1993

Urinary creatinine and 3-methylhistidine as indices of body composition

Kathy Ann Burk
Iowa State University

Order Number 9334966

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Burk, Kathy Ann, Ph.D.

Iowa State University, 1993

U·M·I
300 N. Zeeb Rd.
Ann Arbor, MI 48106

Urinary creatinine and 3-methylhistidine as indices of body composition

by

Kathy Ann Burk

A Dissertation Submitted to the

Graduate Faculty in Partial Fulfillment of the

Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Department: Food Science and Human Nutrition
Major: Nutrition

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa

1993

To

My father, who nurtured this dream but
could not share in its achievement

and

My mother, who has sustained it

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INTRODUCTION

Body composition is of both academic and practical interest to health practitioners. For example, health professionals must be concerned with body composition because of its direct relationship to nutrient requirements and to drug utilization. The size of the body's muscle mass is also an important consideration in nutritional assessment, and body composition data are vital in studies of growth and development, aging, physical conditioning, sex differences, and tissue-wasting diseases. Many techniques for assessing body composition have been proposed during the past 50 years, but no one technique has yet proved to be definitive. Among the methods that have been proposed for estimating lean body mass are measurement of urinary creatinine (Crn) excretion and measurement of urinary 3-methylhistidine (3-MH) excretion.

As early as 1913, urinary Crn was known to be proportional to total body creatine content (Myers and Fine, 1913). Crn, the end product of creatine degradation, is excreted in the urine. Because 98 percent of the body creatine is contained in skeletal muscles, the amount of creatine degraded, and therefore Crn excreted, should be directly proportional to muscle mass, which is the primary component of lean body mass. Although Crn excretion during

controlled diet periods may represent a constant proportion of the creatine pool, the body's creatine pool size may not be under strict metabolic control (Lukaski, 1987). Factors such as dietary levels of Crn and creatine and physical exercise can also directly influence urinary Crn excretion (Heymsfield et al., 1983). Extensive Crn excretion data are available but there is no consensus on the validity of such data for estimating body composition.

Urinary 3-MH has been used as an index of muscle protein turnover. After being incorporated into the protein chain, certain histidine residues are methylated. When these proteins are degraded, 3-MH is released and not reutilized but excreted quantitatively in the urine. The concentration of 3-MH is highest in skeletal muscle, with little variation between body sites or between individual subjects (Elia et al., 1979). Excretion levels are dependent on 3-MH content of the diet (Elia et al., 1980) and also on the physiological state (Young et al., 1973; Long et al., 1977; Iapichino et al., 1985; Fitch and King, 1987). Urinary 3-MH should be proportional to muscle mass whenever dietary intake is constant and muscle protein synthesis and catabolism are in balance. Until recently, the emphasis of studies in which urinary 3-MH was measured has been on estimating rates of muscle breakdown. Little research has been done to establish expected 3-MH excretion

under constant conditions in normal adults (Fitch et al., 1986; Peters et al., 1989).

The objectives of the present study were to examine three specific aspects of urinary Crn and 3-MH excretion: (1) the degree to which urinary Crn and 3-MH reflect dietary intakes of these compounds and their precursors, (2) the daily variation of Crn and 3-MH excretion under controlled conditions, and (3) the relationships among urinary Crn, 3-MH, and body composition. Healthy college women were used as the experimental subjects in two controlled studies.

These studies were supported by Project 2571, a contributing project to the North Central Regional Research Project NC-167, "Dietary modifications designed to affect lipid metabolism." All work was supported by the Iowa Agriculture and Home Economics Experiment Station.

REVIEW OF LITERATURE

The literature pertaining to body composition is voluminous, if one includes papers discussing theoretical bases, methods of measurement, and experimental data. The present review does not attempt to critique the available body composition data but instead provides a representative overview of papers relevant to the present study.

Densitometry

The major components of the human body are water, fat, protein, and minerals. The assessment of body composition by whole-body density assumes that water, protein, and minerals comprise the fat-free mass and that their relative proportions are constant. The density of this fat-free body mass in healthy adults is 1.1 g/cc at normal body temperature (Keys and Brozek, 1953; Brozek et al., 1963). To this fat-free mass is added fat, which has a density of 0.900 g/cc. Whole-body density then lies between these extremes and this knowledge can be used to estimate the fat-free and fat masses. The accuracy of this body composition assessment depends on the degree of the body's conformity to the assumptions used in the mathematical derivation of the fat-free mass.

A measure of body volume is needed to calculate body

density. Two of the available techniques for measuring body volume involve submersion of the subject in water. Hydrostatic weighing is based on Archimedes' principle in which the difference between body weight in air and body weight in water is the apparent body volume. The water displacement technique measures the actual volume of water displaced. In both of these methods, residual lung volume must be measured at the time of submersion. Gastrointestinal gas volume in not measured in either method (Buskirk, 1961).

An alternative technique for determining body volume measures the concentration of a known volume of a gas introduced into a closed chamber in which the subject sits. Siri (1956a) designed a closed-chamber apparatus into which helium gas is introduced; no correction is necessary for air in the lungs and respiratory passages because the helium comes to equilibrium in these spaces in less time than is required for complete mixing of the gases in the chamber. The volume of gas in the gastrointestinal tract compensates for the volume of helium absorbed into tissues and fluids (Siri, 1956b). Corrections are made in the calculations for the temperature difference between the helium and chamber air and for water vapor. The effect of body heat, and the accumulation of carbon dioxide and the consumption of oxygen from respiration, are compensated for by extrapolation on the chart recording. Body density of the subjects is

calculated as body weight divided by body volume. Body weights taken just before and after the body volume determinations give the best estimate of body mass. Percent body fat (F) can be readily calculated from body density (BD). Brozek et al. (1963) proposed: $F = (457.0/BD) - 414.2$. For subjects with less than 30 percent body fat, this equation gives estimates that agree, within one percent body fat, with the equation developed by Siri (1956b): $F = (495/BD) - 450$.

Urinary Creatinine

Urinary Crn is derived from creatine in the muscles. Although other tissues take up creatine, skeletal muscles contain 98 percent of the body creatine pool, and most of this creatine is in the form of phosphocreatine. Crn can be formed from creatine either indirectly, by the loss of phosphoric acid from the intermediate phosphocreatine, or directly, by splitting off a molecule of water from creatine (Borsook and Dubnoff, 1947).

Folin (1905) was the first to propose a relationship between urinary Crn and body composition. Although a relationship between Crn excretion and muscle mass is generally accepted (Miller and Blyth, 1952; Doolan et al., 1962; Boileau et al., 1972; Forbes and Bruining, 1976), various factors have been identified that may influence

daily Crn excretion and complicate proposed relationships between urinary Crn and muscle mass. A review of the use of urinary Crn to estimate human body composition has been presented (Heymsfield et al., 1983).

