Urinary 3-methylhistidine excretion in man: the role of protein-bound and soluble 3-methylhistidine

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(*Received 11 August 1982 – Accepted 11 October 1982*)

1. The influence of dietary meat and meat stock intake on urinary excretion of 3-methylhistidine (3MH) was examined in human adults.

2. In the absence of 3MH ingestion for 48 h, the study subjects adjusted to an intrinsic urinary 3MH: creatinine value. If the meat and meat stock-free diet was maintained on subsequent days, only minute diurnal variations occurred, and the values of random urine samples during the day were representative of the 24 h 3MH: creatinine value.

3. The mean 3MH: creatinine value (SD) for a group of adults (n 7) was $0{\cdot}105 \pm 0{\cdot}023$ (μmol of 3MH/mg creatinine), which is approximately 35% lower than the corresponding value in healthy growing infants ($0{\cdot}148 \pm 0{\cdot}039$) (Seashore *et al.* 1981).

4. Ingestion of meat soup and meat causes different patterns of urinary excretion of 3MH which are consistent with the finding that meat extracts, such as soup and stock, contain considerable amounts of 3MH. The 3MH contents of beef, chicken and turkey were $3{\cdot}8 \pm 0{\cdot}15$, $3{\cdot}0 \pm 0{\cdot}09$ and $2{\cdot}3 \pm 0{\cdot}29$ μmol/g dry wt meat respectively. All three meats contained a water-soluble 3MH-fraction (% total 3MH: beef 8, chicken 21, turkey 23). Amino acid analysis of the soluble fraction with or without hydrochloric acid hydrolysis demonstrated free 3MH in chicken and turkey (5·2 and 2·8% of the total respectively) but not in beef.

5. Patients undergoing urinary 3MH measurements should maintain a diet that is free not only of solid meats, but also of meat stock. The ingestion of commercial food products (e.g. frozen or canned meals, sauces, pizza, etc.) may impair the validity of such measurements because of their meat-stock content.

6. A dietary regimen is presented which is based on a shorter 12 h urine collection. The shorter collection time is satisfactory in the light of the steady rate of 3MH-excretion after 2 d of a diet free of meat and meat stock.

The unusual amino acid 3-methylhistidine (3MH) is present as a single residue in the peptide chains of myosin and actin (Huszar & Elzinga, 1971; Elzinga *et al.* 1973). Upon breakdown of these muscle proteins, 3MH is not re-utilized, but is rapidly and quantitatively excreted in the urine (Young *et al.* 1973; Long *et al.* 1975). It is increasingly apparent that the measurements of urinary 3MH excretion and of the ratio of urinary 3MH:creatinine (cr), which is proportional to the muscle mass (Graystone, 1968), are valuable in the assessment of protein catabolism, although the relative contributions of 3MH from actin and myosin in different tissues are under investigation (Millward *et al.* 1980; Harris, 1981). The 3MH:cr value has already been successfully validated in various animal and human studies (e.g. Wannemacher *et al.* 1975; Gross *et al.* 1978; Young & Munro, 1978; Griggs *et al.* 1980; Elia *et al.* 1981; Long, Birkhahn, Geiger, Betts *et al.* 1981; Seashore *et al.* 1981). Controversy persists, however, with respect to basal or normal values for urinary 3MH excretion and 3MH:cr values in human subjects (reviewed in part by Lukaski *et al.* 1981). We believe that most of the inconsistent values are due to the vague definition of the meat-free or flesh-free diet ingested by the study participants during the experiments. Although interference with 3MH:cr measurements as a result of ingestion of meat has been detected by several authors (Tomas *et al.* 1979; Griggs *et al.* 1980; Long, Birkhahn, Geiger, Betts *et al.* 1981; Lukaski *et al.* 1981), the quantitative implications of small diet errors or inadvertent ingestion of meat-free foods which actually contain meat stock have not been recognized.

