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ORIGINAL ARTICLE

Diagnosis and management of trimethylaminuria (FMO3 deficiency) in children

R. A. Chalmers · M. D. Bain · H. Michelakakis · J. Zschocke · R. A. Iles

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Summary Persistent trimethylaminuria in children is caused by autosomal recessively inherited impairment of hepatic trimethylamine (TMA) oxidation due to deficiency of flavin monooxygenase 3 (FMO3) secondary to mutations in the *FMO3* gene. Trimethylaminuria or 'fish odour syndrome' is due to excessive excretion into body fluids and breath of TMA derived from the enterobacterial metabolism of dietary precursors. The disorder is present from birth but becomes apparent as foods containing high amounts of choline or of trimethylamine *N*-oxide (TMAO) from marine (sea or salt-water) fish are introduced into the diet. In our experience, trimethylaminuria (FMO3 deficiency) in children is rare. We have compared the dynamics and diagnostic efficacy of choline loading with marine fish meals in six children with trimethylaminuria. Loading with a marine fish meal provides a simple and acceptable method for confirmation of diagnosis of suspected trimethylaminuria in children, with the effects being cleared more quickly than with a choline load test. However, oral loading with choline bitartrate allows estimation of residual oxidative capacity *in vivo* and is a useful adjunct to molecular studies. Patients homozygous for the 'common' P153L mutation in the *FMO3* gene showed virtual complete lack of residual TMA *N*-oxidative capacity, consistent with a nonfunctional or absent FMO3 enzyme, whereas a patient with the M82T mutation showed some residual oxidative capacity. A patient compound heterozygous for two novel mutations, G193E and R483T, showed considerable residual *N*-oxidative capacity. A further patient, heterozygous for two novel sequence variations in the *FMO3* gene, consistently showed malodour and elevated urinary TMA/TMAO ratios under basal conditions but a negative response to both choline and marine fish meal loading. Comparison of the effects of administration of antibiotics (metronidazole, amoxicillin, neomycin) on gut bacterial production of trimethylamine from choline showed they all reduced TMA production to a limited extent, with neomycin being most effective. 'Best-practice' diagnostic and treatment guidelines are summarized.

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R. A. Chalmers (✉) · M. D. Bain
Paediatric Metabolism Unit, St George's Hospital Medical School, London, UK
e-mail: rachalmers@cimoa.org.uk

H. Michelakakis
Institute of Child Health, Aghia Sophia Children's Hospital, Athens, Greece

J. Zschocke
Institute of Human Genetics, University of Heidelberg, Heidelberg, Germany

R. A. Iles
Department of Diabetes and Metabolic Medicine, Barts and the London School of Medicine and Dentistry, Queen Mary College, London, UK

Present address:
R. A. Chalmers
CIMOA, London BioScience Innovation Centre, 2 Royal College Street, London NW1 0NH, UK

Trimethylaminuria or 'fish odour syndrome' (McKusick 602079) was first described briefly by Humbert and colleagues (1970) and in more detail by Lee and colleagues (1976) and is characterized by a distinctive body odour reminiscent of decaying fish, due to excessive excretion into the urine, sweat and breath of the malodorous volatile aliphatic amine trimethylamine (TMA). TMA is derived from the enterobacterial metabolism of dietary precursors such as trimethylamine *N*-oxide (TMAO), choline, lecithin and

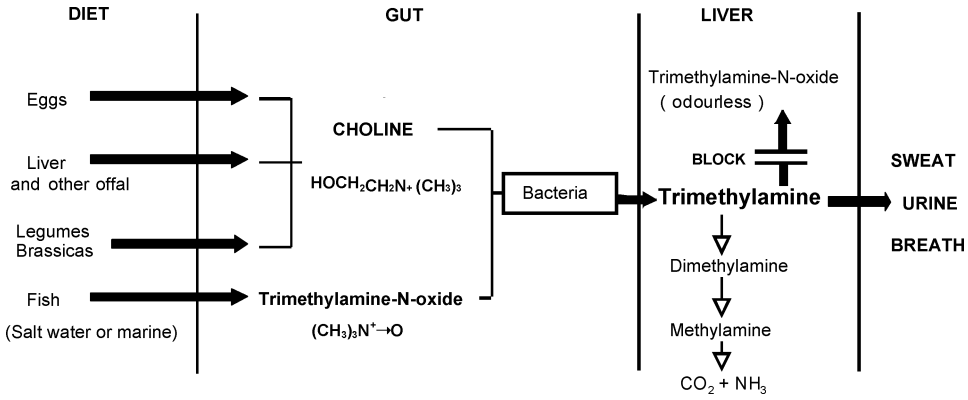

Fig. 1 Origins and metabolism of trimethylamine in humans

possibly carnitine and other betaines (Fig. 1) and is normally cleared effectively by hepatic *N*-oxidation and urinary excretion of the odourless TMAO. Persistent trimethylaminuria in otherwise healthy children is caused by autosomal recessively inherited impairment of hepatic TMA oxidation (Ayesh et al 1993; Higgins et al 1972) due to deficiency of flavin monooxygenase 3 (FMO3) (EC 1.14.13.8; McKusick *136132). The disorder is present from birth but the condition becomes apparent as the child is weaned and foods containing high amounts of choline, for example eggs, liver and other offal, or trimethylamine *N*-oxide (from marine (sea or saltwater) fish) are introduced into the diet. Patients are unable to effectively oxidize the ingested trimethylamine and the malodorous free amine is excreted into the urine, sweat and breath. In this paper, 'TMA' refers to free, unoxidized, trimethylamine and 'TMAO' to *N*-oxidized TMA.

