Increase in Human Exposure to Methylamine Precursors of N-Nitrosamines after Eating Fish

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ABSTRACT

Consumption of fish has been encouraged recently because it may prevent mortality due to heart disease. Fish contains methylamines, which are precursors of N-nitrosamines. Nitrosamines can act as potent carcinogens in a wide variety of animal species, and there is no reason to assume that humans are resistant. Human subjects (n = 5) ingested a diet of known methylamine content for 2 days. On Day 3, they ate fish at the luncheon and dinner meals. On Day 4, they again ate the control diet. A single portion of fish contained as many methylamines as were normally excreted by the human in 2 days. Urinary excretion of mono-methylamine remained constant (1.3 to 1.5 μmol/24 h/kg of body weight) throughout the study. Dimethylamine excretion increased more than 4-fold after fish was eaten (from 5.6 to 24.1 μmol/24 h/kg of body weight), while trimethylamine excretion increased more than 8-fold (from 0.2 to 1.6 μmol/24 h/kg of body weight).

We conclude that the consumption of fish significantly increased exposure to methylamines, particularly to dimethylamine. Although there is potential for the in vivo conversion of dimethylamine to nitrosodimethylamine, a carcinogen, we know of no studies that have determined that the ingestion of fish increases the risk of cancer. This should be carefully investigated prior to recommending that humans change their eating habits.

INTRODUCTION

DMA3 and TMA are important because they are precursors of NDMA (1). N-Nitrosamines can act as potent carcinogens in a wide variety of animal species, and there is no reason to assume that humans are resistant (2, 3). The National Academy of Sciences has recommended that exposure of humans to N-nitroso compounds should be reduced (3). Although a causal relationship between nitrosamine exposure and cancer in humans has not been rigorously proved, epidemiological studies linking nitrosamines in tobacco products to oral cancer in humans strongly suggest such an association (2).

Fish, as consumed by humans, contains significant quantities of TMA and DMA (4, 5). TMAO is an end-product of nitrogen metabolism in fish (5, 6). It replaces urea as the major route for nitrogen excretion. TMAO is metabolized to form TMA by bacteria once fish have been killed (6). TMA gives fish its characteristic "fishy" odor. DMA is formed by the action of endogenous tissue enzymes upon TMAO and occurs maximally at temperatures below the freezing point of fish (−5 to −10°C) (5, 7).

Epidemiological evidence, chiefly from studies on Eskimos of Greenland, Japanese on Okinawa, and Dutch men in the village of Zutphen, suggests that there is an inverse relationship between fish consumption and mortality from coronary heart disease (8, 9). A mechanism for the effects of fish upon heart disease has not been proven, but it has been suggested that the effect is related to the ω3-polysaturated fatty acids present in fish oils (10). On the basis of available epidemiological and experimental data, it has been recommended that humans increase their intake of fish (11).

Physicians need to consider the risk/benefit ratio when recommending that humans change their eating habits. Exposure to increased amounts of nitrosatable methylamines and therefore possible exposure to nitrosamines are a risk of eating more fish. For this reason we examined the effect that eating fish has upon the exposure of humans to MMA, DMA, and TMA.

MATERIALS AND METHODS

Experimental Protocol. Five healthy, normal subjects (1 male, 4 females), ages 23 to 35 yr, weighing 52.3 to 72.7 kg, gave their informed consent for participation in this study. For 2 days they ingested a control diet of known methylamine content. On Day 3 of the study, fish was substituted as the chief constituent of the luncheon and dinner meals. On Day 4, the subjects returned to the control diet. The diets ingested by the subjects are described in Table 1.

Sample Collection. Urine was collected by all subjects in 24-h blocks. Collection vessels contained 50 ml of 3 N hydrochloric acid (to inhibit bacterial growth and to trap methylamines). Urine was refrigerated during the collection period. Urine volumes were recorded, and aliquots were frozen at −90°C until assayed. Collection and storage under these conditions did not affect methylamine concentrations when known amounts of methylamines were spiked into the collection vessels.

