Nutrition Discussion Forum

Urine nitrogen as an independent validatory measure of dietary intake: potential errors due to variation in magnitude and type of protein intake

In work aimed at developing methodologies for validation of estimates of the dietary intake of free-living individuals, Bingham and colleagues have examined the use of urinary nitrogen (UN) excretion as an index of protein intake (Bingham & Cummings, 1985; Bingham, 1994; Bingham *et al.* 1995). The basis of this approach is that in subjects in N equilibrium, N intake is assumed equal to N excretion. Thus, if N excretion is measured it should indicate N and dietary protein intakes. In practice, since most N excretion occurs via the urine, which is relatively simple to collect, Bingham and colleagues examined the relationship between 24 h UN and N intake (DN). They argued that in subjects on typical UK diets UN should bear a fixed relationship to DN, and measured it carefully in a group of subjects. They showed that in a group of eight individuals in which intake and N excretion were measured meticulously, UN was 81% DN (SD 2, range 78–83%). They argued that measurement of this ratio could be used to assess the validity of food intake measurement and concluded 'In healthy individuals eating normal western diets, 24 h urine N from an 8 day collection verified for its completeness by the PABA check method, should establish urine N to within 81(SD 5)% of the habitual dietary intake, range 70–90%. If the dietary assessment from 18 days of records or 24 h recalls or the diet history falls within these limits, it can be stated that there is no evidence of interference with normal dietary habits, or of reporting errors...' (Bingham & Cummings, 1985). Subsequently this ratio has been used to validate weighed records and other methods of dietary assessment (Bingham *et al.* 1995), where N intake is discussed in relation to 'the expected physiological limits of 80 (SD 5)% urine N excretion,' and most recently in a report to the Nutrition Society of a study to assess the degree of under-reporting of food intakes involving validation of dietary intakes against both 24 h UN and energy expenditure by doubly-labelled water (Black *et al.* 1997).

There is no doubt that measurement of UN is an extremely useful validation measure, especially in studies such as that of Bingham *et al.* (1995) where it allows identification of subjects who are grossly under-reporting as indicated by UN exceeding DN. However, I am concerned with the potential misuse of this approach in attempting to calculate the degree of under-reporting by comparing the measured ratio of UN:DN with 0·81 on the assumption that 0·81 is a biological constant. It is not and will vary with a number of factors, especially the digestibility of the dietary protein and the protein intake level.

Bingham & Cummings (1985) deal with the digestibility issue saying that faecal N varies with the dietary fibre intake and so this fraction may be greater in areas of the world where fibre intakes are higher than in the west. In fact FAO/WHO (1991) report values for the true digestibilities of diets ranging from 78% for Indian rice + beans, maize and beans and Brazilian mixed diets, 88% for Filipino mixed diets, and up to 96% for American mixed diets. The data reported by Bingham & Cummings (1985) indicate true digestibilities of 0·92 (SD 0·02), range 0·88–0·95, on the basis of their measured intakes and faecal N excretion (FN). Furthermore, according to the Dietary and Nutritional Survey of British Adults (Gregory *et al.* 1990) as analysed by Jackson & Margetts (1993), in the

UK, protein from less digestible sources (cereals and other vegetables) accounts for on average 33% of intakes with values of $> 50\%$ identified in some of the subjects surveyed. For these individuals the digestibility will be lower than those where most protein derives from animal sources, and in a group which includes vegans the range may be expected to vary from > 80 to $< 95\%$ on the basis of the FAO data above.

