DIURNAL AND DAILY VARIATION IN CREATININE EXCRETION
AMONG PREADOLESCENT GIRLS CONSUMING THREE LEVELS
OF DIETARY PROTEIN

by

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# INTRODUCTION

Creatine is defined as "a white crystalline compound found especially in the muscles of vertebrates both as phosphocreatine and in the free form and also in the blood and obtained in meat extracts" (1). It was isolated from meat extracts by Chevreul as early as 1832 and creatinine was discovered by Rosenthal in 1870 (2). Creatine formation probably begins when the amide group of arginine is transferred, in the kidney, to glycine, forming glycocyamine, or guanidinoacetic acid, which is normally found in the urine of children. Transmethylation of the glycocyamine in the liver involving methionine or choline results in the formation of creatine (3). Synthesized creatine diffuses into the vascular system and is transported to various cells, particularly to those of muscle, where it is phosphorylated. Both phosphocreatine and creatine are spontaneously converted into creatinine. Creatine is not usually found in adult urine (4), but creatinuria appears in children, as a result of methyltestesterone administration, in eunuchoidism, in hyperthyroidism, in pregnancy, in diabetes, possibly in old age, in muscular dystrophy and in poliomyelitis (3).

Other than the general consideration of it being an end product of the mammalian metabolism, the function of creatinine remained unquestioned for many years. Creatinine as an internal anhydride of creatine was characterized as early as 1886. Since that time the function of this substance has baffled each generation of physiologists and biochemists. Lis and Bijan (4) advanced the hypothesis that formation of creatinine in the muscle may be a simple nonenzymatically operated organism protective mechanism: as fatigue of the muscle increases and lactic acid accumulates the energy supply can be shut off by the formation of creatinine from phosphocreatine or creatine. Consequently, the more popular theory has been that creatinine (which cannot be utilized for any other purpose) is excreted in the urine in quantities which are independent of the diet. Later, Lis et al. (2) repeat that creatinine has been considered essentially a metabolic end product (or waste) without further function, and that the nonenzymic acid-catalyzed conversion is a lactate-dependent mechanism. They go on to say that creatinine may be responsible for control of torpor induction, i.e., drowsiness, to allow the muscle cell time for recovery of energy-containing creatine-phosphate. The nonenzymic formation of creatinine from creatine-phosphate makes this mechanism more feasible in view of the nocturnal-diurnal variation of activity and recovery.

Nitrogen entering the body of mammals as food is ultimately stored in the form of body protein or eliminated, chiefly through urine and feces, as urea, ammonia, creatinine, uric acid, amino acids and other known nitrogenous substances (5). The use of creatinine excretion as an index of endogenous protein metabolism was introduced by Folin (6). The "creatinine coefficient", defined as the milligrams of creatinine or creatinine nitrogen excreted per kilogram of body weight, has been variously interpreted as proportional to or an index of (a) the amount of active protoplasmic tissue (6) or (b) muscular mass and efficiency (7). The effects of such factors as diet, exercise and sex upon urinary creatinine have been controversial for more than 55 years. Folin (6,8) stimulated considerable interest in this subject when he pointed out that the amount of creatinine excreted in the urine by a given individual receiving a meat-free diet is a constant quantity, but that it may be different for other individuals and wholly independent of the quantitative changes in the total amount of nitrogen eliminated. This assumption of constancy of creatinine excretion has been widely accepted by nutritional survey teams who have used creatinine excretion as a reference standard against which other metabolites may be compared. In the present study the reliability of the urinary nitrogen/creatinine ratio as an index of dietary protein level was investigated.

## REVIEW OF LITERATURE

### Constancy of Creatinine Excretion

Since Folin s classic studies on protein metabolism, the concept that the creatinine excretion in adult animals and man was essentially constant and not subject to change as a result of dietary protein manipulation has been widely accepted (9). Folin in 1905 also came to the conclusion that the daily creatinine output of a given individual is more or less constant, is influenced by the body weight, and independent of a diet which is free of creatinine or creatine (2). Hunter (10,11) also indicated that creatinine is independent of dietary protein levels.

Kertz et al. (12) studied the effects of varying nitrogen intake and the relationship of diurnal variation to creatinine excretion and to the creatinine/nitrogen ratio (milligrams of creatinine per milliliter of urine/milligrams of nitrogen per milliliter of urine) in bovine urine. They found that total urinary creatinine and the creatinine coefficient were not significantly affected by treatment, period, or by their interaction, but that increased nitrogen intake does significantly increase creatinine and nitrogen concentrations in urine.

Albin and Clanton (13) studied the factors contributing to the variation in urinary creatinine in beef cattle. They found that urinary nitrogen and the creatinine/nitrogen ratio were affected by rations in all experiments where studied, but that creatinine did not fluctuate for a given individual with respect to rations. There was no significant difference in urinary creatinine, nitrogen or the creatinine/nitrogen ratio in the trials where daily variation was studied. A small variation in diurnal urinary creatinine values was observed. This is in agreement with a report by De Groot and Aafjes (14). Albin and Clanton (13) found that urinary nitrogen was excreted in a diurnal cycle similar to the pattern shown for urinary creatinine. Variation for the diurnal urinary nitrogen mean values was larger than the variation for urinary creatinine.

Butcher and Harris (15, 16) illustrated the dependence of urinary nitrogen in contrast to the independence of urinary creatinine upon level of protein intake. As a result, total daily excretion of urinary nitrogen and the urinary/creatinine nitrogen ratio were inversely associated with dietary protein when energy intake was relatively constant. However, they did report a diurnal variation in urinary creatinine.

The assumption that creatinine excretion is constant in short collection periods is based mainly on the work of Shaffer (7), who found a remarkably constant hourly excretion in three persons followed at 1- to 5-hour intervals during several 24-hour periods and in four persons on whom 2 or 3 collections were made for different periods within a single day. Chotkowska and Rakowska (17) found that the difference in average creatinine in urine of ten children fed 55.5 and 26.9 grams of protein per day was statistically insignificant.

Despite wide-spread acceptance of Folin's theory of constancy of creatinine excretion, the results of a number of studies do not support such a conclusion. Commenting immediately on Folin's original observations, Paton (18) reported a marked change in the creatinine excretion of dogs fed protein at varying levels. During the 1930's and early 1940's Beard (19) published a series of papers as well as a review monograph in which he criticized Folin's concept of a constant creatinine excretion and gave considerable evidence in support of a lack of constant creatinine excretion. Beard's studies have often been quoted, but dismissed as insignificant. A report by Nakagawa et al. (20) on the amino acid requirements of children shows not only a clear variation, but also a distinct pattern in urinary creatinine excretion upon changing the subjects from their "normal diet" to the experimental diet. Paterson (21) found that for most subjects, the 24-hour creatinine output was not sufficiently constant in the same individual from day to day, or in relation to body weight or surface area to justify its use as a physiological reference standard against which the excretion of other substances ought to be compared, except in circumstances where accurately timed urine collections are not possible. Vestergaard and Leverett (22) found that there were very large individual variations in creatinine excretion in the 24-hour periods and also in the shorter collection periods.

