The effects of chronic cassava consumption, cyanide intoxication and protein malnutrition on glucose tolerance in growing rats

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Intraperitoneal glucose tolerance tests were performed at 4-week intervals in groups of weanling rats before and after feeding with maize- or cassava-based diets with and without adequate protein and sub-lethal cyanide supplementation. Weaning weights were doubled (increase of about 50 g) after 4 weeks on control (maize-based with adequate protein) and protein-replete diets. Weight gain on the protein-deficient diets was much less (22 g or 50%), a pattern maintained by the rats on these diets until the age of 12 weeks. Plasma thiocyanate levels were identical at weaning and after 8 weeks of the control diet but increased by 200–300% after 4 weeks intake of the cassava or cyanide-supplemented feeds. Levels returned to normal in all groups after a further 4 weeks feeding with the control diet. Glucose tolerance (as assessed by the area under the 2 h glucose *v.* time curve) was impaired to a varying extent in the rats after 4 weeks on the various diets: protein-replete cassava and protein-deficient maize diets by 50%, protein-deficient cassava diet by 300%, and cyanide-supplemented protein-deficient maize diet by 150%. The derangement in the rats on the protein-replete cassava diet was unaffected by a further 4 weeks intake of the control diet, unlike in the other groups where there was significant improvement in the glucose tolerance indices at the same time. It is concluded that in growing rats: (1) cassava intake and protein malnutrition may have independent and additive effects on the genesis of glucose intolerance, (2) cyanide supplementation of a cassava-free protein-replete diet has no effect on glucose tolerance.

Cassava: Diabetes mellitus: Tropical medicine: Cyanide: Protein–energy malnutrition

Malnutrition-related diabetes mellitus (MRDM) was indicated as a separate sub-class of diabetes by the World Health Organization Study Group (1985). It is believed to account for up to 80% of all cases of diabetes seen in some tropical countries (Ekoe, 1985; Abu-Bakare *et al.* 1986). Various reports suggest that MRDM might be causally related to malnutrition alone, or in combination with cassava consumption and cyanide intoxication (Rao, 1984; Ekoe, 1985; World Health Organization Study Group, 1985). However, it still remains unclear whether MRDM should be considered a distinct clinical entity; more so as many epidemiological studies have not unequivocally established correlations between its prevalence and cassava consumption patterns or protein malnutrition (Lester, 1984; Abu-Bakare *et al.* 1986; Teuscher *et al.* 1987; Akanji, 1990; Akanji *et al.* 1990).

This prompted the World Health Organization Expert Committee on Diabetes to suggest in its report (World Health Organization Study Group, 1985) that the relationship between MRDM and high cassava consumption, especially in the background of protein malnutrition, needs to be defined, including the mechanisms whereby malnutrition in early life results in partial loss of pancreatic beta cell function. Furthermore, the World Health Organization Study Group (1985) called for a search for other food toxins and causative factors (other than cassava) that may be important in the genesis of MRDM. This is necessary to expedite development of intervention strategies.

Table 1. *Composition of the control and experimental diets (g/kg)*

Diet...	Control	A	B	C	D	E
Maize meal	685	—	—	685	908	908
Cassava flour	—	556	747	—	—	—
Soya-bean meal*	221	350	161	221	—	—
Groundnut oil	50	50	50	50	50	50
Oyster shell	10	10	10	10	10	10
Bone meal	20	20	20	20	20	20
Vitamin and mineral mix†	10	10	10	10	10	10
Sodium chloride	2	2	2	2	2	2
Methionine	2	2	—	2	—	—
Potassium cyanide‡	—	—	—	1·9	—	1·9

* Extruded soya-bean meal containing 450 g crude protein (nitrogen × 6·25)/kg.
† Composition according to Syme (1982).
‡ Analar grade (BDH Chemicals, Poole, Dorset, UK).

The present study was, therefore, designed to assess, in rats at various stages of growth and development, the separate and combined effects on glucose tolerance of: (1) cassava ingestion with and without protein malnutrition, (2) cyanide supplementation of maize-based (cassava-free) diets with and without adequate protein content. Maize is also widely consumed in tropical areas, often to a similar extent to that of cassava. The objective was to define whether the glucose intolerance after chronic cassava ingestion was due to its cyanide content or some other factors peculiar to that food crop. This experimental model was used because manipulations which control for one factor while another is being investigated, as in the present study, cannot for ethical reasons be adopted in humans. Furthermore, it is possible using rats to follow development from adolescence (weaning) through adulthood within a time period of 3 months. We believe our results could be extrapolated to the human situation.