The level of protein intake will also influence the value. The reason for this is because as the intakes fall, the obligatory losses account for an increasing fraction of total loss, and for the obligatory N losses (ONL), UN is only 0·61 of total N excretion (TN) (mean values for OUN, 32 mg N/kg; OFN, 13 mg N/kg; and miscellaneous losses, 8 mg N/kg (FAO/WHO/UNU, 1985). Thus as DN falls, TN will fall and UN/TN will fall. Fig. 1 shows calculated values for individuals on protein intakes ranging from 0·6 to 2 g/kg body weight per d with digestibilities from 0·78 to 1·0. Each value is calculated from the expression:

$$UN/TN = (DN - 8 - 13 - (1 - d) \times DN)/DN,$$

where values are (mg N/kg), UN is urinary N, TN is total N excretion, 8 and 13 are obligatory surface and faecal losses (FAO/WHO/UNU, 1985), and d is true digestibility.

Thus vegans, who may be expected to have digestibilities as low as 78% from the above, and many of whom have protein intakes below the current UK RNI of 0·75 g/kg (Jackson & Margetts, 1993) could have UN:TN ratios of $< 0·6$ with a maximum value of $< 0·7$. Furthermore, there is some evidence that some subjects habituated to plant-based diets do not behave as expected in that UN and FN are inversely correlated, the opposite of what is usually observed (see Millward *et al.* 1989), so that for these subjects even lower ratios might be observed. Subjects with digestibilities of 0·85 will have values ranging from 0·63–0·78 according to intakes. The data reported by Bingham & Cummings (1985)
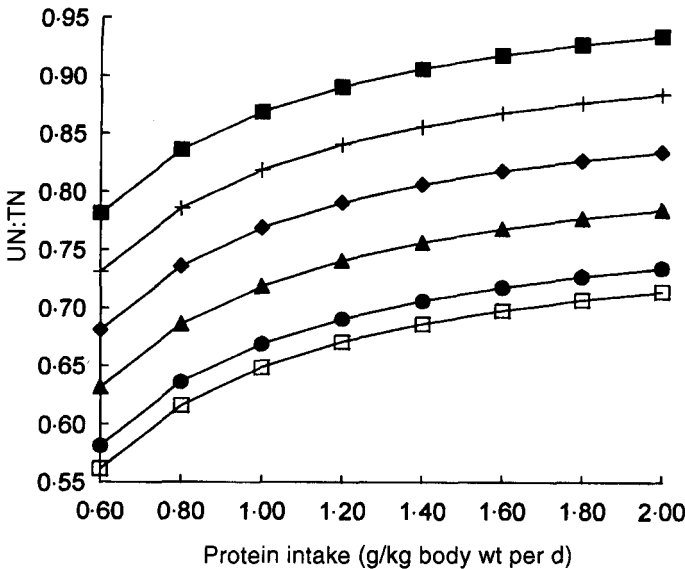

Fig. 1. Predicted relationship between urinary N (UN) excretion as a fraction of total N (TN) excretion, and protein intake (g protein/kg body weight per d) at digestibility values of 1·0 (■), 0·95 (+), 0·9 (◆), 0·85 (▲), 0·8 (●) and 0·78 (□). Values calculated from published values of obligatory urinary, faecal, and surface N losses, as discussed in the text.

indicated true digestibilities of 0·92 at a mean intake of 1·44 g protein/kg. From the above expression this would be predicted to give a mean value of 0·829 for UN:TN, which is close to the reported value of 0·81 and which verifies that the subjects examined by Bingham & Cummings (1984) were behaving as expected in relation to their N excretion.

Thus on the basis of the above, if UN is to be used to predict TN and hence DN as a validation of protein intake, some recognition of this expected variability should be taken into account. It is inappropriate to argue, as in a recent report (Black *et al.* 1997), 'For all subjects, the mean ratio UN:NI was 0·87 (SD 0·13) compared with an expected mean ratio of 0·81 (Bingham & Cummings, 1985), indicating a bias to under-reporting of about 7% for N'.

