Research Article

Cancer Epidemiology, Biomarkers & Prevention

Urinary Biomarkers of Meat Consumption

Amanda J. Cross, Jacqueline M. Major, and Rashmi Sinha

Abstract

Background: Meat intake has been positively associated with incidence and mortality of chronic diseases, including diabetes, heart disease, and several different cancers, in observational studies by using self-report methods of dietary assessment; however, these dietary assessment methods are subject to measurement error. One method to circumvent such errors is the use of biomarkers of dietary intake, but currently there are no accepted biomarkers for meat intake.

Methods: We investigated four analytes (creatinine, taurine, 1-methylhistidine, and 3-methylhistidine) specifically found in meat and excreted in urine. Twenty-four–hour urine samples were collected from 17 individuals on controlled diets containing varying levels of meat: vegetarian (0 g/d), low red meat (60 g/d), medium red meat (120 g/d), and high red meat (420 g/d), as part of two randomized crossover feeding studies.

Results: When compared with the low red meat diet or the vegetarian diet, the urinary levels of all four analytes were significantly higher in urine samples collected after 15 days of a high red meat diet ($P < 0.0001$). Only urinary 1-methylhistidine and 3-methylhistidine were statistically significantly different for every diet type, increasing as the amount of meat in the diet increased ($P < 0.01$ for 1-methylhistidine and $P < 0.05$ for 3-methylhistidine). Furthermore, urinary excretion of 1-methylhistidine and 3-methylhistidine elevated with increasing meat intake in every individual.

Conclusion: Urinary 1-methylhistidine and 3-methylhistidine may be good biomarkers of meat intake.

Impact: To determine the public health impact of red meat on cancer risk, biomarkers are crucial to estimate true intake; these potential biomarkers should be further investigated in free-living populations. *Cancer Epidemiol Biomarkers Prev; 20(6); 1107–11. ©2011 AACR.*

Introduction

Meat intake has been associated with an elevated risk of several chronic diseases, including diabetes (1), heart disease (2), and cancers of the colorectum, stomach, esophagus, prostate, breast, and pancreas (3), as well as all-cause mortality (4); although there are some inconsistencies in the data. Observational epidemiologic studies, in which diet is usually assessed by self-administered food frequency questionnaires, are associated with measurement error; therefore, the public health impact of a diet high in red and processed meat may have been underestimated. Currently, there are no available biomarkers of meat to provide a more accurate measure of intake that is independent of memory of the subject and ability to describe foods consumed. We identified 4 analytes that are all present in meat and excreted in urine: creatinine (5) and 3-methyhistidine (6) are both released on muscle or protein breakdown; and taurine (7) and 1-methylhistidine (8) are mainly from foods of animal origin and meat, respectively. In this study, we investigated these 4 analytes as potential biomarkers of meat intake.

Materials and Methods

We analyzed 24-hour urine samples from 2 randomized, crossover, dietary studies; detailed methodology for these feeding studies have previously been described (9). In brief, healthy male volunteers, age range of 24 to 74 years, were recruited to live in a metabolic suite where all food and drinks were provided. Each volunteer signed a consent form after receiving a detailed explanation of the study protocol.

In study I, a 2-way crossover study, 9 volunteers were randomized to the following 2 diets: a 60 g/d red meat and a 120 g/d red meat diet; the protein contents of these diets were 63 and 77 g/d, respectively. In study II, a 3-way crossover study, 8 volunteers were randomized to each of 3 diets: a 60 g/d red meat; 420 g/d red meat; and a high-protein vegetarian diet (0 g/d meat), in which meat was substituted with egg, peanuts, low-fat cheese, kidney

Authors' Affiliations: Nutritional Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, Department of Health and Human Services, Rockville, Maryland

Corresponding Author: Amanda J. Cross, Division of Cancer Epidemiology and Genetics, National Cancer Institute, 6120 Executive Boulevard (EPS), Suite 320, Rockville, MD 20852. Phone: 1-301-496-4378; Fax: 1-301-496-6829. E-mail: crossa@mail.nih.gov.

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beans, and green lentils; the protein contents of these 3 diets were 65, 149, and 143 g/d, respectively. Within both studies, each diet was consumed for 15 consecutive days with no washout phase between diets. All dietary components were weighed to the nearest gram per day. Furthermore, with the exception of the meat component, all diets were comprised of similar foods, including cereal, bread, milk, fruits, and vegetables, and were isocaloric and matched for fat and fiber content and adjusted for the energy needs of each individual with extra bread, low-fat margarine, and marmalade.