MATERIALS AND METHODS

Groups of in-bred strains of rats were obtained from a local breeding colony and subjected to experimental and control diets (for details, see below). The procedures were approved by the local Animal Research Ethics Committee.

Diets. Two sets of experimental diets (one cassava-based and the other maize-based) were devised. The cassava-based diet contained a cassava flour base with either 165 g protein (diet A, adequate protein) or 80 g protein (diet B, protein deficient)/kg. A control diet with a maize base and adequate in protein (165 g/kg) was also prepared. The compositions of these diets are indicated in Table 1. The maize-based diet contained a maize-meal base with 165 g protein (diet C) or 80 g protein (diets D and E)/kg. Additionally, diets C and E were supplemented with a sub-lethal dose (750 mg/kg) of potassium cyanide. The control diet was similar to that used for rats on the cassava-based diet. The compositions of these diets are indicated in Table 1.

The pelleted semi-synthetic experimental diets were prepared as previously described (Tewe, 1975). Methionine was added to the protein-replete diets (control, diets A and C) to obviate acute deficiencies of this sulphur-containing amino acid. The loss in energy due to reduction of the protein content in diets B, D and E was compensated by increasing the carbohydrate (cassava or maize) content; the control and experimental diets were, thus, isoenergetic. The protein content of diets D and E was similarly contributed by this

increased maize constituent. The total individual rat feed intake was not assessed; it was, however, noted that rats fed on the protein-deficient diets reduced their feed intake significantly (see Swenne *et al.* 1987).

Animals. Male Wistar albino rats (28 d old) were obtained from the Clinical Animal House of the University of Ibadan, College of Medicine. They were caged in groups of three, and housed in a room with controlled temperature ($26 \pm 2°$), humidity ($50 \pm 10\%$) and light (07.00–19.00 hours) throughout the study. The rats were weighed weekly and weaned on to the control diet or any of the experimental diets. The diets and water were consumed *ad lib.* for 4 weeks, after which all the rats (at age 8 weeks) changed to the control diet for another 4 weeks (when aged 12 weeks).

Glucose tolerance tests. An intraperitoneal glucose tolerance test was performed when the rats were aged 4, 8 and 12 weeks. All food was withdrawn overnight for 12–14 h before each study in the morning at about 09.00 hours. Each rat was then injected intraperitoneally with a glucose solution (200 g/l), at a dose of 2 g glucose/kg body-weight (Swenne *et al.* 1987). Blood samples were obtained from the cut tip of the tail, orbital veins or cut cervical vessels after death by cervical dislocation, as appropriate, to obtain the required volume of blood for the various laboratory estimations. Six rats were usually investigated on each occasion, and were randomly drawn from the group of rats on each specific diet; they were usually killed after each study.

Laboratory analyses. Blood samples were collected before glucose injection (0 min) and at 60 and 120 min after glucose injection, into fluoride oxalate tubes for glucose assay. Heparinized samples for the estimation of plasma thiocyanate were collected at 0 min only. Plasma was usually separated within 1 h of sample collection and kept frozen at $-20°$ until assayed within 1 month of collection. Plasma glucose was measured by a glucose oxidase (*EC* 1.1.3.4) method (Trinder, 1969) and fasting plasma thiocyanate by a spectrophotometric method (Bowler, 1944). The intra- and inter-assay coefficients of variation for both assays was always less than 2·5%.

Statistics. The results are expressed as means and standard deviations. The index of glucose tolerance used was the area under the 2 h glucose *v.* time curve normalized for the 0 min value (incremental area) calculated by the trapezoidal rule. The differences in body weight, glucose incremental areas and fasting plasma thiocyanate levels between the control and experimental diets, were explored by unpaired *t* tests and ANOVA as appropriate. The level of statistical significance was $P < 0{\cdot}05$.

RESULTS

Cassava-based diet

Body weight gain. Table 2 shows that body weight increased by about 50 g (i.e. approximately doubled) in the rats on both the control diet and diet A, with no significant difference in weight gain on either. The rats on diet B after 4 weeks failed to gain as much weight, only about 28 g. When the 8-week-old rats changed to the control diet for another 4 weeks (Table 3) there was further weight gain in all the groups, although to a much lesser extent in the rats initially fed on the protein-deficient diet B (mean weight gain of 43 g from weaning). Those rats on the control diet throughout the 8-week feeding period were the heaviest at 12 weeks (weight gain from weaning of about 90 g), although final weights did not differ significantly from those of rats initially on the protein-replete cassava diet A (weight gain from weaning of 74 g).