A sample of each food in the diet (100 mg) was added to 2 ml of 0.1 N hydrochloric acid and mixed thoroughly. This was allowed to stand on ice for 1 h. The mixture was centrifuged at 3000 × g for 10 min at 4°C, and the supernatant was saved for methylamine analysis.

Analysis of Methylamines. MMA, DMA, and TMA were measured using a gas-liquid chromatography method (1). An aliquot of the acidified biological fluid (urine or tissue extract) was placed into a sealed vial (Teflon-lined WISP septum; Waters Instruments, Milford, MA). 2-Propanol was added into each vial, along with an internal standard of tripropylamine. Potassium hydroxide (65%) was injected into the vial to bring the pH to 13. Vials were vigorously shaken, heated at 60°C for 30 min to volatilize the amines, and then subjected to centrifugation at 1000 × g for 5 min at −4°C. An aliquot was drawn up into a syringe, followed by 1 μl of air and 1 μl of 30% ammonium hydroxide. This was injected into the glass-lined injection port (pretreated with potassium hydroxide to inactivate any binding sites for methylamines) of the gas chromatograph (Sigma 2000; Perkin Elmer, Norwalk, CT). We used a 2-m-long (2-mm inner diameter) glass column packed with 60/80 CarbopackB/0.8% KOH on 4% Carbowax 20M (Supelco, Bellefonte, PA) and eluted methylamines with helium (25 ml/min) and a temperature gradient (75°C for 2 min, then rising to 200°C at 32°C/min). Amines were detected with a nitrogen-phosphorus detector (NPD; Perkin Elmer), and peaks were integrated using a computing integrator (LCI-100; Perkin Elmer). During each sample run, we injected 20 μl of 30% ammonium hydroxide onto the column when the temperature reached 150°C, and the temperature program was then continued to 200°C. This markedly reduced "ghosting" of methylamines on subsequent runs.

This method had a precision (SD/mean × 100) for MMA of 1.6%, for DMA of 2.2%, and for TMA of 2.0%. The assay was linear for...
FISH AND METHYLAMINES IN HUMANS

Table 1. Data are expressed as the mean of triplicate determinations.

<table>
<thead>
<tr>
<th>Diet</th>
<th>MMA (µmol/100 g food)</th>
<th>DMA (µmol/100 g food)</th>
<th>TMA (µmol/100 g food)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ham steak</td>
<td>ND*</td>
<td>6.7</td>
<td>40.8</td>
</tr>
<tr>
<td>Haddock</td>
<td>ND*</td>
<td>197.2</td>
<td>233.3</td>
</tr>
<tr>
<td>Cod</td>
<td>ND*</td>
<td>78.2</td>
<td>114.9</td>
</tr>
<tr>
<td>Milk</td>
<td>7.9</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Cheese pizza</td>
<td>2.9</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Green beans</td>
<td>4.9</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

* ND, not detected.

Table 2. Methylamine-containing foods in diet

<table>
<thead>
<tr>
<th>Food</th>
<th>MMA (µmol/100 g food)</th>
<th>DMA (µmol/100 g food)</th>
<th>TMA (µmol/100 g food)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Snacks</td>
<td>600 g ginger ale</td>
<td>600 g ginger ale</td>
<td>70 g cookies</td>
</tr>
<tr>
<td></td>
<td>70 g cookies</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>* Frozen haddock, baked prior to ingestion.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>* Fresh cod, broiled in butter.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RESULTS

Some of the foods ingested as part of the experimental diets contained MMA (milk, pizza, and beans). Fish and ham were the only foods which contained DMA and TMA (Table 2). On control days, subjects ingested 31.6 µmol of MMA, 6.7 µmol of DMA, and 40.8 µmol of TMA per day. On the day fish was eaten, subjects consumed 18 µmol of MMA, 754 µmol of DMA, and 956 µmol of TMA per day.