A variety of disease-causing mutations in the *FMO3* gene have been identified (Ackerman et al 1999; Dolphin et al 1997; Hernandez et al 2003; Treacy et al 1998) that result in deficient activity of hepatic FMO3 and what has been termed 'primary' trimethylaminuria. Polymorphic variations and sequence variations in the *FMO3* gene do not commonly lead to overt trimethylaminuria in children, but in adult life these may result in reduced ability to deal with increased amounts of trimethylamine derived from the diet, leading to transient or intermittent trimethylaminuria (Zschocke et al 1999). This may occur especially in association with bacterial overgrowth syndromes and other bowel disorders leading to chronically enhanced metabolism to TMA of choline-containing foodstuffs in the bowel and of trimethylamine *N*-oxide in marine fish by gut bacteria, leading to 'secondary' trimethylaminuria (Fraser-Andrews et al 2003; Mitchell and Smith 2001). Overt malodour in such patients is difficult to detect and they may not show abnormal responses to acute choline loading. In our (UK) experience, none of the adults presenting with apparent or perceived malodour whom we have examined have been proven to have primary trimethylaminuria or any evidence for enterobacterial overgrowth.

Trimethylaminuria in children is a rare inborn error of metabolism, but the true incidence may possibly be underestimated owing to poor clinical awareness and the need for access to specialized diagnostic facilities. The large majority of cases with primary FMO3 deficiency will present in early childhood and accurate diagnosis is essential for appropriate genetic counselling and their long-term management. Clinically, whether in the child or adult, trimethylaminuria cannot be considered a benign or 'social' condition. Problems develop as the child attends early school, with embarrassment and ridicule, and the resulting low self-esteem, social exclusion and isolation leading to anxiety and depression. In later life, it becomes difficult to form personal relationships or to hold regular employment, and isolation and depression increase. The condition may be intermittently exacerbated by increased trimethylamine production during periods of excessive sweating and exercise, stress on emotional upset and, in females, just before and during menstruation. In addition, deficient activity of the underlying enzyme, FMO3, may have wider-reaching clinical consequences, leading to abnormal metabolism of nitrogen and sulphur-containing drugs and other compounds including neurotransmitters and to hypertension or increased cardiovascular risk (Zschocke et al 1999).

Early diagnosis is important in children with trimethylaminuria so that appropriate dietary therapy may be introduced as soon as possible. Although initial indications of the disorder may be obtained by analysis of a single urine sample, this is not always reliable, especially when the child is ingesting a diet low in trimethylamine precursors. Unequivocal confirmation of the diagnosis is important, partly to exclude transient secondary trimethylaminuria and other malodour syndromes. Oral loading with trimethylamine has been employed as a diagnostic tool in early studies of the condition (Al-Waiz et al 1988) but is unsatisfactory for investigations in young children because of the number of enteric-coated capsules that need to be taken and the relatively massive dose of TMA employed. Dietary loading with foods with a high choline content such as eggs has also been used (Spellacy

et al 1979). However, much of the choline content in eggs is esterified and not readily available for metabolism and a large number of eggs (>9–26 mmol choline (if available)) is required to obtain usable results; most definitive studies have employed oral choline loads to confirm diagnoses (Spellacy et al 1979). Marine (sea or saltwater) fish contain high amounts of trimethylamine *N*-oxide as an osmolyte (Yancey et al 1982); thus, feeding a meal containing marine fish offers an alternative loading method that provides a direct load of trimethylamine (*N*-oxide) (Spellacy et al 1979) and is a useful alternative and differential to choline loading. Loading with a fish meal may also be more suitable and acceptable to young children (and their parents).

In the present study we compared the dynamics and diagnostic efficacy of choline loading with the use of marine fish meals in children with trimethylaminuria. We also compared the effect of administration of antibiotics on gut bacterial production of trimethylamine from choline as an adjunct to dietary management of the condition. Some of these results have been presented elsewhere in poster and abstract form (Chalmers et al 2003).

Methods, patients and procedures

Analytical methods: Urinary trimethylamine (TMA) and trimethylamine *N*-oxide (TMAO) were measured directly and simultaneously in untreated urine samples using proton magnetic resonance spectroscopy (MRS) (Holmes et al 1997; Murphy et al 2000). Samples of urine (0.5 ml) were placed in 5 mm diameter NMR tubes together with 50 μl ^2H_2O (as a 'lock' signal to maintain stability of the magnetic field) containing 20 mmol/L 3-(trimethylsilyl)-2,2,3,3-deuteropropionate ($TSPd_4$) as an internal reference. Sample pH was adjusted to 2.5 where necessary to confirm the identity of the TMA and TMAO proton resonances. (See Note below.) Each sample was analysed at room temperature using Bruker AM (400 MHz) or Jeol GSX (500 MHz) NMR (nuclear magnetic resonance) spectrometers using 45° radiofrequency pulses with a pulse recycle time of 5 s. Total acquisition times were between 5 and 11 min (64–128 scans). Suppression of the water pulse was achieved by selective irradiation during the relaxation delay. Resonances were assigned by reference to our own spectral database of standard chemical shifts, making adjustments where necessary to reflect the urine pH. Metabolites in urine were quantified by measuring relative peak heights. Results are expressed as mmol/mol creatinine and individual and total amounts (μmoles) of TMA and TMAO excreted were calculated for choline loads by summation of the amounts in each urine collection over the study period.

(Note: At physiological pH, the protons of the trimethyl group of TMA resonate at 2.90 ppm as a singlet. At pH values below 3, the proton on the adjacent nitrogen atom causes the trimethyl proton resonances to split into a doublet. The corresponding resonance for TMAO remains as a singlet at low pH but its resonance frequency shifts to the left due to the changing ionization status of the nitrogen proton ($pK = 4.65$).)