Glucose tolerance. Tables 2 and 3 indicate that glucose tolerance, as assessed from the incremental area under the 2 h glucose *v.* time curve, did not differ significantly after the weanling rats were fed on the control diet for up to 8 weeks. There was some deterioration

Table 2. *Body weight, pre- and post-glucose injection plasma glucose and fasting plasma thiocyanate levels in groups of rats at weaning and after 4 weeks on the control and cassava-based experimental diets*

(Mean values and standard deviations for 6 rats/group)

Treatment group*...	Weaning		Control diet		Diet A		Diet B	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Weight (g)	48·1[a]	6·8	96·0[bc]	12·1	109·8[b]	6·1	70·4[ac]	9·6
Glucose (mmol/l) at								
0 min	4·1	0·6	3·8	0·5	4·6	0·5	6·0	1·9
60 min	6·5	0·9	5·8	0·6	7·3	1·2	12·7	3·3
120 min	5·0	0·9	4·3	0·7	6·3	1·5	9·8	2·9
Incremental area (mmol/l per h)	2·86[a]	0·93	2·64[a]	1·19	3·56[ab]	1·34	8·64[b]	2·11
Fasting SCN (mmol/l)	0·058[a]	0·023	0·068[a]	0·030	0·192[b]	0·014	0·176[b]	0·025

[a, b, c] Mean values with unlike superscript letters were significantly different ($P < 0·01$).
A, Adequate protein; B, protein deficient.
* For details of treatments and diets, see pp. 270–271 and Table 1.

Table 3. *Body weight, pre- and post-glucose-injection glucose levels and fasting thiocyanate levels in the groups of rats fed on cassava-based diets after a further 4 weeks on the control diet*

(Mean values and standard deviations for 6 rats/group)

Initial diet*...	Control		A		B	
	Mean	SD	Mean	SD	Mean	SD
Weight (g)	140·4[a]	19·6	122·0[a]	12·8	91·4[b]	9·0
Glucose (mmol/l) at						
0 min	3·8[a]	0·2	4·7[ab]	0·6	5·4[b]	0·6
60 min	5·9[a]	0·4	7·5[ab]	1·0	9·8[b]	2·0
120 min	5·2[a]	0·8	6·5[ab]	1·2	8·2[b]	1·3
Incremental area (mmol/l per h)	2·80	1·20	3·63	0·60	5·86	1·90
Fasting SCN (mmol/l)	0·067	0·026	0·073	0·034	0·089	0·043

[a, b, c] Mean values with unlike superscript letters were significantly different ($P < 0·05$).
A, Adequate protein; B, protein deficient.
* For details of diets and treatments, see pp. 270–271 and Table 1.

at age 8 weeks (compared with weanling rats and rats on control diets) in rats fed on diet A for 4 weeks, with about 50% increase in values for incremental area. This defect persisted until 12 weeks of age, even after the rats had changed to the control cassava-free diet for another 4 weeks. In the rats on diet B for 4 weeks (Tables 2 and 3) the impairment of glucose tolerance was much worse, with about 250% deterioration, compared with weanling rats and rats on the control diet. By 12 weeks of age, after 4 weeks on the control diet, glucose tolerance in these diet B rats had improved significantly, although still abnormal, but values were not significantly different from those obtained in the rats initially on diet A.

Plasma thiocyanate levels. Plasma thiocyanate levels did not differ significantly in rats at weaning and after consuming the control diet but increased in the rats on diets A and B by

about 300% (0·065–0·185 mmol/l), with the greater numerical increase on diet A (although not significantly different from diet B because of the wider scatter of levels in the latter). When these rats changed to the control diet the fasting plasma thiocyanate levels returned to control levels.

Maize-based diet

Body weight gain. Table 4 shows that body weight increased by about 50 g (i.e. approximately doubled) in the rats on diet C (protein replete) to a similar extent to that with the control diet. However, body weight gain on the protein-deficient diets D and E after 4 weeks was much less (about 20 g or 40%). When the 8-week-old rats changed to the control diet for another 4 weeks (Table 5), there was further weight gain in all the groups (about 70–90 g total weight gain), with no significant difference in the final weight of any of the groups at age 12 weeks, although those rats on the control diet throughout the 8 week feeding period tended to be the heaviest (mean weight gain from weaning of about 90 g).

