

Combination of cassava flour cyanide and urinary thiocyanate measurements of school children in Mozambique

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The maximum daily cassava flour intake of children may be calculated from determination of the total cyanide content of cassava flour and urinary thiocyanate levels of school children in samples collected at the same time and place. Four sites, two with and two without recent konzo cases, were chosen for study. In two sites with recent konzo cases, 84% and 93% of school children consumed cassava the previous day, and the calculated maximum daily consumption of cassava was over 700 g. In two sites without recent konzo cases, about 50% of school children consumed cassava the previous day and the calculated daily consumption of cassava flour was less than 150 g. By measurements of cyanide in flour and urinary thiocyanate we are therefore able to distinguish between communities whose diet is almost totally reliant on cassava, and who are therefore susceptible to konzo, and those who have a broader diet and are free from konzo. In another calculation it is shown that 4–23% of the essential S-containing amino acids in the cassava flour consumed by children is used up to detoxify and flour cyanide to thiocyanate. This depletion of methionine and cystine may lead to protein deficiency and may contribute to onset of konzo.

Introduction

Konzo is a spastic paraparesis with sudden onset that occurs particularly in children and women of childbearing age (Howlett *et al.*, 1990; Tylleskar *et al.*, 1993). In Mozambique large epidemics of konzo occurred from 1981

to 1993 in the northern provinces of Nampula and Cabo Delgado (Ministry of Health Mozambique, 1984; Cliff *et al.*, 1997). Konzo has persisted since 1993 in Mogincual District of Nampula Province (Ernesto *et al.*,

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2002). The occurrence of konzo in Mozambique and elsewhere in East and Central Africa is associated with a high intake of cyanide from cassava flour and roots, and a low intake of S-amino acids (Cliff *et al.*, 1985; Cliff, 1994; Howlett *et al.*, 1990; Ernesto *et al.*, 2002).

Some of the ingested cyanide in the form of the cyanogenic glucosides present in cassava (linamarin and a small amount of lotaustralin) is absorbed by the body (Carlsson *et al.*, 1999) and converted to thiocyanate, which is excreted in the urine. The sulphur used in this process comes from the S-containing amino acids cystine and methionine (Osuntokun, 1981; Schulz, 1984; Westley, 1988). High total cyanide intake may therefore cause depletion of essential amino acids in a situation where the diet is already short of protein (Cliff *et al.*, 1985; Tylleskar *et al.*, 1992).

In a longitudinal study from 1996 to 1999 in Mogincual District, we monitored total cyanide levels of cassava flour using a simple picrate kit (Egan *et al.*, 1998; Bradbury *et al.*, 1999; Cardoso *et al.*, 1998). After development of a urinary thiocyanate picrate kit (Haque & Bradbury, 1999) we were also able to measure urinary thiocyanate levels of school children in the same sites. In 2001, we added Niboia in Ile District, Zambezia Province (size of a recent konzo outbreak) and two sites in Erati District in Nampula Province, where konzo had not been reported recently.

The main objective of this paper is to combine the information from flour cyanide and urinary thiocyanate concentrations in 2001 to calculate cassava flour intake. Another objective is to calculate the fraction of S-containing amino acids from cassava flour that is used up in detoxification of cyanide to thiocyanate. For both sets of calculations, we aimed to compare results from sites where new cases of konzo have recently occurred, with control sites in Erati District.

Materials and methods

Study sites and collection sites

We chose four sites for study: Mujocojo in Mogincual District, Niboia in Ile District,

and Munhacuco and Namango in Erati District (Figure 1). Mujocojo has reported persistent konzo since 1993 (Ernesto *et al.*, 2002), while Niboia in Ile District in Zambezia Province had recently reported a small konzo outbreak. Nine cases had onset between 1998 and 2000; six of these had onset in the last 4 months of 2000. Erati District in Nampula Province had suffered large konzo epidemics in 1981 and 1988 associated with drought and war. Munhacuco, on the outskirts of the district capital, had suffered an epidemic in 1988 during war and Namango had reported a case in 1994. No cases have been reported since. World Vision is currently implementing an intervention project in the district to promote cassava safety and improve family nutrition.

In July 2001 we administered a questionnaire and collected 30 cassava flour samples from houses in each site. The questionnaire asked about food consumed in the past 24 h above and about cassava processing methods. In November and December 2001, we again collected in the same sites in Erati and Mogincual Districts. We used the same house selection method and questionnaire as previously (Cardoso *et al.*, 1998), except that in November and December flour was brought to a central point, and smaller numbers of samples were obtained. In July, we also obtained 30 samples of urine from school children in the same sites on the same day, together with information on the proportion of school children who had consumed cassava on the previous day.