Patients: Five unrelated children with trimethylaminuria were studied, 1 boy and 4 girls, age range 5 years to 13 years at the time of study (Table 1). A sixth patient was a girl, aged 5 years, with a malodour syndrome, who was shown not have a known disease-causing mutation of *FMO3* (see below). A further child (patient 1a) was diagnosed from mutation analysis shortly after birth, following the diagnosis of her elder brother (patient 1), but was not studied further in this work. A basal urine sample was referred from patients on the basis of overt malodour observed both by parents and by the referring

Table 1 Basal biochemical data on children with trimethylaminuria at referral

Patient	Age at referral (years)	Sex	Ethnic origin	Basal metabolite levels (mmol/mol creatinine (unrestricted diet)		
				TMA	TMAO	TMA/TMAO ratio
1	5.5	M	Scottish	160	60	2.67
1a	(0.08)	F	Scottish	NT[a]	NT	NT
2	6.3	F	Irish	33	22	1.5
3	12.7	F	English	5	15	0.33
4	4.5	F	English	25	1	25
5	1.8	F	Filipino	5	2	2.5
6	5.2	F	Scottish	40	20	2.0
Healthy controls (unrestricted diet)[b]				<10	50–1000	<0.1

[a]NT, not tested
[b]TMAO levels in healthy controls are dependent upon dietary intake (and on the proximity of dietary intake to urine collection) of choline-containing foods and of marine fish; thus values of TMAO may be proportionately high on occasion

paediatrician. At the time of sampling, the patients were receiving a normal unrestricted diet for their age; follow-up studies were undertaken when the ratio of TMA to TMAO exceeded 0.1 (Table 1).

The use of healthy children as controls for these studies was precluded for ethical reasons and 'controls' used were adults (all female, age range 30–53 years at referral) referred with apparent malodour who underwent fish or choline load tests as part of their diagnostic investigations. None of these adult patients was shown to have primary trimethylaminuria on the basis of choline and/or fish loading tests.

Procedures: These studies were approved by the Wandsworth Local Research Ethics Committee and complied with the Declaration of Helsinki recommendations. Informed consent was obtained from the patient's parents and simplified informed consent from the children themselves prior to each stage of these studies.

Patients were placed on a diet restricted in choline and trimethylamine (restriction of eggs, liver, kidney and other offal, peas, peanuts and other legumes, and of marine fish) for 3 days prior to loading tests. Two timed (8 h or 12 h) basal urine collections were made. An oral choline load (10 g choline bitartrate, equivalent to 39.5 mmol choline, dissolved in water or orange juice; for adults 15 g, equivalent to 59.3 mmol choline) or a meal of marine fish (e.g. cod) was given and timed (8 h or 12 h) urine collections were made for the following 72 h or more. Sample volumes were measured and aliquots were stored at −20°C until analysed.

In some studies, while subjects were still receiving a diet restricted in choline, metronidazole (7.5 mg/kg body weight twice daily for one week, followed by 7.5 mg/kg per day for three weeks) was administered and the choline load test was repeated while subjects were still receiving the antibiotic. Metronidazole administration was then stopped for one month and, while subjects were still on the choline-restricted diet, amoxicillin (125 mg three times daily for one week followed by 125 mg once per day for three weeks) was administered and the choline load test was repeated while subjects were still receiving the antibiotic. One child was also treated with neomycin 25 mg/kg three times per day for seven days and the choline load test was repeated while the child was still receiving neomycin. If there was any adverse reaction to the first choline load test, the repeated loads were carried out using smaller amounts of choline bitartrate (5 mg, 3.5 mg or 2.5 mg, equivalent to 19.8, 13.8 and 9.9 mmol choline, respectively).

Not all of these studies could be carried out on all patients for ethical, practical or clinical reasons, and the data presented below are representative of the results obtained in the cohort of young patients.

Results

Load tests

Choline load test

Figure 2 shows the effects of 10 g oral choline bitartrate loading on the excretion of trimethylamine (TMA) and trimethylamine *N*-oxide (TMAO) by two of the children with trimethylaminuria (FMO3 deficiency) and of 15 g loading in one unaffected adult control patient. Patient 1 shows a marked rise in excretion of TMA with minimal increase in TMAO

