The Use of Nitrogen to Creatinine Ratios in Random Urine Specimens to Estimate Dietary Protein[1]

RICHARD C. POWELL, IRVIN C. PLOUGH AND EUGENE M. BAKER III
United States Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver, Colorado

Proper evaluation of dietary protein is one of the important problems confronting nutrition survey teams today. A biochemical test which would aid in judging the adequacy of protein intake, then, would be helpful. Such a test would have to be easily and rapidly performed for use when surveying large populations. For many years it has been known that daily urinary nitrogen excretion varies with nitrogen intake (Folin, '05). This assumes, of course, nitrogen balance. Equally well-established is a diurnal variation of nitrogen excretion such that when the daily diet is divided into three equal meals, maximum excretion occurs in the late afternoon and early evening hours (Forsgren and Schnell, '34). Thus, while we would expect 24-hour urinary nitrogen excretion to reflect protein intake, the determination of 4- and 6-hour urinary nitrogens would add the problem of diurnal variation.

Experience with nutrition surveys has taught us that timed urine collections are difficult to obtain, and the accuracy of these collections has been questioned. For this reason certain vitamin excretions have been measured in random urine samples, the results being expressed per gram of creatinine contained in the sample (Adamson et al., '45; Aykroyd et al., '49; Interdepartmental Committee on Nutrition for National Defense [ICNND], '57, '58; Lowry, '52; Plough and Consolazio, '59). Following the same line of reasoning, the present study was designed to evaluate the use of nitrogen/creatinine ratios (grams of nitrogen per gram of creatinine) in random urine samples to estimate dietary protein. Partial instead of random urine samples were collected, however, so that the ratios could be compared with timed urinary nitrogen excretion. In addition, the diurnal variation of nitrogen and creatinine excretion was examined. Because methods for measuring total nitrogen and creatinine in the urine are rapidly and easily performed, this technique can be readily adapted to field survey work. Although the accuracy may not be satisfactory for studying individuals, it is hoped this method of estimating dietary protein will be useful when applied to large populations.

METHODS

The subjects were 6 healthy young men, 18 to 23 years old, whose average weight was 74.8 kg (range 62.0 to 92.1 kg). They were housed in a metabolic ward, but were permitted off the ward for supervised activities. During the study they completely consumed weighed test diets as the only source of food. The daily water intake which exceeded two liters for each subject, provided adequate urine specimens. Timed urine samples were collected throughout the study at 3-, 4-, and 5-hour intervals (5 to 8 A.M., 8 to 12 A.M., 12 to 5 P.M., 5 to 9 P.M., 9 to 1 A.M., and 1 to 5 A.M.). The volume of each urine sample was recorded, and each specimen was analyzed for total nitrogen using a microKjeldahl method (Hawk and Bergeim, '37) and for creatinine using the Jaffe reaction (Knowlton et al., '55). This permitted us to express urinary nitrogen excretion both as grams of nitrogen per collection period and as grams of nitrogen per gram creatinine.

The study was divided into three periods differing in dietary protein (17, 13 and 13 days respectively). In the first period all 6 subjects consumed a daily diet containing by analysis: 84 gm of protein, 135 gm of fat, and 359 gm of carbohydrate. In the

Received for publication August 11, 1960.
[1] Presented at the Annual Meeting of the American Institute of Nutrition, Chicago, 1960.

second and third periods the subjects were divided into two groups of three men. In the first group a protein supplement of wheat gluten was added to the previous diet and served as gluten bread; in the second group a protein supplement of meat was added. Macronutrients were adjusted to keep the total calories relatively constant at 3000 per man per day. The gluten group in the second period consumed a diet containing by analysis: 100 gm of protein, 122 gm of fat, and 339 gm of carbohydrate; in the third period: 113 gm of protein, 111 gm of fat, and 340 gm of carbohydrate. The meat group in the second period consumed a diet containing by analysis: 100 gm of protein, 120 gm of fat, and 354 gm of carbohydrate; in the third period: 128 gm of protein, 124 gm of fat, and 351 gm of carbohydrate. The diet in the first period contained approximately 25 gm of protein as meat. Diet composites were also analyzed for preformed creatinine and creatine.

