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Effects of the dietary supplements, activated charcoal and copper chlorophyllin, on urinary excretion of trimethylamine in Japanese trimethylaminuria patients

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Abstract

Trimethylaminuria (TMAU) is a metabolic disorder characterized by the inability to oxidize and convert dietary-derived trimethylamine (TMA) to trimethylamine *N*-oxide (TMAO). This disorder has been relatively well-documented in European and North American populations, but no reports have appeared regarding patients in Japan. We identified seven Japanese individuals that showed a low metabolic capacity to convert TMA to its odorless metabolite, TMAO. The metabolic capacity, as defined by the concentration of TMAO excreted in the urine divided by TMA concentration plus TMAO concentration, in these seven individuals ranged from 70 to 90%. In contrast, there were no healthy controls examined with less than 95% of the metabolic capacity to convert TMA to TMAO. The intake of dietary charcoal (total 1.5 g charcoal per day for 10 days) reduced the urinary free TMA concentration and increased the concentration of TMAO to normal values during charcoal administration. Copper chlorophyllin (total 180 mg per day for 3 weeks) was also effective at reducing free urinary TMA concentration and increasing TMAO to those of concentrations present in normal individuals. In the TMAU subjects examined, the effects of copper chlorophyllin appeared to last longer (i.e., several weeks) than those observed for activated charcoal. The results suggest that the daily intake

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of charcoal and/or copper chlorophyllin may be of significant use in improving the quality of life of individuals suffering from TMAU.

Keywords: Trimethylamine; Trimethylamine *N*-oxide; Fish-like odor syndrome; Trimethylaminuria; Charcoal; Chopper chlorophyllin; Flavin-containing monooxygenease; Japanese

Introduction

Trimethylaminuria (TMAU), or fish-like odor syndrome, is a genetic disease characterized by excretion of trimethylamine (TMA) (Al Waiz et al., 1987; Ayesh et al., 1993; Cashman et al., 2003). The odor of TMA is recognizable as "fishy", however at low concentrations it may only be recognized as foul or "garbage-like." In normal humans, malodorous TMA is metabolized to trimethylamine *N*-oxide (TMAO). TMAO is more polar than TMA, is non-odorous, and readily excreted in the urine (Cashman and Zhang, 2002). Patients suffering from TMAU have a reduced capacity to oxidize free TMA to TMAO. The inability to efficiently oxidize TMA may lead some individuals to emanate a body odor, that in the extreme form, may be unpleasant and fish-like. This offensive odor is caused by excess, unoxidized TMA present in the systemic circulation that makes its way into urine, sweat, and breath. Consequently, some affected individuals may express a foul or fishy malodor that leads to social problems.

TMAU is an autosomal, recessive inborn error of metabolism. The severe forms are caused by mutations in the flavin-containing monooxygenase (*FMO3*) gene. Severely deleterious FMO3 mutations are quite uncommon and non-randomly distributed in the population (Treacy et al., 1998; Zschocke et al., 1999). TMAU-affected individuals have been relatively well-documented in British, Australian, and American populations (Akerman et al., 1999; Mitchell and Smith, 2001). In addition, TMAU has been reported in Thailand (Thithapandha, 1997; Kubota et al., 2003) and Hong Kong (Lee et al., 2000), however, to date, no Japanese affected by TMAU have been reported in the literature (Mitchell and Smith, 2001). More common inter-individual FMO3 variation arises from the milder forms caused by a "spectrum" of single nucleotide polymorphic changes in the *FMO3* gene (Cashman et al., 2003; Lattard et al., 2003).

For normal individuals, the metabolic capacity of the FMO3 enzyme converts over 95% of TMA to TMAO (Cashman and Zhang, 2002; Cashman, 2002). Diagnosis has been performed by determining urinary TMA and TMAO concentrations. Individuals showing FMO3 metabolic capacity lower than 90% conversion of TMA to TMAO are considered to be suffering from TMAU (Zhang et al., 1995; Zschocke et al., 1999).