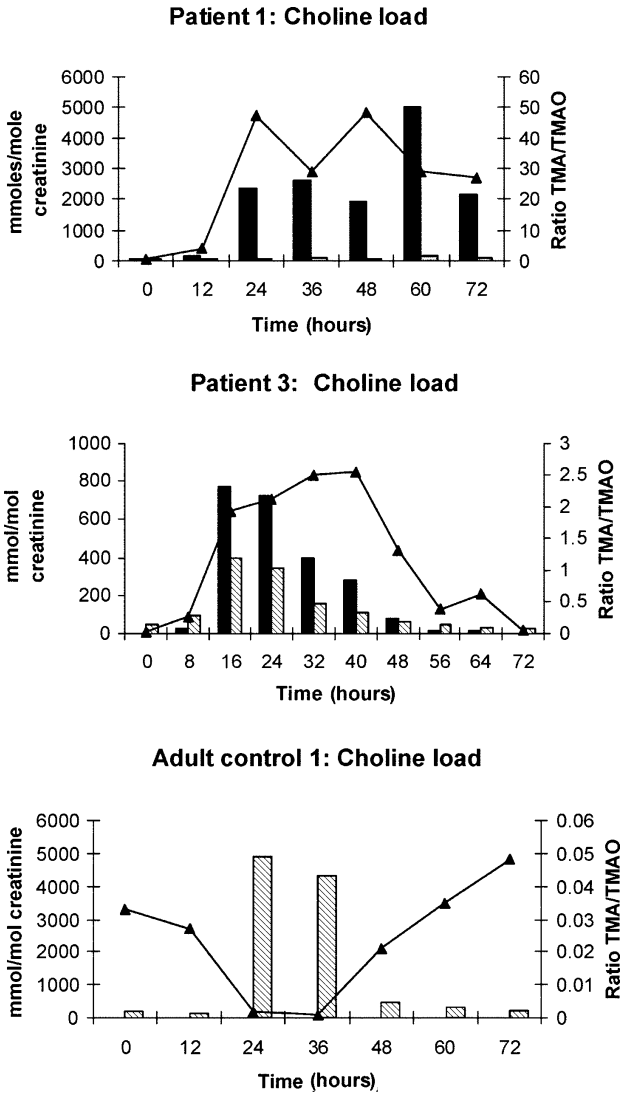

Fig. 2 Effects of oral loading with choline bitartrate on the urinary excretion of trimethylamine (TMA) and trimethylamine *N*-oxide (TMAO) in two children with trimethylaminuria (FMO3 deficiency) and in an unaffected adult. Children received 10 g choline bitartrate (39.5 mmol choline) and adults 15 g (59.3 mmol choline). Levels are expressed as mmol/mol creatinine. Note differences in scales used. Solid bars, TMA; tinted bars, TMAO; –▲–, TMA/TMAO ratio

excretion, with a corresponding rise in the TMA/TMAO ratio. This patient showed about 72% conversion of choline into TMA+TMAO, excreting more than 70% as TMA, suggesting very little residual FMO3 activity *in vivo*. TMA was still present in his urine more than 72 h after loading. Patient 3, in comparison, again showed a marked increase in the excretion of free TMA but with a significant rise in TMAO excretion; about 70% of the choline dose was converted into TMA+TMAO, with 59% being excreted as TMA, suggesting some residual activity of FMO3. Levels of both metabolites had returned to basal levels within 72 h of loading. Similar degrees of conversion of choline into trimethylamine were observed in other children studied. In contrast, the unaffected adult showed only a marked increase in TMAO excretion with virtually no TMA appearing; 72% of the dose was converted into TMA+TMAO with less than 0.5% as TMA.

Patient 1 showed an adverse clinical reaction to the 10 g choline bitartrate load, with fever and vomiting, and exhibited the same symptoms during episodes of excessive malodour. No adverse effects were observed in other patients or subjects given oral choline bitartrate loads. The amount of choline bitartrate was reduced to 2.5 g in subsequent choline loads in patient 1 while he was taking antibiotics.

Fish meal load test: Figure 3 shows the effects of feeding a marine fish meal to three children with trimethylaminuria and to an unaffected adult control patient. Patients with trimethylaminuria show an initial rise in TMAO followed by a marked rise in TMA that persists for more than 72 h after the meal. All three patients produced a maximal excretion of TMA at around 18 h post meal, with the levels achieved being comparable. No adverse effects of a fish meal (other than malodour) were observed in any of the children studied. The unaffected adult control patient showed only an increase in TMAO and no increase in TMA during the subsequent 72 h. The results show that much of the TMAO contained in marine fish is converted into TMA in the large bowel, the TMA being absorbed and, in the normal subject, re-oxidized in the liver to TMAO. The initial rise in urinary TMAO is presumably due to direct absorption of the intact compound from ingested fish prior to its reaching the colon, followed by rapid excretion into the urine.

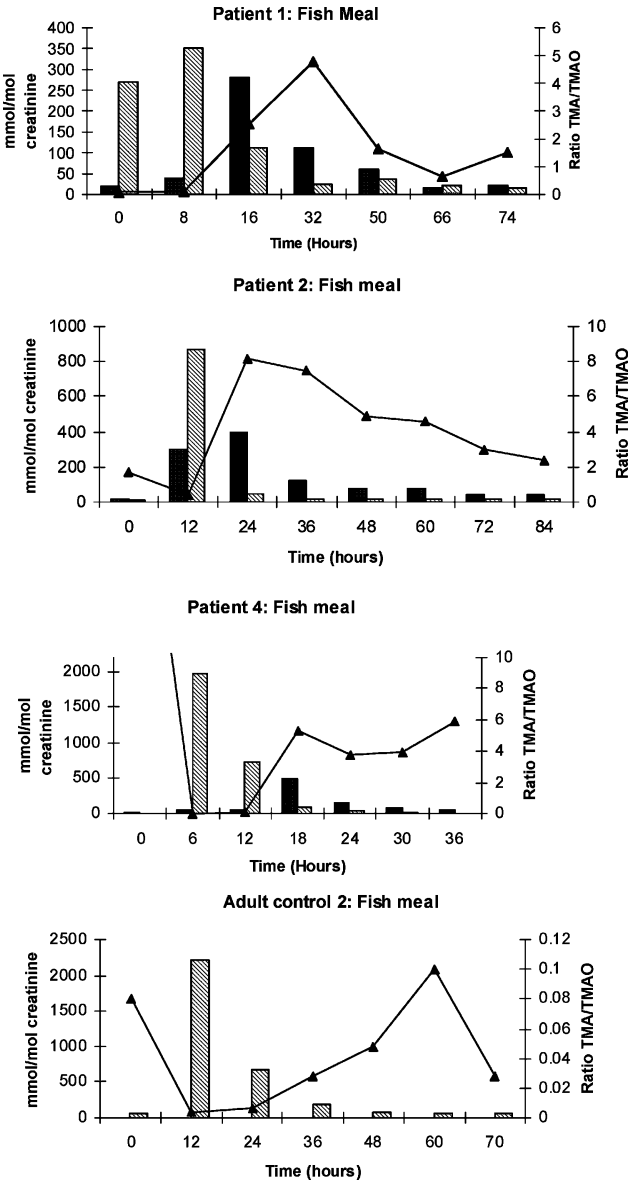

Fig. 3 Effects of a marine fish meal on the urinary excretion of trimethylamine (TMA) and trimethylamine *N*-oxide (TMAO) in three children with trimethylaminuria (FMO3 deficiency) and in an unaffected adult. Levels are expressed as mmol/mol creatinine. Note differences in scales used. Solid bars, TMA; tinted bars, TMAO; –▲–, TMA/TMAO ratio