Laboratory methods

Flour and urine samples were stored for less than 48 h at room temperature or in cool boxes and were then analysed or refrigerated at -20°C until analysed. Single analyses of each of the cassava flour and urinary thiocyanate samples were made using the picrate method as previously described (Cardoso *et al.*, 1998, 1999; Ernesto *et al.*, 2002). Results were obtained using the colour chart method in July and the spectrophotometer method in December (Bradbury *et al.*, 1999). The thiocyanate content was reported in micromoles per litre, which equals parts per million (ppm) multiplied by 17.2. Duplicate samples

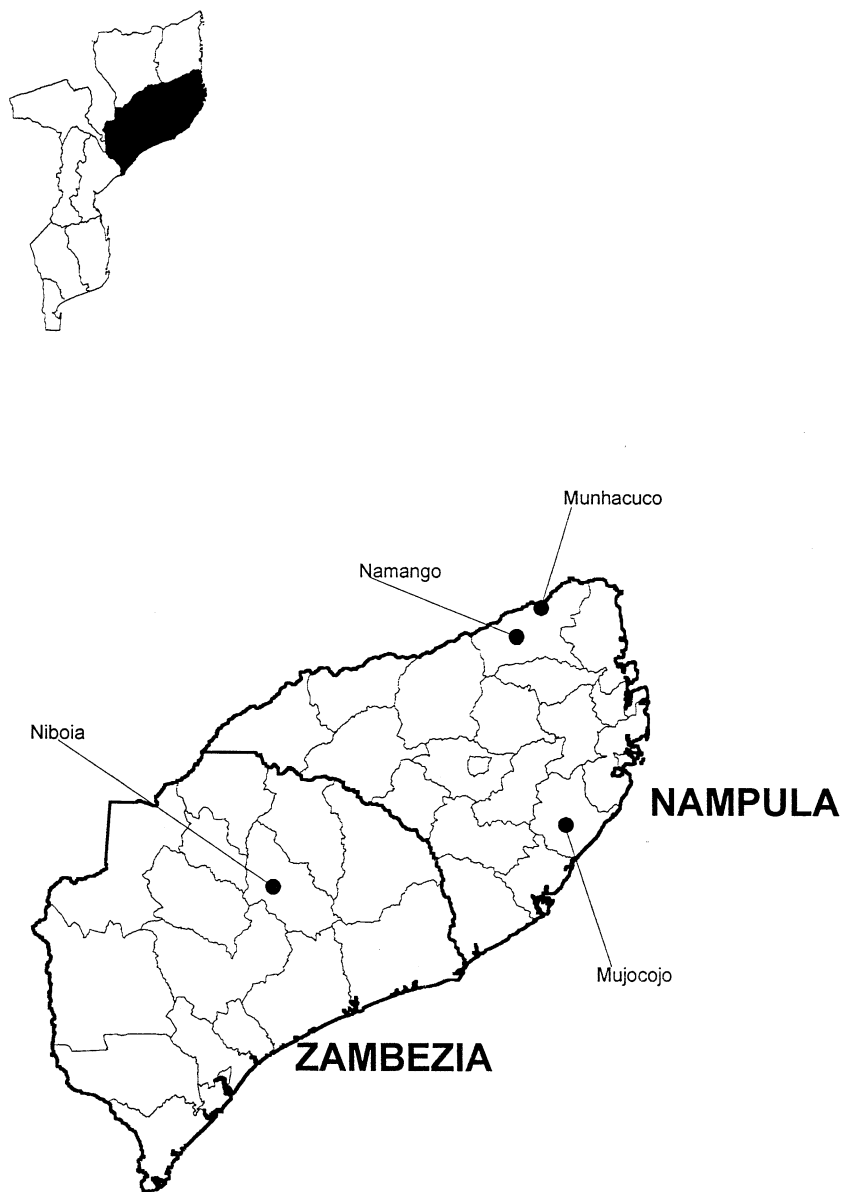


Figure 1. Map of Mozambique (inset), and detailed map of Nampula and Zambezia Provinces showing the location of study sites.

of three lots of cassava flour were dried to constant weight at 75°C to determine their moisture content.