The daily ration was divided into three equal parts and served at 8 A.M., 12 noon, and 5 P.M. The following exceptions are noted. On the 11th day of each period 4 meals (divided equally) were served at 8 A.M., 12 noon, 5 P.M. and 9 P.M. On the 12th and 13th days of each period two meals were served at 12 noon and 5 P.M. On the 4th day of the study 1.8 gm of creatinine was given orally before breakfast to each subject in order to evaluate its effect on urinary creatinine excretion.

RESULTS

The diets were tolerated well by all subjects. Each man in the gluten group (average weight 64 kg) gained approximately 0.5 kg during the study. The subjects in the meat group (average weight 86 kg), on the other hand, each lost approximately 0.7 kg. Daily urinary nitrogen and creatinine excretions were obtained by adding the results of the 6 collection periods (5 A.M. to 5 A.M.). The 24-hour urine excretions of all subjects in each group were averaged using the last 5 days of each period. These results, together with nitrogen intake, are shown in figure 1.

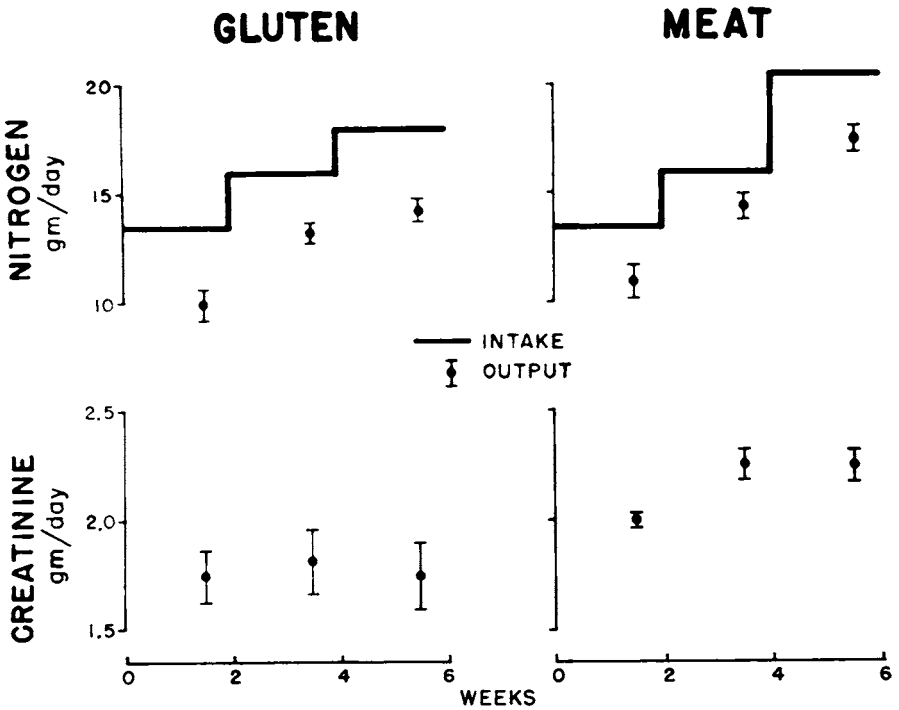

Fig. 1 Nitrogen intake, and average 24-hour urinary nitrogen and creatinine excretions in each period with one standard deviation. Three subjects in each group were averaged using the last 5 days of each diet period.

When 1.8 gm of creatinine was given orally to each subject before breakfast, an additional 1.0 gm of creatinine on the average was recovered in the urine over the next 24 hours. Diet analyses for preformed creatinine averaged 0.16 gm per day in the control and gluten diets, and averaged 0.24 gm per day in both meat-supplemented periods. Analyses for dietary creatine averaged 0.21 gm per day in the control and gluten diets, and 0.36 gm per day in both meat-supplemented periods.