Effect of antibiotics

The effects of antibiotics on trimethylamine production could be studied in detail in only two of the children. Figure 4 shows the effects of choline load before and during metronidazole and amoxicillin treatment in patient 1. There was a delay in peak excretion of TMA following choline loading while receiving both metronidazole and amoxicillin, with an overall modest reduction (approximately 20%) of TMA+TMAO excretion over the same timescale observed in choline loading under basal conditions. Similar results were observed in patient 5, with amoxicillin being apparently slightly more effective than metronidazole in reducing TMA excretion, the amount of choline converted into TMA reducing from 39% before antibiotic treatment to 31% while receiving metronidazole and to 24% while receiving amoxicillin. Patient 5 was also given a choline load test while on treatment with neomycin and receiving a low-choline and low-trimethylamine diet. Neomycin reduced her TMA production from choline to about 50% of basal production over

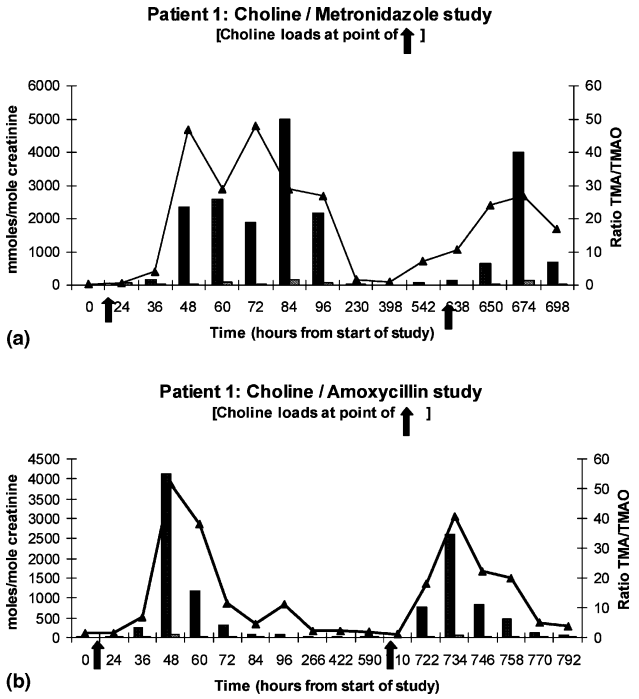

Fig. 4 Effects of oral loading with choline bitartrate on the urinary excretion of trimethylamine (TMA) and trimethylamine *N*-oxide (TMAO) in a child with trimethylaminuria (FMO3 deficiency) before and during treatment with metronidazole (a) and amoxicillin (b). Metronidazole treatment (7.5 mg/kg body weight twice daily for 1 week followed by 7.5 mg/kg per day for 3 weeks) commenced at about 100 h from the start of the study and continued throughout the second load test (a). Urine collections after 698 h of study (or more than 50 h after the second choline load) were incomplete. However, levels returned to basal values in due course as the effects of the load diminished. Amoxicillin treatment (125 mg three times daily for 1 week followed by 125 mg once per day for 3 weeks) commenced at about 100 h from the start of the study and continued throughout the second load test (b). The first choline load in each figure was given before antibiotic treatment, the second during treatment. 10 g choline bitartrate (39.5 mmol choline) was given in the first load test (a), 2.5 g (9.9 mmol choline) in subsequent loads (see text). Levels are expressed as mmol/mol creatinine. Note differences in scales used. Solid bars, TMA; tinted bars, TMAO; –▲–, TMA/TMAO ratio

a shorter timescale (Fig. 5), but with continued production of TMA (70% of TMA+TMAO) and moderate alleviation of the associated malodour.

Patient 6

Patient 6 presented with malodour and showed a consistently elevated TMA/TMAO ratio (Table 1). However loading both with a marine fish meal and with choline bitartrate resulted in a major increase in TMAO excretion, with only minimal increase in TMA and a sharp fall in the TMA/TMAO ratio (Fig. 6). She thus did not have a major problem in conversion of trimethylamine into trimethylamine *N*-oxide but continued to show elevated TMA/TMAO ratios and malodour under basal conditions while receiving a normal diet.

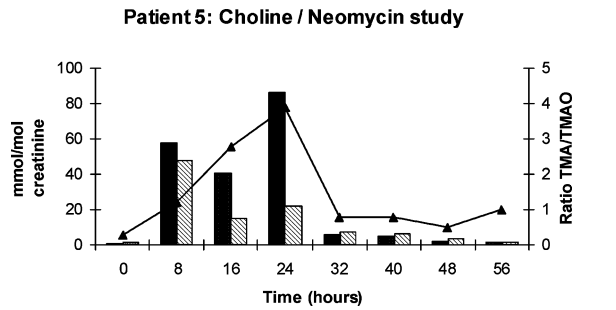

Fig. 5 Effects of oral loading with choline bitartrate on the urinary excretion of trimethylamine (TMA) and trimethylamine *N*-oxide (TMAO) in a child with trimethylaminuria (FMO3 deficiency) during treatment with neomycin. (Neomycin was administered 25 mg three times a day for 7 days prior to the load test and continued throughout the study period.) 2.5 g choline bitartrate (9.9 mmol choline) was given. Levels are expressed as mmol/mol creatinine. Note differences in scales used. Solid bars, TMA; tinted bars, TMAO; –▲–, TMA/TMAO ratio