In examining this question, we found that (1) wide fluctuations in urinary 3MH:cr values occur during the day in patients on their regular diets; (2) a period of at least 2 d abstinence from meat and meat stock is necessary before dietary 3MH clears and subjects attain a basal level of 3MH excretion; (3) after meat ingestion 3MH quickly appears in the urine, which indicates that some of this amino acid is present in a soluble protein-bound fraction of meats. This observation has been confirmed by measurement of meat extracts and of urinary 3MH excretion in experimental subjects; (4) various meat-free or flesh-free foods may actually contain substantial amounts of 3MH from the added meat stock; and (5) patients who participate in 3MH excretion studies should ingest well-defined diets. We have devised a meat-free, protein-substituted diet that satisfies both the protein and energy requirements of patients.

EXPERIMENTAL DESIGN AND METHODS

3MH:cr values in fractional urines. Adults on their normal diet were asked to collect 24 h urine samples and to save approximately 2 ml urine from three to four voids during the day.

Expt 1. The 8 d experiment was carried out as follows. Healthy adult subjects were studied (three females, four males, with weights (mean ± SD) 54 ± 5 and 81 ± 5 kg, respectively) age between 25 and 60 years. Subjects ingested a meat-free, protein-substituted diet until 18.00 hours on the fourth experimental day, when six of the seven ate roast beef and roast turkey until satisfied. Subject D (see Table 1, p. 290) ate cold-cuts. On days 5–8, the subjects resumed their usual diets. Urine specimens (24 h) were collected for 8 d. Two of the seven subjects also collected fractional urines (1–2 ml) on days 3, 4, 5 and 6. All urine samples were stored at −20°.

Expt 2. Soup–meat experiment. The experiment was carried out in two men (ages 40 and 25 years, weights 84 and 75 kg). The subjects remained on the meat-free, protein-substituted diet for 11 d, except for ingesting soup in the late evening of day 5 and meat in the evening of day 8. Soup and meat were prepared as follows. Lean beef (1·68 and 1·5 kg or 2% of participant's body-weight) was sliced to a thickness of approximately 10 mm, and two consommes were cooked in 3 vol. of water overnight with vegetables and spices. The soups were concentrated by boiling, then refrigerated, with the meat, until consumption. Daily 24 h urine collections were carried out for eleven study days; urine samples were stored at −20°.

Determination of soluble and protein-bound 3MH in meat. Chicken breast, turkey breast and round roast of beef were used. Six independent determinations in each source were carried out as follows: (*a*) aliquots of the meat were hydrolysed in 6 M-hydrochloric acid and total 3MH per g muscle weight was determined; (*b*) further samples of the meats were chopped and cooked (3 vol of water at 90° for three periods of 6 h). The suspensions were centrifuged, pooled, dried and hydrolysed in 6 M-HCl. The meat pellets were also hydrolysed in 6 M-HCl. Both the soup and meat samples had been subjected to 3MH determinations. Thus, total 3MH as well as soluble and insoluble 3MH were determined for all three meats. Samples of the soluble fractions were also applied to the amino acid analyser without acid hydrolysis to determine free *v.* peptide-bound 3MH. Dry weights of the meats were determined after lyophilization (48 h).

3MH content of foods. Samples of pizza sauces, various soups and gravies and sauces of baked beans were hydrolysed in 6 M-HCl and subjected to 3MH determination.

Amino acid analysis. Urine and food samples were hydrolysed in 6 M-HCl acid after evacuation at 100° for 18 h. Each sample was dried and dissolved in 0·2 M-citrate buffer, pH 2·2, filtered, and applied to a Beckman 119 automated amino acid analyser. The elution

programme for the basic and methylated amino acids was developed as previously published (Huszar & Elzinga, 1971). Recovery of 3MH (> 95%) was monitored throughout the study.

Creatinine determinations. Jaffe's (1886) procedure was used. Urine (1 ml) was mixed with 8 ml picric acid (1 g/l) and 0·06 ml sodium hydroxide (100 g/l). After colour development for 15 min, absorbance was determined at 520 mm and compared to a standard curve.

RESULTS

3MH:cr values in fractional urines

In preliminary experiments, 3MH:cr ratios were measured in urine samples of experimental subjects consuming their regular diet throughout the day and in 24 h urine collections from the same day. Ratios varied by as much as 200% among the samples of spot urines and the 24 h urine collections (e.g. 0·280, 0·542, 0·260 in the fractional urines *v.* 0·408 for 24 h collections; 0·264, 0·318, 0·410 in the fractional urines *v.* 0·352 for 24 h collections; 0·146, 0·180, 0·340 in the fractional urines *v.* 0·210 for 24 h collections).