Calculation of daily consumption of cassava flour

Part of the cyanide ingested as linamarin from cassava flour is converted to thiocya-

nate and excreted in the urine, and the remainder is removed as linamarin and by other mechanisms (Osuntokun, 1981). Carlson *et al.* (1999) found that on average 27% is converted to thiocyanate. Thus the total daily amount of cyanide ingested from cassava is equal to the total amount of cyanide removed from the body as thiocyanate (corrected for the small amount found

in non-smoking, non-cassava eating populations) multiplied by 100/27. The calculation involves the known cassava flour cyanide content, the known urinary thiocyanate concentration of village school children and an estimate of their daily urine volume (600 ml).

Let the unknown daily intake of cassava flour be W g, containing C mg HCN/kg cassava flour (ppm). Let the urinary thiocyanate concentration be T $\mu\text{mol/l}$, which is reduced by 25 $\mu\text{mol/l}$, the average urinary thiocyanate concentration of non-smoking, non-cassava-eating control persons (Haque & Bradbury, 1999).

Cyanide in cassava flour

$$= C \text{ mg HCN/kg flour}$$

$$= C/27 \text{ mmol HCN/kg flour}$$

Daily cyanide intake

$$= CW/27000 \text{ mmol HCN/day}$$

Cyanide converted to thiocyanate

$$= (27/100) (CW/27000)$$

$$= 10^{-5} CW \text{ mmol thiocyanate/day} \quad (1)$$

Urinary thiocyanate concentration from cassava cyanide $= (T - 25)/1000$ mmol/l

Urinary thiocyanate/day

$$= 600(T - 25)/10^6 \text{ mmol thiocyanate} \quad (2)$$

Since the cyanide converted to thiocyanate equals the urinary thiocyanate per day, Equations (1) and (2) are equated and solved for the unknown quantity W , which gives

$$W = 60(T - 25)/C \quad (3)$$

Because of the possibility of cyanide intake from other sources such as fresh or cooked cassava roots, the calculated value of W is a maximum value.

Calculation of fraction of S-containing amino acids from cassava used to detoxify cyanide

The daily intake of the essential S-containing amino acids (cystine and methionine) obtained from consumption of cassava flour is partially used up in detoxification of ingested cyanide to produce thiocyanate (Osuntokun,

1981; Schulz, 1984; Westley, 1988). Since we know the daily consumption of cassava flour (W g, from Equation (3)) and the amino acid analysis of cassava roots and some cassava products (Ekpenyong, 1984; Bradbury & Holloway, 1988; Yeoh & Truong, 1996; Firmin & Kamenan, 1996; Ngudi *et al.*, 2002), we can calculate the total amount of S-amino acids obtained daily from cassava flour. From Equation (2) we can calculate the daily amount of S-amino acids used up in detoxification of cyanide to urinary thiocyanate. In this way the fraction of S-amino acids from cassava used up in detoxification of cyanide to thiocyanate may be calculated.

The calculation is based on the amino acid composition of cassava roots obtained from four different sources, which are reported in different units that have been converted to milligrams of amino acid per gram of nitrogen. The sun-drying or heap fermentation methods of processing cassava roots to flour practised in Nampula Province (Cardoso *et al.*, 1998) are unlikely to change the amino acid content of the flour. However, the process used widely in Cote d'Ivoire in which peeled roots are steeped in water for some days is found to cause a considerable reduction in the nitrogen content of the product and changes in the amino acid balance (Firmin & Kamenan, 1996), and for this reason the result for processed cassava products are not included. The mean values (standard deviations in brackets) for methionine and cystine in cassava roots from the South Pacific (Bradbury & Holloway, 1988), Nigeria (Ekpenyong, 1984), Cote d'Ivoire (Firmin & Kamenan, 1996) and Philippines (Yeoh & Truong, 1996) are 115(43) and 85(66) mg amino acid/g N, respectively. Thus, there is 115/149 and 85/240 mmol/g N methionine and cystine present, where 149 and 240 are the molecular weights of methionine and cystine, respectively.

The total amount of S/g N $= (115/149 + 2 \times 85/240)$ mmol S/g N (since there are two atoms of sulphur present per molecule of cystine), which equals, 1.48 mmol S/g N.

If we take 100 g cassava root, which has an average moisture content of 62.8% (Bradbury & Holloway, 1988), the amount of dry flour produced would be 37.2 g and of fresh

flour (containing 7.2% moisture) would be 39.9 g. The protein content of cassava root averaged from the four sources is 0.91 (0.42)%. Thus, 100 g cassava root contains 0.91 g protein. For proteins, % N = % protein/6.25, thus 100 g cassava root contains $0.91/6.25 = 0.146$ g nitrogen. The amount of S present in 100 g cassava root will be $1.48 \times 0.146 = 0.216$ mmol S. The amount of S present in 100 g fresh cassava flour = $0.216 \times 100/39.9 = 0.541$ mmolS/100 g flour.