MMA excretion in urine was similar on the day when fish was eaten compared to the control days (1.5 ± 0.2 versus 1.3 ± 0.1 µmol/24 h/kg of body weight; mean ± SE; no significant difference; Fig. 1). DMA excretion was more than 4 times greater on the day that fish was eaten than on the control days (24.1 ± 3.5 versus 5.6 ± 0.2 µmol/24 h/kg of body weight; mean ± SE; P < 0.01; Fig. 2). On the day after fish was eaten, DMA excretion remained elevated, being 2.8 times greater than on the control days (15.9 ± 2.8 µmol/24 h/kg of body weight; mean ± SE; P < 0.05; Fig. 2). TMA excretion was more than 9 times greater on the day that fish was eaten than on the control days (1.6 ± 0.3 versus 0.17 ± 0.03 µmol/24 h/kg of body weight; mean ± SE; P < 0.01; Fig. 3). On the day after fish was eaten, TMA excretion remained elevated, being 5 times greater than on the control days (0.86 ± 0.14 µmol/24 h/kg of body weight; mean ± SE; Fig. 3).

DISCUSSION

Ingestion of fish increased exposure to methylamines many-fold. A 100-g portion of fish contained as many methylamines as were normally excreted by humans in a day. Fresh fish contained fewer methylamines than did frozen fish. Humans excreted 866 µmol of methylamines (MMA + DMA + TMA) in their urine during the first 2 days when ingesting the control...
diet. Much of this was probably derived from endogenous (nondietary) sources of DMA (1). The increase in excretion of total methylamines (1811 µmol) during Days 3 and 4 was almost identical to the methylamine content of the fish (1710 µmol) that was eaten. Much of the ingested TMA must have been converted to DMA within the human, as most of the increase in urine methylamine excretion was in the form of DMA. In rats, it has been established that TMA can be metabolized to form DMA (13).

The methylamines in the fish must have been absorbed from the intestine into blood in order to be excreted in urine. DMA is readily transported from blood to gastric fluid (14). The other precursor needed for the formation of nitrosamine is nitrite, which is formed from dietary nitrate in the oral cavity and stomach by reactions mediated by bacterial enzymes (15, 16). People ingest from 10 to several hundred, mg of sodium nitrate per day in food and water (16–18). The acidic conditions of the stomach favor the formation of nitrous anhydride and nitrosyl compounds which nitrosate amines to form nitrosamines (19). NDMA is rapidly formed when DMA and nitrite are mixed with gastric juice in a test tube or in the stomach of the dog (20, 21). The in vivo formation of nitrosamines has clinical significance, as the same tumors are produced in experimental animals that are fed amine plus nitrite, as are formed in those fed the corresponding nitrosamine (5). The formation of NDMA in vivo in humans has not been well documented because this nitrosamine is rapidly metabolized. However, there is direct evidence that the nonmetabolizable nitrosamine, nitrosoproline, is synthesized in humans following the ingestion of an amine (proline) and nitrate (22, 23). Although it appears that most of the ingested methylamine was rapidly excreted by humans, it is possible that significant amounts of NDMA could have been formed in vivo, as nitrosamines are of concern at very low concentrations (ppm) (5).

The methylamine content of fish varies with its freshness, storage conditions, and species (5–7). DMA concentrations as high as 1640 µmol/100 g (21 times greater than in our fish) have been reported in frozen cod, while fresh spotted trout contained only 15 µmol of DMA per 100 g (5). Fish also contains several other nitrosatable amines, such as morpholine and di-N-propylamine; however, these amines are present in much smaller quantities than is DMA (5).

We have documented a risk associated with increased ingestion of fish in humans: the exposure to increased amounts of nitrosatable amines and therefore the possible exposure to nitrosamines. The relative importance of this risk, compared with the potential for benefits to the cardiovascular system, is not known. We know of no data which suggest that the ingestion of fish is associated with an increased incidence of cancer. However, there are treatment options which might entail less exposure to methylamines. For example, the beneficial ingredients in fish may consist of certain fatty acids, and these could be administered in purified form. Before recommendations are made for changes in the American diet, potential risks should be thoroughly evaluated, so that optimal dietary interventions can be designed.

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REFERENCES

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