Expt 1. Influence of meat ingestion on 3MH:cr value

The 8 d experiment included 3 d of a meat-free diet, 1 d of a meat load, and 4 d of a regular diet.

(*a*) *3MH:cr values* showed a well-defined pattern (Table 1). In the absence of meat intake, ratios declined in the first 3 d until they reached (mean ± SD) 0·105 ± 0·023. After meat ingestion on days 4 and 5, the mean 3MH:cr value increased by three times the basal value. If the meat-free diet was maintained for several days, only minute variations occurred in the daily 3MH:cr value of a particular individual. The importance of a meat-free diet is well demonstrated in terms of the extent of elevation of 3MH:cr following meat ingestion on day 4. The individual differences in the increases were due to variations in the amounts and in the preparation of the poultry and beef ingested. On days 1, 6, 7 and 8, reflecting the subjects' *ad lib.* diet, the mean 3MH:cr values were similar.

(*b*) *Daily 3MH and creatinine excretion* (Table 2) values follow the pattern of 3MH:cr values. 3MH excretion declined in the first 3d of the experiment and increased after meat ingestion on the 4th and 5th days. The mean (± SD) 3MH excreted on day 3 by this study group was 138 ± 51 μmol/24 h. When the subjects' weights were considered, mean (± SD) 3MH excretion was 1·95 ± 0·44 μmol (2·15 for men, 1·68 for women)/kg body-weight per 24 h. The latter value was similar to those reported by Young *et al.* (1973), Gross *et al.* (1978), Griggs *et al.* (1980) and Winterer *et al.* (1980).

Creatinine excretion during the 8 d period did not change considerably (Table 2). The excretions were somewhat lower on day 3, and somewhat higher on days 6 and 7, than the 8 d mean value. The slight increase in cr excretion due to meat ingestion was delayed compared to the 3MH excretion. The mean cr excretion for the group was 22 mg/kg body-weight per 24 h, which corresponds well with values published by other investigators (McKeran *et al.* 1978; Long, Birkhahn, Geiger, Betts *et al.* 1981).

Expt 2. The soup-meat experiment

The rapid rise in 3MH:cr values after the meat meal suggested that meats contain a soluble pool of 3MH that is quickly absorbed and appears in the urine before the digestion of muscle proteins. Thus, Expt 2, consisting of four meat-free days, one soup day, two meat-free days, one meat day and three meat-free days, was performed with daily 3MH and cr measurements throughout. The 3MH:cr values were initially high and stabilized at the basal level by day 3 (Table 3). After the consumption of soup on day 5, 3MH:cr values and 3MH excretion increased on day 6 and declined on days 7 and 8. After the ingestion

Table 1. *Expt 1. Influence of meat ingestion on 3-methylhistidine:creatinine values (μmol/mg) in an 8 d experiment*

Day of experiment... Subjects	1	2	3	4	5	6	7	8
A	0·224	0·146	0·100	0·373	0·436	0·255	0·248	0·130
B	0·197	0·149	0·107	0·142	0·325	0·170	0·198	0·191
C	0·274	0·099	0·126	0·490	0·429	0·288	0·326	0·556
D	0·308	0·155	0·062	0·192	0·587	0·418	0·301	0·256
E	0·276	0·220	0·119	0·204	0·141	0·185	0·165	0·155
F	0·473	0·184	0·094	0·562	0·270	0·171	0·295	0·238
G	0·335	0·172	0·131	0·171	0·315	0·169	0·201	0·196
Mean ± SD	0·298 ± 0·09	0·160 ± 0·037	0·105 ± 0·023	0·304 ± 0·169	0·357 ± 0·142	0·236 ± 0·092	0·246 ± 0·063	0·246 ± 0·143

Table 2. *Expt 1. Influence of meat ingestion on the excretion of 3-methylhistidine (μmol/24 h) and creatinine (mg/24 h)*