Consecutive 24-hour urine collections were made on the final 3 days of each 15-day dietary period. Boric acid (1 g/L) was added to the urine samples as a preservative and then the samples were aliquoted into 28-mL tubes and frozen at −20°C. Para-aminobenzoic acid (PABA) tablets were taken by the volunteers and a colorimetric method was used to determine the completeness of collection of the 24-hour urine samples (10). Urinary nitrogen was measured in all samples by Kjeldahl analysis to determine the level of dietary compliance (11). Urine samples collected on day 15 of each dietary period were analyzed for creatinine, taurine, 1-methylhistidine, and 3-methylhistidine as potential biomarkers of meat intake. Blinded quality control samples from 2 different individuals on free-living diets were inserted into the batches at a level of 10%.

Creatinine was measured by an Olympus AU600 automated chemistry instrument (Olympus America, Inc.) at Quest Diagnostics; the method involved a kinetic modification of the Jaffe procedure, in which creatinine reacts with picric acid at alkaline pH to form a yellow-orange complex, the rate of change in absorbance (measured at 520/800 nm) is proportional to the creatinine concentration in the sample (12). Taurine, 1-methylhistidine and 3-methylhistidine were measured by an ion exchange chromatography technique involving protein precipitation with sulfosalicylic acid, separation of the amino acids on a cation exchange column under acidic conditions, followed by treatment with ninhydrin, which reacts with the primary or secondary amino groups to form colored derivatives that are detected colorimetrically at 440/570 nm (Quest Diagnostics).

We used mixed linear regression (PROC MIXED in SAS, SAS institute), a type of random effects model that accounts for within-subject correlation to determine the effect of diet type on each of the potential urine biomarkers. First, we combined the data from both studies to determine whether there was an overall effect of diet on the urinary analytes, while controlling for study. Second, we compared the means across diet types within each study to determine where the differences occurred, while adjusting for multiple comparisons by using the Tukey method. Two-tailed probability results of 0.05 or less significance level were regarded as statistically significant.

Results

Of a total of 42 urine samples (9 volunteers on 2 different diets and 8 volunteers on 3 different diets), all urine samples were considered complete by the PABA-check method, in that 85% or more was recovered in the 24-hour urine collection (10). Dietary compliance, estimated by the correlation between dietary and urinary nitrogen, was also high ($r = 0.99$ and 0.71 for study I and II, respectively).

An overall effect of diet on the urinary excretion of all 4 analytes was evident (all $P < 0.0001$), with each analyte increasing with the dose of red meat in the diet in a dose-dependent manner (all $P_{trends} < 0.0001$, Table 1). Pairwise

Table 1. Distribution of urinary analytes for each diet type

Urinary analyte	Vegetarian $n = 8$ (all from study II)	60 g/d red meat $n = 17$ (9 from study I and 8 from study II)	120 g/d red meat $n = 9$ (all from study I)	420 g/d red meat $n = 8$ (all from study II)	P_{trend}[a]
Creatinine (mmol/d)					
Mean (SD)	14.5 (1.6)	14.2 (2.2)	15.9 (1.0)	21.0 (3.8)	<0.0001
Median (IQR)	14.4 (13.2–16.0)	14.5 (13.0–16.1)	16.0 (15.3–16.5)	19.8 (18.7–21.6)	
Taurine (μmol/d)					
Mean (SD)	543.1 (235.6)	544.8 (339.0)	671.9 (383.2)	1,675.5 (621.4)	<0.0001
Median (IQR)	517.3 (389.0–740.8)	482.9 (346.0–719.0)	603.8 (469.9–729.3)	1,695.1 (1,187.6–1,887.8)	
1-Methylhistidine (μmol/d)					
Mean (SD)	37.8 (11.5)	91.3 (13.3)	166.1 (15.0)	457.2 (56.2)	<0.0001
Median (IQR)	34.0 (31.2–40.5)	95.1 (82.6–98.5)	166.7 (155.0–172.7)	449.2 (425.6–473.7)	
3-Methylhistidine (μmol/d)					
Mean (SD)	184.1 (21.8)	238.0 (40.0)	306.4 (21.3)	552.8 (86.9)	<0.0001
Median (IQR)	184.7 (166.6–199.2)	248.8 (217.2–262.6)	305.4 (295.5–320.8)	529.2 (497.8–576.4)	

[a] P_{trend} test.