The greatest problem with using urinary Crn to estimate body composition is the large normal daily variability of Crn excretion within individual subjects (Table 1). Some people do seem to excrete uniform amounts of Crn; Bingham and Cummings (1985) reported coefficients of variation of Crn excretion ranging from 1 to 7 percent over 4 days while their subjects consumed a uniform diet, and an increase only to 7 to 11 percent during self-selected diet periods. Cho and Yi (1986) reported coefficients of variation of Crn excretion ranging from 1.6 to 6.9 percent in 12 Burmese adults on self-selected diets. For other individuals consuming unrestricted diets, the coefficients of variability have ranged up to 20 percent (Greenblatt et al., 1976; Forbes and Bruining, 1976; Waterlow, 1986). Controlling diet does not decrease the excretion variability in all individuals. Coefficients of variation of 5 to 22 percent were reported for urinary Crn in subjects consuming a constant diet for 28 days (Edwards et al., 1969). Because Crn undergoes renal filtration and secretion, differences in processing of Crn by the kidney may be responsible for variations in Crn excretion in the same individual, as well

Table 1. Variability of urinary creatinine during constant and varied diets

Reference	n	sex	Range of within person C.V.[a]	Diet conditions
Bingham and Cummings, 1985	8	M	1 - 7	Constant, meat containing; 4 d
	8	M&F	7 - 11	Self-selected; 28 d
Cho and Yi, 1986	12	M&F	1.6 - 6.9	Self-selected; 5 d
Greenblatt et al., 1976	8	M	10.5 - 14.4	Self-selected; 54-94 24-hr samples in 10 months
Forbes and Bruining, 1976	21	M&F	1.7 - 19.5	Self-selected; 3 d
Waterlow, 1986	6	M	7.0 - 17.0	Self-selected; 24 d
Edwards et al., 1969	5	M&F	5.6 - 22.3	Constant, meat containing; 28 d

[a] C.V. = Coeffecient of variation = Standard deviation as a percent of the mean.

as among individuals (Materson, 1971).

Diet composition can both directly and indirectly affect daily Crn excretion levels. Dietary Crn in meat is absorbed and quantitatively excreted, so urinary Crn includes the dietary amount, as well as that derived from creatine (Hunter, 1922). Dietary intake also affects Crn excretion indirectly by providing creatine and its precursors, the amino acids arginine and glycine. Calloway and Margen (1971) and Crim et al. (1975) noted decreased urinary Crn when creatine-free diets were fed to human subjects. This decrease was reversed when creatine alone was added to the diet. These changes were independent of the total dietary protein level but were influenced only by dietary creatine levels. Adding arginine and glycine to the creatine-free diet led to increased urinary Crn; no increase was seen when alanine was added (Crim et al., 1975). Hoogwerf et al. (1986) also found that urinary Crn reflected dietary levels of meat protein but not total protein. Because urinary Crn is dependent not only on dietary Crn but also on dietary creatine and its precursors, the response to a dietary change is a function of time and the degree of dietary change (Lykken et al., 1980).

Very strenuous exercise may increase urinary Crn excretion by up to 10 percent. In a series of four experiments by Srivastava et al. (1967), the daily Crn

output was higher during the days of exercise than during control days, but in only two of these experiments were the differences significant. Slonim (1961) found, however, that there was no significant effect of moderate exercise on Crn excretion, and Van Pilsum and Seljeskog (1958) reported only a slight increase in urinary Crn with physical exertion.

Some researchers have presented formulas for calculating muscle mass from urinary Crn; in these formulas 1 gram of urinary Crn per day is assumed to equal 16 to 20 kg of muscle (Talbot, 1938; Graystone, 1968; Kreisberg et al., 1970). The variation in formulas in part reflects differences in the ages and nutritional status of the subjects, methodological differences in determining body composition, and differences in the mass being estimated (whole wet muscle or fat-free muscle).

Urinary 3-Methylhistidine

The principal sites of 3-MH production are in the actin of all muscle fibers and in the myosin of white muscle fibers. After synthesis in which histidine is incorporated into the muscle protein chain, certain histidine residues are methylated. As protein breakdown occurs during turnover and repair, the released 3-MH is neither recycled into protein nor oxidized but is excreted quantitatively in the urine (Young and Munro, 1978). Urinary 3-MH should be

proportional to muscle mass whenever muscle protein synthesis and catabolism are in balance.

Many of the factors that may affect Crn excretion, such as diet and exercise, also affect 3-MH excretion. Meat and fish in the diet contain 3-MH; this dietary 3-MH is absorbed and excreted quantitatively, but there is a lag time of 3 days between ingestion and excretion (Elia et al., 1980; Lukaski et al., 1981) During the first day on a meat-free diet, six healthy men showed a 66 percent decrease in 3-MH (Tomas et al., 1979). McKeran et al. (1978) reported a 64 percent reduction in 3-MH excreted by five healthy men after the first day of a meat-free diet. After switching 14 healthy men to a meat-free diet, Lukaski et al. (1981) reported 61 percent of exogenous 3-MH was eliminated on the first day; more than 80 percent of the exogenous 3-MH had been excreted after 2 days.

The effect of exercise on 3-MH excretion is not as conclusive as is the effect of diet. When five untrained young men ran on a treadmill for 1 hour per day for 2 weeks, urinary 3-MH was significantly less than when this exercise was not performed (Radha and Bessman, 1983). During 1 day in which exercise on a cycle ergometer was included, urinary 3-MH in eight healthy non-athlete men and women was not significantly different than when exercise was not done (Calles-Escandon et al., 1984). Dohm et al. (1982) reported

3-MH excretion was increased in ten trained men on the day of a 10-12 mile run but was not changed in four men performing their customary one hour weight-lifting routine. When 13 men performed a 36-minute weight-lifting exercise, urinary 3-MH was significantly increased by the workout (Hickson and Hinkelmann, 1985). Thus, the magnitude of the effect of activity may depend on the type and intensity of the exercise and the fitness level of the subjects.

Until recently, the emphasis of studies in which urinary 3-MH was measured has been on estimating rates of muscle breakdown under varying conditions of age (children or adults) and nutritional state and physiological stress level (pregnancy, acute injury, cystic fibrosis; Long et al., 1977; Ballard et al., 1979; Miller et al., 1982; Iapichino et al., 1985; Fitch and King, 1987). Little research has been done to establish expected 3-MH excretion under constant conditions in normal adults. In 14 men consuming a meat-free diet, the mean coefficient of variation of urinary 3-MH for 5 consecutive days was 4.5 percent, ranging from 2.2 to 7.0 percent (Lukaski et al., 1981). During a different study, eight men and six women collected urine samples twice for 3 consecutive days 6 weeks apart. The coefficients of variation of 3-MH excretion for the men were 2.5 and 5.1 percent, and for the women, 4.8 and 8.0 percent for the two periods, respectively (Peters et

al., 1989). In four other women, however, the mean individual coefficient of variation was 20.9 percent (Fitch et al., 1986).

No studies have reported formulas for estimating fat-free mass from 3-MH excretion, but some studies suggest an association of urinary 3-MH with lean mass (Table 2). Fat-free mass calculated from body density was significantly correlated ($r=0.89$, $p<0.001$) with the urinary 3-MH excreted by 16 men (Lukaski and Mendez, 1980). This result is similar to a correlation of 0.93 ($p<0.01$) between fat-free mass and 3-MH excretion in a group of 12 men (Mendez et al., 1984). Lukaski et al. (1981) measured total body potassium and nitrogen to estimate muscle and non-muscle components of the fat-free mass. The correlation between muscle mass and urinary 3-MH ($r=0.91$, $p<0.001$) was greater than that between fat-free mass and 3-MH ($r=0.81$, $p<0.001$). A low correlation ($r=0.33$, $p<0.05$) existed between non-muscle fat-free mass and 3-MH excretion (Lukaski et al., 1981). Densitometrically determined fat-free weight was not different in wrestlers and non-athletes, but the amount of 3-MH excreted by the wrestlers was significantly greater than that of the non-athletes (Mendez et al., 1983). These data suggest that the proportions of muscle and non-muscle in the fat-free body mass may differ considerably but may not be differentiated by density measurements.