Best et al. (23) had their five normal adult male subjects of average build, while following their usual diet and activities, collect six 4-hour urine specimens daily over a period of 14-20 days. The lowest mean excretion occurred from 2-6 a.m. The highest ratio fell between 2 p.m. and 10 p.m. with a mean peak at 2-6 p.m. Lis et al. (2) studied the variation in the quantitative output of creatinine in the urine of six individuals for ten consecutive days and found it to be associated with a diurnal-nocturnal cycle. The excretion of creatinine in the same individual varied from voiding to voiding within the same day and from day to day. They proposed that nocturnal-diurnal variation in creatinine excretion is associated with muscle cell recovery. Lewis et al. (24) added to the ever-increasing body of evidence showing that variation of creatinine within 24 hours is quite significant. They tested the voidings of nine children to determine if the excretion of creatinine and riboflavin in individual voidings during a 24-hour period varied to an extent that a vitamin/creatinine ratio would be questionable. Results showed such variation in creatinine that for these children it was not a reliable basis for comparison of riboflavin in casual urine samples or for determining completeness of 24-hour collections. Briscoe and Ragan (25) found creatinine to be less in night urine samples than in day samples and concluded that under normal living conditions, diurnal changes in diet and physical activity are the basic causes of the diurnal rhythms in excretion.

Scott and Hurley (26) compared the relative constancy of 24-hour urine volume and creatinine output in a situation where accuracy of urine collection was guaranteed by observing the rate of isotope excretion during protein turnover studies. They found that 24-hour urinary creatinine output is insufficiently constant between individuals, or in the same individual from time to time, to justify its use as an accurate index of completeness of collections. Though diets were not controlled throughout her experiment, Chen (27) observed that creatinine output was high for subjects with high-protein diets, whereas low in persons consuming low-protein foods. She also found significant diurnal variation in individual subjects. Ritchey et al. (28) found that as nitrogen intake increased among preadolescent girls, urinary creatinine also increased.

## The Use of Ratios Between Creatinine and Nitrogen As An Index for Balance Studies

One of the more widely used reference works in biochemistry states: "In the adult male it was shown . . . the daily excretion of creatinine is constant over long periods, not only under standard conditions, but under the most varied circumstances. It is little affected by diet, exercise or large variations of urine volume. Apparently the creatinine excretion is characteristic for a given individual in health and is determined chiefly by his size. Because of this relationship the daily excretion of creatinine is often used to check the accuracy of 24-hour collections (29)."

If indeed this is true, creatinine may be used as an index material in urine for balance studies, implying a major application for the use of urinary creatinine values. Evaluating the adequacy of dietary protein is one of the important problems confronting nutritional survey teams. Because of the large population sampled, it is necessary that any test for this purpose be easily and rapidly performed.

Since creatinine has been assumed to be relatively constant for a given individual, a ratio can be used between creatinine and any other constituent in urine. Many studies have shown that 24-hour urine total nitrogen values vary with dietary protein levels (30). If the ration does not alter creatinine excretion, a given urine sample high in nitrogen would have a low creatinine/nitrogen ratio (Cr/N). If the nitrogen content were low, the Cr/N would be high. It is commonly accepted that the ration affects urinary nitrogen. Therefore, as dietary intake fluctuates with respect to nitrogen or calories or both, the urinary Cr/N would be expected to fluctuate accordingly. This expression of urinary creatinine per gram of nitrogen circumvents the difficulties involved in collecting a timed urine specimen. It is important to remember, however, that the use of urinary creatinine as a reference material in metabolism studies depends upon a uniform and constant excretion of creatinine (29, 13). Vestergaard and Leverett (22) also report that the constancy of creatinine excretion in 24-hour periods has been assumed by a number of investigators to be true for short term collection periods, and, in addition, creatinine excretion has been frequently used for the formation of ratios; the advantage of such ratios when used to judge the effect of stimuli on the numerator of the ratios (assuming a constant creatinine excretion) is that the effect of incompleteness of urine collection or inaccuracy is the timing of collection periods would be minimized.

A number of authors mentioned earlier have used ratios. The study of Powell et al.(31) was designed to calculate the use of N/Cr (grams of nitrogen per gram of creatinine) in random urine samples to estimate dietary protein. They employed linear regression equations in order to allow a comparison of timed urinary nitrogen and N/Cr when used to predict nitrogen intake. The objective of Kertz et al. (12) was to study the effects of varying nitrogen intake and the relationship of diurnal variations, total body weight, and total urine volume to urinary creatinine, urinary nitrogen and their ratio on a relatively constant energy ration. Butcher and Harris (15) were able to illustrate the dependence of daily urinary nitrogen in contrast to the independence of daily urinary creatinine upon level of protein intake via use of Cr/N and significant diurnal variations in Cr/N. Albin and Clanton (13) also investigated the possibility of estimating dietary protein and energy imbalances from the urinary Cr/N, and Chen (27) calculated the ratio of urinary nitrogen to the corresponding creatinine in order to determine stability of creatinine excretion in adults.

## MATERIALS AND METHODS

Fifteen healthy preadolescent girls were recruited from the local area to serve as subjects for this study. They ranged in age from 7.5 - 9.5 years with an average age of approximately 8 years. Each subject was examined by a physician prior to the study to insure normal health and absence of food allergies. Parents and subjects were made aware of the objectives of the study and procedures to be utilized by written information and through discussion. Consent was obtained from the parents of each participant. The subjects were housed in a semi-metabolic unit for the study, and were under supervision at all times, with medical supervision available. A nurse checked the subjects on a daily basis for minor illnesses and/or problems, and a physician was available for the treatment of illnesses. The weights and heights of the subjects were recorded on the first and last days of the experiment and on the first and sixth days of each period. This study was conducted similarly to a camp experience for the subjects. Scheduled hours of rest, sleep and activity were maintained to correspond as closely as possible to the habits typical of this age group.