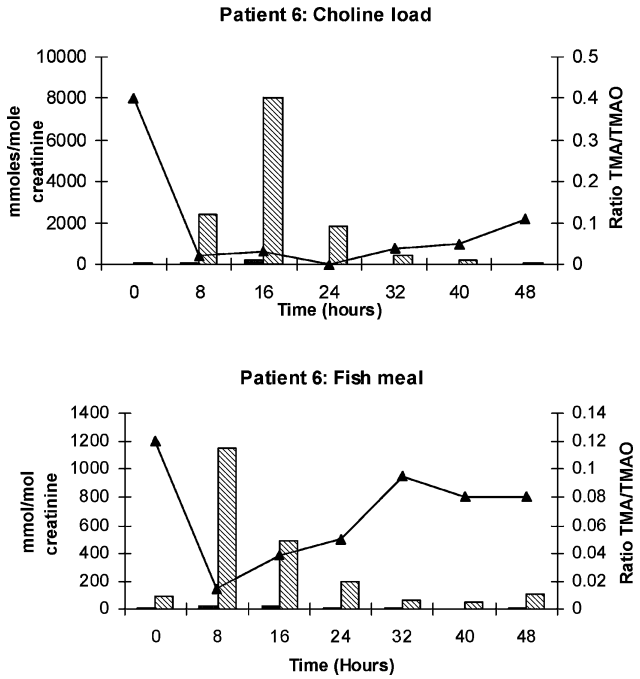

Fig. 6 Effects of oral loading with choline bitartrate (10 g; 39.5 mmol choline) and a marine fish meal on the urinary excretion of trimethylamine (TMA) and trimethylamine *N*-oxide (TMAO) in a child with trimethylamine malodour and *FMO3* mutations of uncertain functional significance. Levels are expressed as mmol/mol creatinine. Note differences in scales used. Solid bars, TMA; tinted bars, TMAO; –▲–, TMA/TMAO ratio

Mutation analysis

Subsequent to diagnosis from loading tests, mutation analysis was carried out on five of the six patients and their families by PCR amplification and fluorescent sequencing of all coding exons of the *FMO3* gene and adjacent intron sequences.

Table 2 *FMO3* mutations in children with trimethylaminuria

Patient	Ethnic origin	Identified mutation(s)
1	Scottish	Homozygous for P153L Father also heterozygous for E158K polymorphism
1a	Scottish	Homozygous for P153L Father also heterozygous for E158K polymorphism
2	Irish	Homozygous for P153L Mother also heterozygous for E158K polymorphism
3	English	Compound heterozygous for G193E and R483T Mother heterozygote for G193E
4	English	Not tested
5	Filipino	Homozygous for M82T (Murphy et al 2000)
6	Scottish	Compound heterozygote for two sequence variations of uncertain functional relevance: paternal intronic variant IVS5+10C>G (in *cis* with E158K polymorphism); maternal exonic variant c.441C>T

Genetic variants known or presumed to affect FMO3 function were identified in all patients in whom positive responses to loading tests had been observed (Table 2), with compound heterozygosity for two novel mutations being found in patient 3. Patient 6 was found to be a compound heterozygote for two novel sequence variations of uncertain functional relevance (Table 2). Mutation analysis in patient 5 has been described previously (Murphy et al 2000) and full molecular details on patients 1 to 4 and patient 6 will be presented elsewhere (Zschocke et al, in preparation). None of the heterozygous parents, siblings or other relatives of the affected children showed any clinical symptoms or malodour.

Discussion

The results presented here demonstrate the use of an oral load of choline (bitartrate) to provide confirmation of trimethylaminuria (presumed FMO3 deficiency) in affected children and a measure of any residual oxidative capacity. This is illustrated in Fig. 2, where patient 3 clearly shows significant oxidative capacity and may be presumed to be less affected by moderate amounts of choline in her diet. In contrast, patient 1 shows virtually no residual oxidative capacity and continued to excrete TMA more than 72 h after the load. Patient 1 also showed adverse reaction to choline loading, with fever and vomiting, and has also been observed to exhibit the same symptoms on a number of occasions during periods of excessive malodour. Adverse reactions to choline loading were not observed in other patients (all female) though they been observed in young adult male control subjects but not female control subjects (R.A. Iles and G.J. Snodgrass, unpublished). About 70% of an oral load of choline (bitartrate) is converted into TMA and TMAO both by affected children and by adult 'control' subjects, with 85–97% of TMA+TMAO occurring as TMA in affected children in comparison to <0.5% as TMA in adult 'controls'.

A marine fish meal is also clearly diagnostic in the affected patients (Fig. 3) and avoids the possibility of the adverse reaction to choline (none was observed in patient 1, for example). Marine fish contain relatively large amounts of trimethylamine *N*-oxide as a protein-stabilizing osmolyte (Seibel and Walsh 2002; Yancey et al 1982) and a marine fish meal provides a direct load of trimethylamine through conversion of TMAO in the ingested fish into free TMA by gut bacteria, followed by re-conversion to TMAO by hepatic *N*-oxidation. It thus provides a direct measure of the endogenous capacity for trimethylamine *N*-oxidation but possibly provides less differentiation between patients as some of the fish-derived TMAO may be absorbed prior to exposure to colonic bacteria so that TMA/TMAO ratios may require some care in interpretation. Fish meals are more acceptable to children and parents and the malodour in affected children is cleared more quickly than with a choline load test. Thus a meal of marine (sea) fish can be recommended as the simplest and most acceptable method for confirmation of the diagnosis in children with suspected trimethylaminuria. A recommended 'best-practice' approach to the diagnosis of trimethylaminuria is shown in Box 1.