Table 2. Reported correlations of urinary 3-methylhistidine and body composition

Body Component	n[a]	sex	r[b]	p[c]	Reference
Fat-free mass by densitometry	16	M	0.89	0.001	Lukaski and Mendez, 1980
Fat-free mass by densitometry	12	M	0.93	0.01	Mendez et al., 1984
Muscle mass by total body potassium and nitrogen	14	M	0.91	0.001	Lukaski et al., 1981
Fat-free mass by densitometry	14	M	0.81	0.001	Lukaski et al., 1981

[a] n = number of experimental subjects.
[b] r = correlation coefficient between urinary 3-MH and body component.
[c] p = the probability of a value of r this large or larger when the true correlation equals zero.

Because the biochemical bases for estimating lean mass from either urinary Crn or 3-MH are parallel, and because extensive Crn excretion data are available, 3-MH excretion has been compared to urinary Crn. In 16 men, densitometrically determined fat-free mass was more closely correlated with 3-MH excretion ($r=0.89$, $p<0.001$) than with urinary Crn ($r=0.67$, $p<0.01$; Lukaski and Mendez, 1980). Similarly, the correlation between fat-free mass and urinary 3-MH ($r=0.93$, $p<0.01$) was greater than that between fat-free mass and Crn ($r=0.63$, $p<0.05$). The correlation between urinary Crn and 3-MH, two measures of muscle mass, was 0.69 ($p<0.05$; Mendez et al., 1984). When muscle mass was estimated from total body potassium and nitrogen, 3-MH excretion was more closely correlated ($r=0.91$, $p<0.001$) with muscle mass than was Crn ($r=0.79$, $p<0.01$). Urinary 3-MH and Crn were also significantly correlated ($r=0.87$, $p<0.001$; Lukaski et al., 1981).

METHODS

Experimental Design

Experiment 1

Twenty college women volunteered to participate in this study. They were selected to include both physically active and inactive individuals of various body sizes (Table 3). All subjects were in good health as determined by physical examination. The study protocol was approved by the University Committee on the Use of Human Subjects in Research.

During phase 1, ten subjects were fed each of three diets for 4 days. Diet 1 was strict vegetarian, eliminating dietary sources of Crn and 3-MH. Diet 2 was ovo-lacto-vegetarian, including eggs and dairy products but no meat. This diet included trace or small amounts of dietary Crn and 3-MH. Diet 3 included meat selections, providing levels of dietary Crn and 3-MH typically contained in the American diet. Subjects selected amounts and particular items from a variety of foods prepared to meet the specifications of each diet. Because of personnel constraints, feeding multiple diets simultaneously was not possible. At the end of each diet period, the subjects resumed their customary eating for 3 days before beginning the next diet. All meals and snacks were prepared and served in the metabolic unit by the

Table 3. Ages, heights, and weights of 20 subjects in Experiment 1

Subject	Age yr	Height cm	Weight kg
Phase 1			
50	20	170.5	62.5
51	21	161.3	56.8
52	21	170.5	74.2
53	20	167.7	57.0
54	20	165.6	56.5
55	20	164.5	65.5
56	21	169.9	66.2
57	19	171.3	79.3
58	22	170.0	55.9
59	22	162.2	61.9
Phase 2			
60	20	169.0	65.4
61	22	176.0	58.8
62	19	164.5	56.2
63	19	165.0	56.9
64	20	168.1	60.5
65	22	157.7	69.1
66	21	168.8	68.0
67	19	155.8	51.5
68	20	168.0	61.5
69	19	163.7	62.6
Mean	20.4	166.5	62.3
S.D.	±1.1	±4.7	±6.6

research staff. There were no discernable weight changes during each diet period. Subjects collected complete 24-hour urine samples for three days during each diet period, beginning the morning of the second day. No sample collections were made on the first day of feeding to allow for clearance from the body of Crn and 3-MH in the previous diet (Tomas et al., 1979; Lukaski et al., 1981; Elia et al., 1980). This overall pattern of feeding was repeated in phase 2 with another ten subjects.

Experiment 2

Data in this experiment were collected as part of a regional project undertaken to investigate lipid metabolism. Ten healthy young women participated in a 10-week feeding study (Table 4). During the first and last weeks, the subjects ate self-selected diets. During the first week, Crn excretion was not different from the eight experimental weeks; during the last week, half of the subjects had mean urinary Crn that was less than those during the experimental period. For 3-MH, seven of the ten subjects had initial excretion levels that were higher than during the controlled diet periods; the 3-MH means during the last week were not different from the experimental period. These data are not included in the present study because the diet was not controlled. Two nutritionally adequate diets were fed for

Table 4. Ages, heights, and weights of 10 subjects in Experiment 2

Subject	Age yr	Height cm	Weight kg
70	20	167.7	66.5
71	19	164.0	47.3
72	23	169.7	67.0
73	19	164.2	64.9
74	22	184.5	88.0
75	19	168.0	61.7
76	19	165.8	55.8
77	22	171.4	63.9
78	19	175.0	76.2
79	19	163.0	63.6
Mean	20.1	169.3	65.5
S.D.	±1.6	±6.3	±10.8

the remaining 8 weeks (Table 5). Each subject ate one diet for 28 days and then switched to the other diet for the remaining 28 days. Meat was not eliminated from the diets (two frankfurters were included in the evening meal on both diets) but because the same foods in the same amounts were served every day on each diet, dietary intakes of creatine, Crn, and 3-MH were constant during each 28-day period. Descriptions of subjects and other details of the present study have been published (Garcia et al., 1991; Manatt et al., 1991). Complete daily urine samples were collected throughout the study.

Experimental Methods

Collection and analysss of urine

Subjects collected each voiding into separate 16 oz. plastic containers with tight-fitting lids. Samples were kept cold until brought to the laboratory. The metabolic unit was located within the dormitory complex. Subjects reported to the unit for all meals, and were provided opportunities for easy delivery of samples to the laboratory.

Daily total urine volume was measured with a glass-stoppered graduated cylinder. Collection containers were rinsed with distilled water and the rinsings were added to the total. Duplicate daily aliquots were stored in tightly-

Table 5. Composition of the constant diets fed during Experiment 2

Dietary Component	Usual U.S. diet[a]	Modified diet[b]
Energy, kcal	2029	2032
Protein, g	76	87
Carbohydrate, g	241	296
Fat, g	92	66
Saturated fatty acids, g	35.0	18.6
Oleic acid, g	34.4	19.9
Linoleic acid, g	10.4	19.2
Cholesterol	687	98
Fiber, g	9.6	11.4
K, mg	3119	4133
Na, mg	3526	3761
Ca, mg	1282	1314
P, mg	1648	1880
Fe, mg	26	28
Thiamin, mg	1.7	2.2
Riboflavin, mg	2.4	2.2
Niacin, mg	15.1	24.6
Ascorbic acid, mg	171	228
Vitamin A, IU	7964	7569

[a] Usual U.S. diet was based on the HANES I survey (Abraham and carrol, 1979) and calculated to supply 40% of energy from fat with a P:S ratio of 0.30 and 600 mg of cholesterol.