Diets were provided from the diet kitchen of the Department of Human Nutrition and Foods of the university. Supervision was provided during the consumption of meals and snacks to insure that loss of food was kept to a minimum. All foods were weighed or measured to insure that accurate intakes were obtained. Unavoidable losses of food or food refused were recorded, and if possible, were weighed and subtracted from the intake. The diets provided all essential nutrients at levels recommended by the Food and Nutrition Board, National Research Council, 1968, with the exception of protein (32, 33, 34, 35). Vitamin and mineral supplements were given as needed to insure that all subjects consumed adequate amounts of essential nutrients. The isocaloric diets of approximately 2,000 kilocalories were written in order that dietary protein would provide 30 grams (approximately 1.0 gram per kilogram of body weight), 60 grams (approximately 2.0 grams per kilogram of body weight), or 90 grams (approximately 3.0 grams per kilogram of body weight) daily. The Recommended Daily Allowance for protein of the Food and Nutrition Board, National Research Council, for this group is 40 grams (32). Upon laboratory analysis approximately 4.5 - 5.0 grams, 8.2 - 8.4 grams, or 12.7 - 12.9 grams daily of nitrogen was provided. The dietary protein was of mixed sources and ranged from medium to high biological value, and was as nearly alike in the three dietary protein levels as possible. Water source was restricted to deionized water, but the amount of intake was not limited.

The subjects were divided among 3 groups of 5 members each, designated as Groups A, B, and C (Table 1). The groups were rotated in a random manner, enabling each to consume all levels of protein. Each group of subjects consumed each diet for one entire period. The first two periods were 10 days each, and the last period was 11 days. The optimal duration of balance periods has long been a point of discussion. Some investigators have claimed adequacy for periods as short as one day, while others have urged the need for test periods of 3 - 4

Table 1. Schedule Enabling Each Subject to Consume All Levels of Dietary Protein.

| Protein (grams) | Periods in Which Protein Levels Consumed | | |
|---|---|---|---|
| | Group A (Subjects 100-104) | Group B (Subjects 105-109) | Group C (Subjects 110-114) |
| 30 | I | III | II |
| 60 | II | I | III |
| 90 | III | II | I |

weeks (36). Three days were used as an adjustment period initial to the experiment, with all subjects consuming 30 grams of protein. On Day 3 of each of the three dietary periods, consecutive urine samples were collected for the following 4 intervals: 8 - 12 a.m., 12 - 4 p.m., 4 - 8 p.m., and 8 p.m. - 8 a.m.

The urine was collected in portable units. Daily urine collection began with the second voiding on the morning of Day 1 of Period I and continued through the first voiding on the morning of Day 12 of Period III. Each unit was emptied before the second voiding of the morning and replaced, with 10 milliliters of 4N HCL added. The urine was then transported to the laboratory where the volumes were recorded. The accuracy of the creatinine and nitrogen determinations was increased by adjusting 24-hour urine volumes to 2000 milliliters with distilled water, thus giving AutoAnalyzer peak heights within a fairly close range for each individual (20). The diurnal samples were collected in plastic lidded cups at the end of each interval, and the units replaced with 2, 2, 2 and 4 milliliters of 4N HCL, respectively. Urine volumes for each interval were recorded. In Periods II and III the diurnal volumes were diluted to the nearest convenient volumes, which were also recorded. On rare occasions, a few subjects did not have voidings during an interval and these were recorded as zero excretion. Daily nitrogen and creatinine values and volumes for Day 3 were derived by summing the values and volumes of the intervals. Urine specimens were immediately analyzed for creatinine and those not immediately analyzed for nitrogen were stored in the freezer at a temperature of -15 degrees F. For every 24 hours, food composites were prepared. Aliquots were taken from these composites for nitrogen and creatinine analysis.

Urinary creatinine determinations were made in duplicate by the alkaline picrate method (Jaffe reaction) using the Technicon Auto-Analyzer II (37, 38, 39, 40, 41). Daily and diurnal nitrogen determinations and daily dietary nitrogen determinations were also made in duplicate by a modified Kjeldahl-Arnold method which uses copper and selenium catalysts (42).

## Statistical Analyses

The mean values of creatinine and urinary nitrogen excretion were calculated for each subject on each level of dietary protein. Ratios of urinary nitrogen to creatinine were also calculated for each subject on each level of dietary protein in each collection interval on Days 3 of the three periods. Means and standard deviations of creatinine and urinary nitrogen were calculated for all subjects on each level of dietary protein. Means and standard deviations of the ratios of urinary nitrogen to creatinine were calculated for all subjects at each interval on Days 3 in the three periods.

An analysis of variance, using the repeated-measures design, was performed to determine the statistical significance of the difference in creatinine excretion of subjects consuming the three levels of protein. Analyses of variance were also performed to determine the significance of the diurnal differences in the ratios of nitrogen to creatinine excreted on Day 3 of each of the three periods (43).

# RESULTS AND DISCUSSION

The mean weight for all subjects was 29.11 kilograms and ranged from 23.30 to 38.95 kilograms. The mean height for all subjects was 132.9 centimeters and ranged from 124.0 to 144.0 centimeters (Table 2).

The mean daily creatinine excretion for each subject on each level of dietary protein and the mean values and standard deviations for all subjects on each level of dietary protein are given in Table 3. The mean for all subjects on 30 grams of protein was 0.657 ± 0.079 grams per day; on 60 grams of protein, 0.672 ± 0.079 grams per day; and on 90 grams of protein, 0.737 ± 0.083 grams per day. Analysis of variance showed that the difference in daily creatinine excretion among subjects consuming the three levels of protein was significant at the 1.0% level (F=29.67, Table 4). This would imply that the excretion of creatinine by the subjects in this study varied in the same direction as dietary protein levels.

Table 5 summarizes the mean daily urinary nitrogen excretion for each subject on each level of dietary protein and the mean values and standard deviations for all subjects on each level of dietary protein. The subjects consuming 30 grams of protein excreted a mean value of 4.11 ± 0.56 grams of nitrogen per day; consuming 60 grams of protein, 6.70 ± 0.49 grams of nitrogen per day; and consuming 90 grams of protein, 10.70 ± 0.64 grams of nitrogen per day. No analysis of variance was performed here; urinary nitrogen is assumed to increase with increasing levels of nitrogen in the diet.

Table 2. Weights (kilograms) and Heights (centimeters) of Subjects.

| Subjects | Kilograms | Centimeters |
|---|---|---|
| 100 | 31.70[1] | 133.5 |
| 101 | 38.95 | 144.0 |
| 102 | 26.20 | 134.0 |
| 103 | 25.95 | 128.5 |
| 104 | 24.45 | 127.8 |
| 105 | 30.80 | 133.3 |
| 106 | 27.10 | 139.5 |
| 107 | 23.30 | 125.8 |
| 108 | 23.30 | 124.0 |
| 109 | 32.20 | 137.8 |
| 110 | 33.30 | 133.0 |
| 111 | 31.45 | 139.5 |
| 112 | 24.30 | 127.5 |
| 113 | 37.55 | 137.8 |
| 114 | 26.15 | 127.3 |
| Mean | 29.11 | 132.9 |

[1] Mean of values at beginning and end of the study.