The most obvious approach is to assume normal behaviour in terms of the partition between UN and FN in relation to intake and digestibility and to use a computed value of UN/DN for the subjects after assessing or estimating in some way intakes and digestibility. As might be expected and is confirmed by further analysis of the data reported by Bingham & Cummings (1985), such a calculation would only be of value for grouped data, given intra-individual variation in obligatory losses and the general relationship between faecal and urinary N excretion routes. Thus whilst calculation of the predicted value of UN:TN for the data set indicates good correspondence in terms of the mean (0·83 (SD 0·02, range 0·79–0·86) predicted, compared with 0·81 (SD 0·02, range 0·78–0·83) observed) individual values were not significantly correlated (r 0·15). Clearly measurement of total N excretion would avoid these difficulties but as a practical tool such measurements are unrealistic.

There are other issues which further complicate the relationship between UN and DN in adults in overall balance. Surface losses can be markedly increased with profuse sweating so that extreme physical activity will reduce UN/TN. In addition, the assumed equality of N intake and loss is often not observed in weight-stable subjects, with an increasing tendency for TN to be less than DN at high intakes (Hegsted, 1976) which may not just be a matter of systematic overestimation of intake and underestimation of loss. However, in each case these factors are variable with no obvious way in which corrections can be made.

What is most important is that UN/TN and hence UN/DN are variables, not biological constants, and use of the principle of UN as a validation of measured DN should take this into account. The formula above for predicted UN/DN is valid on the basis that the values for the obligatory faecal and surface losses are valid and that the usually assumed partition between FN and UN as a function of the food protein occurs. Clearly calculation of the extent of under-reporting in subjects from the UN:DN ratio can only be made after taking into account the expected limits of variability of intakes and digestibilities in the populations examined. From the values shown above this will probably rule out detection of anything other than gross errors.

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Urine nitrogen as an independent validatory measure of protein intake

Professor Millward's recognition that 'There is no doubt that measurement of UN is an extremely useful validation measure' in dietary surveys is pleasing. This technique has proven particularly valuable in validating methods for the assessment of dietary intake of individuals in large cohort studies, where the prime requirement is to place individuals in the correct part of a distribution of dietary exposure, in order that relative risks can be established in relation to disease outcome. In the paper published in the *British Journal of Nutrition*, for example, it was shown that weighed records remained the most accurate method of dietary assessment, and only estimated 7 d diary records were able to approach this accuracy (Bingham *et al.* 1995). Other methods used in epidemiological research, such as the 24 h recall and the food-frequency questionnaire, were less accurate although the particular questionnaire investigated has since been modified, and accuracy can be improved by the technique of energy adjustment (Bingham & Day, 1996).

To develop the UN technique, studies were carried out in a metabolic suite in order that daily N excretion in faeces, blood, skin and urine, together with N intake (DN), from daily duplicate collections, could be measured in eight individuals consuming their normal *ad libitum* diet containing mixed foods over a 28 d period. Day-to-day variation in N intake was extensive (CV 21% within individuals on average), as was the variation in urine N excretion (CV 13%), and these variations were the major determinants of the relationship between dietary N intake and N excretion. For example, 'On any one day individuals may be in positive or negative balance of as much as 4 g N' ... and 'over short periods of time urine N cannot validate estimates of dietary N with any degree of precision' (Bingham & Cummings, 1985). For acceptable precision in comparing intake with output, at least 8 d of urine collections and 16 d of weighed records were found to be necessary (Bingham & Cummings, 1985). The 24 h urine collections must also be shown to be valid in themselves, and a marker was developed for this purpose (Bingham & Cummings, 1983).

In order to correct for extrarenal losses of N, a figure of 2 g is sometimes added onto the 24 h urine output to account for faecal and skin losses (Isaksson, 1980). However, in

individuals it was apparent that UN underestimated DN to a greater degree at higher levels of intake (Bingham, 1983), a phenomenon that is well recorded in the literature (Hegsted, 1976). This has variously been attributed to errors in the metabolic technique (Wallace, 1959; Forbes, 1973), denitrification by bacteria in the colon (Costa *et al.* 1968), increased urea losses from skin and expired ammonia (Calloway *et al.* 1971), and nitrate in food and urine which is not measured by the Kjeldahl technique (Kurzer & Calloway, 1981). However, it is probable that the phenomenon is also at least partly explained by endogenous NO production via NO synthase (Anggard, 1994). This is oxidized to nitrate, which is excreted in urine, or diffuses into the colon where it is reduced to ammonia and nitrite (MacFarlane & Cummings, 1991). Nitrate excretion and faecal N-nitrosocompound levels increase in response to increased protein, nitrate and meat intakes (Rowland *et al.* 1991; Bingham *et al.* 1996).