The similarity of the diurnal variations of nitrogen and creatinine excretion is well demonstrated in figure 2. The average of all subjects for the last 5 days in the first period is graphed. Note also the diurnal variation of nitrogen/creatinine ratios (fig. 2). Similar results were obtained in the other periods. Changing mealtimes did not affect the diurnal variation of creatinine excretion. Administration of ¼th of the daily ration at 9 P.M. did not change nitrogen excretion the following morning. When breakfast was omitted on two consecutive mornings (the total daily ration remaining constant) there was a tendency for nitrogen excretion to reach a peak later in the day. This was noticeable only in the third period when nitrogen intake was at its highest level and was statistically significant only during collection periods from 9 P.M. to 5 A.M. ($P < 0.025$). The nitrogen/creatinine ratios were not significantly altered by changing mealtimes under the conditions of this experiment.

Linear regression equations were calculated for nitrogen intake as a function of urinary nitrogen excretion (table 1). These equations and correlation coefficients are shown, first expressing urinary nitrogen as grams of nitrogen per collection period, and then as grams of nitrogen per gram creatinine. Only the last 5 days of each period were included. Thus, each equation was calculated from 5 urine specimens collected at the appropriate time of day from each subject in each period (a total of 90 specimens). Nitrogen/creatinine ratios were calculated for each of the 90 specimens and these data were used to obtain the second set of equations. The 24-hour urinary excretions were obtained by adding the results of the 6 collection periods (5 A.M. to 5 A.M.). Prediction of daily nitrogen intake from nitrogen/creatinine ratios is shown graph-

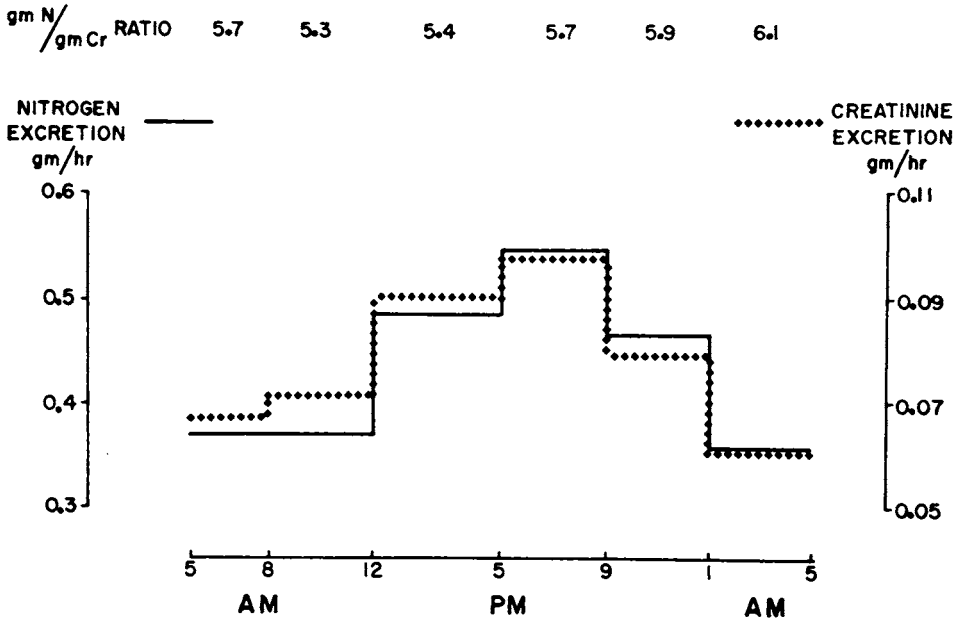

Fig. 2 Diurnal variation of nitrogen and creatinine excretion. Variation of the nitrogen/creatinine ratios is shown numerically at the top. The values for 6 subjects were averaged using the last 5 days of the first period.