Considering the mechanism by which TMA is formed in the large intestines, it seemed possible to reduce the amount of TMA available to the liver by dietary supplements. Because the production of TMA from food constituents such as phosphatidyl choline or TMAO is mediated by an enzyme(s) present in gut flora, one method is to reduce intestinal bacteria by administration of bactericidal agents (Treacy et al., 1995; Zhang et al., 1999). The other is to sequester TMA once it is produced in the gut. We hypothesized that over-the-counter dietary supplements such as activated charcoal or copper chlorophyllin would diminish free amine compounds like TMA (Dashwood et al., 1996). Charcoal has a high surface area and is commonly used by municipal water utilities to adsorb small, odorous molecules (Suffet and Wable, 1995). In addition, copper chlorophyllin has been shown to strongly bind

amine-containing compounds in the gut (Dashwood et al., 1996). These treatments should leave only small amounts of TMA un-sequestered and lessen the burden on the FMO3 enzyme thereby reducing free TMA-related odor symptoms.

We report herein that activated charcoal and copper chlorophyllin when added to the diet of Japanese suffering from TMAU improved their apparent metabolic capacity to convert TMA to TMAO by decreasing the concentration of free TMA excreted in the urine.

Materials and methods

Volunteers and treatment

The ethics committee of Hokkaido University approved this study. Informed consent was obtained from every subject. We asked for volunteers via an Internet article describing the screening of urinary TMA and 19 males and 8 females ranging from 19 to 52 years of age responded to the request. Twenty individuals (22–56 years old; 28 ± 8 yrs: mean ± SD) constituted healthy controls.

Most of the TMAU-positive subjects in the Japanese cohort reported that they started to suffer from apparent TMAU-symptoms in their teens. The age of onset for TMAU symptoms can vary considerably (Cashman et al., 2003); however, many of the severe cases reported in the literature have childhood onset. Subject 1 (male, 28 years old) and Subject 2 (female, 19 years old) were given 3 tablets (250 mg × 3) containing active charcoal (Super Carbon Diet 20, Noguchi Medical Institute, Tokyo, Japan) twice a day according to the manufacturer's description for 10 days. Subject 1, Subject 3 (male, 29 years old), and Subjects 4 (male, 26 years old) took commercial copper chlorophyllin tablets (4 tablets; Saclophyl®, Eisai, Tokyo, Japan) after each meal three times per day (for a total of 180 mg copper chlorophyllin administered per day) for three weeks according to the maximum approved dose as an over-the-counter medication. This is also below the allowable maximum amount (300 mg/day) of copper chlorophyllin approved for use by the Food and Drug Administration (of the U.S.A.) for incontinent individuals (Federal Register, 1990).

Analysis of urine samples

After an overnight fast, the study participants collected their first urine samples which were acidified with 1 M HCl to pH ~ 2–3 for this study. Urinary TMA and TMAO concentrations were determined by gas chromatography using a flame thermoionic detector as described previously (Tjoa and Fennessey, 1991; Kubota et al., 2003). Briefly, TMA concentration in the urine was directly analyzed by a head-space gas chromatography after basicifying with 10 M NaOH and preheating at 95 °C for 20 min. TMAO concentrations were calculated by subtraction the free TMA concentrations from total TMA (= free TMA + TMAO) concentrations after chemical reduction of TMAO to TMA using $TiCl_3$. Intra- and inter-assay variation for free and total TMA were within 5% using the previously described gas chromatography conditions (Kubota et al., 2003). The detection limit for TMA concentration was 0.01 μg/ml of urine.

FMO3 metabolic capacity to convert TMA to TMAO was defined as the ratio of TMAO to total TMA (% of TMAO/(TMA + TMAO)). The values were shown as the averages of at least two samples obtained from first morning void urine.

To evaluate the effects of active charcoal and copper chlorophyllin, urinary concentrations of free TMA or total TMA (μmol/ml of urine) were corrected by creatinine excretion (mmol/ml) from individual subjects to compare the changes of daily TMA concentrations and to account for urine volume. Creatinine concentrations in the urine were measured by a commercially available diagnostic reagent (BML, Tokyo, Japan).