comparisons within study I revealed significantly higher urinary excretion of 1-methylhistidine ($P < 0.0001$), 3-methylhistidine ($P = 0.0003$), and creatinine ($P = 0.02$), when individuals consumed the 120 g compared with the 60 g/d red meat diet; taurine excretion, however, was not different when these 2 diets were compared ($P = 0.24$). Pairwise comparisons within study II revealed that urinary excretion of all 4 analytes was significantly higher when individuals consumed the 420 g/d red meat diet compared with the vegetarian or the 60 g/d red meat diet ($P < 0.0001$, all comparisons). Furthermore, during the 60 g/d red meat diet, urinary excretion of 1-methylhistidine ($P = 0.009$) and 3-methylhistidine ($P = 0.03$) was increased compared with the vegetarian diet, but excretion of taurine ($P = 0.95$) and creatinine ($P = 0.88$) was not statistically different between these 2 diet types.

Although there was individual variation, urinary excretion of 1-methylhistidine and 3-methylhistidine was lowest on the vegetarian diet and increased in every individual with increasing doses of red meat. Figure 1 shows individual data for 1-methylhistidine. All individuals were assigned to a 60 g/d red meat as part of study I and study II, and the urinary excretion of 1-methylhistidine in all 17 of these people was at a similar level when they were consuming this diet (Fig. 1). When both studies were combined, the median urinary excretion of 1-methylhistidine and 3-methylhistidine during the 60 g/d red meat diet was 95.1 μmol/d [inter-quartile range (IQR): 82.6–98.5] and 248.8 μmol/d (IQR: 217.2–262.6), respectively (Table 1). Analyzing these data by study, revealed that median excretion of 1-methylhistidine was consistent between study I (95.1 μmol/d, IQR: 78.4–99.7) and study II (95.1 μmol/d, IQR: 83.9–97.8), as was 3-methylhistidine between study I (230.4 μmol/d, IQR: 217.2–258.0) and study II (254.1 μmol/d, IQR: 228.3–279.1).

Discussion

All 4 urinary analytes examined were able to distinguish between the diets with the extreme levels of meat, but only urinary 1-methylhistidine and 3-methylhistidine was statistically different for each dose of meat in the diet. Urinary 1-methylhistidine excretion in our study increased with increasing dietary red meat, these differences were statistically significant for each of the 4 diet types. The majority of 1-methylhistidine excreted in the urine is from dietary sources, particularly from meat (8), and because 1-methylhistidine is not a marker of muscle catabolism and there is minimal endogenous formation (13), this could be a good potential biomarker of meat intake. Measuring 1-methylhistidine in urine was sufficient to predict vegetarian status in a study of 126 individuals (14). Although a study of 5 individuals found a strong linear association between intake of chicken, pork, or plaice and urinary excretion of both 1-methylhistidine and 3-methylhistidine (8), a study comparing meat eaters to lactovegetarians reported a more pronounced effect on urinary 1-methylhistidine than 3-methylhistidine (15).

Our study noted significantly elevated urinary excretion of 3-methylhistidine with increasing red meat consumption; however, there was considerable variation in 3-methylhistidine excretion between individuals on the same diet, perhaps because it also reflects muscle catabolism and muscle mass (16). Meat contains 3-methylhistidine in a soluble form as well as bound to the muscle proteins actin and myosin (6), breakdown of these