Table 3. Mean Creatinine Excretion (grams/24 hours) Values for Subjects Consuming 30, 60, and 90 Grams of Dietary Protein.

| Subject | 30 Grams | 60 Grams | 90 Grams |
|---|---|---|---|
| 100 | 0.683 | 0.599 | 0.772 |
| 101 | 0.766 | 0.829 | 0.930 |
| 102 | 0.605 | 0.644 | 0.716 |
| 103 | 0.612 | 0.615 | 0.695 |
| 104 | 0.574 | 0.621 | 0.671 |
| 105 | 0.750 | 0.751 | 0.787 |
| 106 | 0.724 | 0.737 | 0.744 |
| 107 | 0.604 | 0.567 | 0.639 |
| 108 | 0.583 | 0.606 | 0.690 |
| 109 | 0.676 | 0.713 | 0.716 |
| 110 | 0.638 | 0.614 | 0.704 |
| 111 | 0.722 | 0.724 | 0.831 |
| 112 | 0.589 | 0.666 | 0.692 |
| 113 | 0.794 | 0.787 | 0.841 |
| 114 | 0.541 | 0.606 | 0.622 |
| Means ± Standard Deviations | 0.657±0.079 | 0.672±0.079 | 0.737±0.083 |

Table 4. Analysis of Variance of Creatinine Excretion Between Protein Levels for Fifteen Subjects.

| Source | SS | df | ms | F | P |
|---|---|---|---|---|---|
| Total | 0.3245 | 44 | ------ | ----- | ---- |
| Subjects | 0.246 | 14 | ------ | ----- | ---- |
| Treatments | 0.0534 | 2 | 0.0267 | 29.67 | $\ll 0.01$ |
| Error | 0.0251 | 28 | 0.0009 | ----- | ---- |

Table 5. Mean Urinary Nitrogen Excretion (grams/24 hours) Values for Subjects Consuming 30, 60, and 90 Grams of Protein.

| Subject | 30 Grams | 60 Grams | 90 Grams |
|---|---|---|---|
| 100 | 3.70 | 5.80 | 11.00 |
| 101 | 3.50 | 6.60 | 11.70 |
| 102 | 3.50 | 7.20 | 10.30 |
| 103 | 3.60 | 6.10 | 11.60 |
| 104 | 3.40 | 6.70 | 12.00 |
| 105 | 4.60 | 7.10 | 10.70 |
| 106 | 4.00 | 6.90 | 10.40 |
| 107 | 4.20 | 6.00 | 10.10 |
| 108 | 4.00 | 6.40 | 10.50 |
| 109 | 5.10 | 6.70 | 10.30 |
| 110 | 5.20 | 6.30 | 9.90 |
| 111 | 4.40 | 7.20 | 10.60 |
| 112 | 4.20 | 7.50 | 10.00 |
| 113 | 4.50 | 6.60 | 10.90 |
| 114 | 3.80 | 7.00 | 10.30 |
| Means±Standard Deviations | 4.11 ± 0.56 | 6.70 ± 0.49 | 10.70 ± 0.64 |

The diurnal creatinine excretions on Days 3 of the three periods are listed for each subject in Tables 6, 7, and 8. The mean values and standard deviations for all subjects are also given. Tables 9, 10, and 11 summarize the diurnal urinary nitrogen excretions for each subject and the mean values and standard deviations for all subjects. The values for diurnal creatinine excretions of each subject range from no excretion (Subjects 100, 110 and 113, 8 - 12 a.m.) to 0.547 grams (Subject 100, 12 - 4 p.m.) when all subjects consumed 30 grams of protein; from no excretion (Subjects 100, 109, and 113, 8 - 12 a.m., Subjects 103 and 113, 12 - 4 p.m., Subject 111, 4 - 8 p.m., and Subjects 101 and 102, 8 p.m. - 8 a.m.) to 0.449 grams (Subject 111, 8 - 12 a.m.) when all subjects consumed 60 grams of protein; and from no excretion (Subject 100, 8 - 12 a.m. and 8 p.m. - 8 a.m., Subject 109, 12 - 4 p.m.) to 0.455 grams (Subject 102, 8 - 12 a.m.) when all subjects consumed 90 grams of protein. The values for diurnal urinary nitrogen excretion of each subject range from no excretion (Subjects 100, 110 and 113, 8 - 12 a.m., Subject 104, 12 - 4 p.m. and 8 p.m. - 8 a.m.) to 2.9 grams (Subject 100, 12 - 4 p.m.) when all subjects consumed 30 grams of protein; from no excretion (Subjects 100 and 113, 8 - 12 a.m., Subjects 103 and 113, 12 - 4 p.m., and Subjects 101 and 102, 8 p.m. - 8 a.m.) to 3.80 grams (Subject 109, 8 p.m. - 8 a.m.) when all subjects consumed 60 grams of protein; and from no excretion (Subject 100, 8 - 12 a.m. and Subject 109, 12 - 4 p.m.) to 7.03 grams (Subject 102, 8 p.m. - 8 a.m.) when all subjects consumed 90 grams of protein.

Table 6. Mean Diurnal Creatinine Excretion (grams) Values for Subjects Consuming 30 Grams of Protein.

| Subject | 8 - 12 a.m. | 12 - 4 p.m. | 4 - 8 p.m. | 8 p.m. - 8 a.m. |
|---|---|---|---|---|
| 100 | 0.00 | 0.547 | 0.157 | 0.278 |
| 101 | 0.148 | 0.157 | 0.131 | 0.336 |
| 102 | 0.282 | 0.150 | 0.088 | 0.301 |
| 103 | 0.227 | 0.129 | 0.172 | 0.250 |
| 104 | 0.234 | 0.00 | 0.252 | 0.002 |
| 105 | 0.128 | 0.123 | 0.133 | 0.328 |
| 106 | 0.462 | 0.127 | 0.096 | 0.086 |
| 107 | 0.244 | 0.085 | 0.086 | 0.256 |
| 108 | 0.122 | 0.126 | 0.076 | 0.290 |
| 109 | 0.137 | 0.126 | 0.138 | 0.059 |
| 110 | 0.00 | 0.096 | 0.078 | 0.377 |
| 111 | 0.120 | 0.045 | 0.126 | 0.039 |
| 112 | 0.093 | 0.077 | 0.107 | 0.268 |
| 113 | 0.00 | 0.236 | 0.194 | 0.349 |
| 114 | 0.065 | 0.076 | 0.118 | 0.244 |
| Means ± Standard Deviations | 0.151 ± 0.124 | 0.140 ± 0.125 | 0.130 ± 0.048 | 0.231 ± 0.122 |

Table 7. Mean Diurnal Creatinine Excretion (grams) Values for Subjects Consuming 60 Grams of Protein.