Statistical analysis

A statistical analysis for frequency distribution of the metabolic capacity to convert TMA to TMAO was done by unpaired t test with Welch correction ($p < 0.05$) using a software InStat (GraphPad software, San Diego, CA, USA).

Results

Urinary Ratios of TMAO to total TMA

FMO3 metabolic capacity was determined in both the self-reported TMAU-affected individuals and control subjects (Fig. 1). Frequency distribution of the FMO3 metabolic capacity to convert TMA to TMAO was significantly different as determined by an unpaired t test with Welch correction ($p = 0.002$) between the self-reported TMAU individuals (Fig. 1A) and the control subjects (Fig. 1B). A lower metabolic capacity (mean, 92.6%; n = 27) and larger variation (SD, 7.3) were observed for the group of self-reported TMAU-affected individuals (Fig. 1A) compared with controls (Fig. 1B). The frequency of subjects who showed less than 90% of the FMO3 metabolic capacity was 26 % (7 individuals out of 27

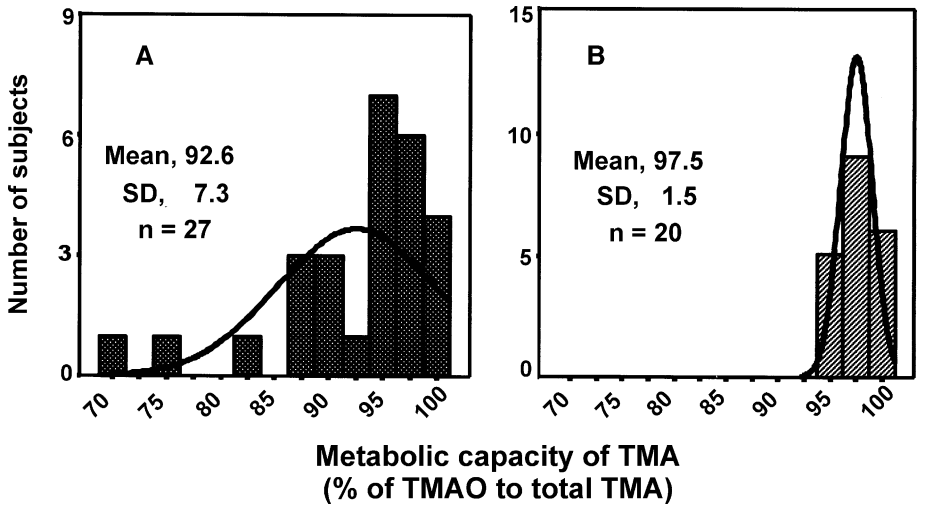

Fig. 1. Frequency distribution of 27 Japanese volunteers who suffered from self-reported malodor (A) and 20 Japanese control subjects (B). Metabolic capacity (% of TMAO/(TMA + TMAO)) was calculated by determination of urinary TMA excretion after analysis by gas chromatography. The distribution in these two groups were significantly different ($p = 0.002$) examined by unpaired t test with Welch correction for their different variances.

volunteers); this suggested that several individuals who thought they were TMAU-affected were actually affected (Fig. 1A). In contrast, 20 of the individuals examined were found to have a metabolic capacity of >95% for conversion of TMA to TMAO (Fig. 1). These 20 individuals were categorized as control subjects.

Effects of charcoal and copper chlorophyllin on the metabolic capacity of FMO3

To investigate whether activated charcoal or copper chlorophyllin was effective in decreasing TMA excretion in the urine, three volunteers that had low metabolic capacities to convert TMA to TMAO were asked to test these agents. The effects of charcoal ingestion on urinary TMA concentrations are shown in Fig. 2. Charcoal clearly decreased the urinary TMA concentration when subjects took 1.5 g of charcoal per day for 10 days. Concomitantly, the metabolic capacity of FMO3 to convert TMA to TMAO increased to over 90% in both Subject 1 (Fig. 2A) and Subject 2 (Fig. 2C). After terminating the