Glucose tolerance. Tables 4 and 5 indicate that glucose tolerance, as assessed from the incremental area under the 2 h glucose *v.* time curve, did not differ significantly after the weanling rats were fed on the control diet and diet C for 4 weeks. Rats fed on diets D and E for the same period had 40 and 150% deterioration respectively in their glucose tolerance compared with values for weanling rats and rats on the control diet. The incremental areas did not change in the diet D group but were significantly reduced in the diet E rats 4 weeks after changing to further control diet treatment; these values remained higher than at weaning or on the control diet.

Plasma thiocyanate levels. Plasma thiocyanate levels did not differ significantly in rats at weaning and after 4 weeks on the control diet and diet D but increased in the rats on the cyanide-supplemented diets C and E to a similar extent by about 120% (0·060–0·150 mmol/l). All the groups of rats had similar fasting thiocyanate levels on return to the control diet for 4 weeks.

DISCUSSION

Our study reports a slower rate of weight gain in the protein-malnourished rats, irrespective of the principal dietary constituent, cassava or maize. Glucose tolerance was also impaired with this state of protein malnutrition, and was only partly corrected on returning to adequate protein feeding. These observations are in agreement with results observed elsewhere (Becker *et al.* 1972; Milner, 1972; Swenne *et al.* 1987). Cyanide supplementation of a protein-rich maize diet (C) in the weanling rats produced no significant change in glucose tolerance over 12 weeks. However, prolonged cassava intake alone, unassociated with malnutrition, caused a significant impairment of glucose tolerance which was not ameliorated even after changing to a cassava-free diet. Indeed, the rats earlier fed on the protein-deficient cassava diet failed to achieve normal adult weight even with later control diet feeding, unlike those earlier fed on the protein-deficient maize diet. This might imply that previous feeding of cassava flour has a deleterious effect on subsequent weight gain, as it did on glucose tolerance.

There was a slight impairment of glucose tolerance by about 40% in the malnourished rats on maize-based diets when they did not ingest extra cyanide (diet D), but this became gross (about 150% deterioration) when cyanide supplementation accompanied dietary protein deficiency (diet E).

The plasma thiocyanate level closely reflects total dietary cyanide intake (Osuntokun, 1970; Bourdoux *et al.* 1978; Casadei *et al.* 1990). In the present study, plasma thiocyanate levels increased significantly (by 150–300%) on the cassava diets (A and B) and the cyanide-supplemented maize diets C and E indicating some degree of cyanide intoxication (Osuntokun, 1970; Bourdoux *et al.* 1978; Akanji *et al.* 1990; Casadei *et al.* 1990). As

Table 4. *Body weight, pre- and post-glucose-injection plasma glucose and fasting plasma thiocyanate levels in groups of rats at weaning and after 4 weeks on the different maize-based diets**

(Mean values and standard deviations for 6 rats/group)

Treatment group†...	Diet C		Diet D		Diet E	
	Mean	SD	Mean	SD	Mean	SD
Weight (g)	99·2[a]	9·4	68·0[b]	4·7	71·2[b]	7·8
Glucose (mmol/l) at						
0 min	3·9	0·5	5·1	0·6	4·7	0·7
60 min	5·9[a]	1·1	8·0[ab]	1·2	9·9[b]	1·4
120 min	4·4[a]	0·5	7·1[b]	0·9	7·4[ab]	1·3
Incremental area (mmol/l per h)	2·65[a]	1·00	3·91[ab]	1·42	6·56[b]	1·31
Fasting SCN (mmol/l)	0·149[d]	0·016	0·060[e]	0·034	0·151[d]	0·022

[a, b, c, d, e] Mean values with unlike superscript letters were significantly different ($P < 0·01$). Additionally, all differed significantly from weaning values; [b, c, d] also differed significantly from control values ($P < 0·01$).

C, Adequate protein; D, E, protein deficient; C, E, additional cyanide.

* Mean values at weaning and on control diet are on Table 2.

† For details of treatments and diets, see pp. 270–271 and Table 1.

Table 5. *Body weight, pre- and post-glucose-injection glucose levels and fasting thiocyanate levels in the groups of rats initially fed maize-based diets after a further 4 weeks on the control diet**

(Mean values and standard deviations for 6 rats/group)

Initial diet†...	C		D		E	
	Mean	SD	Mean	SD	Mean	SD
Weight (g)	133·2	14·9	129·0	18·0	123·2	21·9
Glucose (mmol/l) at						
0 min	4·3	0·4	5·0	0·4	4·9	0·5
60 min	6·4	0·5	8·4[a]	0·4	8·2[a]	1·0
120 min	5·0	0·6	6·4[a]	0·7	6·3[a]	0·8
Incremental area (mmol/l per h)	2·59	0·81	3·50[a]	0·33	4·02[a, b]	1·63
Fasting SCN (mmol/l)	0·054[b]	0·019	0·064	0·022	0·069[b]	0·019

[a] Mean values significantly different from values on Diets A, B ($P < 0·05$).