Since W g cassava flour is consumed per day (Equation (3)), then:

Total $\mu\text{mol S}$ consumed per day

$$= 0.541 \times 1000 \times W/100$$

$$= 5.41 \times W \mu\text{mol S/day.}$$

The amount of S from methionine and cystine used up to convert cyanide to thiocyanate equals $T \times 600/1000 \mu\text{molS/day}$, where T is the urinary thiocyanate (SCN) concentration ($\mu\text{mol/l}$).

% methionine and cystine (from cassava) to

$$\text{produce SCN} = 100 \times 0.6T / (5.41 \times W)$$

$$= 11.1 T/W \quad (4)$$

Results and discussion

Food sources

Table 1 shows that at the time of the survey in July 2001, families in Mujocojo had a less varied diet than those in Munhacuco and Namango, and in particular families in

Mujocojo and Niboia (sites with recent konzo cases) rarely consumed beans, the most common curry ingredient in Munhacuco and Namango. Although the fish consumed in Mujocojo does add variety to the diet, the amounts consumed were probably small, as they were traded with the coast for cassava. More telling is that 31% of surveyed households consumed a wild root 'minani' (*Dioscorea schimperiana* Hochst. Ex. Kunth) as the main ingredient of their curry. Analysis of minami roots using the pierate kit showed that they do not contain cyanide. Bitter cassava leaves predominated as the main curry ingredient in Niboia (which contributed to their cyanide intake), and were also consumed to a much smaller extent in Mujocojo, and only slightly in Munhacuco and Namango. Also, the percentage of school children who had consumed cassava the previous day was higher in Mujocojo and Niboia (Table 2), than in Munhacuco and Namango, where more millet and sorghum was being grown.

Cyanide in flour, urinary thiocyanate and estimated daily consumption of cassava flour

As presented in Table 2, the sites recently affected by konzo showed much higher urinary thiocyanate concentrations than the sites affected in the past. The latter, however, showed higher mean total cyanide concentrations in flour than those with recent konzo. This apparent paradox is explained by the care that women in the recently affected sites were taking to process cassava,

Table 1. Main ingredients of curry consumed by households in study sites, Mozambique, July 2001

| Main curry ingredient | Percentage of total households* with main curry ingredient | | | |
|-------------------------------------|--|--------|-----------|---------|
| | Mujocojo | Niboia | Munhacuco | Namango |
| Beans | 0 | 6.5 | 70 | 60 |
| Fish | 51.7 | 3.2 | 16.7 | 3.3 |
| Cassava leaves | 13.8 | 67.6 | 6.7 | 6.7 |
| Pork | 0 | 9.7 | 3.3 | 6.7 |
| Chicken/Pigeon | 0 | 6.5 | 3.3 | 3.3 |
| Sweet potato/pumpkin leaves/cabbage | 3.5 | 6.5 | 0 | 16.7 |
| Minani (wild tuber) | 31 | 0 | 0 | 0 |
| Other | 0 | 0 | 0 | 3.3 |

*Number of households studied from Manhacuco and Namango was 30, from Mujocojo was 29 and from Niboia was 31.

Table 2. Daily consumption of cassava, urinary thiocyanate in school children and cyanide in cassava flour in konzo affected sites in 2001

| Site | Month | % ate cassava on previous day | Mean total cyanide in flour, C (ppm) | Mean urinary thiocyanate, T ($\mu\text{mol/l}$) | Maximum cassava flour intake, W(g) (Equation (3)) | % S-amino acids to detoxify cyanide (Equation (4)) |
|--------------|----------|-------------------------------|--------------------------------------|---|---|--|
| Recent konzo | | | | | | |
| Mujocojo | July | 84 | 18 | 301* | 920 | 3.6 |
| Niboia | July | 93 | 27 | 351* | 720 | 5.4 |
| Old konzo | | | | | | |
| Munhacuco | July | 42 | 39 | 72 | 58 | 14 |
| Munhacuco | November | – | 58** | 47 | 23 | 23 |
| Namango | July | 60 | 26 | 85 | 140 | 6.7 |
| Namango | November | – | 41 | 57 | 47 | 13 |

*One very high value of 1720 $\mu\text{mol/l}$ solidus 1 in each of these data-sets skewed the mean values.