The use of a proportional value, UN/DN, overcame this underestimation of extrarenal losses of N at high intakes, thus eliminating the need to measure faecal and other losses (Bingham, 1983; Bingham & Cummings, 1985). Faecal excretion was a constant proportion of dietary N intake, but this is probably not because faecal N losses are directly related to dietary protein intake. Ileal N losses are directly related to protein intake so that ileal losses may be as great as 4 g N when dietary N intake is increased to 20 g/d (Silvester & Cummings, 1996). However, within the colon there are many types of proteolytic bacteria so that the nitrogenous residue leaving the ileum is extensively metabolized to a variety of products including ammonia, of which about 4 g/d is transported from the colon (MacFarlane & Cummings, 1991). Faecal ammonia levels increase in response to high protein diets, but in normal mixed diets total faecal N excretion does not increase in response to increases in dietary protein intake (Cummings *et al.* 1979; Bingham *et al.* 1996).

The major determinant of faecal N output is biomass arising from fermentation in the colon by bacteria (Stephen & Cummings, 1979; Stephen, 1980; Bingham, 1983). Fermentation and biomass production is dependent on dietary non-starch polysaccharide (NSP) and other fermentable carbohydrate intake (Cummings *et al.* 1992). This interrelationship is further complicated by the fact that in normal mixed diets, dietary protein and NSP are correlated (Bingham *et al.* 1994), leading to an apparent effect of total protein in normal mixed diets on faecal N levels. However, the changes in faecal N are relatively small, less than 10%, in the context of total dietary N intake.

Taking day-to-day variability into account, and the necessity for validation of urine collections, and the fact that extrarenal losses are a relatively constant fraction of dietary N intake, intake in individuals could be estimated by eight 24 h urine collections to within 81 (SD 5, range 70–90)% of dietary N (Bingham & Cummings, 1985). Is this ratio inappropriate for population mean estimates as Professor Millward suggests? As we stated, the ratio may be too high for high-NSP-consuming populations (Bingham & Cummings, 1985), and this would include vegans and vegetarian populations, since they are likely to have high intakes of fermentable carbohydrate such as NSP, which will increase the colonic biomass, and hence the faecal N excretion. However, vegans make up a small fraction of the UK population as a whole. Less than 2% (29 out of 1844) individuals in the study of the UK representative population sample quoted by Professor Millward were vegetarian and no vegans were reported to be present (Jackson & Margetts, 1993). The small number of vegetarian individuals might contribute at most 1% to average faecal N values and therefore 1% to the average ratio of UN:DN in the UK population as a whole. In addition, there are many individuals within the UK population consuming low intakes of NSP who will thus have low faecal outputs and low daily faecal N levels. There therefore

does not seem to be a good argument that the 80 (SD 5)% ratio UN:DN should not be applied to UK population estimates, provided that the published provisos are adhered to.

Can the ratio be used on an individual basis? Millward argues that 'as DN falls, total N (TN) excretion falls, and UN/TN will fall' and as a result of this, and variation in digestibility, the ratio is too variable to detect other than 'gross errors'. However, digestibility is calculated from faecal N, and in free-living individuals eating normal mixtures of food and varying NSP intakes, faecal N is not dependent on dietary N intake. In our study where TN was measured, the correlation between UN/TN and protein intake was not significant, r 0.338, $P = 0.413$ (Bingham & Cummings, 1985).