| Subject | 8 - 12 a.m. | 12 - 4 p.m. | 4 - 8 p.m. | 8 p.m. - 8 a.m. |
|---|---|---|---|---|
| 100 | 0.00 | 0.182 | 0.167 | 0.373 |
| 101 | 0.061 | 0.224 | 0.148 | 0.00 |
| 102 | 0.377 | 0.184 | 0.114 | 0.00 |
| 103 | 0.096 | 0.00 | 0.152 | 0.281 |
| 104 | 0.405 | 0.076 | 0.108 | 0.215 |
| 105 | 0.123 | 0.128 | 0.117 | 0.288 |
| 106 | 0.084 | 0.152 | 0.108 | 0.275 |
| 107 | 0.240 | 0.138 | 0.145 | 0.070 |
| 108 | 0.065 | 0.129 | 0.113 | 0.241 |
| 109 | 0.00 | 0.157 | 0.148 | 0.409 |
| 110 | 0.157 | 0.125 | 0.093 | 0.277 |
| 111 | 0.449 | 0.105 | 0.00 | 0.125 |
| 112 | 0.123 | 0.090 | 0.142 | 0.241 |
| 113 | 0.00 | 0.00 | 0.105 | 0.345 |
| 114 | 0.116 | 0.103 | 0.080 | 0.233 |
| Means ± Standard Deviations | 0.153 ± 0.148 | 0.120 ± 0.062 | 0.116 ± 0.040 | 0.225 ± 0.125 |

Table 8. Mean Diurnal Creatinine Excretion (grams) Values for Subjects Consuming 90 Grams of Protein.

| Subject | 8 - 12 a.m. | 12 - 4 p.m. | 4 - 8 p.m. | 8 p.m. - 8 a.m. |
|---|---|---|---|---|
| 100 | 0.00 | 0.274 | 0.111 | 0.00 |
| 101 | 0.058 | 0.265 | 0.182 | 0.383 |
| 102 | 0.455 | 0.118 | 0.102 | 0.293 |
| 103 | 0.136 | 0.095 | 0.102 | 0.249 |
| 104 | 0.109 | 0.090 | 0.143 | 0.280 |
| 105 | 0.133 | 0.146 | 0.152 | 0.323 |
| 106 | 0.099 | 0.132 | 0.122 | 0.156 |
| 107 | 0.042 | 0.016 | 0.043 | 0.207 |
| 108 | 0.105 | 0.123 | 0.124 | 0.279 |
| 109 | 0.038 | 0.00 | 0.139 | 0.429 |
| 110 | 0.117 | 0.099 | 0.144 | 0.296 |
| 111 | 0.159 | 0.135 | 0.125 | 0.396 |
| 112 | 0.066 | 0.149 | 0.130 | 0.361 |
| 113 | 0.156 | 0.136 | 0.099 | 0.414 |
| 114 | 0.083 | 0.132 | 0.093 | 0.294 |
| Means ± Standard Deviations | 0.117 ± 0.104 | 0.110 ± 0.072 | 0.113 ± 0.032 | 0.292 ± 0.111 |

Table 9. Mean Diurnal Nitrogen Excretion (Grams) Values for Subjects Consuming 30 Grams of Protein.

| Subject | 8 - 12 a.m. | 12 - 4 p.m. | 4 - 8 p.m. | 8 p.m. - 8 a.m. |
|---|---|---|---|---|
| 100 | 0.00 | 2.90 | 0.86 | 1.70 |
| 101 | 0.75 | 0.80 | 0.60 | 1.40 |
| 102 | 1.58 | 0.90 | 0.80 | 1.50 |
| 103 | 1.15 | 0.80 | 1.05 | 1.40 |
| 104 | 1.23 | 0.00 | 1.80 | 0.00 |
| 105 | 0.80 | 1.20 | 1.20 | 2.50 |
| 106 | 2.70 | 1.37 | 0.97 | 0.80 |
| 107 | 2.40 | 0.90 | 0.98 | 2.15 |
| 108 | 0.82 | 2.06 | 1.10 | 3.50 |
| 109 | 0.81 | 1.24 | 1.50 | 0.40 |
| 110 | 0.00 | 0.81 | 0.60 | 2.30 |
| 111 | 0.82 | 0.36 | 0.90 | 0.43 |
| 112 | 0.65 | 0.63 | 0.80 | 1.72 |
| 113 | 0.00 | 1.60 | 1.40 | 1.80 |
| 114 | 0.38 | 0.62 | 0.90 | 1.70 |
| Means ± Standard Deviations | 1.02 ± 0.80 | 1.08 ± 0.71 | 1.03 ± 0.33 | 1.56 ± 0.90 |

Table 10. Mean Diurnal Nitrogen Excretion (grams) Values for Subjects Consuming 60 Grams of Protein.

| Subject | 8 - 12 a.m. | 12 - 4 p.m. | 4 - 8 p.m. | 8 p.m. - 8 a.m. |
|---|---|---|---|---|
| 100 | 0.00 | 1.32 | 1.70 | 3.40 |
| 101 | 0.44 | 1.84 | 1.30 | 0.00 |
| 102 | 3.50 | 1.84 | 0.93 | 0.00 |
| 103 | 0.97 | 0.00 | 2.10 | 2.80 |
| 104 | 3.40 | 1.05 | 1.50 | 3.20 |
| 105 | 1.08 | 1.50 | 1.30 | 3.20 |
| 106 | 0.65 | 1.50 | 1.33 | 3.11 |
| 107 | 2.50 | 1.44 | 1.70 | 0.70 |
| 108 | 0.60 | 1.30 | 1.54 | 2.60 |
| 109 | 0.00 | 1.25 | 2.64 | 3.80 |
| 110 | 1.40 | 1.50 | 1.50 | 3.25 |
| 111 | 3.90 | 1.24 | 1.70 | 1.80 |
| 112 | 1.20 | 1.43 | 2.80 | 3.05 |
| 113 | 0.00 | 0.00 | 1.50 | 3.20 |
| 114 | 1.50 | 1.30 | 1.60 | 3.40 |
| Means ± Standard Deviations | 1.40 ± 1.32 | 1.23 ± 0.62 | 1.68 ± 0.50 | 2.50 ± 1.26 |

Table 11. Mean Diurnal Nitrogen Excretion (grams) Values for Subjects Consuming 90 Grams of Dietary Protein.