TABLE 1

Prediction of daily nitrogen intake (Y) from timed urinary nitrogen excretion (X)

Collection time	Regression equation	Correlation coefficient
5– 8 A.M.	Y=8.4+6.0X	0.68
8–12 A.M.	Y=8.3+4.1X	0.74
12– 5 P.M.	Y=6.3+3.3X	0.79
5– 9 P.M.	Y=7.6+3.0X	0.80
9– 1 A.M.	Y=9.6+2.8X	0.70
1– 5 A.M.	Y=8.7+4.0X	0.76
24-hour[1]	Y=4.3+0.89X	0.92

Prediction of daily nitrogen intake (Y) from nitrogen creatinine ratios (X)

Collection time	Regression equation	Correlation coefficient
5– 8 A.M.	Y= 6.6+1.4X	0.65
8–12 A.M.	Y= 6.7+1.5X	0.69
12– 5 P.M.	Y= 8.3+1.2X	0.63
5– 9 P.M.	Y= 9.2+1.0X	0.62
9– 1 A.M.	Y=10.6+0.76X	0.59
1– 5 A.M.	Y= 8.2+1.1X	0.62
24-hour[1]	Y= 4.2+1.8X	0.80

[1] The 24-hour excretion was obtained by adding the results of the 6 collections (5 A.M. to 5 A.M.).

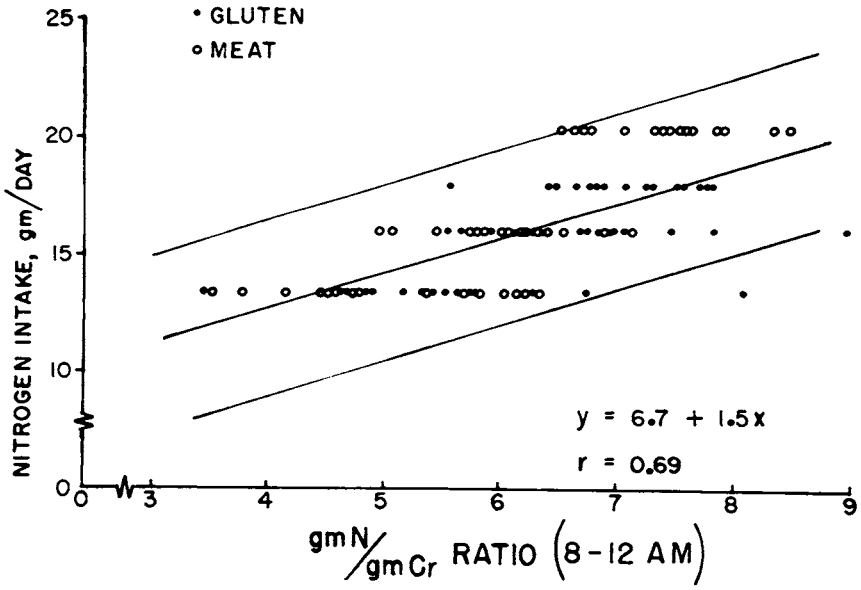

Fig. 3 Prediction of nitrogen intake from nitrogen/creatinine ratios using the 8 to 12 A.M. collection period. The equation, correlation coefficient, and 95% confidence limits are shown.

ically in figure 3. The ratios obtained from the 8 to 12 A.M. collection period are plotted, and are representative of our data. The 95% confidence limits are also shown.

DISCUSSION

Even under the ideal conditions of our experiment we did not, of course, find a perfect correlation between nitrogen intake and nitrogen excretion. This is readily seen in figure 1 where nitrogen excretion was identical in period 3 for the gluten group and period 2 for the meat group, although nitrogen intake differed by two grams (12.5 gm of protein). A possible difficulty is failure of the subjects to achieve nitrogen balance. We believe this was minimized by excluding the first 8

days, or more, of each period from the calculation to allow for diet adjustment. In fact, our data showed that nitrogen excretion was stabilized within 4 days after a diet change. Although greater changes in nitrogen intake would require more time for nitrogen equilibrium, such changes have rarely been noted in dietary surveys. An adequate caloric intake is also important for nitrogen balance. In this study each subject consumed approximately 3000 Cal. daily regardless of his size. There was a mimimal weight gain in the gluten group, and a slight weight loss in the meat group. These small weight changes may not be significant; however, it is possible that there may have been a greater caloric demand in the meat group, thus affecting nitrogen utilization.

