and *in vivo* experiments evaluated the capacity of whole strawberries, garlic and kale juices formation and preservation of DMA and TMA. The concentrations of DMA in TD1, TD2, TD3, TD4 and TD5 diets after 1 h simulated digestion were 4.7±0.3, 6.7±0.2, 7.9±0.2, 7.1±0.2 and 0.3±0.1 mg/kg, respectively (Table 2). We observed that the addition of whole strawberries, and garlic and kale juices to the meals under simulated gastric condition increased *in vitro* DMA concentrations by 42.6, 68.1 and 51.1%, respectively, compared with TD1 (Fig. 1). In the study of Tanaka et al., the catechins in green tea extracts were shown to strongly inhibit the N- nitrosation of DMA (19).

Effect of whole strawberries, and garlic and kale juices on urinary excretion of DMA and TMA in humans

We studied the urinary excretion of DMA and TMA after taking nitrate (400 mg/day) in combination with an amine-rich diet and drinking water (200 mL, TD1), either alone or with whole strawberries (300 g, TD2), garlic juice (200 mL; 75 g garlic juice in drinking water, TD3) or kale juice (200 mL; TD4). TD5 was a low amine and nitrate diet (Fig. 2). The consumption of TD1~TD4 diets increased urinary TMA and DMA excretion compared with

Table 2. The concentrations of DMA and TMA in experimental diets following simulated gastric digestion

Diets groups	DMA (mg/kg)[1)]	TMA (mg/100 g)[1)]
CD (Control)	9.2±0.4	3.9±0.2
TD1	4.7±0.3	5.3±0.1
TD2	6.7±0.2	4.3±0.2
TD3	7.9±0.2	4.4±0.1
TD4	7.1±0.2	4.1±0.2
TD5	0.3±0.1	0.3±0.1

[1)]Results are averages of three experiments, mean±SD (standard deviation). Reaction condition were as follows: TD1: amine-rich diet (Table 1)+nitrate (400 mg/day)+drinking water (200 mL)+simulated saliva (200 mL) and gastric juice (800 mL), TD2: amine-rich diet (Table 1)+whole strawberries (300 g)+simulated saliva (200 mL) and gastric juice (800 mL), TD3: amine-rich diet (Table 1)+nitrate (400 mg/day)+garlic juice (200 g; 75 g garlic juice in drinking water)+simulated saliva (200 mL) and gastric juice (800 mL), TD4: amine-rich diet (Table 1)+nitrate (400 mg/day)+kale juice (200 mL)+simulated saliva (200 mL) and gastric juice (800 mL), Control: amine-rich diet+drinking water (200 mL)+simulated saliva (200 mL) and gastric juice (800 mL). The incubation was performed at 37°C for 1 hr.

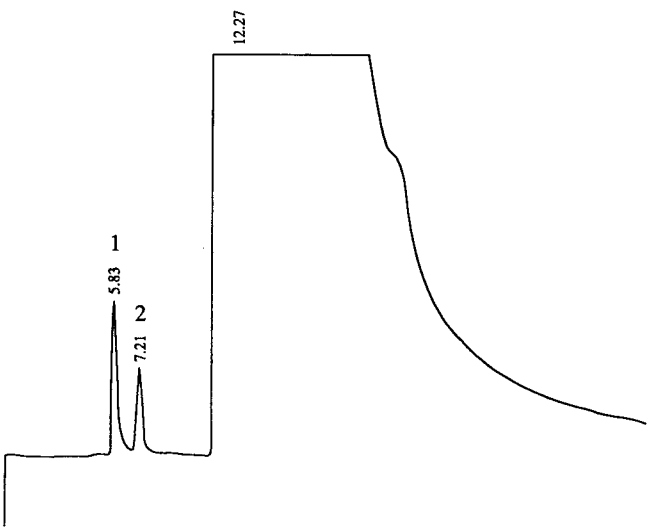

Fig. 1. GC-chromatograms of urinary dimethylamine (1) and trimethylamine (2).

the consumption of the TD5 diet. TD2, TD3 and TD4 diets also increased urinary DMA and TMA excretion above that of the TD1 diet. Whole strawberries, garlic and kale juices significantly increased the excretion of urinary DMA and TMA. The urinary excretion of DMA after consumption of the experimental diets (TD1~TD5) was 22.0±5.0, 28.3±4.3, 29.2±4.1, 27.4±4.5 and 20.4±3.1 mg/18 hr, respectively and the urinary excretion of TMA after consumption of experimental diets (TD1~TD5) was 111.1±36.0, 207.0±65.2, 226.2±77.0, 173.1±49.1 and 66.3±14.0 mg/18 hr, respectively.