| Subject | 8 - 12 a.m. | 12 - 4 p.m. | 4 - 8 p.m. | 8 p.m. - 8 a.m. |
|---|---|---|---|---|
| 100 | 0.00 | 3.70 | 2.44 | 0.04 |
| 101 | 0.54 | 4.20 | 3.50 | 6.03 |
| 102 | 3.80 | 2.40 | 2.50 | 7.03 |
| 103 | 1.92 | 2.00 | 2.96 | 5.80 |
| 104 | 1.43 | 1.96 | 4.20 | 5.90 |
| 105 | 1.43 | 2.11 | 1.92 | 4.80 |
| 106 | 1.64 | 2.16 | 1.80 | 2.23 |
| 107 | 1.70 | 0.27 | 2.40 | 4.23 |
| 108 | 1.30 | 1.99 | 2.10 | 5.13 |
| 109 | 1.20 | 0.00 | 2.00 | 6.94 |
| 110 | 1.23 | 1.42 | 2.23 | 5.30 |
| 111 | 1.64 | 2.00 | 1.50 | 5.10 |
| 112 | 0.90 | 1.90 | 1.90 | 5.10 |
| 113 | 2.22 | 2.04 | 1.70 | 6.20 |
| 114 | 1.28 | 1.20 | 2.03 | 5.30 |
| Means ± Standard Deviations | 1.48 ± 0.84 | 1.15 ± 1.07 | 2.34 ± 0.72 | 5.01 ± 1.79 |

The ratios of grams of urinary nitrogen to grams of creatinine excreted were calculated for each subject in each interval of Day 3 of each period, i.e., on each protein level. These ratios are listed with the mean ratios and standard deviations for all subjects in each interval in Tables 12, 13 and 14. The mean ratios increased with increased levels of dietary protein, suggesting that the concomitant increase in creatinine excretion was smaller in relation to the increase in urinary nitrogen. Among subjects consuming 30 grams of protein, the ratios ranged from no excretion (Subjects 100, 110 and 113, 8 - 12 a.m. and Subject 104, 12 - 4 p.m. and 8 p.m. - 8 a.m.) to 16.35 (Subject 108, 12 - 4 p.m.). Subjects consuming 60 grams of protein yielded ratios ranging from no excretion (Subjects 100, 109 and 113, 8 - 12 a.m., Subjects 103 and 113,12 - 4 p.m., Subject 111, 4 - 8 p.m., and Subjects 101 and 102, 8 p.m. - 8 a.m.) to 20.00 (Subject 114, 4 - 8 p.m.). Among subjects who consumed 90 grams of protein, the ratios ranged from no excretion (Subject 100, 8 - 12 a.m., Subject 109, 12 - 4 p.m., and Subject 100, 8 p.m. - 8 a.m.) to 40.48 (Subject 107, 8 - 12 a.m.). The lowest mean ratios fell between 8 a.m. and 12 noon in each period; the muscular activity of the subjects was likely to have been greatest in these hours.

The diurnal difference in the ratios of urinary nitrogen to creatinine among subjects consuming 60 grams of protein was not significant at the 5.0% level (F=2.781, Table 16). However, the diurnal difference in the ratios was significant at the 5.0% level among subjects consuming 30 grams of protein (F=5.625, Table 15) and also among subjects consuming

Table 12. Mean Diurnal Values of Ratios of Grams Nitrogen/Grams Creatinine for Subjects Consuming 30 Grams of Protein.

| Subject | 8 - 12 a.m. | 12 - 4 p.m. | 4 - 8 p.m. | 8 p.m. - 8 a.m. |
|---|---|---|---|---|
| 100 | 0.00 | 5.30 | 5.55 | 6.10 |
| 101 | 5.10 | 5.10 | 4.60 | 4.20 |
| 102 | 5.60 | 6.00 | 9.09 | 4.90 |
| 103 | 5.10 | 6.20 | 6.10 | 5.60 |
| 104 | 5.26 | 0.00 | 7.10 | 0.00 |
| 105 | 6.25 | 9.76 | 9.00 | 7.60 |
| 106 | 5.64 | 10.79 | 10.10 | 9.30 |
| 107 | 9.84 | 10.60 | 11.40 | 8.40 |
| 108 | 6.72 | 16.35 | 14.40 | 12.10 |
| 109 | 5.90 | 9.84 | 10.90 | 6.80 |
| 110 | 0.00 | 8.44 | 7.70 | 6.10 |
| 111 | 6.83 | 8.00 | 7.10 | 11.02 |
| 112 | 6.99 | 8.20 | 7.50 | 6.40 |
| 113 | 0.00 | 6.78 | 7.20 | 5.20 |
| 114 | 5.85 | 8.16 | 7.63 | 6.90 |
| Means ± Standard Deviations | 5.01 ± 2.84 | 7.97 ± 3.59 | 8.36 ± 2.53 | 6.71 ± 2.89 |

Table 13. Mean Diurnal Values of Ratios of Grams Nitrogen/Grams Creatinine for Subjects Consuming 60 Grams of Protein.

| Subject | 8 - 12 a.m. | 12 - 4 p.m. | 4 - 8 p.m. | 8 p.m. - 8 a.m. |
|---|---|---|---|---|
| 100 | 0.00 | 7.25 | 10.18 | 9.12 |
| 101 | 7.22 | 8.21 | 8.78 | 0.00 |
| 102 | 9.36 | 10.00 | 8.16 | 0.00 |
| 103 | 10.06 | 0.00 | 13.82 | 9.96 |
| 104 | 8.40 | 13.82 | 13.89 | 14.88 |
| 105 | 8.78 | 11.72 | 11.11 | 11.11 |
| 106 | 7.74 | 9.87 | 12.31 | 11.31 |
| 107 | 10.42 | 10.43 | 11.72 | 10.00 |
| 108 | 9.23 | 10.08 | 13.63 | 10.79 |
| 109 | 0.00 | 7.96 | 17.84 | 9.29 |
| 110 | 8.92 | 12.00 | 16.13 | 11.73 |
| 111 | 8.69 | 11.81 | 0.00 | 14.40 |
| 112 | 9.76 | 15.89 | 19.72 | 12.66 |
| 113 | 0.00 | 0.00 | 9.52 | 9.28 |
| 114 | 12.93 | 12.60 | 20.00 | 14.59 |
| Means ± Standard Deviations | 7.43 ± 4.06 | 9.44 ± 4.45 | 12.45 ± 5.08 | 9.94 ± 4.47 |

Table 14. Mean Diurnal Values of Ratios of Grams Nitrogen/Grams Creatinine for Subjects Consuming 90 Grams of Protein.

| Subject | 8 - 12 a.m. | 12 - 4 p.m. | 4 - 8 p.m. | 8 p.m. - 8 a.m. |
|---|---|---|---|---|
| 100 | 0.00 | 13.50 | 21.98 | 0.00 |
| 101 | 9.30 | 15.55 | 19.23 | 15.79 |
| 102 | 8.35 | 20.34 | 24.51 | 23.99 |
| 103 | 14.12 | 21.05 | 29.02 | 23.29 |
| 104 | 13.12 | 21.78 | 29.37 | 21.07 |
| 105 | 10.75 | 14.45 | 12.63 | 14.86 |
| 106 | 16.57 | 16.36 | 14.75 | 14.29 |
| 107 | 40.48 | 16.88 | 55.80 | 20.43 |
| 108 | 12.38 | 16.31 | 16.94 | 18.39 |
| 109 | 31.58 | 0.00 | 14.39 | 16.18 |
| 110 | 10.51 | 14.34 | 15.49 | 17.91 |
| 111 | 10.31 | 14.80 | 12.00 | 12.89 |
| 112 | 13.64 | 12.75 | 14.62 | 14.13 |
| 113 | 14.23 | 15.00 | 18.89 | 14.98 |
| 114 | 15.42 | 9.10 | 21.83 | 18.03 |
| Means ± Standard Deviations | 14.72 ± 9.64 | 14.81 ± 5.25 | 21.43 ± 10.97 | 16.42 ± 5.66 |