**Mean of four samples only.

both reliant on it as their sole staple and also conscious of its potential to induce konzo. Forty per cent of households in Mujocojo used heap fermentation. As in our previous studies (Cardoso *et al.*, 1998, 1999; Ernesto *et al.*, 2000, 2002), the amount of cyanide in heap fermented flour in Mujocojo (17 HCN/kg) was approximately one-half that of sun-dried flour (30 mg HCN/kg). In Niboia, a slightly different processing method was used, owing to the proximity of a river. The most common processing method involved washing in the river, followed by sun drying. All households in Erati District used sun drying.

Table 2 shows that the maximum daily cassava flour intake, calculated using Equation (3), was much higher in the recently konzo-affected sites. The daily cyanide intake of school children in Munhacuco and Namango is much reduced compared with the recently affected sites because of their broader and better diet. The maximum daily cassava intakes of 920 and 720 g for Mujocojo and Niboia for July 2001 are high, which may be due to: (1) consumption of other cyanide containing cassava products such as raw/cooked roots and leaves, which would have the effect of increasing *T* in Equation (3); (2) urinary thiocyanate levels have been elevated by a single very high result in each case; and (3) the percentage of ingested cyanide converted to thiocyanate may be greater than 27%, the average value obtained from the very variable results from

seven individuals (Carlsson *et al.*, 1999) used in the calculation of Equations (1) and (3). It is probable that the value of 27% may vary from person to person depending on their nutritional status, including the availability of sulphur for thiocyanate formation. Other workers suggested that at least one-half of the ingested cyanide is converted to thiocyanate (Osuntokun, 1981; Schulz, 1984). If the percentage of ingested cyanide converted to thiocyanate was actually 50%, rather than 27%, then the maximum, daily cassava intakes for Mujocojo and Niboia would be reduced to 500 and 390 g, respectively. Data from four sites in October 1999 (Ernesto *et al.*, 2002) gave values of *W* from 350 to 570 g, in some of which konzo had recently occurred.

Calculation of the maximum daily cassava intake allows one to distinguish between populations who are almost totally reliant on cassava and who are therefore at risk of contracting konzo, and those who have a broader, safer diet. For example, comparing the July 2001 results, in Munbacuco the total cyanide content of flour was highest, yet the daily cyanide intake was lowest because of the low intake of cassava flour. Conversely, Mujocojo had the lowest value for total cyanide content in flour, yet the daily cyanide intake was highest. Measurements of total cyanide in cassava flour alone do not necessarily give a good idea of cyanide consumption.

Clearly the combination of flour total cyanide and urinary thiocyanate measurements on samples collected at the same site and time is needed to give a complete picture.

Fraction of S-containing amino acids from cassava required to detoxify cyanide to thiocyanate

Table 2 presents the percentage of S-amino acid intake from cassava needed to detoxify cyanide ingested from cassava. The calculation is based on a wide data-set of protein and amino acid analyses of cassava from Africa, Asia and the South Pacific. Methionine and cystine obtained from foods consumed other than cassava (see Table 1) cannot be included in this calculation because the data such as amino acid contents is not available. Similarly we do not know whether all the S-containing amino acids present in cassava are available for thiocyanate formation. The higher values of 14–23% for Munhacuco in Table 2 result from the relatively small daily consumption of cassava in Munhacuco. Thus, for Namango, as the daily consumption cassava decreased from July to November there was an increase in the percentage of S-amino acids from cassava needed to detoxify cyanide.

In the body the conversion of poisonous cyanide to thiocyanate is a priority, and these calculations have shown a depletion of the S-containing amino acids ingested from cassava flour of 4–23%. These values would be increased further if the percentage of ingested cyanide converted to thiocyanate were to be greater than 27%, since this would decrease *W* and hence increase the percentage of methionine and cystine used to produce thiocyanate in Equation (4). The enzyme

that is used in this reaction (rhodanese) would also be produced in greater amount, to catalyse the detoxification of the continual cyanide load. This would make extra demands on the body's reserves of S-amino acids, which if prolonged may impair the synthesis of many proteins vital for bodily function and lead to protein deficiencies in a diet already short of protein (Padmaja, 1996; Ngudi *et al.*, 2002). The low S-amino acid values found in konzo-affected communities may contribute to the onset of konzo (Cliff *et al.*, 1985; Tylleskar *et al.*, 1992; Padmaja, 1996).

Conclusion

As we showed previously (Ernesto *et al.*, 2002) and have confirmed in this paper, konzo is associated with cyanide intoxication in poor rural areas where the population is dependent on a diet of bitter cassava. The two sites in our study, where konzo has been recently reported, are not suffering from an acute agricultural crisis caused by drought or war. Rather, they are communities suffering from chronic extreme poverty, and are not helped by agricultural policies that favour richer communities.

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