In N balance, N output cannot exceed intake and there was close agreement between urine and dietary N, r 0.87, in 125 free-living individuals designated as having valid weighed intake records (Bingham et al. 1995). This close agreement suggests that in fact the ratio can be a precise estimate of underreporting of protein intake for individuals.

Furthermore, Table 1 shows that the range of protein intake consumed by those individuals whose excretion did not exceed intake was similar in this study to that used in the example of Millward, 0.69 to 1.88 g protein per kg body weight. The Table also shows quartile means of protein intake per kg, and quartile means of the UN:DN ratio. The individual values were negatively correlated as would be expected, $r - 0.221$, $P = 0.013$, but protein intake per kg contributed little (5%) to the variance of the UN:DN ratio. In the thirty-one individuals designated as having underreported, there was a highly significant negative association, due to the fact that urine N exceeded dietary N ($r - 0.624$, $P = 0.000$).

In those whose dietary records were valid, mean ratio was 0.878 in the lowest quartile of protein intake per kg body weight, and 0.820 in the highest quartile (Fig. 1). Hence those with the lowest intakes of protein tended to have high ratios, and individuals with low ratios had high intakes. The individual with the lowest intake of protein per kg (0.69 g/kg) had a ratio of 0.92, whereas the individual with the highest intake of protein per kg (1.88 g/ kg) had a ratio of 0.81. Nevertheless, despite this range in protein intake values, the range in the UN/DN value agrees well with the predicted range of 70–90% (Bingham & Cummings, 1983).

In conclusion, used within the published limitations of the technique, there is little evidence that digestibility and protein intake affect the viability of the UN:DN ratio to establish validity in dietary surveys. In individuals in overall N balance, the main problem in using this technique is day-to-day variation in intake and urine N output so that sufficient

Table 1. *Mean UN:DN ratios in quartiles of the distribution in dietary protein intake in g/kg body weight, for those 125 individuals whose urine excretion did not exceed intake (Bingham* et al. *1995)*

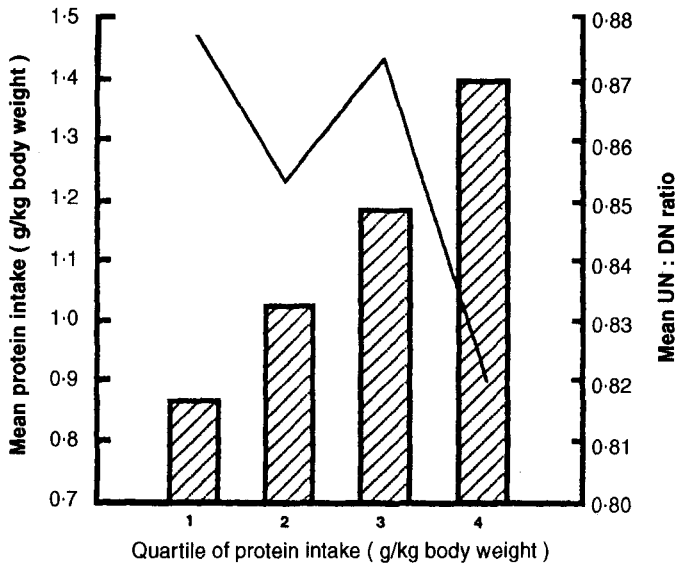| Quartile | n | Protein intake (g/kg body weight) | | Mean UN:DN ratio |
		Mean	Range	
1	31	0.868	0.69–0.963	0.878
2	31	1.026	0.964–1.101	0.852
3	32	1.186	1.106–1.252	0.874
4	31	1.400	1.253–1.881	0.820

Fig. 1. The relationship between protein intake (g/kg body weight) and the UN:DN ratio (———), by quartile, in 125 free-living women whose UN did not exceed intake.

observations on each individual are necessary to introduce acceptable precision into the estimates. The completeness of the 24 h urine collections also needs to be assured.

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