Day of experiment... Subjects	1	2	3	4	5	6	7	8
				3-Methylhistidine				
A	301	256	184	485	669	573	528	199
B	302	216	149	285	476	355	413	334
C	342	119	119	745	571	363	463	551
D	246	141	78	209	1067	520	332	299
E	629	452	158	382	305	338	351	362
F	895	346	208	1148	628	377	547	518
G	434	184	71	136	172	138	225	217
Mean ± SD	449 ± 233	244 ± 118	138 ± 51	484 ± 354	555 ± 287	380 ± 140	400 ± 114	353 ± 137
				Creatinine				
A	1343	1753	1840	1300	1529	2245	2124	1486
B	1532	1449	1392	2000	1460	2090	2088	1746
C	1248	1202	944	1519	1329	1261	1418	991
D	798	909	1258	1084	1814	1242	1102	1166
E	2278	2054	1327	1870	2159	1819	2127	2338
F	1892	1880	2212	2040	2318	2197	1848	2169
G	1295	1069	541	795	545	815	1116	1104
Mean ± SD	1484 ± 479	1473 ± 435	1359 ± 549	1515 ± 480	1593 ± 582	1667 ± 561	1689 ± 467	1571 ± 532

Table 3. *Expt 2. Influence of soup–meat ingestion on urinary creatinine (cr) and 3-methylhistidine (3MH) in an 11 d experiment*

(Subjects consumed a meat-free protein-substituted diet for 11 d except for ingesting soup on day 5 and meat on day 8)

Subject	Day of experiment	Volume (ml)	cr (mg)	3MH (μmol)	3MH:cr value (μmol/mg)
A	1	1300	1860	822	0·442
	2	1100	1650	346	0·210
	3	630	1525	208	0·136
	4	990	1614	221	0·137
	5	1050	1796	232	0·129
	6	810	1725	327	**0·190**
	7	970	1552	198	0·128
	8	1440	1613	162	0·101
	9	1270	1740	398	**0·229**
	10	1200	1642	241	**0·146**
	11	1010	1626	199	0·123
B	1	1000	1360	496	0·365
	2	1950	1482	303	0·205
	3	660	1129	154	0·137
	4	830	1429	211	0·128
	5	1000	1700	207	0·122
	6	1650	1914	371	**0·194**
	7	1318	1843	221	0·120
	8	1130	1684	188	0·112
	9	1080	1750	420	**0·240**
	10	1010	1576	277	**0·176**
	11	880	1560	198	0·127

of meat on day 8, 3MH excretion increased on days 9 and 10. Both 3MH:cr and 3MH excretion declined to basal levels by day 11, the 3rd day after meat consumption.

As soup and meat for each subject had been prepared from meat weighing 2% of his body-weight, the changes in 3MH:cr values may be comparable. Values for 3MH:cr on day 6 were 0·190 *v.* 0·194, and on day 9 were 0·229 *v.* 0·240 in the two subjects. For days 3, 4, 5 and 11, each of which followed the 48 h meat-free period, the mean 3MH:cr value was 0·129. After soup ingestion, 3MH:cr values increased for 1 d, while they increased for 2 d after meat ingestion. In further experiments we determined whether the rapidly-rising soluble 3MH was present in the urine as free 3MH or as 3MH-containing peptides.

3MH content of various meats

3MH content was measured on HCl-hydrolysed samples in turkey breast, chicken breast and beef round. The 3MH concentrations varied by species: $3{\cdot}8 \pm 0{\cdot}15$, $3{\cdot}0 \pm 0{\cdot}09$ and $2{\cdot}3 \pm 0{\cdot}29$ μmol 3MH/g per dry weight in turkey, chicken and beef respectively. The distribution of water-soluble *v.* protein-bound 3MH was also different in the three meats. The soluble (in the cooking liquid) *v.* meat-bound 3MH (expressed as % of the total) was beef 8–92, chicken 21–79, turkey 23–77. The poultry meats contained much higher percentages of soluble 3MH. Amino acid analysis of the soluble fraction with or without HCl hydrolysis gave the amount of 3MH present as free 3MH *v.* peptide-bound 3MH, either in small digestion products of actin or myosin or in the 3MH-containing dipeptide, balenine (Harris & Milne, 1980). In chicken, 25% of soluble 3MH was free 3MH (5·2% of the total in muscle), in turkey the value was 12% (2·8% of total 3MH), whereas in beef none of the 8% soluble 3MH appeared as free 3MH before HCl hydrolysis.