Whole strawberries, and garlic and kale juices in TD2, TD3, and TD4 diets enhanced the urinary excretion of

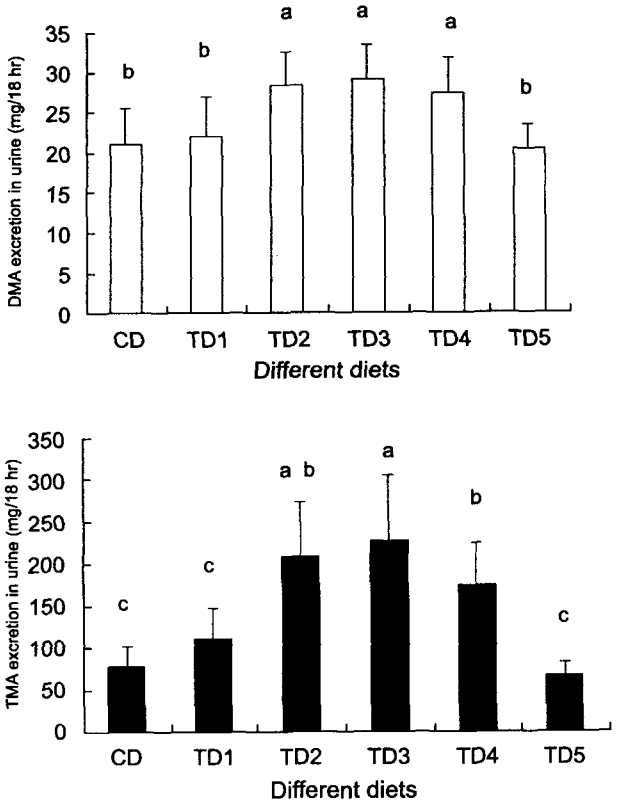

Fig. 2. Dimethylamine and trimethylamine excretion in urine (mg/18 hr) after consumption of CD, TD1, TD2, TD3, TD4 and TD5 diet[1)].

[1)]Values are mean±SD (n=10). Bars with different letters (a, b, c) are significantly different from each other at $p<0.05$ as determined by Duncan's multiple range test. CD: normal diet in the control days. TD1, TD2, TD3 and TD4: amine rich diet (Table 1)+nitrate (400 mg/day)+drinking water (TD1, 200 mL)+whole strawberries (TD2, 300 g), garlic juice (TD3, 200 mL; 75 mL garlic juice in drinking water) and kale juice (TD4, 200 mL), respectively. TD5: without amine and nitrate source in meal.

DMA and TMA compared with the TD1 diet. The effects may be the result of a rapid reaction between nitrite and inhibitors in whole strawberries, garlic and kale juices. Korean green tea and Maesil (*Prunus mume* SIEB. *et* ZACC.) extracts added to an amine-rich diet with added nitrate enhanced the excretion of urinary DMA and TMA while suppressing the excretion of urinary NDMA (20).

Recent studies (10,13,14,20,21) suggest that a low incidence of cancer is associated with the consumption of fresh fruits and vegetables rich in phenolic compounds, fiber, chlorophyll, β-carotene, and antioxidant vitamins such as C and E, which have antimutagenic and/or anticarcinogenic properties.

In our study, the consumption of whole strawberries, garlic juice and kale juice were positively correlated with the urinary excretion DMA and TMA ($p<0.05$). These results suggest that the consumption of whole strawberries, garlic juice, and kale juice may reduce endogenous NDMA formation.

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