Table 15. Analysis of Variance of Diurnal Nitrogen/Creatinine Ratios for Fifteen Subjects Consuming 30 Grams of Protein.

| Source | SS | df | ms | F | P |
|---|---|---|---|---|---|
| Total | 673.21 | 59 | ---- | ---- | ----- |
| Subjects | 354.93 | 14 | ---- | ---- | ----- |
| Treatments | 91.61 | 3 | 30.09 | 5.625 | $<0.05$ |
| Error | 228.01 | 42 | 5.43 | ---- | ----- |

Table 16. Analysis of Variance of Diurnal Nitrogen/Creatinine Ratios for Fifteen Subjects Consuming 60 Grams of Protein.

| Source | SS | df | ms | F | P |
|---|---|---|---|---|---|
| Total | 1434.3 | 59 | ----- | ----- | ----- |
| Subjects | 273.10 | 14 | ----- | ----- | ----- |
| Treatments | 192.42 | 3 | 64.14 | 2.781 | $>0.05$ |
| Error | 968.79 | 42 | 23.07 | ----- | ----- |

Table 17. Analysis of Variance of Diurnal Nitrogen/Creatinine Ratios for Fifteen Subjects Consuming 90 Grams of Protein.

| Source | SS | df | ms | F | P |
|---|---|---|---|---|---|
| Total | 4,168.5 | 59 | ----- | ----- | ----- |
| Subjects | 1,782.1 | 14 | ----- | ----- | ----- |
| Treatments | 447.9 | 3 | 149.3 | 3.235 | $<0.05$ |
| Error | 1,938.5 | 42 | 46.2 | ----- | ----- |

90 grams of protein (F=3.235, Table 17). The diurnal difference in creatinine excretion illustrated by these results suggests that the ratios of nitrogen to creatinine vary in the same individual from voiding to voiding within the same day. This lowers confidence in the reliability of the value of the ratio of nitrogen to creatinine in an individual urine specimen or an isolated aliquot of a daily specimen.

The mean daily creatinine excretion over the entire study ranged from 0.686 to 0.691 grams per day for groups of subjects (Table 18). The lowest group mean excretion was given by the group of subjects who were of the smallest mean weight and height (Group B) and the highest group mean excretion of creatinine was given by the group of largest mean weight (Group C). The positive relationship between both height and weight has been demonstrated by numerous investigators (3, 7, 10, 11, 44).

The lack of significant diurnal difference in the ratios of urinary nitrogen to creatinine among subjects consuming 60 grams of protein is puzzling, in light of the significant diurnal differences among subjects consuming 30 and 60 grams of protein. Reexamination of the feeding schedule revealed that the intermediate level of dietary protein (60 grams) could not have been preceded or followed by a level of protein 30 grams lower or higher. However, the lower and higher levels of dietary protein (30 and 90 grams) were preceded by and followed one another three times during the study, i.e., Groups B and C were exposed to more abrupt changes in dietary protein during the study than Group A, and the intermediate level of protein was exposed to the least amount of change during the study. It is doubtful that this factor contributed

Table 18. Mean Daily Creatinine Excretion (grams) by Groups of Subjects.

| | Group A | Group B | Group C |
|---|---|---|---|
| I | 0.683 | 0.750 | 0.638 |
| | 0.766 | 0.724 | 0.722 |
| | 0.605 | 0.604 | 0.589 |
| | 0.612 | 0.583 | 0.794 |
| | 0.574 | 0.676 | 0.541 |
| II | 0.599 | 0.751 | 0.614 |
| | 0.829 | 0.737 | 0.724 |
| | 0.644 | 0.567 | 0.666 |
| | 0.615 | 0.606 | 0.787 |
| | 0.621 | 0.713 | 0.606 |
| III | 0.772 | 0.787 | 0.704 |
| | 0.930 | 0.744 | 0.831 |
| | 0.716 | 0.639 | 0.692 |
| | 0.695 | 0.690 | 0.841 |
| | 0.671 | 0.716 | 0.622 |
| Means | 0.688 | 0.685 | 0.692 |

to the smaller diurnal differences among subjects consuming 60 grams of protein; no investigation of this matter was found in the literature. However, several authors have commented on the effects of abrupt changes in dietary protein levels on daily creatinine excretion. Fisher (9) observed considerable variation in creatinine excretion of individual rats fed any given diet, but particularly following a change from one diet to another. In his study, he also found that the creatinine excretion decreased significantly during a protein-free feeding period only to double upon continuation of this feeding regime. Van Niekerk et al. (45) discovered that increased outputs of urinary creatinine resulted from abrupt, large increases in protein intake while decreased outputs resulted from starvation. The carry-over of their animals to the control diet was sufficient to indicate that the changes in creatinine output were at least partly attributable to concomitant changes in body composition.

There have been many other investigators who have been able to relate creatinine excretion to the protein content of diets and/or to lean body mass. However, several of these authors admit that the increase in creatinine excretion may be the result of an increased amount of dietary creatinine. Powell et al. (30) and Dominguez and Pomerene (46) were able to recover, within 24 hours, approximately 56% of the oral creatinine administered to their subjects before breakfast. The former felt that increased creatinine excretion among their subjects was the result of a greater meat intake and therefore more preformed creatinine in the daily ration. Bleiler and Schedl (47) found that creatinine excretion in subjects always decreased upon changing from meat-containing diets to formulas which were essentially without creatine and creatinine, even when the daily formulas contained as much as 104 grams of protein. Benedict and Osterberg (48) have shown that ingestion of creatine may lead to an increased output of creatinine in the urine. They performed experiments which seemed to them to establish definitely the origin of urinary creatinine from creatine at a metabolic rate of about 30%. Chanutin (49) also found that the creatinine content of the urine in man increases after ingestion of large doses of creatine. Ritchey et al. (28) agree that increases in creatinine excretion cannot be clearly attributed to increased nitrogen intake, unless the source of nitrogen contains no preformed creatine or creatinine. Garn (50) also realized the necessity for controlling dietary creatine and creatinine to obtain the most accurate results in any group of individuals. Kumar et al. (44) related creatinine excretion to lean body mass in rats, but pointed out the need for using a low-creatinine diet.