[b] Modified diet was designed to meet the U.S. dietary goal recommendations (Dietary Goals for the United States, 1977) calculated to provide 30% of energy from fat with a P:S ratio of 1.04 and less than 300 mg of cholesterol.

capped plastic bottles at -15° C until analyzed for Crn and 3-MH. Individual samples were placed in the refrigerator the afternoon before analysis to allow for thawing. Samples were brought to room temperature on the laboratory counter, mixed well, and aliquots were taken.

Daily urinary Crn was determined by use of the alkaline picrate colorimetric method of Folin (1914) as presented in Hawk's Physiological Chemistry (Oser, 1965, pp 1233-1236). For each sample, duplicate determinations of each of two aliquot sizes were done. If the final total 24-hour urine volume was less than 1300 ml, aliquot sizes of 0.25 ml and 0.50 ml of urine were used; for total volumes greater than 1300 ml, 0.50 ml and 1.0 ml of urine were measured into 100 ml volumetric flasks. Previous experience in this laboratory with Crn analyses indicated that one of these aliquot sizes would give final readings within the standard curve range. Appropriate amounts of distilled water were added to bring the volume in each flask to 1.0 ml. A blank of 1.0 ml water and a set of four standards were prepared. To each flask was added 20.0 ml of saturated picric acid solution followed by 1.5 ml of 10 percent NaOH solution. The flasks were stoppered, swirled to mix and left to sit for 15 minutes. Distilled water was added to bring the volume in each flask to 100 ml. The flasks were stoppered and inverted ten times to mix well. Optical densities of

the sample mixtures were read at a wavelength of 520nm on a Beckman spectrophotometer with a sipper attachment. The concentration of Crn in each sample was calculated from the standard curve. Results from the aliquot reading nearer the middle of the standard curve were used for additional calculations.

3-MH was determined by use of an isocratic high pressure liquid chromatography (HPLC) modification (Li and Wassner, 1981) of an earlier gradient elution method from the same laboratory (Wassner et al., 1980). Into 1.5 ml micro-test tubes were measured 0.75 ml of water, 0.05 ml of 70 percent perchloric acid (PCA) and 0.2 ml of urine. Each tube was vortexed and centrifuged in a microcentrifuge for one minute. Ten standard solutions were made at concentrations from 1.4 nmol 3-MH/ml water to 70 nmol/ml. Seventy-five microliters of the diluted urine or 100 µl of standard were transferred to a 1.5 ml micro-test tube; 0.25 ml of sodium borate solution (0.4 M B_2O_3 adjusted to pH 12.2 with NaOH) was added. The sodium borate solution also contained 0.01 mmol/ml histidinol as an internal standard. While vortexing, 0.25 ml of acetonitrile (AN) was added. The AN contained 160 mg fluorescamine/100 ml. After the tubes sat for 5 minutes, 0.04 ml of PCA were added; the tubes were capped, and placed in a water bath at 80° C for 1 hour. When the tubes had cooled to room temperature, 0.01

ml of 0.5 M morpholinopropanesulfonic acid (MOPS) in 3 M NaOH was added. The pH of each sample was checked; if the pH was above seven, the sample was not injected because precipitates were present. Fifty microliters of sample or standard were injected onto a μBondapak C_{18} reverse phase column (Waters Associates). Isocratic elution was performed with 25% AN in 10 mM sodium phosphate butter (pH 7.5) which was made fresh daily and filtered. Flow rate was 1.5 ml/minute. The 3-MH peak came off between 5 and 6 minutes. Concentrations of 3-MH were calculated from the peak area, which showed a linear response to the concentration of 3-MH in the standards ($r=0.99$, $p<0.0001$) with an intercept which was not different from zero.

Body composition

Duplicate body volume determinations were made by the helium dilution method by use of a Body Volume Determinator as described by Siri (1956a). Each subject was weighed on an Acme platform balance before and after each determination. Whole body density was calculated as body weight divided by body volume. The percentage of body fat (F) was estimated from body density (BD) according to the formula of Siri (1961): $F = (495/BD) - 450$. Lean body mass was calculated from body weight and percent body fat.

Statistical Analyses

The mean, standard deviation, and coefficient of variation were calculated for Crn and 3-MH by diet in Experiment 1 and by week in Experiment 2. Analysis of variance was used to test for significant diet effects in Experiment 1. Linear time trends in Experiment 2 were examined by regression analysis. Simple correlation coefficients and multiple regression equations among the urinary constituents and estimates of body composition were also calculated (SAS Institute Inc., 1985). The usefulness of including variables in the equations was examined by comparing the R^2 and standard errors of estimate (Draper and Smith, 1966, p. 117-120).

RESULTS AND DISCUSSION

Experiment 1

The amount of Crn produced per day is dependent on the creatine pool size, and the length of time required for the size of the creatine pool to stabilize after dietary change is dependent on the extent of change (Lykken et al., 1980). Because of these complex interactions, urinary Crn levels of the subjects in Experiment 1 may not represent excretion levels from a constant creatine pool. Urinary 3-MH has been shown to stabilize by the third day after dietary changes (Tomas et al., 1979; Lukaski et al., 1981; Peters et al., 1989), so urine samples collected during day 2 of the diets were discarded. In addition, some values were not included because urine collections were known to be incomplete. The results in Tables 6 and 7 represent the average of days 3 and 4 when both days data were available, or the excretion level on day 3 or on day 4.

Diets 1 and 2 contained no or insignificant levels of creatine, Crn, or 3-MH, and during Diet 3 the subjects could select foods containing these compounds. If urinary excretion of Crn and 3-MH do indeed reflect dietary intake, there should be no difference between Diets 1 and 2, and an increase in both Crn and 3-MH excretion levels during Diet 3. Analysis of variance for these Crn data indicated excretion levels on Diet 1 were significantly higher than on

Table 6. Urinary creatinine (Crn, g/d) of college women consuming different diets

	Diet[a]		
Subject	1	2	3
50	1.23	0.57	1.14
51	-	1.08	0.97
52	0.96	0.62	1.00
53	1.13	1.28	1.16
54	1.11	0.78	0.73
55	1.14	1.31	0.85
56	0.96	0.98	1.36
57	1.05	1.01	0.61
58	1.04	0.80	0.87
59	1.02	0.70	1.02
60	1.09	1.00	0.96
61	1.17	1.05	1.14
62	1.00	0.74	1.11
63	1.00	0.76	0.88
64	0.91	0.79	0.91
65	1.21	1.26	1.03
66	1.07	0.91	0.94
67	0.98	0.90	0.94
68	0.93	0.71	0.80
69	1.28	1.12	0.98
Mean	1.06	0.92	0.97
S.D.	+0.12	+0.25	+0.19

[a] Diet 1 was vegetarian, providing no dietary Crn and 3-MH; Diet 2 was ovo-lacto vegetarian, providing small amounts of Crn and 3-MH, and Diet 3 was typical American diet with meat included, providing usual amounts of dietary Crn and 3-MH.