Table 4. *3-Methylhistidine (3MH) collection and diet outline for metabolic studies influencing 3MH measurements*

This is a meat-free, protein-substituted diet. The experimental period is 3 d.
Please observe the following schedule:

Night before		You may eat moderate amounts of meat
Day 1	Breakfast	
	Lunch	No meat
	Dinner	
Day 2	Breakfast	
	Lunch	No meat
	Dinner	
Day 3	Breakfast	
	Lunch	No meat
	Dinner	Have dinner after 18.00 hours. Before dinner void one more time. Stop collecting and you may resume your regular diet.

You should ingest approximately 80 g protein/d. The following amounts of non-meat foods represent one-fourth of your daily protein: three eggs, 85 g cheese, 0·85 l milk (three cups), 170 g cottage cheese, 6 tablespoons peanut butter. Any combination of four portions is satisfactory. For example:

(*a*) Three eggs, 0·85 l milk, 170 g cottage cheese, 85 g cheese.
(*b*) Six eggs, 6 tablespoons peanut butter, 0·85 l milk.
(*c*) 1·7 l milk, three eggs, 170 g cottage cheese.
(*d*) 340 g cottage cheese, six eggs.

Feel free to select vegetables, fruits, breads, cereal, pastry, and other non-meat products as desired. Please do not eat prepared soups, frozen meals, pizza or processed food of any kind, as they may contain meat juices.

3MH content of meat-free foods and diet protocol for 3MH:cr measurements

In the course of our metabolic studies, and in reviewing results from other laboratories, we found that subjects on meat-free or flesh-free diets sometimes showed unexpectedly high urinary 3MH:cr values. In investigating the 3MH content of the foods ingested by our study subjects, we found that a number of the supposedly meat-free processed foods (e.g. baked beans, cheese pizza, vegetable soup, mushroom gravy) contained considerable amounts of 3MH from the added meat stock. Some examples (expressed as nmol 3MH/100 g food) include brown sauce, 416, mushroom gravy 1120, won ton soup 340, chunky vegetable soup 672, baked beans 516, pizza and spaghetti sauces 300–700, consomme (home-made) 2200. To eliminate this problem with self-selected meat-free diets, we designed a protein-substituted, meat and meat stock-free diet that our experimental subjects (Huszar & Golenwsky, 1978; Huszar *et al.* 1982) used successfully (Table 4).

DISCUSSION

Since 3MH has been recognized as a unique marker of protein catabolism, the 24 h 3MH measurement has been utilized in a number of clinical studies of hospitalized or out-patient subjects (for example, see Wannemacher *et al.* 1975; Gross *et al.* 1978; Young & Munro, 1978; Griggs *et al.* 1980; Long, Birkhahn, Geiger & Blakemore, 1981; Elia *et al.* 1981; Seashore *et al.* 1981). Certain patients were given either a meat-free diet or specified amounts of dietary meat, whereas others received intravenous alimentation. The influence of dietary meat intake on urinary 3MH excretion was not considered in some of the studies. An early report (Block *et al.* 1965) failed to find a close relationship between these two factors. We have measured urinary 3MH:cr values in evaluating the metabolic state of infants (Seashore *et al.* 1981) and of diabetic (Huszar *et al.* 1982) and obstetrical patients (Huszar & Golenwsky, 1978). The infants (ingesting formula or mother's milk)

presented no problems with respect to diet, but with the adult outpatients, it has been difficult at times to obtain reliable serial measurements. In some cases, we found unexplained high 3MH:cr values, even though we were certain that the subjects abstained from meat.

The relationship between dietary meat intake and variability in urinary 3MH excretion and 3MH:cr values was demonstrated in the fractional urine samples and in the 8 d experiment in the present study. During the first 2 d of the meat-free diet, the 3MH:cr values of the seven subjects decreased to the basal level. The amounts of meat ingested before commencement of the meat-free diet did not influence the 48 h period necessary to reach endogenous 3MH excretion. For instance, the 3MH:cr value for subject F declined from 0·473 to 0·094. We found, in similar studies, that if the meat-free diet was maintained for several days longer, only minute variations occurred in the daily 3MH:cr values. In the absence of meat or meat-stock ingestion, the variations in 3MH:cr value also diminished, and the ratios in fractional urines during the day became true reflexions of the 24 h value.