# CONCLUSION

In these preadolescent girls daily excretion of creatinine increased significantly as protein consumption increased. However, by the end of the study the heaviest group of subjects had excreted a larger mean daily amount of creatinine than the others, and the smallest and shortest group had excreted the smallest mean daily amount. It is also worthy of note that the lowest mean ratios of urinary nitrogen to creatinine fell in the interval which began at 8 a.m. and ended at noon, the hours when the muscular activity of these preadolescent subjects was likely to have been the greatest. This gives impetus to the suggestion that preformed muscular creatine and creatinine may contribute to creatinine excretion, making individual lean body mass an important factor in the determination of the amount of creatinine excreted, and indicates a need for control of preformed creatine and creatinine in the diet in order to gain a greater understanding of the true effect of increased dietary protein on creatinine excretion. This could be accomplished through the administration of a meat-free diet or by analysis of meat-containing diets daily for preformed creatine and creatinine, using the conversion factor of Powell et al. (31) and Dominguez and Pomerene (46).

Several authors have described changes in creatinine excretion with abrupt changes in dietary protein levels. This suggests a need for very gradual changes from one protein level to the next when fed under experimental conditions.

Although dietary protein variation appeared to affect creatinine excretion, the daily N/Cr ratio did reflect dietary intake, increasing with increased levels of dietary protein, and therefore may be useful in the evaluation of protein nutriture. However, the significant diurnal differences in N/Cr ratios observed among subjects in two of the three dietary periods would preclude the possibility of using a random, or casual, urine sample as a reliable index material for nutrient balance studies. Neither creatinine output from day to day nor the N/Cr ratio from voiding to voiding is sufficiently constant in the same individual to justify its use as a physiological reference standard against which the excretion of other substances ought to be compared. Vestergaard and Leverett (22) also found very large individual variations in variability of creatinine excretion in 24 hour periods and in short collection periods of 2 hours and 8 hours overnight. They state that creatinine excretion should not be used for checking purposes in short collection periods or incorporated in ratios unless a preliminary study of variability in excretion is done on the individual. Lewis et al. (24) found that creatinine varied so much that it was not a reliable basis for comparison of riboflavin in casual urine specimens from the children who served as their subjects.

# SUMMARY

Creatinine, an anhydride of creatine, is a nitrogenous substance excreted in the urine as a metabolic end product. The use of creatinine excretion as a reference index of protein metabolism was introduced by Folin when he pointed out that the amount of creatinine excreted in the urine by a given individual is a constant quantity and is independent of the changes in the amount of nitrogen eliminated. This concept of the constancy of creatinine excretion has been widely accepted and implies a major application for the use of urinary creatinine values in evaluating the adequacy of dietary protein. A ratio could also be used between creatinine and any other constituent in urine.

Fifteen healthy preadolescent girls served as subjects for this 34-day study. During the study all subjects consumed three levels of dietary protein (30, 60, and 90 grams), each for periods of 10 or 11 days. Total urine was collected for each subject daily. Urine volume was measured and the urine then analyzed for creatinine and nitrogen, using, respectively, the alkaline picrate method and the Arnold-Kjeldahl method. On Day 3 of each period the urine was collected at 12 noon, 4 p.m., 8 p.m., and 8 a.m. the following morning for the purpose of determining any diurnal differences in creatinine and nitrogen excretion.

The mean daily creatinine and nitrogen excretions were calculated for each subject on each level of dietary protein. The mean values of creatinine and nitrogen excretion and standard deviations for all subjects on each level of dietary protein were also calculated. The diurnal

creatinine and nitrogen excretions on Days 3 of each period are listed for each subject along with the mean values and standard deviations for all subjects.

The ratios of grams of urinary nitrogen to grams of creatinine excreted were calculated for each subject in each interval on Day 3 of each period, i.e., on each protein level. These are listed with the mean ratios and standard deviations for all subjects. Analysis of variance was performed to determine the significance of the difference in daily creatinine excretion among subjects consuming the three levels of protein. Analyses of variance were also performed on the ratios of grams of urinary nitrogen to grams of creatinine excreted during Day 3 of each period to determine the significance of diurnal differences in ratios among subjects.

The increased dietary protein levels fed in this experiment resulted in a significant increase in mean daily creatinine excretion for all subjects. Significant diurnal differences in the ratios of urinary nitrogen to creatinine were observed. These results indicate that the diurnal N/Cr ratio is insufficiently constant, among these girls, from voiding to voiding for the casual urine sample to be considered a reliable indicator of protein nutritional status.

This author's contention is that in these preadolescents, lean muscle mass was a contributing factor to the amount of creatinine excreted, since the greatest mean value of creatinine was given by the heaviest group of subjects, and the smallest mean value was given by the subjects who were the smallest in height and weight. The creatinine values given by these subjects increased significantly with increasing dietary protein levels, but, in order to gain a clearer understanding of the effect of dietary protein level on creatinine excretion, there is a need to administer diets which are free of preformed creatine or creatinine or are of known creatine and creatinine content, since there is a possibility that these food substances will contribute significantly to creatinine excretion. There is also a need to change experimental protein levels less abruptly, as several authors have described changes in creatinine excretion with abrupt changes in dietary protein levels.

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DIURNAL AND DAILY VARIATION IN CREATININE EXCRETION

AMONG PREADOLESCENT GIRLS CONSUMING THREE LEVELS

OF DIETARY PROTEIN

by

Patillo Elizabeth Donald

(ABSTRACT)

Fifteen healthy preadolescent girls served as subjects in this 34-day study. The subjects were equally divided into 3 groups which were rotated through a feeding schedule enabling each subject to consume 30, 60, and 90 grams of protein for periods of 10 or 11 days. All urine was collected daily throughout the experiment and analyzed for nitrogen and creatinine. On the third day of each period, urine was collected from 8 - 12 a.m., 12 - 4 p.m., 4 - 8 p.m., and 8 p.m. - 8 a.m.

The mean amounts of creatinine excreted daily by subjects consuming 30, 60, and 90 grams of protein differed significantly at the 1% level. There was also significant diurnal variation at the 5% level in the urinary nitrogen/creatinine ratio among subjects consuming both 30 and 90 grams of protein. The heaviest group of subjects excreted the greatest amount of creatinine, and the lightest group of subjects, the lowest mean amount of creatinine, in each period. The greatest mean diurnal creatinine excretion of subjects on Day 3 of each period occurred from 8 - 12 a.m.

In the author's opinion, urinary nitrogen/creatinine ratio is insufficiently constant from voiding to voiding to serve as a reliable index

of protein nutriture in metabolic studies. The apparent contribution of lean body mass to creatinine excretion points to the need for experimental diets which are controlled for preformed creatine and creatinine in order to gain a greater understanding of the true effect of dietary protein level on creatinine excretion.