Table 7. Urinary 3-methlyhistidine (3-MH, mg/d) of college women consuming different diets

	Diet[a]		
Subject	1	2	3
50	30.7	16.3	38.7
51	-	23.6	51.5
52	25.3	18.6	47.3
53	26.8	39.5	70.1
54	26.6	20.7	36.4
55	24.6	38.0	49.3
56	23.5	33.9	70.8
57	25.5	27.4	28.9
58	30.6	23.0	36.0
59	32.0	29.1	52.0
60	33.2	27.0	39.9
61	39.8	32.4	47.8
62	28.3	20.7	52.3
63	31.8	18.1	38.2
64	29.3	23.3	38.8
65	32.9	26.6	48.1
66	31.5	25.2	44.0
67	21.3	27.5	43.4
68	31.0	20.7	30.2
69	42.1	32.3	43.0
Mean	29.8	26.2	45.8
S.D.	+ 6.1	+ 7.5	+11.8

[a] Diet 1 was vegetarian, providing no dietary Crn and 3-MH; Diet 2 was ovo-lacto vegetarian, providing small amounts of Crn and 3-MH, and Diet 3 was typical American diet with meat included, providing usual amounts of dietary Crn and 3-MH.

either Diet 2 or Diet 3 ($p<0.05$; see Table 6). This response may be caused by the lag time needed after a dietary change for the creatine pool to stabilize. During Diet 1, which contained no dietary creatine or Crn, the creatine pool should have been decreasing, but the 4 days of feeding was not long enough for stabilization at a new, decreased level. When Diet 2 was fed, the dietary creatine and Crn were again minimal. Urinary Crn excretion was less during Diet 2 than Diet 1, reflecting a decreasing creatine pool during this period. Three days of customary food intake between diet periods did not replace the creatine lost from the pool during 4 days of Diet 1. Although more dietary Crn and creatine present in Diet 3, Crn excretion during Diet 3 remained significantly ($p<0.03$) less than during Diet 1 and not greater ($p<0.32$) than during Diet 2. This difference suggests a delayed response in urinary Crn to increases in dietary Crn, as well as a lag time when Crn is eliminated from the diet.

Individually, only subject 56 followed the expected pattern of Crn excretion. Rather than being the highest, urinary Crn during Diet 3 was actually the lowest in five subjects (54, 55, 57, 65, 69). Some subjects excreted Crn equally during Diets 1 and 3 with lesser amounts during Diet 2 (50, 52, 59, 61, 62, 63, 64), and other subjects excreted more during Diet 2 and less during Diets 1 and 3 (53, 55).

Subject 67 had remarkably constant urinary Crn during the three diets. The other subjects (58, 60, 66, 68) generally had the highest Crn excretion on Diet 1 with equal and lower levels on Diets 2 and 3.

The means and standard deviations for Crn excretion were Diet 1, 1.06 ± 0.12 g Crn/d; Diet 2, 0.92 ± 0.25 g Crn/d; and Diet 3, 0.97 ± 0.19. These means are less than 1.23 ± 0.12 g Crn/d in nine women observed by Fitch and King (1987). Fitch and King's subjects were consuming diets containing meat, which may account for some of the difference in urinary Crn. Also, the subjects in the 1987 study were heavier (mean weight 67.9 kg) than those in the present study (mean weight 62.3 kg).

Urinary 3-MH responded more quickly to dietary changes and reflected dietary 3-MH levels more closely than urinary Crn reflected dietary Crn. Excretions during Diets 1 and 2 were significantly lower ($p<0.0001$) than 3-MH excretion during Diet 3 (see Table 7). Urinary 3-MH during Diet 1 was greater ($p<0.055$) than during Diet 2. This difference suggests that, in some subjects, more time may be needed to allow for clearance of 3-MH from the previous diet or that the subjects were under stress as they began the experiment. Although it is difficult to quantitate it or its effect, stress is believed to influence the rate of protein turnover and, therefore, the level of urinary 3-MH (Buskirk and

Mendez, 1985).

The expected pattern of urinary 3-MH during the three diets was seen in the majority of the subjects (51, 52, 53, 54, 55, 56, 59, 60, 61, 62, 64, 65, 66, 67); in six of these subjects (54, 60, 61, 64, 65, 66), however, the increase in 3-MH excretion during Diet 3 was slight enough that it may not be detectably different from diets 1 and 2 on an individual basis. Subject 57 had little change in urinary 3-MH on the three diets. The remaining subjects (50, 58, 63, 68, 69) showed some variation in urinary 3-MH but most of the differences were relatively small.

Means and standard deviations of 3-MH excretion were Diet 1, 29.8 ± 6.1 mg 3-MH/d (176.3 ± 36 μmol/d); Diet 2, 26.2 ± 7.5 mg 3-MH/d (154.8 ± 44.1 μmol/d); Diet 3, 45.8 ± 11.8 mg 3-MH/d (270.5 ± 69.7 μmol/d). Peters et al. (1989) measured urinary 3-MH excreted by six women during 4 weeks. During 2 weeks, the subjects consumed their usual diet; during the other 2 weeks the subjects ate a diet free of 3-MH. Mean values during the weeks of usual dietary intake were 250 ± 71 and 225 ± 69 μmol 3-MH/d. When the diet was free of 3-MH, daily mean 3-MH excretion ranged from 209 to 221 μmol/d. Three of these subjects were vegetarians so the average 3-MH excretion measured during usual dietary intake was less than might be expected among omnivores. Thus, in the present study, mean urinary 3-MH during Diet 3 (270.5

μmol/d) seems to be comparable to that found by Peters et al. (1989) when their subjects were not on a 3-MH-free diet. When the diets were meat-free, however, urinary 3-MH excretion by the subjects in the present study was less than that reported by Peters et al. (1989) This difference cannot be explained by the subject's body size differences because the average weight of the women in the Peters et al. (1989) study was 60.9 kg compared to 62.3 kg in the present study.

The correlation coefficient between Crn and 3-MH for all three diets was 0.52 ($p<0.001$). Although this correlation is highly significant, the r^2 of 0.27 indicates that either of these two measures of muscle mass can vary independently of the other. The within diet correlation between Crn and 3-MH was 0.74 ($p<0.0001$), which is of similar magnitude to the 0.69 ($p<0.05$) reported by Mendez et al. (1984). The correlation in Experiment 1 (0.87; $p<0.001$) is similar to the correlation by Lukaski et al. (1981), who measured Crn 3-MH excreted by male subjects consuming meat-free diets.

Experiment 2

Means by week and for the entire 56 days for urinary Crn and 3-MH in subjects on the constant diets in Experiment 2 are presented in Tables 8 and 9. Regression analysis

Table 8. Urinary creatinine (Crn, g/d) of college women consuming a constant diet