Patient 1 is homozygous for the 'common' P153L mutation in the *FMO3* gene and showed virtually complete lack of residual TMA *N*-oxidative capacity on choline loading, consistent with a nonfunctional or absent FMO3 enzyme. The P153L mutation has previously been shown to abolish FMO3 catalytic activity *in vitro* (Dolphin et al 1997; Treacy et al 1998). Patient 2 is also homozygous for this mutation. The results of marine fish loading suggests that patients 1 and 2 have a similar severe reduction in FMO3 oxidative capacity. The homozygous M82T mutation in patient 5 has also been shown to abolish FMO3 catalytic activity *in vitro* (Murphy et al 2000), although choline loading suggests some residual oxidative capacity *in vivo* (Fig. 5). Patient 3, who is compound heterozygous for two novel mutations G193E (c.578G>A) and R483T (c.1448G>C), also showed considerable residual

Box 1 Diagnosis of trimethylaminuria

Fish load test

1. Administer a diet low in choline and trimethylamine for 3 days.
2. Collect one or more timed urine collections (bottles containing no preservative or dilute (4 mol/L) hydrochloric acid); measure volumes and store 20 mL aliquots at −20°C for analysis.
3. Feed a meal containing marine (sea) fish (e.g. cod and chips); collect all urine samples passed during the following 48 h or more, noting times of collections; measure volumes and store aliquots as above.
4. Analyse all samples for creatinine, TMA and TMAO; calculate TMA and TMAO concentrations in terms of mmol/mol creatinine and plot these and the TMA/TMAO ratio against time to determine response.

A child with trimethylaminuria (FMO3 deficiency) will show an initial rise in TMAO followed by a greater increase in TMA while TMAO concentrations fall (see e.g. Fig. 3). An unaffected child should show a marked rise in TMAO but will show virtually no increase in TMA concentrations.

Choline load test

1. Administer a diet low in choline and trimethylamine for 3 days.
2. Collect one or more timed urine collections (6 h or 12 h) with no preservative or into bottles containing dilute (4 mol/L) hydrochloric acid; measure volumes and store 20 ml aliquots at −20°C for analysis.
3. Give an oral choline load (as choline bitartrate, dose in table below) dissolved in water or orange juice (e.g. with breakfast) and collect all urine specimens passed during the following 72 h, noting times of collections; measure volumes and store aliquots as above.
4. Analyse all samples for creatinine, TMA and TMAO; calculate TMA and TMAO concentrations in terms of mmol/mol creatinine and plot these and the TMA/TMAO (or TMA/TMA+TMAO) ratio against time to determine response. Also calculate the total TMA and TMAO excreted by summation of the amounts in each urine collection post load and calculate the percentage of choline converted into TMA, TMAO and TMA+TMAO.

A child with trimethylaminuria (FMO3 deficiency) will show a rapid increase in TMA following the choline bitartrate load with a smaller rise in TMAO and an increase in the TMA/TMAO ratio (see e.g. Fig. 2). An unaffected child will show an increase in TMAO only, accompanied by a sharp fall in the TMA/TMAO ratio.

Choline load dosage table

Patient age (years)	Choline bitartrate (g)	Equivalent choline (mmol)
<4	2.5	9.9
4–6	4	15.8
7–9	6	23.7
10–14	10	39.5
>15	15	59.3

or 250 mg/kg body weight for all ages from 1 year (to a maximum of 15 g choline bitartrate)

N-oxidative capacity on choline loading (Fig. 2) and it may be presumed that these particular mutations result in formation of an FMO3 enzyme with reduced rather than absent function. Marine fish meal loading in patient 4 suggests this patient also has *FMO3* mutations resulting in some residual oxidative capacity, although unfortunately this could not be tested further.

Patient 6 is of considerable interest, consistently showing malodour and elevated urinary TMA/TMAO ratios under basal conditions but with a negative response to both choline (bitartrate) and marine fish meal loading, indicating that this child is able to completely oxidize exogenous TMA in the liver and should therefore have normal hepatic FMO3 activity. Nevertheless, she is apparently unable to fully metabolize TMA from other sources or in other organs. The girl is heterozygous for two novel sequence variations in the *FMO3* gene—a paternally inherited intronic variant IVS5+10C>G (in *cis* with E158K, a common polymorphism associated with a discrete reduction in enzyme activity *in vitro*), and a maternally inherited exonic variant c.441C>T (S147S). Both variants are predicted to leave the amino acid composition of the enzyme unchanged and would be regarded as

silent. However, it cannot be excluded that these variants affect mRNA splicing (possibly in some organs only) and may result in an FMO3 enzyme that shows reduced function under basal conditions that can be overcome at higher substrate levels. The possibility of deficient activity of other tissue-specific FMO isoenzymes contributing to a minor but clinically apparent trimethylaminuria and malodour under basal conditions also cannot be excluded. It is possible that some other patients with mild trimethylaminuria and *FMO3* variants (Zschocke et al 1999) may show similar responses to choline and marine fish loading.

Patients with trimethylaminuria may generally be managed by use of dietary restriction of foods with a high trimethylamine (*N*-oxide) or high choline content and use of soaps with a pH value of 5.5–6.5 to remove any traces of free trimethylamine from the skin. Marine (sea or saltwater) fish (including cephalopods and crustaceans) should especially be avoided, particularly deep-sea fish, in which the TMAO content is very high (Seibel and Walsh 2002). Foods with relatively high content of choline include eggs, liver, kidney and other offal, and peas, beans, peanuts, soya products and other legumes (see Box 2).

The choline in most foodstuffs is present in the form of phosphatidylcholine and may be of limited direct availability, although this may be altered by food processing. Unless the phosphatidylcholine is degraded and the choline is metabolized in the intestine, the amount of free trimethylamine released may be limited; thus in a normal healthy individual (without significant bacterial overgrowth), in whom most phosphatidylcholine is absorbed in the small intestine, the ingested amount of a particular food needs to be relatively high to provide an increased load of trimethylamine. Some studies have suggested that esterified choline in foods is not a precursor of trimethylamine (Zhang et al 1999), but the amounts of foodstuffs ingested were relatively small (227 g). Only minute amounts of trimethylamine are necessary in an individual with FMO3 deficiency to produce malodour and a small degree of metabolism of ingested esterified choline in an affected individual may have clinical consequences. Choline also occurs in brassicas, usually as aromatic esters that are easily hydrolysed by enterobacteria, with the choline being subsequently degraded to trimethylamine; these and their products (e.g. rapeseed products) may need to be avoided by some patients.