[b] Mean values significantly different from values on Table 4 ($P < 0·05$).

C, Adequate protein; D, E protein deficient; C, E additional cyanide.

* Mean values for the rats initially on the control diet are indicated on Table 3.

† For details of treatments and diets, see pp. 270–271 and Table 1.

expected, the rats on the control diet and protein-deficient maize diet (D) had no significant change in plasma thiocyanate levels. The thiocyanate levels returned to normal in all rat groups on changing to the control diet, thus also indicating that they were no longer exposed to cyanide.

Many previous workers have suggested that childhood malnutrition, as induced in the weanling rats here, causes pancreatic acinar damage from ductal blockage by inspissated secretions (DeLange, 1974; Nwokolo & Oli, 1980). This, in turn, results in impairment of glucose tolerance and insulin secretory capacity (Kajubi, 1972; Milner, 1972; Rao &

Raghuramulu, 1972; Weinkove *et al.* 1977). Likewise, the cyanogenetic glycosides present in cassava, linamarin and linostraulin, could be hydrolysed during processing or in the small intestine, to produce cyanide which has been described to be toxic to the pancreatic islets whether in rats (Handler, 1945; McMillan & Geevarghese, 1979) or in humans (Vannasaeng *et al.* 1982). One could, thus, speculate that the cyanide ingested by the rats fed on cassava and cyanide-supplemented maize diets should be toxic to their islet cells.

However, the results observed differed slightly between the groups fed on cassava and those given maize. In both groups protein malnutrition definitely caused a partly reversible impairment in glucose tolerance. Cassava intake, however, was associated with a persistent impairment of glucose tolerance (diet A) which continued even after protein repletion (diet B). Cyanide supplementation of maize-based protein-replete diets had no influence on glucose tolerance, and it is likely that the persistent impairment after protein feeding in rats on diet E represents lingering effects of the early protein malnutrition. This was in spite of the increased plasma thiocyanate levels on both diets (although slightly higher on the cassava diets), indicating some degree of cyanide intoxication. It is unlikely that the rats ate less of the cyanide-supplemented meals than the cassava meals as the weight gains were similar on either diet (with and without an adequate protein content). One might, therefore, speculate that the diabetogenic effect of cassava is much more than that of a cyanide-supplemented maize diet, despite nearly similar degrees of cyanide exposure on both, because cassava possesses an additional diabetogenic property. Indeed, in the protein-malnourished rats in the present study, the degree of glucose intolerance induced, with and without dietary cyanide supplementation, was much lower than that observed with identical cassava-based diets. This observation has important implications, particularly in tropical populations consuming a predominantly cassava-based diet.

It would, therefore, appear that cassava consumption and protein malnutrition have separate and additive effects in the development of glucose intolerance, possibly not directly related to the cyanogenetic potential of cassava. The nature of the additional diabetogenic factors specific to cassava remains unknown. However, as indicated earlier, the cyanide content of cassava is naturally complexed in the glycosides linamarin and linostraulin (Tewe, 1975) and only released during processing of the fresh cassava root or digestion in the mammalian gut. This manner of presentation may influence its cyanogenic potential. Also, cassava is inherently nutritionally poor, and its prolonged intake, to the exclusion of other foodstuffs richer in vitamins and trace elements, may have an additional influence on its effect on glucose utilization. Maize is nutritionally more balanced in this respect.

These findings need to be interpreted cautiously, especially in relation to human diabetes. It is unlikely that humans, even in areas of endemic cassava consumption, will consume cassava continuously to the exclusion of other foodstuffs. Moreover, processing of fresh cassava root before human consumption results in significant loss of cyanide content (Tewe, 1975; Akanji *et al.* 1990). Additionally, adequate protein intake can moderate the toxic effects of prolonged cassava intake.

In conclusion, we report that our studies in growing rats suggest that: (1) continuous cassava intake and protein malnutrition have independent and additive effects on the development of glucose intolerance which are not consistently reversible, (2) sub-lethal cyanide supplementation of maize diets impaired glucose tolerance only in the protein-malnourished rats, an effect only partly reversible, (3) chronic cassava intake may have effects on the development of glucose intolerance which are independent of the degree of cyanide intoxication.

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