Subject		Week 1	2	3	4	5	6	7	8	Mean for 56 days
70	mean	1.33	1.37	1.25	1.29	1.25	1.39	1.39	1.27	1.32
	S.D.[a]	0.29	0.08	0.10	0.05	0.19	0.16	0.08	0.13	0.15
	C.V.[b],%	21.7	5.9	7.8	3.8	15.0	11.2	5.8	9.9	11.5
71	mean	1.17	1.22	1.15	1.17	1.11	1.11	1.04	1.05	1.13
	S.D.	0.04	0.30	0.05	0.04	0.05	0.07	0.07	0.03	0.12
	C.V.,%	3.6	24.3	4.3	3.7	4.4	6.2	7.2	2.9	10.9
72	mean	1.15	1.21	1.17	1.21	1.08	1.31	1.08	1.08	1.16
	S.D.	0.12	0.04	0.09	0.04	0.23	0.28	0.12	0.17	0.17
	C.V.,%	10.6	3.4	7.6	3.3	21.6	21.2	11.5	16.0	14.3
73	mean	1.15	1.24	1.29	1.27	1.20	1.18	1.13	1.11	1.20
	S.D.	0.11	0.06	0.16	0.07	0.06	0.06	0.11	0.10	0.11
	C.V.,%	9.6	4.8	12.1	5.8	5.1	5.3	9.5	9.3	9.0
74	mean	1.71	1.79	1.63	1.66	1.65	1.73	1.53	1.64	1.67
	S.D.	0.11	0.07	0.12	0.07	0.06	0.10	0.11	0.23	0.13
	C.V.,%	6.1	3.9	7.5	4.2	3.7	5.6	7.3	13.9	7.9
75	mean	1.20	1.28	1.11	1.20	1.16	1.25	1.08	1.15	1.18
	S.D.	0.13	0.08	0.17	0.05	0.05	0.22	0.32	0.19	0.17
	C.V.,%	10.9	6.0	15.3	4.6	4.6	17.6	29.7	16.8	14.8
76	mean	1.40	1.37	1.37	1.34	1.27	1.34	0.97	1.13	1.29
	S.D.	0.10	0.16	0.05	0.04	0.06	0.18	0.36	0.07	0.20
	C.V.,%	7.3	11.7	4.0	2.9	4.3	13.5	37.5	5.5	15.6
77	mean	1.22	1.34	1.20	1.23	1.21	1.17	1.14	1.15	1.21

	S.D.	0.10	0.07	0.26	0.09	0.09	0.09	0.22	0.10	0.14
	C.V.,%	7.9	5.0	21.3	7.7	7.3	7.9	19.2	8.6	12.0
78	mean	1.26	1.38	1.22	1.23	1.24	1.24	1.15	1.17	1.24
	S.D.	0.15	0.17	0.11	0.06	0.09	0.12	0.20	0.14	0.14
	C.V.,%	12.3	12.5	9.1	4.8	7.4	9.8	17.1	12.2	11.6
79	mean	1.21	1.33	1.22	1.24	1.23	1.30	0.95	1.22	1.22
	S.D.	0.17	0.11	0.13	0.10	0.13	0.05	0.30	0.22	0.18
	C.V.,%	14.1	8.6	10.3	8.3	10.4	3.5	31.1	18.3	15.1

[a] S.D. = standard deviation.
[b] C.V. = Coeffecient of variation = Standard deviation as a percent of the mean.

Table 9. Urinary 3-methylhistidine (3-MH, mg/d) of college women consuming a constant diet

Subject		Week 1	2	3	4	5	6	7	8	Mean for 56 days
70	mean	44.5	43.9	53.5	48.0	49.1	58.6	56.7	51.6	51.1
	S.D.[a]	13.8	8.3	12.0	8.9	11.1	13.0	16.1	9.5	12.1
	C.V.[b],%	31.1	19.0	22.4	18.5	22.6	22.1	28.3	18.3	23.6
71	mean	44.0	42.4	38.3	45.3	38.2	42.4	28.3	33.3	38.9
	S.D.	4.4	4.3	15.7	8.4	5.7	5.0	6.7	6.6	9.3
	C.V.,%	9.9	10.0	41.1	18.6	15.0	11.8	23.5	19.9	23.9
72	mean	42.6	38.9	44.4	50.1	39.1	50.7	34.3	31.2	41.4
	S.D.	6.7	6.1	5.9	9.0	11.5	8.6	10.4	12.5	10.8
	C.V.,%	15.8	15.7	13.3	17.9	29.3	17.0	30.3	40.0	26.1
73	mean	42.6	37.7	45.4	44.8	41.9	37.6	33.3	25.1	38.6
	S.D.	9.0	3.3	7.8	7.6	5.1	6.5	5.7	6.4	9.0
	C.V.,%	21.1	8.7	17.2	17.0	12.2	17.3	17.1	25.6	23.3
74	mean	65.9	59.0	54.1	53.9	53.3	63.5	58.4	49.9	57.2
	S.D.	10.7	6.8	7.7	9.1	9.5	7.1	7.8	17.1	10.7
	C.V.,%	16.3	11.6	14.3	17.0	17.8	11.2	13.3	34.2	18.6
75	mean	47.0	34.6	36.5	34.6	41.3	45.8	36.0	38.0	39.2
	S.D.	8.4	4.3	6.3	6.6	4.9	10.7	13.7	8.0	9.1
	C.V.,%	17.9	12.3	17.4	19.1	11.9	23.3	38.1	21.2	23.2
76	mean	56.9	34.1	51.6	51.9	44.4	45.2	32.8	34.6	44.3
	S.D.	10.4	6.6	6.8	5.2	6.1	5.4	16.5	10.0	12.2
	C.V.,%	18.2	19.4	13.1	10.0	13.6	12.0	50.3	28.8	27.5

77	mean	48.3	43.3	37.9	46.2	36.7	36.5	35.9	35.0	39.8
	S.D.	11.8	7.8	8.6	6.6	3.6	6.6	11.5	9.2	9.3
	C.V.,%	24.5	18.1	22.6	14.2	9.7	18.1	32.1	26.3	23.3
78	mean	47.6	42.5	45.3	43.4	44.6	48.1	39.9	41.5	44.1
	S.D.	7.6	8.6	7.6	4.8	10.9	5.6	10.0	9.8	8.3
	C.V.,%	15.9	20.2	16.7	11.0	24.5	11.6	25.1	23.5	18.7
79	mean	40.7	37.9	45.1	42.4	50.2	51.4	35.0	42.3	43.3
	S.D.	9.2	9.0	8.2	7.7	9.4	5.2	9.6	9.9	9.6
	C.V.,%	22.6	23.8	18.3	18.2	18.6	10.1	27.3	23.3	22.2

[a] S.D. = standard deviation.
[b] C.V. = Coeffecient of variation = Standard deviation as a percent of the mean.

indicated a decrease in both Crn and 3-MH excretion over the 8 weeks. Values during week 1 may have been elevated urinary clearance of Crn and 3-MH from the self-selected diet before week 1. When week 1 is omitted from the regression analysis, urinary Crn significantly decreases ($p<0.023$) with time during weeks 2 through 8. This trend over time is less evident ($p<0.134$) for 3-MH. This decrease in Crn over time supports the model presented by Lykken et al. (1980) in which the creatine pool size may not stabilize for quite some time because of the various sources of creatine precursors and lack of strict metabolic control over creatine synthesis. The release of 3-MH, however, is not only dependent on the supply of precursors but also on the rate of protein turnover. Emotional stress and physical trauma are believed to affect the rate of protein turnover and, therefore, the amount of 3-MH released (Buskirk and Mendez, 1985). These factors are difficult to control and the effects have not been quantified. The decline in 3-MH excretion during weeks 2 through 8 may reflect the lessening of stress as the subjects became accustomed to the experimental protocol.

For some subjects excretion levels of Crn among days were quite variable as indicated by the coefficients of variability (C.V.) but other subject's data were remarkably constant. In 7 of the 8 weeks, subjects 71, 73, and 74 had

C.V. less than 10 percent for Crn. Subjects 76 and 77 were almost as consistent, with C.V. less than 10 percent for 5 and 6 of the 8 weeks, respectively. During week 7, three subjects (75, 76, and 79) showed unusually high variability (C.V. of 29.7, 37.5, and 31.1 percent). In 50 of the 80 subject-weeks observed, the C.V. were less than 10 percent and in an additional 22 subject-weeks, the C.V. were between 10 and 20 percent. An average of all 56 days of Crn data probably provides the best estimate of both excretion and variability that could be expected from these subjects. The range of C.V. became 7.9 to 15.6 percent when all 56 days were averaged. This C.V. approximates the 11 percent variation Lukaski and Mendez (1980) claimed is present when controlled diets are fed. The present results support the work of Edwards et al. (1969), who found C.V. for urinary Crn of 5 to 22 percent in subjects consuming a constant diet for 28 days. If the present data included only week 4 data, during which the C.V. ranged from 2.9 to 8.3 percent for the 10 subjects, the results would agree with those of Bingham and Cummings (1985) who found C.V. of 1 to 7 percent over 4 days in subjects consuming a constant diet.