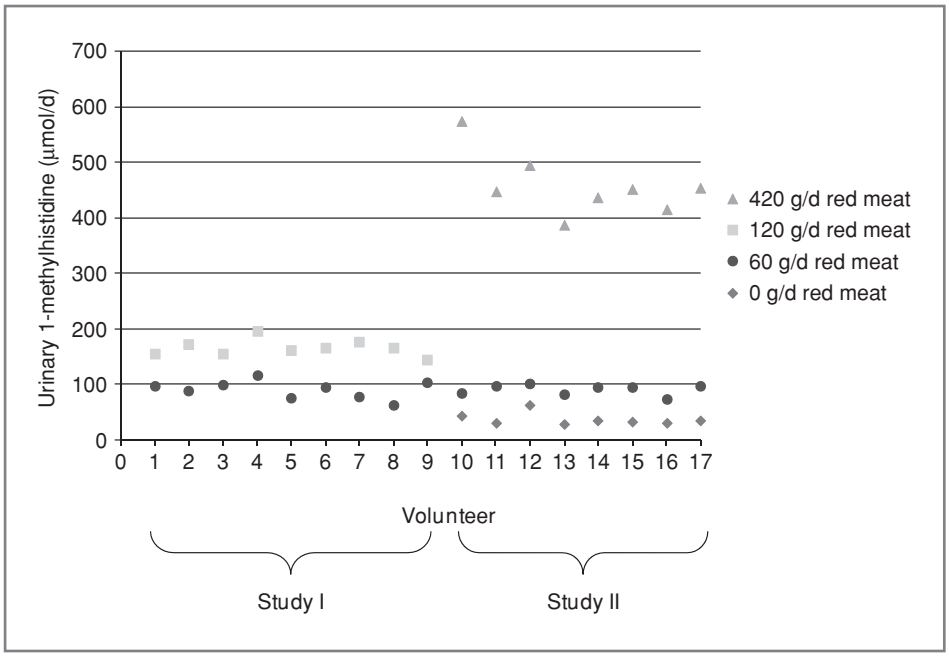

Figure 1. Urinary 1-methylhistidine for each volunteer according to diet type

proteins consequently results in urinary excretion of 3-methylhistidine (17, 18). Several studies have reported an increase in urinary 3-methylhistidine after meat consumption (6, 15, 16, 19). A small study found an approximate quantitative relationship between ingested 3-methylhistidine and that excreted in the urine in 5 subjects (19). Furthermore, 3-methylhistidine has been correlated with meat intake reported by dietary recall 1 year previously ($r = 0.77$) in free-living individuals (20).

Creatinine is a muscle breakdown product and previous studies have reported higher urine creatinine levels in individuals who consume a diet high in red meat (5). Our study noted statistically significant differences for creatinine only between the extreme doses of meat. Furthermore, there are caveats of creatinine as a biomarker of meat intake, which include the associations of urinary creatinine with the muscle mass of an individual (21) and renal function, because the kidney filters creatinine.

Even though a small amount may be synthesized in humans (22, 23), the main source of taurine is from ingestion of foods of animal origin (7), this is reflected in the low levels of taurine excretion in vegans (22). However, vegans can excrete more taurine than they consume, which suggests that endogenous taurine synthesis can result in urinary excretion even when dietary intake is low (22). A previous study showed that increasing taurine intake leads to an equivalent increase in urinary taurine excretion (24). Although we did observe higher urinary taurine excretion when individuals were consuming the high red meat diet, compared with the low red meat diet, excretion during the vegetarian diet was not lower than excretion during the low red meat diet. Furthermore, the consumption of specialized supplements and energy drinks, which sometimes contain taurine, could preclude its potential as a good biomarker of meat intake in free-living individuals.

On the basis of our findings, both 1-methylhistidine and 3-methylhistidine may be potentially good biomarkers of meat intake; although 1-methylhistidine may be superior because it is independent of muscle mass and catabolism. We observed a dose-dependent effect of red meat in the diet on urinary excretion of both 1-methylhistidine and 3-methylhistidine. In addition to a targeted biomarker approach, some studies have reported data from metabolomics analyses to identify potential biomarkers of meat intake; however, this approach generally identifies patterns (or profiles) that distinguish between diet types rather than excretion levels of a particular metabolite. Nevertheless, a recent review on biomarkers and metabolomics (25) noted 2 studies that had reported trimethylamine-*N*-oxide as a urinary metabolite that differed according to meat in the diet; therefore, this potential biomarker should also be investigated in future studies.

Our data are from randomized, highly controlled, crossover studies in which individuals were their own controls, the optimum study design for investigating dietary biomarkers. However, our study design did not consider the potential of these analytes as long-term biomarkers of intake, especially if meat is episodically consumed. It is likely that 1-methylhistidine and 3-methylhistidine may be good short-term biomarkers of meat intake, and that a single urine sample may not be sufficient to assess "usual" intake; a very small study reported that the half-lives of 1-methylhistidine and 3-methylhistidine were 11.7 and 12.6 hours, respectively (8). Nevertheless, these biomarkers could be used in studies in which repeat samples are available and in calibration studies for measurement error correction. In addition, good short-term biomarkers of intake can be used in conjunction with existing self-reported questionnaires to improve the accuracy of exposure assessment (26). Because our results are from a controlled feeding study, they must be replicated in free-living individuals to determine whether the excretion of these compounds can categorize individuals according to their usual meat intake irrespective of other components of the diet. Despite tight control of the diets in our study, there remained a degree of variation in urinary excretion of all the analytes between individuals on the same diet, which may indicate that this variation would be even greater in free-living populations.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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