Zhang and colleagues (1999) suggested that carnitine (4-*N*-trimethylamino-3-hydroxybutyrate) was a precursor of trimethylamine, but in their studies used D,L-carnitine. Our studies of oral loading with L-carnitine in a patient with FMO3 deficiency showed no production of trimethylamine or trimethylamine *N*-oxide (Holmes et al 1997) and it is most likely that the increased trimethylamine (*N*-oxide) observed by Zhang and colleagues (1999) originated from D-carnitine metabolized by gut bacteria, consistent with their failure to observe increased TMA(O) excretion after ingestion of meats other than offal. It is, however, possible in some patients receiving chronic treatment with L-carnitine that their gut bacteria become adapted to utilize the 3-hydroxybutyryl moiety of L-carnitine, with cleavage of the trimethylamine moiety, resulting in increased production of trimethylamine. In such patients, any trimethylamine absorbed should normally be oxidized to TMAO in the liver, but if the patients concerned also have functionally important polymorphic variants of FMO3 activity, this could conceivably result in some malodour. It is of interest in this context that patients with medium-chain acyl-CoA dehydrogenase (MCAD) deficiency and with isovaleric acidaemia (disorders of medium-chain fatty acid metabolism) given L-carnitine consistently excrete much greater amounts of TMAO than other patients with different metabolic disorders treated similarly (Holmes et al 1997). The cilia of the intestinal wall depend on short- and medium-chain fatty acids for their function; in the presence of a generalized disorder of

Box 2 Treatment of trimethylaminuria

1. Diet low in choline and trimethylamine:
   Avoid foodstuffs with a *high* choline content including eggs, liver, kidney and other offal, soya bean products, beans, peas, peanuts and other legumes, brassicas including rape products.
   (Do not over-restrict choline intake in young developing infants and children (or in women during pregnancy or lactation) and supplement with folate if required, through diet if possible, e.g. dark green leaf vegetables, fortified bread and cereals, orange juice etc.)
   Avoid marine (sea or saltwater) fish (including cephalopods and crustaceans), especially deep-sea fish. (Freshwater fish may be eaten freely.)
2. Use of soaps and body lotions with a low pH (5.5–6.5)
3. Elimination of excess intestinal TMA production where necessary (e.g. for clinical or social reasons):
   (a) Copper–chlorophyllin tablets (available from pharmacies with health food counters) to modulate gut flora activity and complex TMA.
   (b) Intermittent oral antibiotics (e.g. metronidazole or a broad-spectrum antibiotic) to reduce and modulate gut flora activity (as prescribed by a physician).

medium-chain fatty acid metabolism, activity of these cilia may be reduced, resulting in malabsorption and bacterial overgrowth.

The enterobacterial production of trimethylamine has led to the suggestion and use of antibiotics as a form of treatment for patients with trimethylaminuria. Following a brief report of the effects of neomycin on one patient (Danks et al 1976), some short-term but clinically effective studies have been made with metronidazole and neomycin in patients with trimethylaminuria (Fraser-Andrews et al 2003; Treacy et al 1995). The observations reported here show that use of antibiotics may to a limited extent reduce the production of trimethylamine from choline as precursor and also slow the rate of production. The antibiotics used here included metronidazole, amoxicillin and neomycin, all with differing target organisms. Neomycin was the most effective in reducing TMA production from choline, with amoxicillin being rather more effective than metronidazole. Neomycin is recognized clinically for its efficacy in 'sterilizing' the intestinal flora more effectively than amoxicillin and metronidazole has anti-anaerobic activity only. Thus a broad-spectrum antibiotic would be of more value in treatment of this condition. However, none of the antibiotics studied completely prevented formation of trimethylamine from choline, the most effective (neomycin) resulting in a 50% reduction in excretion of TMA. Use of these antibiotics does not appear to prevent or produce major reductions in colonic bacterial degradation of choline to trimethylamine but they may extend the process over a longer timescale, thereby moderately alleviating the malodour produced. This is consistent with these antibiotics not removing all metabolically active bacteria but modulating their activity, analogous to similar observations in patients with disorders of propionate metabolism given metronidazole (Boriello et al 1987). Antibiotics should thus be regarded as an adjunct to dietary management of patients with FMO3 deficiency (or 'primary' trimethylaminuria). They could be of especial value during periods of excessive stress, exercise, infection, emotional upset and menstruation, when trimethylamine production appears to increase, and when dietary restriction needs to be relaxed for any reason. Antibiotics would also be of value in other patients with secondary trimethylaminuria associated with bacterial overgrowth syndromes. The possibility of development of resistance to metronidazole (Treacy et al 1995) may be overcome by alternating bi-weekly therapy of different antibiotics. Modulation of the enterobacterial production of trimethylamine and sequestration of free trimethylamine by use of orally administered chlorophyllin–copper complex has also been suggested as an adjunct therapy (Yamazaki et al 2004). The optimal evidence-based treatment of trimethylaminuria is summarized in Box 2.

In our experience, (primary) trimethylaminuria (FMO3 deficiency due to *FMO3* mutations) in children is rare. We have shown here that loading with a meal of marine fish provides a simple and acceptable method for confirmation of diagnosis of suspected trimethylaminuria (FMO3 deficiency). Oral loading with choline bitartrate allows estimation of residual oxidative capacity *in vivo* and is a useful adjunct to molecular studies. Treatment of trimethylaminuria should be primarily by dietary restriction, especially of marine (sea or saltwater) fish and foods with a high choline content, with occasional and periodic use of antibiotics and copper–chlorophyllin where necessary to reduce and modulate gut flora activity.

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