Although the diets were not creatine- and Crn-free in the Experiment 2, daily intakes of these compounds were constant at least during each 28-day period, if not over the entire 56 days. The intra-subject correlation for daily Crn

excretion was 0.84 ($p<0.01$), indicating good repeatability of this measure in each subject under these controlled conditions but also implies a lack of specificity between muscle mass and urinary Crn levels. Differences between subjects accounted for 49.4% variability in Crn excretion and 50.6% was accounted for by variations within subjects.

If large daily Crn excretion variability within individuals is a drawback to using Crn to estimate body composition, daily variation must also be considered a problem when using urinary 3-MH to calculate body composition. The within subject variability of 3-MH excretion was 75.0% of the total, leaving 25% from between subject differences. In only four instances (subject 71, week 2; subject 72, week 6; subject 76, week 6; and subject 79, week 7) were the C.V. for urinary 3-MH less than those for Crn. The C.V. for 3-MH excretion ranged from 8.7 up to 20 percent in 50 of the 80 subject-weeks that were studied, and from 20 to 30 percent in an additional 22 subject-weeks. This degree of variability is comparable with that found by Fitch et al. (1986), who reported an average C.V. of 20.9 percent in four women. Much less variation has been reported by other investigators. For example, in 14 men, C.V. of 3-MH excretion ranged from 2.2 to 7.0 percent (Lukaski et al., 1981), and Peters et al. (1989) found C.V. of 4.8 and 8.0 percent in six women. The range in C.V. in

the present study was 18.6 to 27.5 percent when calculated over all 56 days, which is approximately twice the variability observed for Crn excretion. The intra-subject correlation for daily 3-MH excretion was 0.65 ($p<0.01$), less than the intra-subject correlation for Crn (0.84). These correlations indicate that during the controlled conditions of Experiment 2, urinary 3-MH excretion was not as constant as Crn excretion.

Just as there were eight subject-weeks during which the C.V. for Crn were unusually large (exceeding 20 percent), there were eight subject-weeks in which the C.V. for 3-MH were also large (greater than 30 percent). The subject-weeks with the greatest C.V. for Crn were not necessarily those with the largest C.V. for 3-MH; in only three of the eight subject-weeks did large variation in Crn coincide with high variation in 3-MH (subject 70, week 1; subject 75, week 7; and subject 76, week 7). Using daily values for all subjects, the within subject correlation coefficient between Crn and 3-MH was 0.48 ($p<0.0001$). Again, this is a very significant correlation, but the r^2 of 0.23 indicates that these two indices of muscle mass can vary independently and that factors other than muscle mass and dietary components influence their excretion levels. The correlation coefficient is less than the correlation coefficient of 0.69 ($p<0.05$) reported by Mendez et al. (1984), and the

correlation coefficient of 0.87 ($p<0.001$) reported by Lukaski et al. (1981).

Body Composition, Creatinine, and 3-Methylhistidine

Because of technical problems with the Body Volume Determinator, body composition could not be measured while the subjects were participating in both Experiments 1 and 2. Body density was measured within a year after the first feeding experiment. Only 20 of the 30 subjects were available because of graduations and transfers. Because the subjects in Experiment 2 were consuming meat, each subject's mean excretions of Crn and 3-MH during Experiment 2 were put in a data set with the mean values from the subjects on Diet 3 in Experiment 1, which also included meat.

The means, standard deviations, and ranges for the body composition measures are presented in Table 10. For all variables, the ranges are quite large, meaning that the subjects had bodies of different sizes and compositions.

The correlation coefficients between Crn and 3-MH excretion and the estimates of body composition are presented in Table 11. A correlation of 0.57 ($p<0.009$) was found between Crn and 3-MH; this was less than that of 0.87 ($p<0.001$) reported by Lukaski et al. (1981). Urinary Crn was more highly correlated with the more easily measured body weight ($r=0.57$, $p<0.008$) or height ($r=0.60$, $p<0.005$)

Table 10. Body composition measures of 20 subjects

	Mean ± S.D.	Range limits
Body weight, kg	63.3 ± 9.2	46.8 - 89.7
Height, cm	167.3 ± 5.6	155.8 - 184.5
Height/weight ratio	2.68 ± 0.32	2.06 - 3.50
Body density, g/cc	1.003 ± 0.020	0.977 - 1.057
Fat, %	43.8 ± 9.6	18.3 - 56.5
Lean body mass, kg	35.2 ± 5.7	24.3 - 50.7
Fat mass, kg	28.1 ± 8.2	9.0 - 39.2
Urinary Crn, g/d	1.09 ± 0.22	0.73 - 1.65
Urinary 3-MH, mg/d	44.6 ± 8.9	30.2 - 70.1
Crn coefficient, mg/kg	17.4 ± 3.1	12.5 - 23.7
3-MH ratio, mg/kg	0.71 ± 0.15	0.52 - 1.17

Table 11. Correlation coefficients (r) between urinary creatinine (Crn) and 3-methylhistidine (3-MH) and estimates of body composition

	Crn, g/d		3-MH, mg/d		Ht/Wt ratio	
	r	p[a]	r	p	r	p
Urinary Crn, g/d	-	-	0.57	0.009	-0.43	0.06
Body weight, kg	0.57	0.008	0.35	0.13	-0.95	0.0001
Height, cm	0.60	0.005	0.29	0.21	-0.64	0.002
Ht/wt ratio	-0.43	0.06	-0.30	0.20	-	-
Body density, g/cc	-0.32	0.17	-0.18	0.44	0.48	0.03
Lean body mass, kg	0.22	0.35	0.16	0.50	-0.38	0.10
Fat mass, kg	0.49	0.03	0.28	0.22	-0.81	0.0001

[a] The probability of a value of r this large or larger when the true correlation equals zero.

than with either body density ($r=-0.32$, $p<0.17$) or lean body mass ($r=0.22$, $p<0.35$) The correlation between fat mass and Crn was also significant ($r=0.49$, $p<0.03$). These data indicate that in these subjects urinary Crn seemed to be more dependent on body size than on body composition. Increases in body weight were more associated with increases in fat mass ($r=0.79$, $p<0.0001$) than increases in lean body mass ($r=0.49$, $p<0.03$). These relationships are reflected in the greater correlation between fat mass and Crn than between lean mass and Crn. Urinary 3-MH was less correlated with the body composition measures than was Crn. There were greater correlations for 3-MH with measures of body size, such as body weight ($r=0.35$, $p<0.13$) and height/weight ratio ($r=-0.30$, $p<0.20$), than there were with body composition estimates. These correlations between estimates of body composition and urinary Crn or 3-MH are much less than those between lean mass and 3-MH reported by Lukaski et al. (1981; $r=0.81$, $p<0.001$) or Mendez et al. (1984; $r=0.93$, $p<0.01$).

