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Dimethylamine and diet

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Abstract

Forty-six different foods eaten by six healthy male volunteers were investigated as potential sources of the aliphatic secondary amine, dimethylamine. None that were representatives from the fruit and vegetable, meat, dairy and grain produce categories afforded any measurable elevation in urinary dimethylamine output following ingestion. All of the statistically significant increases occurred after consumption of fish and seafoods. However, within this category a wide variation was observed. The highest values were obtained for coley, squid and whiting with cod, haddock, sardine, skate and swordfish also producing substantial increases. Freshwater trout, plaice and prawns gave no discernable effect. It seems that not all fish and seafoods may be treated equally with regards to human dimethylamine exposure and that the situation is more complicated than at first appears.

Keywords: Dimethylamine; Amines; Food; Fish; Seafoods; Urine

1. Introduction

Despite having been recognised as a normal component of human urine for over 70 years (Asatoor and Simenhoff, 1965; Loffler, 1935; Wacek and Loffler, 1934) a paucity of information still exists regarding the origin of dimethylamine, its possible physiological roles and potential pathological associations. Dimethylamine has been shown to interfere with cell growth; causing a decrease in overall size, nucleic acid and protein content of cultured mouse embryos (Guest and Varma, 1991), having an unspecified toxic effect on tumour cells in culture (Simenhoff, 1975) and inhibiting the oxidation of succinate in brain tissue (Young and Wootton, 1964). This latter observation lends credence to the suspicion that the amine may act as a neurotoxin in uraemic patients (Ihle et al., 1984) where it occurs in higher than normal concentrations in the intestine, blood, cerebrospinal fluid and brain tissues (Simenhoff et al., 1963, 1976, 1977; Baba and Watanabe, 1988). However, it has been the growing concern over the potential for undergoing nitrosation to dimethylnitrosamine, a potent carcinogen (Magee and Barnes, 1956) which is excreted in trace amounts in normal human urine (Garland et al., 1986) that has fuelled most of the literature reports regarding this secondary amine.

Fish and other seafoods consumed by humans have been shown to contain significant quantities of short chain aliphatic amines (Lin et al., 1984) and ingestion of these foods presumably will expose humans to elevated amounts of these substances. Any possible problems that this exposure may precipitate are at variance with the potential health benefits of fish meals especially advocated with respect to cardiovascular disease (Zeisel and DaCosta, 1986). Radiotracer work in man has shown that orally administered dimethylamine is absorbed readily and excreted rapidly and almost quantitatively unchanged within the urine. The measurement of urinary levels of dimethylamine, therefore, provides a reasonable indication of the body exposure to this amine (Zhang et al., 1994). Exploiting this approach, a study in which volunteers ingested a fish diet estimated to contain about

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40 mg dimethylamine showed that the subsequent urinary excretion of dimethylamine increased 4-fold (Zeisel and DaCosta, 1986). Contrariwise however, investigations with three groups of male volunteers possessing different fish consumption habits demonstrated that urinary levels of trimethylamine and its N-oxide were significantly associated with the weekly fish intake but no such relationship was evident for urinary dimethylamine values (Svensson et al., 1994). Against the background of such conflicting reports, the present study has been undertaken to investigate a range of dietary materials, including non-fish products, as potential sources of human urinary dimethylamine.

2. Materials and methods

2.1. Volunteer studies

Six healthy male volunteers were recruited (age range 28–40 years, 32 ± 5) for the study. None were smokers and four took moderate amounts of alcohol (approx. 15 units/week), but not immediately before or during the test periods. All the subjects were free from medication during the test periods and, fortuitously, no-one was required to take antibiotics at any time during the entire study. The protocols were fully explained to the volunteers before commencement of the investigations and ethical approval was obtained from the local ethics committee.

The volunteers fasted overnight and discarded all urine voided in the morning. A light breakfast was eaten and the following 0–8 h urine collected, during which time only water was allowed. This acted as a control (background) urine sample. The next day an identical light breakfast was consumed plus the particular food (227 g, 8 oz) under investigation, followed by the 0–8 h urine collection. This procedure was repeated for the 46 foods that were purchased from local stores and were cooked, where appropriate, in the normal manner. At least 1 week separated each individual food study.