Less easily explained was the variation in creatinine excretion (fig. 1). The average excretion of those in the meat group was 0.25 gm higher in the first period when all subjects were eating the same diet. This difference we feel is the result of a discrepancy in body size between groups. Endogenous creatinine excretion tends to vary with body size or lean body mass (Miller and Blyth, '52), and the subjects in the meat group weighed an average of 20 kg more than those in the gluten group. The increased creatinine excretion in the meat group during periods 2 and 3, we feel, is a result of a greater meat intake and therefore more preformed creatinine in the daily ration. Note the diet analyses for preformed creatinine. When 1.8 gm of creatinine was consumed before breakfast, we recovered approximately 56% in the urine within 24 hours. This recovery for oral creatinine agrees with the results of Dominguez and Pomerene ('45).

Variation in creatinine excretion, as would be expected, caused changes in the nitrogen/creatinine ratios independent of the protein intake. For example, during the second period when nitrogen intake was the same for all subjects, the average ratio using 24-hour urine specimens for the gluten group was 7.32, and for the meat group, 6.41. In period three, the ratios were 8.21 and 7.81, respectively, even though the nitrogen intake was 2.5 gm higher by those in the meat group. This is also shown in figure 3. Note that the meat group (open circles) tends to fall to the left (smaller ratios) of the regression line. We feel this tendency for smaller nitrogen/creatinine ratios in the meat group can be explained by the greater creatinine excretion. In an attempt to partially correct for this, we studied our data by expressing creatinine excretion per kilogram of body weight. This manipulation, however, seemed to overcorrect and did not improve the results. It would probably be more satisfactory if creatinine was expressed per unit of lean body mass, although even this would not account for changes in dietary creatinine.

The diurnal variation of creatinine and nitrogen excretion was similar (fig. 2). This tended to cause less variation in the nitrogen/creatinine ratios with respect to time of day, a possible advantage in the use of ratios. With our data it was possible to combine the results on specimens collected from 5 A.M. to 9 P.M. in predicting nitrogen intake without loss of accuracy. Although admittedly we have not tested large shifts in nitrogen intake, changes in mealtimes under conditions of this experiment did not significantly alter our results.

The linear regression equations (table 1) allow a comparison of timed urinary nitrogen and nitrogen/creatinine ratios (X) when used to predict nitrogen intake (Y). As we might expect, 24-hour nitrogen excretion yields the best correlation coefficient. However, it is our feeling that there is little to choose from when comparing 4-hour urinary nitrogen excretion with nitrogen/creatinine ratios under these conditions. The use of ratios to predict nitrogen intake is shown graphically in figure 3. The 90 specimens collected from 8 to 12 A.M. are plotted. Note also the 95% confidence limits. Over our range of data these limits expressed as grams of nitrogen intake are ± 3.66 gm. Using 4-hour urinary nitrogen collected from 8 to 12 A.M. to predict nitrogen intake, the confidence limits are ± 3.37 gm. These limits define the predictability of dietary nitrogen from one urine specimen in one man. When more than one individual is sampled, the average nitrogen intake for the group can be predicted more accurately.