Multiple regression analyses were performed to see if combining variables would increase the predictability of body composition measures; body density, lean body mass, and fat mass were each estimated from Crn, 3-MH, and Ht/Wt ratio. Because percent lean and percent fat were calculated from body density using constants, these equations gave parallel results. The equations for body density, lean body

mass, and fat mass are presented in Table 12. As expected from the correlation coefficients in Table 11, the Ht/Wt ratio was the single best predictor of body composition. For all body components, the Ht/Wt ratio gave the highest R^2 and the smallest standard error. When Crn and 3-MH were included in the equation with the Ht/Wt ratio, the R^2 increased slightly in the equations for all body components. If Crn and 3-MH were significantly contributing to the prediction, addition of them to the equations should increase the regression sums of squares while decreasing the residual or error sums of squares, resulting in a smaller error mean square when compared to the single variable equations. However, the error mean square is larger when Crn and 3-MH are included with the Ht/Wt ratio in the equations. This indicates there is no improvement in the accuracy of the predictions by including Crn and 3-MH.

In the present studies, body composition determinations were not done at the same time urinary Crn and 3-MH were measured. The subjects' body weights were not different at the time of body density determinations than they were during the experimental periods, however, and the subjects reported no important physical activity changes during the time between the feeding studies and the body composition measurements. There may have been unidentifiable changes such as seasonal variation during Experiments 1 and 2 that

Table 12. Equations for predicting body composition from urinary Crn, 3-MH, and Ht/Wt ratio[a]

Predicted Component	Equation	R^2	S.E.E.[b]
Body Density (gm/cc)	1.0352 - 0.0296 Crn	0.102	0.0195
	1.0212 - 0.0004 3-MH	0.034	0.0202
	0.9217 + 0.0302 Ht/Wt	0.233	0.0180
	0.9433 - 0.0141 Crn + 0.0001 3-MH + 0.0267 Ht/Wt	0.248	0.0189
Lean Body Mass (kg)	28.87 + 5.82 Crn	0.049	5.67
	30.70 + 0.10 3-MH	0.026	5.74
	53.10 - 6.66 Ht/Wt	0.141	5.39
	49.18 + 1.58 Crn + 0.01 3-MH - 6.07 Ht/Wt	0.146	5.70
Fat Mass (kg)	7.69 + 18.65 Crn	0.243	7.30
	16.45 + 0.26 3-MH	0.081	8.04
	83.51 - 20.65 Ht/Wt	0.652	4.95
	72.31 + 7.71 Crn - 0.05 3-MH - 18.80 Ht/Wt	0.678	5.05

[a] Crn=urinary creatinine, g/d; 3-MH=urinary 3-methlyhistidine, mg/d; Ht/Wt ratio=Height, cm/Weight, kg.

[b] Standard error of the estimate; square root of error mean square.

obscured the relationship between lean mass and 3-MH excretion.

SUMMARY AND CONCLUSIONS

Urinary creatinine (Crn) and 3-methylhistidine (3-MH) were measured in healthy college women during two metabolic studies. Twenty subjects participated in Experiment 1. Three diets providing increasing levels of Crn (and its precursors) and 3-MH were fed to the subjects. Urine samples collected during days 3 and 4 of each diet were analyzed for Crn and 3-MH. In Experiment 2, ten women ate a constant diet for 28 days, then switched to a different diet for an additional 28 days. Although these diets were not Crn- and 3-MH-free, the dietary intakes were constant. Daily urine collections were analyzed for Crn and 3-MH. Body composition was estimated in 20 of the 30 participants by use of densitometry.

For Experiment 1, analysis of variance indicated significantly higher Crn excretion during Diet 1 than during either Diet 2 or Diet 3. Urinary 3-MH excretion during Diet 3 was significantly higher than during either Diet 1 or Diet 2; this difference reflected the changes in dietary 3-MH. The patterns of excretion among the subjects for the three diets varied more for Crn than for 3-MH.

Both urinary Crn and 3-MH excretion were variable during the 56 days on the constant diets of Experiment 2. Coefficients of variation (C.V.) were calculated by week for

each of the 10 subjects. No subject had a C.V. less than 10 percent for all 8 weeks. Urinary 3-MH was approximately twice as variable as Crn. The weeks with the greatest variability for Crn were not those with the most variability for 3-MH. Both urinary Crn and 3-MH decreased over the experimental feeding period. The decrease of Crn was more evident ($p<0.023$) than the decrease in 3-MH ($p<0.134$).

Urinary Crn was more highly correlated with the more easily measured body weight ($r=0.57$, $p<0.008$) and height ($r=0.60$, $p<0.005$) than the measured estimates of body composition. The correlation between 3-MH excretion and body size and composition was less than the correlation between Crn and body size and composition.

In conclusion, urinary 3-MH responded to dietary changes within 3 days but Crn was not stabilized within this time. Crn excretion on a constant diet was less variable than urinary 3-MH. The extended time required for Crn excretion to stabilize may negate its usefulness as an index of body composition. The problem of variability in 3-MH excretion may be overcome by collecting multiple consecutive 24-hour urine samples to improve the estimate of an individual's urinary 3-MH.

LIMITATIONS AND RECOMMENDATIONS

Although any report of observations on human subjects is valuable, each has its limitations that restrict the interpretation of the data. Each indirect method for estimating body composition, whether complex or simple, is based on a number of assumptions. The estimates of body components are, therefore, only as valid as the assumptions inherent in the method.

Experiment 1 was carried out to examine the responsiveness of Crn and 3-MH excretion to changes in dietary intake of these constituents and their precursors. There are three possible ways to redesign this experiment to improve the interpretability of the data. First, each diet was fed for only 4 days. Because of the time required to excrete 3-MH and Crn from previous diets from the body, a longer feeding period is recommended so that there are multiple days of urine collections from which daily excretion levels of Crn and 3-MH could be estimated. Second, all subjects were fed the diets in the same order. Thus, it is not possible to ascertain whether some of the differences seen in this study are caused by the dietary changes or to the order of the diets fed. Additional experiments incorporating a cross-over design might clarify the causes of these differences. Third, the study was

conducted in two phases. Although seasonal variation is not believed to affect urinary Crn or 3-MH, this variable should be controlled when possible.

The focus of Experiment 2 was to determine the variability in Crn and 3-MH excretion on a constant diet. The major factors believed to affect Crn and 3-MH levels (diet and activity) were controlled. All subjects were healthy, so physical stress and trauma were minimized. The great variability in urinary Crn and 3-MH found during the controlled conditions of this study indicate a need to identify other factors that may influence the excretion of these compounds.

Because of the time between the measurement of urinary Crn and 3-MH and the determination of body composition for both Experiment 1 and 2 subjects, the relationships among body composition estimates and urinary Crn and 3-MH found in both Experiments 1 and 2 may not describe the real situation. Dietary intakes change with seasons, and activity levels change with weather conditions. Body composition indices should be measured concurrently if comparisons among them are to be valid.

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ACKNOWLEDGEMENTS

Sincere appreciation is expressed to Dr. Pilar Garcia, Dr. Mary Jane Oakland, Dr. Donald Hotchkiss, Dr. David Cox, and Dr. Dean Zimmerman for their advice and support in this endeavor. Gratitude also goes to Dr. Wayne Bidlack for his assistance.

Special thank yous are given to Robin Orr and Meg Manatt for their understanding, encouragement, and participation in completing this project.