The mean basal ($\pm$SD) 3MH:cr value for the experimental group was $0{\cdot}105 \pm 0{\cdot}023$ μmol 3MH/mg cr. This value of approximately 0·110 was confirmed in our other studies (Huszar *et al.* 1982) and was approximately 35% lower than the corresponding value for healthy, growing, premature infants ($0{\cdot}148 \pm 0{\cdot}039$; Seashore *et al.* 1981). We believe that this phenomenon is related to the higher rate of protein turnover in growing infants. As found by other investigators, there were also sex-related differences in 3MH-excretion when expressed as μmol/3MH per kg body-weight per day (2·15 for men, 1·68 for women). In light of these developmental and sex-related differences, as well as the age-related changes reported by others (Bilmazes *et al.* 1978; Tomas *et al.* 1979), we have used control subjects matched for age and sex in our recent studies.

Another notable observation was the sudden rise in 3MH excretion shortly after meat ingestion. Measurements of spot urine samples indicated a two- to threefold increase in 3MH:cr value in urine samples after the ingestion of meat on the evening of the 4th day of the 8 d experiment. This rapid rise, which is not consistent with proteolytic digestion of actin and myosin, indicated the presence of a soluble 3MH fraction in the meat. Soluble 3MH is presumably absorbed and excreted quickly, whereas protein-bound 3MH appears in the urine only after the meat has been digested.

The existence of soluble and protein-bound 3MH was demonstrated by the excretion patterns of the soup–meat experiment. On day 5, after the ingestion of soup, the 3MH excretion rose briefly in the two subjects: from 203 and 197 μmol/24 h (the average of days 3, 4, 5, 7, 8 and 11) to 327 and 371 μmol/24 h respectively. After meat ingestion on day 8 the ratios were higher: 398–420 and 241–277 μmol/24 h on days 9 and 10 respectively. These findings demonstrate that cooked-meat products lose part of their 3MH content to the cooking liquid; hence meat stock contains 3MH.

The 3MH content of meats, as well as the distribution of soluble *v.* protein-bound 3MH, differs in the various species. The 3MH content of chicken (3·0 μmol/g dry weight) corresponds well with recent values (Hillgartner *et al.* 1981). The soluble 3MH fraction is approximately three times higher in poultry than in beef (22 *v.* 8%). Free 3MH is not present in beef. The free 3MH content of chicken is higher than that of turkey (5·2 *v.* 2·8%). Free 3MH represents catabolic products, as simple amino acids are not substrates for methylating enzymes (Young *et al.* 1972; Hillgartner *et al.* 1981). The 3MH liberated by HCl hydrolysis orginates in soluble peptides that are either digestion products of actin and myosin or of the β-alanine-3MH dipeptide, balenine. The balenine contents of various meats have been recently reported (Harris & Milne, 1980, 1981).

Several meat-free food products enriched with meat-stock (primarily from chicken) contained 3MH, due to the soluble 3MH fraction. Although this 3MH may not be a major component in some foods (e.g. baked beans, won ton soup), others (e.g. consomme or pizza

sauces) may contain substantial amounts of 3MH and thus interfere with the validity of the meat-free diets. Also, the variability of the stock content of various products (e.g. pizza sauce) makes the urine collections unpredictable. It was reassuring to know, however, that none of the three commercial vegetarian food products contained 3MH.

Our meat-free, protein-substituted dietary regimen is useful, although the participants are limited in the self-selection of foods. The 60 h programme includes a 48 h meat-free period and a 12 h urine collection for the measurements. The regimen is well-defined, and the protein content is adjustable for special needs (e.g. diabetes or pregnancy). The shorter urine collection increases compliance. The validity of the 3MH:cr ratio is assured by the 48 h meat-free period and the programme allows the subjects to consume meat for dinner on the third experimental day.

This research has been supported by the NICHD (Grant HD-12183).

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Printed in Great Britain