The greatest drawback in the use of nitrogen/creatinine ratios to estimate the protein intake of a population does not

appear to be variation in the ratio *per se*, but variation in the actual output of creatinine. The equations in table 1 were calculated from men excreting about 2.0 gm per day of creatinine. Using the equation for the 8 to 12 A.M. collection period, an observed ratio of 5 gm nitrogen per gram creatinine indicates a daily intake of 14.2 gm nitrogen. If an individual is consuming this amount of nitrogen but excreting only 1.0 gm of creatinine (to use an extreme example), the ratio would be 10, and the estimated dietary intake 21.7 gm. It may be possible in surveys to obtain some 24-hour urine collections for creatinine, or perhaps make a rough estimate of creatinine excretion from body size, and use this to adjust the equation. For a daily excretion of 1.0 gm creatinine, the 8 A.M. to 12 A.M. equation becomes $Y = 6.7 + 0.75X$. Another limitation should be mentioned. This method for estimating protein intake would be useful only in evaluating adult populations where nitrogen retention is not a large factor. It is our feeling, however, that the use of nitrogen/creatinine ratios merits further consideration. It would be helpful to know whether the same correlation exists for individuals consuming very low and very high protein diets. The effects of changing mealtimes should be more thoroughly evaluated and truly random samples should be examined.

SUMMARY

In this study, we have considered the use of nitrogen/creatinine ratios (grams of nitrogen per gram of creatinine) in random urine samples to estimate dietary protein. With diets varying from 80 to 125 grams of protein, we compared the accuracy of 3-, 4-, and 5-hour timed urinary nitrogen excretion with nitrogen/creatinine ratios determined from the same samples in the prediction of nitrogen intake. Although 24-hour urinary nitrogen gave the best correlation, we found little difference in results when comparing 4-hour urinary nitrogen with the ratios. Difficulties from diurnal variation of nitrogen excretion were reduced by the use of ratios, but the variability in creatinine excretion among individuals raised other problems. It is concluded that for surveys of large groups of individuals the use of nitrogen/creatinine ratios in random urine samples to estimate protein intake merits further consideration.

ACKNOWLEDGMENT

The authors gratefully acknowledge the technical assistance of Gerhard J. Isaac in helping with the statistical analyses of our data.

LITERATURE CITED

Adamson, J. D., N. Joliffe, H. D. Kruse, O. H. Lowry, P. E. Moore, B. S. Platt, W. H. Sebrell, J. W. Tice, F. F. Tisdall, R. M. Wilder and P. C. Zamecnik 1945 Medical survey of nutrition in Newfoundland. Canad. Med. A. J., *52:* 227.

Aykroyd, W. R., N. Joliffe, O. H. Lowry, P. E. Moore, W. H. Sebrell, R. E. Shank, F. F. Tisdall, R. M. Wilder and P. C. Zamecnik 1949 Medical resurvey of nutrition in Newfoundland 1948. Ibid., *60:* 329.

Dominguez, R., and E. Pomerene 1945 Recovery of creatinine after ingestion and after intravenous injection in man. Pro. Soc. Exp. Biol. Med., *58:* 26.

Folin, O. 1905 Laws governing the chemical composition of urine. Am. J. Physiol., *13:* 66.

Forsgren, E., and R. Schnell 1934 On the rhythm of the metabolism. Acta Med. Scand., *82:* 155.

Hawk, P. B., and O. Bergeim 1937 Practical Physiological Chemistry, ed. 11. The Blakiston Company, Philadelphia, p. 708.

Interdepartmental Committee on Nutrition for National Defense 1957 The Republic of the Philippines, report of nutrition survey of the armed forces. United States Government Printing Office, Washington, D. C.

——— 1958 Spain, report of nutrition survey of the armed forces. Ibid.

Knowlton, M., W. H. Horner, D. Seligson and F. L. Iber 1955 Analytical Procedures. Department of Metabolism, Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington, D. C.

Lowry, O. H. 1952 Biochemical evidence of nutritional status. Physiol. Rev., *32:* 431.

Miller, A. T., Jr., and C. S. Blyth 1952 Estimation of lean body mass and body fat from basal oxygen consumption and creatinine excretion. J. Appl. Physiol., *5:* 73.

Plough, I. C., and C. F. Consolazio 1959 The use of casual urine specimens in the evaluation of the excretion rates of thiamine, riboflavin, and N^1-methylnicotinamide. J. Nutrition, *69:* 365.