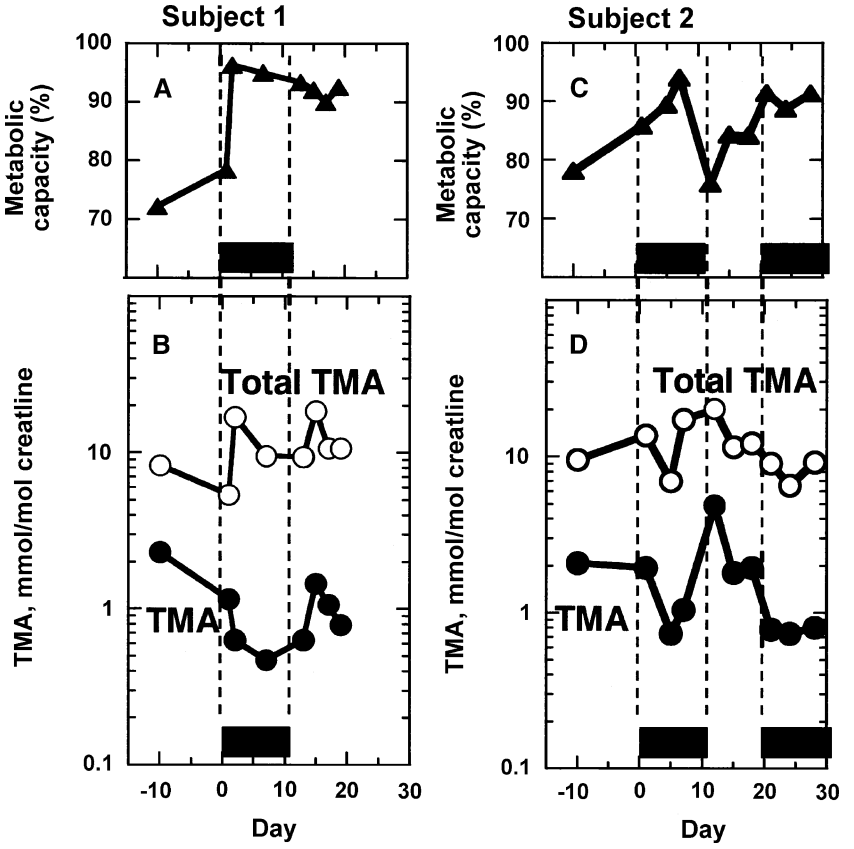

Fig. 2. Decrease of urinary TMA concentration and the increase of apparent FMO3 metabolic capacity by active charcoal in Subjects 1 and 2. Subject 2 received two runs of charcoal treatments as indicated. The metabolic capacity of FMO3 (A, C) is indicated as percent of TMAO to total TMA excreted in the urine as described in the text. Total urinary TMA and free TMA concentrations (B, D) were calculated as mmol TMA/mol creatinine. Bars indicate the periods of active charcoal treatments (10 days).