2.2. Urine collection

Urine from all studies was collected into a plastic container to which hydrochloric acid (6 M, 10 ml) had been added previously to prevent microbial growth and maintain dimethylamine as its water soluble non-volatile hydrochloride salt. The total urine volume was recorded and multiple aliquots (25 ml) stored in the dark at −20 °C until analysis, which was carried out in duplicate as soon as possible.

2.3. Urine analysis

Thawed urine (5 ml), spiked with 0.2% (v/v) isopropylamine (30 μl, 20.8 μg; Sigma–Aldrich Co. Ltd., Dorset, UK) as internal standard, was placed into a screw-capped glass vial (15 ml) and pelleted potassium hydroxide (2 g) added before sealing with an airtight PTFE-lined septum cap and leaving on ice to cool. The vial was then vortex mixed and heated at 90 °C for 20 min in an aluminium heating block, after which an aliquot (2 ml) of the generated head-space gas was injected directly onto the analytical column of a gas chromatograph. The use of authentic dimethylamine hydrochloride (Sigma–Aldrich Co. Ltd.) added to distilled water and to urine permitted the construction of calibration curves (0.1–150 μg/ml), which enabled the quantification of endogenous dimethylamine. All analyses were undertaken in duplicate, urine samples with a high dimethylamine concentration being diluted before analysis as appropriate (Zhang et al., 1992a).

Gas chromatography was performed on a Pye Unicam 4500 series gas chromatograph (Pye Unicam, Cambridge, UK) with a flame ionization detector. The silanized glass column (170 cm × 4mm ID) was packed with 4% (w/w) Carbowax 20 M and 0.8% (w/w) potassium hydroxide on Carbopack B (60–80 mesh) graphitized support (Supelco Inc., Philadelphia, PA, USA). The operating temperatures of the column, injection port and detector unit were 70 °C isothermal, 150 °C and 200 °C, respectively, with a nitrogen carrier-gas flow rate of 60 ml/min (Zhang et al., 1992a).

3. Results

The value obtained (6.67 ± 1.75 mg/8 h) for the amount of dimethylamine excreted in control urine (276 urine samples in total) was in alignment with those previously published for daily urine estimated by a variety of methods (8.4–22.2 mg/24 h; Asatoor and Simenhoff, 1965; Blau, 1961; DaCosta et al., 1990; Dowden, 1938; Loffler, 1935; Zeisel and DaCosta, 1986) and a more recent and extensive population study (17.4 ± 11.8 mg/24 h; Zhang et al., 1995).

None of the food products examined that were representatives from the fruit and vegetable, meat, dairy and grain produce categories, produced any significant increases in urinary dimethylamine output following ingestion. The level following bread consumption was raised but a wide variation between subjects removed any statistical significance ($P > 0.05$, Student's '*t*'-test); perhaps a larger number of subjects may have enabled statistical significance to be reached. However, with the exception of prawns, plaice and freshwater trout, all of the fish and seafoods investigated gave rise to a statistically significant increase in subsequent urinary dimethylamine excretion. The highest values were obtained for squid, coley and whiting (up to 7-fold increase), with skate, cod, sardine, haddock and swordfish producing substantial (3–4-fold) increases (Table 1).

4. Discussion

Although the urine collection period (0–8 h) may appear short it was the maximum feasible for the subjects within the present experimental protocol designed to permit a direct comparison of ingested foodstuffs to be made. Any prolonged fasting, necessary to prevent ingestion of potentially confounding materials, was deemed undesirable. Ensuing alterations in normal physiology and biochemistry and a probable decrease in volunteer compliance would have served to undermine the results obtained. Previous studies with orally administered [^{14}C]-labelled dimethylamine have shown that half of the material appeared within the urine during first 6 h (Zhang et al., 1994). Indeed, unambiguous positive results have been obtained in the present study with some fish and seafoods. Perhaps interestingly, the increases found after cod and haddock ingestion agreed closely with those reported by Zeisel and co-workers (Zeisel and DaCosta, 1986).

What is apparent from this study is that not all fish and seafoods can be treated equally; the bland statement that fish provide dimethylamine is too simplistic. It is known that the dimethylamine content of fish varies between species (Bocklisch, 1885; Lin et al., 1984) and that within species fresh fish contain less dimethylamine than stored