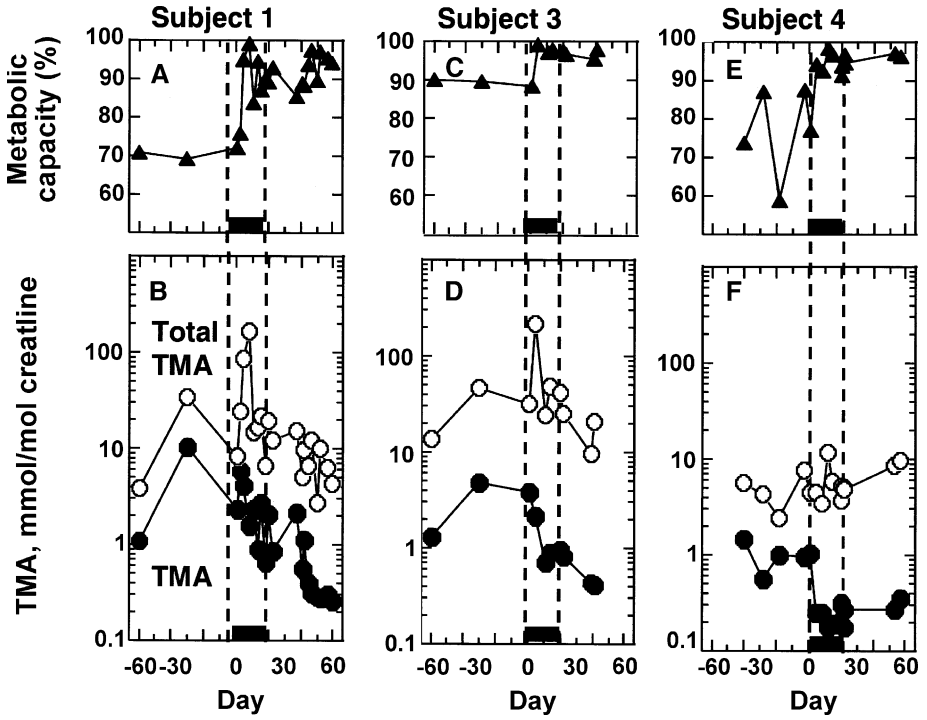

Fig. 3. Decrease of urinary TMA concentration and subsequent increase of metabolic capacity of FMO3 by copper chlorophyllin in Subjects 1, 3, and 4. Bars indicate the period of copper chlorphyllin treatment. See legend of Fig. 2 for details.

charcoal administration, the urinary concentration of TMA and the metabolic capacity of FMO3 returned to previous values especially in Subject 2 (Fig. 2C, 2D).

Copper chlorophyllin was also effective in decreasing the urinary TMA concentration and increasing the metabolic capacity of FMO3 (Fig. 3). While ingesting copper chlorophyllin, the urinary concentration of TMA was decreased in all subjects. The apparent metabolic capacity was also improved by the treatment with copper chlorophyllin (Fig. 3A, 3C, 3E). The beneficial effects of copper chlorophyllin on TMA elimination continued for several weeks after terminating its use (Fig. 3A, 3B).

Discussion

TMAU arises from the decreased capacity of the FMO3 enzyme to form TMAO from TMA. Factors that increase the substrate TMA burden include the microbial formation of TMA from dietary precursors by gut flora and an increase of intestinal absorption of TMA contained in the diet. It should be mentioned that some foodstuff components contain indoles that may competitively inhibit TMA metabolism by human FMO3 in vivo and in vitro (Cashman et al., 1999). The intake of vegetables containing indoles and/or nitrogen-containing drugs may be other factors making the situation worse for individuals affected by TMAU. The consumption of brussels sprouts was reported to inhibit the TMA N-oxidation in vivo (Cashman et al., 1999). Further detailed studies are needed to evaluate the effects of

vegetables on the metabolism of TMA. This is under investigation with some Japanese subjects in this laboratory.

TMAU is considered an autosomal recessive disorder resulting in a deficiency of the FMO3 enzyme (Cashman and Zhang, 2002). Some disease-causing factors have been reported and include: polymorphic changes of the *FMO3* gene in North American and European populations, viral infection and hormonal modulation as well as the intake of competitive FMO3 substrates or the presence of serious liver damage (Mitchell, 1999; Cashman and Zhang, 2002). The subjects of these preliminary studies, presented herein, do not appear to have the previously reported *FMO3* gene mutations that cause severe symptoms and low metabolic capacity of FMO3 (<40% TMAO). However, because of the mild symptoms presented by the individuals examined herein, the subjects may be either heterozygotes for one of the *FMO3* mutations or have one or more of the affected polymorphic changes to the *FMO3* gene (Zschocke et al., 1999; Cashman et al., 2003). Detailed analysis of *FMO3* DNA sequences for the Japanese subjects examined in this study is now under investigation in our laboratory (Fujieda et al., 2003).

In the present study, we observed a very high apparent frequency (i.e., 26% shown in Fig. 1A) for impaired FMO3 metabolic capacity in Japanese, because 7 out of 27 self-reported individuals were recruited by our internet article seeking volunteers suffered from malodor. A frequency of 26% is similar to other self-reporting populations that have been tested for TMAU (Preti et al., 1995; Cashman et al., 2003). In contrast, there were no healthy control Japanese individuals examined that showed low FMO3 activities among 20 control subjects tested (at least <5% frequency, Fig. 1B). The frequency of impaired FMO3 metabolic capacity in a normal Japanese population is not known as yet. The extent of *N*-oxidation of TMA to TMAO in various ethnic groups has been reported in previous studies (Mitchell et al., 1997; Lee et al., 2000): the incidence of deficiency in TMA *N*-oxidation varied from at least 1% (British Caucasian and Chinese) to probably 11% (New Guinean) of the population (Mitchell et al., 1997; Lee et al., 2000).

One report has appeared on the change of metabolic capacity of TMA around the time of menstruation (Zhang et al., 1996). We found 2 Japanese females in our population that showed lower FMO3 metabolic capacity to convert TMA to TMAO during menstruation (e.g., metabolic capacities of 75% versus 82% and 90% versus 98%). Further studies are needed to clarify the mechanisms responsible for the increased TMA excretion during menses.

A 10-day treatment with charcoal decreased urinary TMA concentrations in TMAU-affected patients. In addition, treatment with copper chlorophyllin also decreased free urinary TMA. However, compared to charcoal, the beneficial effects of copper chlorophyllin appeared to continue longer after cessation of use. The mechanism responsible for the effects of charcoal on TMA appears to be adsorption. Because charcoal may adsorb free TMA, the decreased TMA concentration may place less of a burden on the ability of FMO3 to detoxicate TMA in TMAU-affected individuals. Copper chlorophyllin may also chemically sequester free TMA as has been previously documented for other *N*-containing compounds (Dashwood et al., 1996). The effects of copper chlorophyllin lasted for several weeks after termination of administration copper chlorophyllin. The mechanism(s) responsible for causing this prolonged effect is unclear as yet. One possible mechanism might be to retard uptake of foodstuffs that are precursors of TMA or another mechanism may be to eliminate TMA directly in the feces. However, it is also possible that copper chlorophyllin could affect the make-up of gut flora resulting in decreased formation of TMA. It is interesting to note that the total TMA excretion was increased temporally after starting the daily intake of copper chlorophyllin (Fig. 3). The mechanism for this is also unclear. Since copper

chlorophyllin is available as an over-the-counter medication in Japan and the United States, our results suggest that use may provide a significant benefit to improve the lives of TMAU-affected individuals.

Subject 1 was the first patient in our study and was involved in both courses of dietary treatments. Based on our genetic analysis (Fujieda et al., 2003), Subjects 1–4 have suffered from TMAU-related symptoms for much of their lives but do not appear to have the mutations in the *FMO3* gene seen in other non-Japanese TMAU populations. The measures of TMA excretion employed herein were done under normal dietary conditions as previously reported in other populations (Treacy et al., 1995). The other Japanese subjects examined here that possessed lower metabolic capacity to convert TMA to TMAO also had lower urinary TMA than reported by Treacy et al. (1995) (<18 mmol TMA/mol of creatinine). However, the excess urinary TMA excretion found in the subjects reported herein has still produced social problems for the TMAU-affected individuals. It is not known whether Japanese have a decreased olfactory threshold to the odor of TMA but it may differ from Western populations because of different dietary or genetic differences.

In conclusion, we were able to successfully treat Japanese individuals experiencing TMAU with dietary supplements such as activated charcoal or copper chlorophyllin. These supplements decreased the free TMA excreted in urine; consequently, our results suggest that these agents can be used to improve the quality of life of TMAU-affected individuals. In terms of treatments of TMAU-affected individuals, gut flora treatment with antibiotics or limitation of some foodstuff resulting in TMA formation has been reported (Treacy et al., 1995; Cashman et al., 2003). The dietary supplements described in the present study are recommended for use in combination with and/or following the previously published treatments for the TMAU-affected individual after consultation with the individual's physician.

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